REVIEW

REVISED Unveiling the fibrotic puzzle of endometriosis: An overlooked concern calling for prompt action

[version 3; peer review: 2 approved]

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Abstract

Endometriosis is a benign, estrogen-dependent, persistent chronic inflammatory heterogeneous condition that features fibrotic adhesions caused by periodic bleeding. The characteristic ectopic lesions are marked by a widely spread dense fibrotic interstitium comprising of fibroblasts, myofibroblasts, collagen fibers, extracellular proteins, inflammatory cells, and active angiogenesis. Fibrosis is now recognized as a critical component of endometriosis because of which current treatments, such as hormonal therapy and surgical excision of lesions are largely ineffective with severe side effects, high recurrence rates, and significant morbidity. The symptoms include dysmenorrhea (cyclic or noncyclic), dyspareunia, abdominal discomfort, and infertility. The significant lack of knowledge regarding the underlying root causes, etiology, and complex pathogenesis of this debilitating condition, hinders early diagnosis and implement effective therapeutic approaches with minimal side effects presenting substantial hurdles in endometriosis management. Emerging research offer a close relationship between endometriosis and fibrosis, which is believed to be tightly linked to pain, a primary contributor to the deterioration of the patient's quality of life. However, the underlying pathophysiological cellular and molecular signaling pathways behind endometriosis-associated fibrosis are poorly addressed. The available experimental disease models have

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tremendous challenges in reproducing the human characteristics of the disease limiting the treatment effectiveness. Future translational research on the topic has been hindered by the lack of an adequate fibrotic model of endometriosis emphasizing the necessity of etiological exploration. This review article focuses on recent developments in the field and highlight the necessity for novel fibrotic models for early diagnosis, a better understanding the disease's etiology and develop effective anti-fibrotic treatments. By addressing these knowledge gaps, we want to open fresh avenues for a thorough investigation and extended research in the field of endometriosis.

Keywords
Endometriosis, pelvic pain, etiology, animal model, Epithelial-mesenchymal transition, fibrosis

Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 2**

In the current version, changes have been made according to the reviewer's suggestion. The duplicative statements present in the introductions were deleted and sentences were rephrased to improve the clarity. In the section "Endometriotic Models: Importance of Addressing Gaps in Preclinical Animal Models" in addition to the rodent models, nonhuman primate models were included to provide a more complete overview. In the section "Primate Model of Endometriosis", surgically induced endometriosis models in non-human primates, particularly the baboon model were included. As advised by the reviewer, Table 1 was modified to include a more detailed classification of endometriotic fibrotic rodent models, and new references were added. A new table 2 outlines the type of endometriosis tissue used, specific fibrotic markers evaluated, and key pathways. The section "Human Experiment Details" was completely rewritten to include details relevant to human clinical endometriosis. Another section about the Interplay of EMT and MMPs in Endometriosis has been included in the text. A new figure 2, summarizes a comparison of different animal models (non-human primates, mice, rats) and their strengths and weaknesses. Figure 1 has been modified to improve the resolution.

Any further responses from the reviewers can be found at the end of the article

Introduction

Endometriosis is an estrogen-dependent chronic inflammatory disorder resulting from the implantation of viable endometrial, epithelial, and stromal cells (lesions) outside the uterus and is often associated with infertility.[1] The condition affects at least 10% (~247 million) of women worldwide, with Asian women reporting the highest prevalence, with over ~42 million girls and women from India,[2,3] which can negatively affect the outcome of IVF treatments.[4,5] Endometriosis can result in severe dysmenorrhea, dyspareunia, and menorrhagia; exacerbates pelvic/abdominal pain; and eventually leads to infertility due to considerable damage to the structure and function of reproductive organs, even compromising the entire body system through the accumulation of fibrotic tissue.[6] The diagnosis can take 4 to 11 years due to difficulties in classifying and identifying the disease and its peculiar symptoms, as well as a lack of diagnostic indicators.[7] According to Maddern et al., endometriosis has a significant effect on a person's quality of life, reproductive health, and society at large.[8] Currently, the most widely recognized theory explaining how endometriosis begins is "Sampson's theory", which holds that the misplaced viable endometrium-like tissue is transferred onto the pelvic peritoneum by retrograde menstruation via the fallopian tubes.[9] Even after several decades of research, the etiology is still unclear and depends on a few key theories and assumptions, such as retrograde menstruation theory, embryonic remnants, coelomic metaplasia, immune dysfunction, inflammation, oxidative stress, hormones, dysfunctional apoptosis, the microbiome, metabolomics, endocrinology, and genetic expression differences, which fail to explain its pathophysiology[2,9] adequately. Although retrograde menstruation occurs in 90% of reproductive-age women, only 10% develop endometriosis, indicating that additional relevant factors contribute to disease onset and progression within the peritoneal cavity. This disparity suggests that complex networks contribute to the emergence of this challenging condition.[10,11] This entails understanding how cells from the normal lining of the uterus find atypical locations, multiply excessively, escape immune and apoptotic processes, and acquire the necessary blood supply and nutrients that ultimately result in the formation of aberrant fibrotic lesions that contribute to the distinctive symptoms triggered by endometriosis, including excruciating pain and infertility.[12] None of the available theories fully capture the intricacies of fibrotic endometriosis, emphasizing the need for additional studies to identify the pathophysiology of endometriosis.[13] The production of fibrotic tissue comprising fibroblasts, myofibroblasts, collagen fibers, and inflammatory cells is increasingly recognized as a crucial element contributing to disease severity, resistance to treatment, and high recurrence rates. This paucity of understanding of the molecular and cellular mechanisms encouraging fibrotic endometriosis provides an important barrier to the development of effective diagnostic tools and therapeutic strategies.[14] Moreover, the American Society of Reproductive Medicine (rASRM) categorization score approach does not account for pathology-based staging on the basis of fibrosis, which includes epithelial-to-mesenchymal transition (EMT), mesenchymal-to-epithelial transition (MET), or smooth muscle metaplasia (SMM). This means that patients with fibrotic characteristics and adhesions may fail to obtain a reliable diagnosis.[15] Integrating fibrosis-specific indicators into diagnostic standards should increase the reliability of endometriosis diagnosis and staging, allowing for more targeted and successful treatment options.[16] The formation, invasion, and angiogenesis of fibrotic ectopic lesions are also associated with disrupted immunoregulatory processes and a variety of inflammatory markers, including immune cells, cytokines, chemokines, matrix metalloproteinases, and other components associated with the immune system.[17,18] Thus, a thorough understanding of the mechanisms underlying the origin and evolution of fibrotic endometriosis is crucial for managing and evaluating the risks associated with this condition. This review highlights the critical need to investigate and outline the molecular drivers of fibrotic endometriosis. In this review, we intend to address these gaps by providing a detailed understanding of the role of fibrosis in endometriosis, evaluating existing endometriotic models, identifying significant research gaps, and proposing new directions for exploration. We emphasize that an improved understanding of fibrotic pathways in endometriosis may aid in the development of novel therapeutics that target fibrosis, thus improving the prognosis of patients.

Method

We conducted an electronic database literature search of PubMed and Google Scholar for published research articles on endometriosis and endometriotic animal models. The search terms "endometriosis", "endometriosis mouse model", "primate model of endometriosis", "endometriotic patients", and "endometriosis-associated fibrosis" were used. Articles with thorough experimental data and definitive results were considered for inclusion; those with inconclusive research findings were eliminated. We incorporated clinical trials, surveys of endometriosis-affected women, and observational and experimental studies, including animal studies, as references. Research written in languages other than English was not considered. All the graphics were prepared via Biorender software (BioRender.com).

Literature review

Endometriotic models: Importance of addressing gaps in preclinical animal models

Owing to the unavailability of definitive treatments and the limited understanding of endometriosis, researchers have attempted to develop animal models to provide insights into its causes and to identify novel therapeutic targets. The most extensively studied animal models for endometriosis include autologous or syngeneic rodent models, xenotransplantation of human endometrial tissue into immunodeficient mice, and, to a lesser extent, owing to ethical considerations and expensive costs, nonhuman primate models.[19] The most significant distinction between these models is that endometriosis develops spontaneously in nonhuman primates but not in rodents.[19] According to Greaves et al., endometriosis is currently being studied via two basic approaches: human-based *in vitro* samples and experimental *in vivo* animal models.[20] The first type involves experimental *in vitro* research using tissue biopsies and fluids obtained from resected lesions or aspiration biopsies, such as endometrial and peritoneal explants, endometriotic cell lineages, primary endometrial stromal cells, endometrial stem cells, and immune cells.[21] *In vivo* animal models are essential for assessing drug candidates and preclinical trial testing. Our knowledge of the early phases of disease development, including the effects of the peritoneal microenvironment, inflammatory responses, and steroid responsiveness, has improved because of these models.[22] However, for a variety of reasons, it has been difficult to create *in vitro* or *in vivo* models that accurately replicate the features found in endometriotic patients. Endometriosis is complex, multifactorial, and heterogeneous, and the uncertainty underlying its onset further complicates the development of reliable models. Second, the disease manifests in several forms, including peritoneal, deep infiltrative lesions, and ovarian endometriomas, each exhibiting distinct pathological characteristics.[23] Finally, endometriosis cannot be effectively characterized based on a single pathophysiological mechanism. Additionally, this condition is connected with genetic,[24] immunological,[25] environmental,[26,27] and hormonal changes, such as progesterone resistance[28] and estrogen reliance,[29] further challenging the establishment of acceptable animal models (Figure 1). Additionally, most animal models fail to adequately mimic crucial characteristics of human endometriosis, such as persistent chronic fibrosis. These limitations hinder the successful translation of research findings to human disease settings. These findings emphasize the need for a higher-fidelity mouse model that better portrays the complex pathophysiology of endometriosis in humans.[19,30] Despite these constraints, progress has been made in the development of representative endometriosis models, but these existing models have major limitations, emphasizing the need for additional research to bridge this gap in knowledge.

Considering all of these factors and all the possible limitations of rodent models, researchers have focused on nonhuman primates (NHPs), such as baboons (*Papio anubis*) and rhesus monkeys, because they spontaneously develop endometriosis and menstruate in a cyclic pattern. Interestingly, even in NHPs, surgically induced endometriosis reduces fertility, much like it does in humans. Cynomolgus monkeys (*Macaca fascicularis*) with moderate or severe endometriosis have been shown to have lower rates of fertilization and pregnancy following surgery.[31] In addition, subfertility due to endometriosis is tied to disease stage in baboons.[32] The work by Nishimoto-Kakiuchi et al.[33] presents novel and crucial insights from a nonhuman monkey for translational research in endometriosis, where they carefully examined screening, diagnosis, staging, and monitoring in a population of cynomolgus monkeys. They proposed a robust methodology that has the benefit of employing an animal model with a lower body size than baboons do, making it easier to monitor and handle in an experimental setting. However, the major limitation of this model is the reduced incidence rate of endometriosis, which is only 28.7%.[33] In this context, NHP models appear to be the best model animals for endometriosis research owing to their phylogenetic, anatomical, and reproductive similarities to humans. Moreover, they experience spontaneous endometriosis, as observed in humans.[34,35] However, in some species (*Papio anubis*), the menstrual period is nearly every 4 weeks, corresponding to that of humans. Indeed, the diagnosis of spontaneous disease in NHP models is problematic, as a substantial animal number is necessary for induction, and there is a lack of accurate noninvasive tools for early detection.[36] Nonetheless, NHP models are useful for studying the etiology, development, and progression of the disease and possibly evaluating the efficacy of drugs. However, more research is needed to confirm the effectiveness of the "biological response," which is correlated with endometriosis and its symptoms. This could lead to improved diagnostic accuracy and early detection in NHP models, which would be in line with the main goals of clinical endometriosis research in humans.

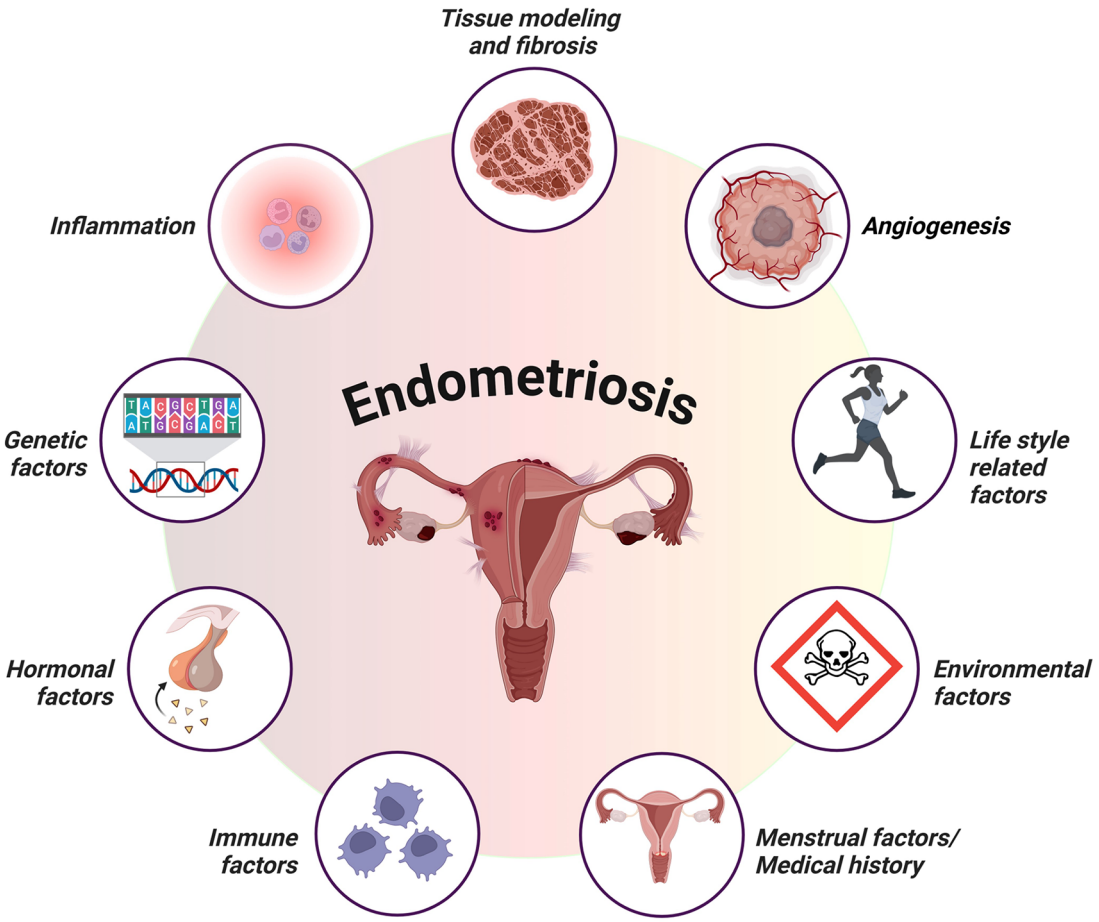

Figure 1. Schematic representation of key factors contributing to the development and progression of endometriosis. The illustration highlights the interplay between genetic factors, hormonal imbalances, immune dysfunction, and inflammation, including lifestyle-related and environmental factors. These factors collectively influence lesion establishment, persistence, and growth, providing a comprehensive overview of the multifactorial nature of endometriosis pathophysiology (created with Biorender.com).

Fibrotic endometriosis overview: knowledge gaps and challenges

Endometriosis is characterized by the persistent occurrence of fibrosis and myofibroblasts within endometriotic lesions, which play critical roles in disease development, making fibrosis a molecular hallmark of endometriosis.[37] Notably, significant scarring is commonly linked to endometriosis.[37] Although the initial onset of endometriosis is associated with the existence of endometrial stroma and glands in abnormal locations, the endometrial components are often soon replaced by fibrotic and smooth muscle components.[38] For example, rectovaginal nodules display glandular epithelium embedded deeply within fibromuscular tissue devoid of any surrounding stroma.[39] Similarly, in 40% of ovarian endometriomas, the endometrial epithelium is not detected, and the interior of the cyst is covered solely by fibrotic tissue.[40] Despite being a crucial pathological feature of this disease, pelvic adhesions generally lack any endometrial components.[41] These adhesions contribute to the pathology of some common symptoms of endometriosis, including chronic pelvic pain, deep dyspareunia, and infertility presumably aggravated by these fibrotic formations.[41] The process by which endometriosis progresses to a malignant condition remains unknown. However, continuous inflammation, immunological dysregulation, and fibrosis, most likely caused by iron-induced oxidative stress, may lead to genetic changes, which may lead to malignant features.[14,42] Fibrosis is believed to be linked to pain, which is the disease's most common symptom and the principal cause of a patient's poor quality of life.[43] Thus, understanding the underlying mechanisms will help to understand why the morphological characteristics of the disease do not match the degree and nature of fibrosis-related pain reported.[44]

Fibrotic tissue is characterized by excessive development of extracellular matrix (ECM) components inside and around inflamed or damaged tissue, and it is a typical and significant phase of tissue repair in all organs. Fibrosis involves activated platelets, macrophages, and myofibroblasts, which results in increased collagen deposition.[45] Fibrosis and

smooth muscle metaplasia are two of the main characteristics of endometriosis in women with fibrosis surrounding endometriotic tissue, and the degree of fibrosis is correlated with the severity of smooth muscle metaplasia.[46] Endometriotic lesions are thought to be "wounds" that undergo repeated tissue injury and repair (ReTIAR), leading to TGF-β1/Smad3-mediated EMT and ultimately resulting in fibrosis as the lesions progress. In essence, regardless of location or subtype, all endometriotic lesions are recognized to be identical to wounds that undergo ReTIAR, ultimately resulting in the fibrotic appearance of both ovarian endometriomas (OMAs) and deep infiltrating endometriosis (DIE).[47,48] This process enables solitary cells to pass through the basement membrane, grow invasively, and metastasize by both intra- and extravasation.[9] However, if the underlying mechanisms are known, they may explain why the disease's morphological characteristics do not match the extent and nature of fibrosis-induced pain sensations.[49] However, there is a paucity of information on the development of preclinical models that define clinically effective endpoints such as chronic fibrosis. Additionally, Modi et al.'s mouse model of endometriosis revealed considerable inflammation but lacked histological signs of fibrosis, with neither EMT nor fibrosis commonly reported in such models.[50] Consequently, studies on the molecular pathways associated with fibrosis or possible targets for therapeutic intervention for fibrosis in endometriosis have been stopped because of the unavailability of an animal model of endometriosis.[50,51] Furthermore, 50–70% of drugs that have advanced to phase II and III clinical trials are unable to show efficacy, indicating the insufficiency of current disease models in the exploration of critical biological processes.[52] These findings suggest that there are no reliable animal models for examining significant cellular processes associated with endometriosis. Given the chronic nature of the disease, we believe that chronic fibrosis may play a major role in the progression of endometriosis, potentially leading to fibrotic adenomyosis. In summary, an optimal model for understanding endometriosis that mimics the cellular and pathophysiological processes and clinical behaviors observed in human patients, notably fibrosis coupled with invasion and metastasis, is needed. Despite these limitations, considerable improvements have been made in the development of endometriotic models for fibrosis-based research studies.

Primate model of endometriosis

Endometriosis is challenging to eliminate because of the inadequate understanding of its genesis and pathophysiology. Controlled experimental investigations on humans are limited because assessing disease prevalence and development necessitates numerous laparoscopies, which are challenging for multiple reasons. Although endometriosis occurs spontaneously in humans, human investigations have been limited for ethical and practical reasons, with one of the primary reasons being the difficulty of studying the disease. As a result, understanding the etiological mechanisms of this disease requires the use of an appropriate animal model. The evidence for fibrosis has primarily been derived from *in vitro* experiments on human endometriotic tissues and *in vivo* studies on nonhuman primates, which are potential candidates for research because of their anatomical and physiological resemblance to humans.[34] Endometriosis is recognized to occur exclusively in menstrual animals, including nonhuman primates, such as rhesus macaques[53] and baboons,[54] because their endometrial morphology, physiology, and menstrual cycle are nearly identical to those of women.[54] Baboons are capable of developing spontaneous endometriosis, which makes them particularly relevant models for investigating this disease.[55] Two types of endometriotic models have been established in baboons. Spontaneously[34] and experimentally generating endometriosis via autologous endometrial transplantation.[56,57] Moreover, induced endometriosis in NHPs closely resembles spontaneous endometriosis that develops in women.[58] It was also reported that iatrogenically induced retrograde menstruation might lead to the onset of endometriosis, validating the concept of Sampson. Endometriosis was experimentally generated in rhesus macaques via surgical diversion of the cervix into the abdomen. However, endometriosis has been identified in only 50% of animals.[59] The first baboon experimental model of nodular endometriosis was established by Donnez et al. in 2023 for the exploration of deeper nodular lesions as well as invasion events connected with nodular lesions.[60] Frequent surgical interventions, however, have been shown to provoke the spontaneous growth of endometriotic lesions and could modify the functionality of the endometrium.[61]

According to Zhang et al., a baboon endometriosis model demonstrated the progressive nature of EMT, FMT, and fibrosis. This led to the expansion of fibrosis from minor fibrosis at three months to highly fibrotic lesions at twelve months after endometriosis induction. This strongly suggests the progressive nature of the disease.[47] Additionally, histological analyses revealed that fibrosis in baboon endometriosis closely mirrors that observed in human cases, making it an appropriate model for investigating disease progression and treatment outcomes in patients with fibrosis. Donnez et al. discovered altered morphology, elevated mitotic activity, and fewer adhesion molecules in invasive glands associated with induced nodular endometriosis, implying that cell migration is involved in the process of invasion of deep fibrotic endometriotic lesions generated in a baboon model.[62] A model of iatrogenic deep nodular endometriotic lesions was developed to construct an experimental model of replicating human deep nodular fibrotic lesions.[60] Deep nodular endometriotic lesions created in the baboon closely mirror spontaneous deep-infiltrating nodules in invasive and noninvasive lesions.[60,63] A recent investigation in baboon models indicated that the overexpression of IL-6 enhances the expression of fibrotic factors, inducing fibrosis via the TGF-β signaling pathway. These findings in baboons closely match those in humans with endometriosis reinforcing the concept that fibrosis is a critical component of the disease course.[64]

Limitations of nonhuman primate models in endometriosis research

The use of NHP in endometriosis research is not free of potential drawbacks or limitations. First, the low incidence rates, i.e., 4.8% and 20.7%, of spontaneous and induced endometriosis, respectively, demonstrate that baboons can cleanse and regenerate their peritoneum, which may decrease the significance of the model.[65] In contrast, in rhesus monkeys,[66,67] the significance of peritoneal cysts in endometriosis pain and discomfort has not been investigated. The cynomolgus monkey[33,68] has been described, with the limitations that deep lesions are difficult to diagnose and that time course changes in the condition are not investigated. Other challenges include a relatively small cohort of endometriotic animals for experimentation, an extended period of gestation for fertility research, a longer duration to develop endometriotic lesions, the difficulty of dealing with conscious baboons, and the high cost of experimentation and maintenance, which require larger doses of medications, specialized infrastructure, logistics, and special training for handling these animals. It is also perceived to be ethically sensitive and expensive.[57,69] Consequently, rodent models are commonly used for preclinical efficacy testing for therapeutic interventions owing to their reduced costs and ease of handling.

Rodent models of endometriosis

Preclinical modeling is crucial for investigations of disease pathogenesis, biomarker development, and preventative and therapeutic discovery. This is particularly true for complex conditions, such as endometriosis, where nonsurgical diagnostic techniques to allow longitudinal clinical study designs remain unavailable. Rodents are frequently employed as preclinical models in biomedical research since they are molecularly well-annotated species. This permits researchers to utilize different interrogative strategies to dissect multifactorial disorders. Their usefulness for examining the molecular foundations of disease pathogenesis lies in the simplicity of genetic modifications and their ability to target potential genes for specialized study.[70] Additionally, given the lack of accessibility and high costs related to nonhuman primates, rodents offer a convenient and inexpensive alternative for researching the origins and course of disorders such as endometriosis. However, because research facilities for primates/nonhumans are limited, nonprimate experimental animal species, such as mice or rats, are regarded as suitable first-line tools for researching the origin of this puzzling disease. Endometriosis is characterized by the recurrent development of new lesions with each menstrual cycle and the advancement of preexisting lesions. Therefore, additional research is needed to understand the natural course and gradual development of endometriosis lesions.[71] There is evidence of gradual lesion clearing, but only a small number of studies using mouse models of endometriosis have investigated disease induction and regression.[71,72] While rodent models have been valuable for researching the disease, especially its pathophysiological and molecular underpinnings, gaps exist in understanding fibrotic lesion progression. Most importantly, owing to the ethical limits of frequent laparoscopic screening of endometriotic patients, rodent models provide essential longitudinal investigations to increase the translational value of preclinical findings.[71]

Mice are the most popular experimental animal models because of their ease of gene manipulation, availability, easy handling, tissue similarity *in vivo*, small size and large litter, which make them cost-effective, and their relatively short gestation, which allows transgenerational examination.[22] On the basis of the available research publications, two types of mouse models have been successfully used to implant endometriotic lesions. The first approach involves suturing, where human endometriotic implants are surgically autotransplanted into the peritoneum of immunocompromised mice.[73–75] The second approach involves the intraperitoneal or subcutaneous implantation of autologous uterine segments into the peritoneum of recipient mice from a syngeneic donor.[76–78] Mouse models have aided in investigating several aspects of this disorder, such as early disease phases,[79] steroid hormone involvement,[80] host inflammatory mechanisms,[81,82] oxidative stress,[83,84] neuroangiogenesis,[76] and infertility.[85] While these methods have enhanced our understanding of disease pathways, challenges persist. For example, immunocompetence is a difficulty when employing human uterine tissue or human endometriotic tissue in a mouse model. Immunocompromised mice may not reflect the environment within the human peritoneal cavity, and the outcomes of the experiment may not correctly reflect disease onset.[86] In ovariectomized mouse models generated with exogenous estrogen, estrogen reliance drives lesion progression in endometriosis; however, these models add surgical factors and off-target effects. Because endometriosis mirrors natural hormonal cycles, hormonally intact mice offer a more realistic representation.[75] However, mice, like other members of the rodent family, typically do not menstruate and hence do not develop endometriosis spontaneously. They also have a closed reproductive system and are highly fragile with respect to dietary needs. Consequently, earlier studies modeling endometriosis utilizing mice required stimulation of menstruation or endometrium transplantation for the development of endometriotic lesions.[70] Hence, there are publications that claim that these lesions do not adequately mirror real endometriosis, as they lack features such as persistent fibrosis.[87]

On the other hand, rats can produce only superficial lesions, which are the most fundamental and possibly least clinically significant types of lesions. Many studies using rodents as a model for endometriosis have investigated the gene expression patterns of ectopic tissue deposits in rats in an attempt to correlate them with human endometriotic lesions. Chronic inflammation, angiogenesis, and extracellular matrix remodeling are common pathways.[86–88] While some

aspects of the disease are replicated in the rodent model, all the modifications involve suturing uterine fragments (endometrium plus myometrium) to different sites, which does not accurately represent the formation of lesions from those shed endometrial tissue or the dissemination of menstrual tissue into the peritoneum. Notably, particularly in terms of understanding its pathophysiology and treatment options, the current rodent models have not been successful in yielding findings that apply to human endometriosis. The inability of any study to recreate fibrotic endometriotic lesions may account for the failure of rat models to yield data relevant to the pathophysiology and treatment of human endometriosis. This situation demonstrates that the preclinical animal studies that have been established are not transferable.[89] Therefore, fibrosis, a mostly disregarded component of human endometriosis, should be taken into consideration.[89,90] We reviewed the existing mouse models in the context of the optimal parameters found in well-evidenced pathophysiologic aspects identified in endometriosis (Table 1). Collectively, these models have yielded critical insights and advanced the replication of the molecular characteristics of this disease. Owing to their ability to model chronic fibrosis, mouse models constitute a powerful resource for translational research in endometriosis. Therefore, developing novel rodent models that mirror the continuous fibrotic process observed in endometriotic patients is essential for improving our understanding of this disease. Emerging research has recently focused on the role that fibrosis plays in clinical-grade endometriosis. On the other hand, little is known about fibrosis treatment strategies. Therefore, developing a fibrotic mouse model of endometriosis, elucidating the regulatory processes underlying fibrosis in endometriosis, and identifying more precise specific biomarkers for this disease are critical. These markers can also be utilized to find effective therapeutic targets and identify endometriosis in its early phases. The successful translation of potential discoveries obtained in a preclinical model to humans is dependent primarily on model fidelity. To mimic the fibrotic scarring observed in endometriosis, many endometriotic fibrotic mouse models have been developed (Table 1).

Table 1. Summary of available mouse models of endometriosis, demonstrating the presence of fibrotic markers. The table includes details on the type of model, approach used for model development, and specific fibrotic markers and pathways explored. This analysis emphasizes the heterogeneity in fibrotic marker expression across different models and provides insights into their relevance for researching the fibrotic elements of endometriosis.

Experimental model	Induction method	Fibrotic genes involved	Mechanism	Inflammatory response	References
BALB/c	Surgical method	TGF-β, COL1A1 and COL3A1, α-SMA	Platelet activation contributing to EMT, FMT, and SMM	Activation of TGF-β1	93
Swiss nude mice	Transplantation of human endometrial tissues	α-SMA, COL1A1, fibronectin, CTGF	Cell proliferation and migration Enhanced collagen gel contraction Wnt/β-catenin signaling	Wnt/β-catenin interaction with TGF-β1	94
C57BL/6	Transplanting shed endometrial tissue from female donor mice into recipient mice	Fibronectin, COL1A1	Shed endometrial tissue as a key source of pro-inflammatory mediators thereby driving fibrosis	IL-6, TNFα, CCL2 and CCL5	76
BALB/c	Intraperitoneal injection of uterine fragments from donor mice	α-SMA, FSP-1/ S100A4, Desmin, vimentin	EMT, FMT, SMM, MMT, EndoMT	SP and CGRP sensory nerve-derived inflammatory mediators	95
BABL/c nude mice	Transplanting endometrial tissue into the peritoneal cavity of mice	Fibronectin, ColA1, α-SMA, and CTGF	Paracrine signaling of eMSCs	Thrombospondin 4	96
Swiss nude mice	Implanting pieces of autologous endometrial tissue into the peritoneal cavity of the mice	A-SMA, Col-I, FN and CTGF	TGF-β signaling	TNF-α, IL-6	97

Table 1. *Continued*

Experimental model	Induction method	Fibrotic genes involved	Mechanism	Inflammatory response	References
BALB/c	Intraperitoneal injection of human eutopic endometrial tissue	Collagen I, α-SMA, and CTGF	CTGF signaling	-	98
C57BL/6	Heterotransplantation with immortalized human endometrial cells	α-SMA, COL1A-I, FN and CTGF	mTOR signaling	-	99
C57BL/6	Donor endometrial tissue fragments transplanted into the recipient	α-SMA, COL1AI, TGF-β1	Platelet activation and fibrosis	CD41	100
Athymic nude mice	Subcutaneous injection of proliferative endometrial fragments	α-SMA, COL1A1, CTGF, FN	Wnt/β-catenin pathway	TGF-β1	101

Alpha-smooth muscle actin (α-SMA), connective tissue growth factor (CTGF), fibronectin (FN), transforming growth factor beta (TGF-β), COL1A1 and A3 (collagen types 1 and 3), epithelial-to-mesenchymal transition (EMT), fibroblast-to-myofibroblast transdifferentiation (FMT), smooth muscle metaplasia (SMM), tumor necrosis factor α (TNFα), monocyte chemoattractant protein chemokine ligands 2 and 5, fibroblast-specific protein 1 (FSP1), S100 calcium-binding protein A4 (S100A4), TNF-α (tumor necrosis factor-alpha) and IL-6 (interleukin-6), the mesothelial-mesenchymal transition (MMT), the endothelial-mesenchymal transition (EndoMT), endosome-derived mesenchymal stem cells (eMSCs), and mammalian target of rapamycin (mTOR).

Limitations of rodent models in endometriosis research

Endometriosis is termed the 'missing disease' because of its ambiguous etiology and discrepancies in its origin, diagnosis, and treatment.[102] Despite a recent surge in endometriosis research, the underlying pathobiology of the disease remains poorly known, implying that animal models of the disorder are crucial for future studies in this field. This ambiguity highlights the need for animal models that precisely mimic human endometriosis and elucidate its conditions, which can provide a basis for subsequent research.[103] One of the most significant obstacles in endometriosis research is the lack of reliable mouse models that characterize the manifestations of this condition in humans.[104] Ideally, a disease model should mirror human disease, allowing researchers to investigate the effects of intrinsic (e.g., genes) and extrinsic (e.g., environment) factors on disease progression. Many previous studies linked fibrosis secondary to the development of endometriosis, and there has not been much research on fibrosis as a primary focus.[15,105] Research from animal models revealed that a percentage of women receiving hormone therapy in human trials do not respond to these drugs[105] and require surgical lesion removal to alleviate symptoms. Women may have endometriotic lesions that have progressed to a fibrotic state by the time they seek medical attention, rendering treatment ineffective. This highlights the urgent need to develop an *in vivo* model that can effectively mimic the development and characteristics of human endometriosis, opening avenues for more effective treatments and a deeper understanding of this disease. These findings will also facilitate the understanding of the connection between the origin of fibrosis in endometriosis, existing medical care, and potential targets for therapy. In conclusion, although the literature emphasizes the importance of fibrosis in the course of endometriosis, gaps remain in understanding the underlying genes and pathways related to the fibrotic aspect of the disease. While existing rodent models highlight certain factors, such as inflammation and immune dysregulation, they often overlook fibrosis, thus poorly reflecting the complexity of the disease. In addition, these models insufficiently depict the degree of severity, traits, and drivers of fibrosis in clinical human endometriosis. Additionally, the complex interplay of signaling mechanisms that promote lesion formation in a fibrotic milieu remains inadequately studied. These limitations highlight the demand for improved fibrotic-based animal models that accurately replicate the disease and offer an in-depth investigation of fibrotic pathways. Although studies have provided insight into genes that contribute to fibrosis in endometriosis, further exploration of the complicated signaling networks underlying this disease remains important. This gap highlights the necessity for future investigations employing advanced methodologies such as knockout animal models, high-throughput RNA sequencing, and omics techniques. These techniques provide greater insights into the mechanisms of fibrotic markers and assist in confirming their function in endometriosis growth, providing strong evidence for the creation of medications that delay, terminate, and reverse fibrosis advancement and benefit endometriotic patients. Additionally, many of the current animal models of endometriosis can be further enhanced by altering them to allow noninvasive *in vivo* monitoring of lesion size, as this approach is desirable for preclinical models of endometriosis.

Human experiment details

After years of relentless advocacy from individuals affected by the condition, endometriosis is gradually gaining increased attention, as evidenced by an increase in research, particularly large-scale controlled human trials and meta-analyses, which have the potential to significantly increase awareness of the condition and its management. Except for several NHPs, animals do not develop endometriosis spontaneously; hence, *in vitro* models employing human tissues have been employed to research the pathophysiology of this medical condition (Table 2). The majority of currently known *in vitro* models utilize several cell or tissue types, including endometriotic cell lines as monolayer culture models, human primary endometrial epithelial and stromal cells, endometrial stem cells, endometrial explant cultures, and coculture models with peritoneal cells and immune cells.[106–108] Each model exhibits unique characteristics and functions and is able to illustrate one or more components of the process of endometriosis. These models are helpful and can be used to explore the origin of endometriosis and the underlying mechanisms of this condition in depth and assist investigators in selecting relevant models for their research.[21] In recent years, researchers have developed different *in vitro* models of varying complexity that provide helpful tools to unravel the processes involved in the etiology of endometriosis. Most cell culture methods are maintained in 2D settings; however, more advanced 3D models are becoming more prevalent to improve the specific endometriosis milieu. They offer the chance to examine endometriotic cell connections with surrounding cells and analyze unique cross-talk between cells.[106] Patient-obtained tissues of ectopic and eutopic endometria or biopsy samples from endometriotic cysts and fluids from women with and without endometriosis undergoing laparoscopy for diverse research goals are being used. However, the protocol variation employed for collecting, processing, and storing samples certainly restricts the compilation and repeatability of data produced at different research institutions.

According to Fan 2020, in addition to studying the origin and mechanisms behind fibrosis in endometriosis, *in vitro* models are a viable tool for investigating therapeutic innovations for the management of endometriosis.[21] The idea that endometriosis is a fibrotic disease has prompted studies to explore how myofibroblasts differentiate and how fibrosis develops in endometriotic lesions. This will lead to the development of new models that can be used to study endometriotic fibrosis. Thus, future studies should concentrate on myofibroblast differentiation and activity in endometriotic lesions. Advances in *in vitro modeling* technology could revolutionize the study of endometriosis pathophysiology and allow the discovery of new targets to develop effective treatment approaches.

Table 2. Overview of *in vitro* studies on endometriosis tissues demonstrating the presence of fibrotic markers. The table outlines the type of endometriosis tissue used, specific fibrotic markers evaluated, and key pathways. This compilation highlights the contributions of *in vitro* systems in unraveling the molecular mechanisms underlying fibrosis in endometriosis.

Sample type	Fibrosis associated markers	Pathway	References
OE/Ovarian cysts	Collagen I, α-SMA, Fibronectin	TGF-β1/Smad signaling	109
DIE or OE	α-SMA, collagen I, CTGF	Wnt/β-catenin signaling	110
DIE with or without OE	AKT and ERK	AKT and ERK signaling	111
Endometriotic ectopic implants	α-SMA, collagen I	ADAM17/Notch signaling	112
OE or DIE	α-SMA, N-cadherin, Vimentin, Snail, Slug, Desmin, Fibronectin, LOX, PAI1	TGF-β1, PDGF, Wnt/β-catenin	113
OE	α-SMA, COL1A1, CTGF, FN	mTOR signaling	99
OE	GLI3, HOXA10 and HOXA9, MAPK8 (JNK1), GATA2, ETS2	TGF-β signaling, MAPK signaling pathway, FoxO signaling pathway	114
Endometriomas	FAK, MCP1, TGF-β1, α-SMA	PI3K/Akt and focal adhesion kinase (FAK) pathways	115

OE - Ovarian endometrioma, DIE - Deep infiltrating endometriosis, Transforming Growth Factor β1 (TGF-β1) Pathway, Platelet-Derived Growth Factor (PDGF) Pathway, Wnt/β-catenin Pathway, α-SMA (alpha-Smooth Muscle Actin), COL1A1 (Collagen Type I Alpha 1 Chain), CTGF (Connective Tissue Growth Factor), FN (Fibronectin), rapamycin (mTOR) signaling, GLI3: GLI Family Zinc Finger 3, HOXC8, HOXA9 and A10: Homeobox C8 and A10, MAPK8: Mitogen-Activated Protein Kinase 8 (also known as JNK1), ETS2: ETS Proto-Oncogene 2, Transcription Factor, GATA2: GATA Binding Protein 2, FAK (Focal Adhesion Kinase), TFAP2C: Transcription Factor AP-2 Gamma, PRDM1: PR/SET Domain 1 (also known as BLIMP-1).

Interplay of EMT and MMPs in endometriosis

Endometriosis is a common benign gynecological disease with a high propensity for migration and invasion. The cell-to-cell or cell-ECM connections allow the cells to migrate, invade, and proliferate in new locations. MMPs are linked to adhesion, invasion, and the severity of endometriosis. These findings indicate that MMPs play a role in extracellular matrix remodeling, which is necessary for the development of ectopic endometriosis lesions.[116] They are also significantly more abundant in the endometrial and peritoneal fluid of endometriosis patients.[117,118] Matrix metalloproteinases (MMPs) are a family of enzymes that are mostly found in the functional layer of the endometrium. They are secreted by resident immune cells and stromal fibroblasts, which facilitate the remodeling of the extracellular matrix, including collagen, elastins, and other glycoproteins, and endometrial disintegration during menstruation. Tissue inhibitors of matrix metalloproteinases (TIMPs) are endogenous antagonists that reduce MMP overexpression, and ovarian steroid hormones are known to control MMP activity.[119] EMT is a process in which epithelial cells lose the polarized structure of the cytoskeleton and acquire the enhanced motility of mesenchymal cells. These modifications are considered necessary for the original formation of endometriotic lesions. While fibrosis has been recognized as a prominent component of endometriosis, its importance is underexplored, particularly in relation to EMT.[37,120] For early clinical studies of EMT, the nude mouse is a suitable model, particularly for the identification of MMP-2 and TIMP-2, proteins that seem to play a significant role in the pathophysiology of EMT. Estrogen specifically increases MMP-2 expression to encourage ectopic implantation of the endometrium. On the other hand, progestin can suppress TIMP-2 expression, increasing the MMP-2/TIMP-2 ratio and increasing the invasiveness of the ectopic endometrium to facilitate implantation.[121] In ovarian endometriosis, MMP7 facilitates EMT; EGF increases MMP7 expression by activating the ERK1–AP1 pathway.[122,123] MMP14 affects the development and function of invadopodia, which in turn modulates the ability of mesenchymal cells to invade and migrate.[124] MMP-2 and MMP-9, two important enzymes involved in the destruction of diverse types of ECM, have been linked to the development of endometriosis by regulating endometrial cell invasion.[125] Both MMP-2 and MMP-9 have been shown to function as biomarkers of both EMT and triggering factors that contribute to the progression of EMT.[126] Despite this, it is apparent that MMPs play crucial roles in the production of collagen, which is necessary for the gradual development of endometriosis fibrosis.[99] These findings suggest that there may be a precise equilibrium between collagen synthesis and breakdown, which should be investigated further. As a result, we hypothesize that MMPs may be crucial in controlling the endometriosis-related EMT process. However, further research is needed to fully understand the connection between MMPs and EMT-induced fibrosis in endometriosis, as there are not enough comprehensive studies on this topic.

Discussion

Endometriosis is an underdiagnosed chronic inflammatory disease that affects millions of people around the world. The primary explanation for endometriosis growth is the transplantation of living endometrial cells that are refluxed after menstruation, thereby attaching to and invading other pelvic organs and leading to inflammation and fibrosis.[2] Despite its broad incidence and importance, endometriosis research has significant limitations.[127] The gaps include a lack of understanding of the disease's etiology, a delay in diagnosis that necessitates invasive treatments, and the difficulties of integrating electronic health records for research, which aids in identifying potential therapeutic tools and reminds us to look beyond endometriotic lesions.[128] Currently, 50 to 70% of endometriotic drugs that have advanced to phases II and III in clinical trials are unable to show efficacy, suggesting an unfulfilled research gap in the development of appropriate animal models.[129] Endometriotic fibrosis shares characteristics with other fibrotic conditions, including increased myofibroblast and smooth muscle cell activity, high levels of fibrotic-associated growth factor and protein production, epithelial–mesenchymal transition, and collagen deposition.[15] There is substantial evidence that fibrosis is a molecular characteristic of endometriosis etiology along with other molecular hallmarks, such as immunological dysregulation, ER expression, progesterone resistance, chronic inflammation, angiogenesis, and epigenetic changes.[15] Interestingly, fibrosis, as a histologic feature of lesions, can progress, most likely due to repeated tissue injury and repair caused by inflammation-induced recurrent menstrual bleeding.[47,127] Thus, a thorough understanding of the disease process is needed for progress in the fields of biomarker identification and nonhormonal therapy. Fibrosis may impair drug administration and efficacy. Rather, a study into the mechanisms that resolve fibrosis will uncover new possibilities by discovering new targets for pharmacologically regulating this condition, notably in the pharmacology of multicomponent medications.[128,130] Because chronic fibrosis plays vital role in various human body systems, robust longitudinal studies are needed to [a] confirm biomarkers and underlying mechanisms linked with fibrosis progression, providing insights into disease causes and potential diagnostic or prognostic tools. [b] To investigate temporal dynamics to record the progression of fibrosis over time, researchers can better comprehend its development from early stages to advanced stages, thereby allowing early intervention and personalized treatment methods. [c] Investigating treatment efficacy, or the effectiveness of various interventions for fibrosis, can provide useful data on long-term outcomes and responses. [d] To better understand the natural course of fibrosis, including its variations among individuals, potential triggers, and variables influencing its progression, preventive and targeted therapeutics should be created. [e] To determine whether the inflammatory environment of endometriosis is involved in fibrosis. The potential pathways by which endometriosis

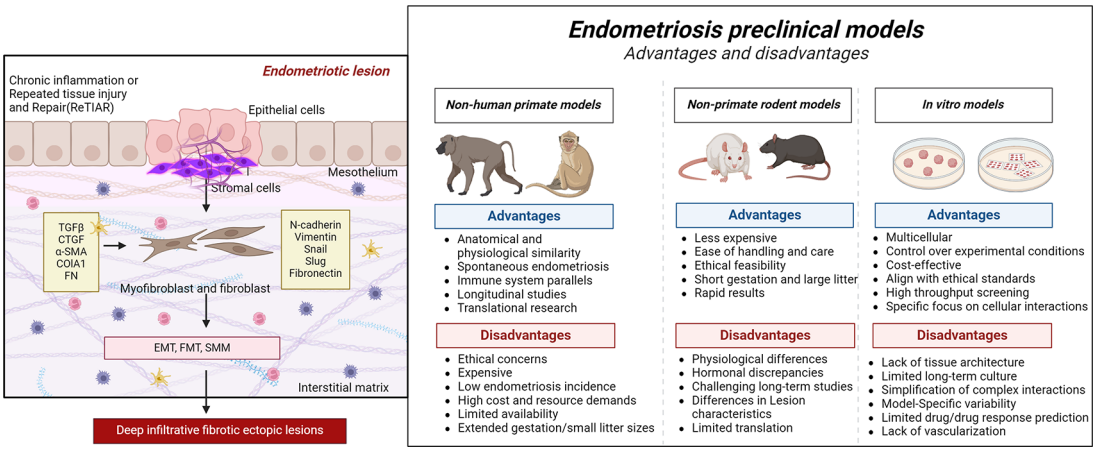

Figure 2. Schematic representation displaying the endometriotic lesion microenvironment and a comparative analysis of nonprimate and nonhuman primate models, emphasizing their advantages and disadvantages in investigating endometriosis. This image shows the importance of selecting appropriate models on the basis of unique research objectives (created with Biorender.com).

participates in fibrosis require additional exploration. Indeed, developing fibrosis-specific treatments for endometriosis remains a major challenge. Therefore, drug repurposing could be a viable approach in this quest. Novel anti-fibrotic drug Pirfenidone has been shown to decrease postoperative adhesion formation following laparoscopic endometriosis surgery.[131] Considerable research has been made in the specific blockade of cytokines or their downstream signaling pathways for the treatment of fibrotic diseases in general. Nonspecific targeting of S100A4 has been tried using Niclosamide that performs blanket targeting of several signalling pathways, including S100A4, mTOR, STAT3, and NF-κB.[132] However, a peptide antagonist of RAGE (ELKVLMEKEL) was developed based on the sequence of the RAGE-binding domain of HMGB1. The antagonist also worked well to prevent interaction between S100A4 and RAGE.[133] The antagonist peptide, named RAGE-antagonist peptide (RAP), has been evaluated as an anti-inflammatory drug in various inflammatory diseases.[134] In previous studies, RAP has been found to bind to RAGE and reduce signal transduction-mediated RAGE. The efficacy of RAP as an antifibrotic intervention was validated in bleomycin-induced pulmonary fibrosis.

Identifying the root cause of endometriosis is more difficult because the disease's missing components, such as persistent fibrosis, are yet to be duplicated in experimental rodent models. Filling these gaps may lead to more accurate patient diagnoses, more effective treatments, and improved information on how the condition affects women's lives. Any therapy that helps lessen the fibrotic element of the disease will have far-reaching repercussions for the individual, the population, and the healthcare system. This study contributes to the careful choice of animal models tailored in line with the research objective or study question to improve our understanding of endometriosis (Figure 2). These findings emphasize the multisystem characteristics of endometriosis, as well as the need for researchers to think beyond only the endometrial lesion. As anticipated, no single cause can entirely explain the onset of endometriosis. However, these investigations emphasize the need for new therapeutic techniques to increase the quality of life of endometriotic patients. The advancement in model development represents a large step forward, delivering promising research with the potential to yield real benefits for patients. Implementing these findings in clinical practice could dramatically shorten diagnostic delays and offer additional insight into the epidemiological elements of the disease.

Conclusion

Endometriosis is a prevalent gynecological condition that significantly affects the physical and emotional well-being of female patients because of its invasive and recurrent characteristics. Fibrosis, as a histological characteristic of lesions, may progress, presumably due to recurrent tissue injury and repair. In a nonhuman primate model of endometriosis, the predominant type of peritoneal lesion transitioned from red vesicular to white fibrotic over the course of time. However, the association between endometriosis and fibrosis is poorly understood. Additionally, EMT may play a role in the etiology of endometriosis through immunological regulation, the production of proinflammatory cytokines, and other mechanisms. Clinical trials have shown that targeting EMT-induced fibrosis can help treat endometriosis, establishing a new research direction and theoretical foundation for the diagnosis and treatment of fibrotic endometriotic patients. As randomized, double-blinded investigations of endometriosis in women are difficult and at times ethically restrictive, animal models for endometriosis have evolved into vital tools for obtaining a mechanical understanding of the etiology

and pathophysiology mechanisms of this complex condition. Thus, it is vital to examine the molecular pathways that drive and sustain fibrosis in endometriosis via a novel fibrosis-based animal model to discover new pharmacological targets and provide creative therapeutics for patients. Furthermore, the research connecting endometriosis and fibrosis has added a further complicating factor to the shared strategy for dealing with endometriotic patients with infertility, as well as a potentially essential concern in the counseling and management of the condition for those desiring future fertility. Well-designed longitudinal studies are needed to improve clinical decision-making in these contexts. Although gynecological surgeons are aware of the complex role of fibrosis in the surgical treatment of endometriosis, the molecular pathways that relate fibrosis to endometriosis-associated pain and infertility remain unknown. Thus, more research is needed to better understand the clinical implications of fibrosis and identify it as a molecular marker of endometriosis etiology, a potentially important element to consider when counseling and managing endometriotic patients who are planning to have children in the future. Well-designed longitudinal studies are needed to make more informed clinical decisions in these contexts. However, the challenges of heterogeneity, diagnostic difficulties, treatment variability, high attrition, and ethical concerns make these studies complex and resource-intensive. Therefore, efforts should be focused on building trustworthy models that incorporate physiologically relevant cells, such as organoids and microfluidics. The continued creation of mouse models to aid in understanding the processes of endometriosis development offers the best chance of creating therapeutic options to prevent or reverse this mysterious disease. This review aims to spark a debate on the need to improve the present understanding by focusing on the fibrotic features of endometriosis pathogenesis. We believe that this approach will shed new light on this condition and suggest areas that need to be investigated further.

Data availability statement

No data are associated with this article.

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Current Peer Review Status: ✔ ✔

Version 3

Reviewer Report 11 December 2024

https://doi.org/10.5256/f1000research.175006.r345346

✔ **Asgerally T Fazleabas**
Michigan State University, Grand Rapids, MI, USA

The authors have responded appropriately to our concerns. I now approve the manuscript.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 12 November 2024

https://doi.org/10.5256/f1000research.171658.r332392

✘ **Asgerally T Fazleabas**
Michigan State University, Grand Rapids, MI, USA
Yong Song
Michigan State University, East Lansing, Michigan, USA

This review, titled "Unveiling the Fibrotic Puzzle of Endometriosis: An Overlooked Concern Calling for Prompt Action," addresses the role of fibrosis in endometriosis and highlights the need for improved animal models to study this aspect of the disease. While the topic is important there are significant omissions that do not make this a comprehensive review as the authors claim.

The specific comments are as follows:

1. In the Introduction: The sentences, "*Retrograde menstruation is prevalent in healthy women, and only a small population of women develop this condition, contributing to the understanding of complex mechanisms that underlie the onset of this challenging condition. While 90% of women of reproductive age undergo retrograde menstruation to the pelvic cavity, only 10% of them develop endometriosis. These findings suggest that the onset and progression of the disease in the peritoneal cavity depend on additional relevant factors*." contain duplicative statements. These could be streamlined to improve the clarity.
2. Section "Endometriotic Models: Importance of Addressing Gaps in Preclinical Animal Models": The preclinical animal models include both rodent and nonhuman primate models, but only rodent models were discussed. Including nonhuman primate models here would provide a more complete overview.
3. Section "Primate Model of Endometriosis": This section only discusses spontaneous endometriosis. It would be beneficial to include surgically induced endometriosis models in non-human primates, particularly the baboon model, which is valuable for simulating disease establishment and progression. Discussing the surgically induced non-human primate model with respect to fibrosis would add depth to this section.
4. Although the mouse model does not fully capture the fibrosis characteristics seen in human endometriotic lesions, it remains essential for exploring fibrosis' mechanisms and therapeutic approaches. This model has been valuable for studying the role of inflammation in fibrosis development and developing the methods that detect endometriotic lesions. A more detailed classification of endometriotic fibrotic rodent models in Table 1 would also strengthen this discussion.
5. Section "Human Experiment Details": The subtitle "Human Experiment Details" is misleading, as this section includes not only human studies but also rodent and non-human primate studies. Consider combining this section with "Interplay of EMT and MMPs in Endometriosis" into a summary of mechanisms contributing to fibrosis in endometriosis. While this section discusses several genes and pathways associated with fibrosis, a more in depth discussion of mechanisms, with specific examples of pathway interactions that promote fibrosis, would enhance the context of the manuscript.
6. The manuscript would benefit from a more detailed comparison of different animal models (non-human primates, mice, rats) and their individual strengths and weaknesses concerning fibrosis research in endometriosis. A table or figure summarizing this information could help readers better understand which models are suitable for addressing specific questions related to this pathology.
7. Table 1 References: Ensure that all references in Table 1 are accurate; for instance, Reference 99 does not involve any animal model.
8. The quality of Figure 2 is not good, and abbreviations should be written out in full in the figure legend for better readability.

Is the topic of the review discussed comprehensively in the context of the current literature?

Partly

Are all factual statements correct and adequately supported by citations?

Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endometriosis

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 18 Nov 2024

Rahul Dutta

Dear Prof Fazleabas
Accept our gratitude for your detailed critical inputs for improving our manuscript. We have attempted to incorporate most of the suggested changes in the current version of the M/S. We have made changes to the tables and added new figures to improve the quality of review. We enlist the changes made in detail below. We once again, thank you for the opportunity to improve the work,

Reviewer comment:

1. In the Introduction: The sentences, “Retrograde menstruation is prevalent in healthy women, and only a small population of women develop this condition, contributing to the understanding of complex mechanisms that underlie the onset of this challenging condition. While 90% of women of reproductive age undergo retrograde menstruation to the pelvic cavity, only 10% of them develop endometriosis. These findings suggest that the onset and progression of the disease in the peritoneal cavity depend on additional relevant factors.” contain duplicative statements. These could be streamlined to improve the clarity.

Author response: Despite retrograde menstruation occurring in 90% of reproductive-age women, only 10% develop endometriosis, indicating that additional relevant factors contribute to the disease's onset and progression within the peritoneal cavity. This disparity indicates complex networks contributing to the emergence of this challenging condition.

Reviewer comment:

1. Section “Endometriotic Models: Importance of Addressing Gaps in Preclinical Animal Models”: The preclinical animal models include both rodent and nonhuman primate models, but only rodent models were discussed. Including nonhuman primate models here would provide a more complete overview.

Author response: The order of sub-sections should be changed here.

- Endometriotic models: Importance of addressing gaps in pre-clinical animal models
- Fibrotic endometriosis overview: knowledge gaps and challenges

- Primate model of endometriosis
- Limitations of non-human primate models in endometriosis research
- Rodent models of endometriosis
- Limitations of rodent models in endometriosis research
- Human experiment details
- Interplay of EMT and MMPs in endometriosis

Changes made:
Endometriotic models: Importance of addressing gaps in preclinical animal models
Added at the beginning,
Due to the unavailability of a definitive treatment and limited understanding of the disease, researchers attempted to develop animal models to provide insights into the disease's causes and to identify novel therapeutic targets. The most extensively studied animal models for endometriosis comprises autologous or syngeneic rodent models, xenotransplantation of human endometrial tissue into immunodeficient mice, and, to a lesser extent due to ethical considerations and expensive costs, nonhuman primate models (19). The most significant distinction between these models is that endometriosis develops spontaneously in non-human primates but not in rodents (19).
Figure and its legend changed here. (Figure is in PPT).
Figure 1: Schematic representation of key factors contributing to the development and progression of endometriosis. The illustration highlights the interplay between genetic factors, hormonal imbalances, immune dysfunction, inflammation, including lifestyle related and environmental factors. These factors collectively influence lesion establishment, persistence, growth, providing a comprehensive overview of the multifactorial nature of endometriosis pathophysiology (created with Biorender.com).
Added primate model details to the same section,
Taking into account all of these factors as well as possible limitations of rodents, researchers focused on non-human primates like baboons (Papio anubis) and rhesus monkeys because they spontaneously develop endometriosis and menstruate in a cyclic pattern. It's interesting to note that even in nonhuman primates, endometriosis that has been surgically induced reduced fertility, much like it does in humans. In fact, cynomolgus monkeys (Macaca fascicularis) with moderate or severe endometriosis have been shown to have lower rates of fertilization and pregnancy following surgery (31). In addition, subfertility due to endometriosis was tied with stage disease also in baboons (32). The work by Nishimoto-Kakiuchi et al., (33) presents novel and crucial insights from a non-human monkey for translational research in endometriosis where they carefully examined screening, diagnosis, staging, and monitoring in a population of cynomolgus monkeys. They proposed a robust methodology and which has the benefit to employing an animal model with lower body size instead of baboons, making easier for monitoring and handling in an experimental setting. But the major limitation here is the reduced incidence rate of endometriosis of only 28.7% (33). In this context, it appears that non-human primates might serve as the best model organisms in endometriosis research, taking into consideration the similarities to humans regarding phylogenetics, reproductive biology and anatomy, also the presence of spontaneous endometriosis which is identical to its humans (34,35). However, only in some species (Papio anubis) the menstrual periods are nearly every 4 weeks corresponding to that of humans. Indeed, diagnosis of the spontaneous disease in non-

human primate models is problematic, as a large number of animals is necessary for the induction and there is a lack of accurate non-invasive tools for the early detection (36). In conclusion, even though non-human primate models are useful for studying the etiology, development, and progression of the disease as well as possibly evaluating the efficacy of drugs, more research is needed to confirm the effectiveness of the "biological response," correlating with endometriosis and its symptoms. This could lead to improved diagnostic accuracy and early detection in non-human primate models, which would be in line with the main goals of clinical endometriosis research in humans.
This paragraph was deleted. (Owing to differences in opinions concerning the etiology of the disease, the EMT route has received less attention in the context of endometriosis than it does in cancer research. Recently, most research on EMT in endometriosis has focused on tissues; very few studies have examined the specific transcription factors involved in EMT signaling that are present in endometriotic cells 35 , 36 EMT-related processes in endometriosis have been reported to be far more prevalent in ectopic endometrial lesions than in eutopic endometria, suggesting that EMT may contribute to the development of endometriosis. 37 For example, in fibrosis of organs such as the lungs, liver, and kidney, the involvement of the TGF-β signaling pathway is well documented. 38 TGF-β is an influential growth factor and a chemical that attracts monocytes and is capable of triggering fibrosis and angiogenesis during abnormal growth and promoting the progression of endometriosis. 39 Compared with those of normal women, the peritoneal fluid of stage III and IV endometriosis patients has greater levels of TGF-β. 40) and 27 Endometriosis research is mostly based on nonhuman primate or rodent models due to the apparent limitations and ethical concerns of human experimentation. The available mouse models have aided in investigating several aspects of the disorder, such as early disease phases, 41 steroid hormone involvement, 42 host inflammatory mechanisms, 43 , 44 oxidative stress, 45 , 46 neuroangiogenesis, 47 and infertility, 48 in mice.)
The last paragraph was changed to this.
In summary, we want to bring attention to the need for an optimal model for understanding endometriosis that mimics the cellular and pathophysiological processes and clinical behaviors observed in human patients, notably fibrosis coupled with invasion and metastasis. Despite these limitations, considerable improvements have been made in the development of endometriotic models for fibrosis-based research studies.

Reviewer comment:

1. Section “Primate Model of Endometriosis”: This section only discusses spontaneous endometriosis. It would be beneficial to include surgically induced endometriosis models in non-human primates, particularly the baboon model, which is valuable for simulating disease establishment and progression. Discussing the surgically induced non-human primate model with respect to fibrosis would add depth to this section.

Author response:
Primate model of endometriosis and limitations (All the references are tagged in the original draft)
(Added this at the beginning). Endometriosis is challenging to eliminate due to the inadequate understanding of its genesis and pathophysiology. It is recognized to occur exclusively in menstrual animals, including nonhuman primates, such as rhesus macaques (Zondervan et al., 2014) and baboons (Dick et al., 2003), and has demonstrated significant relevance in the study of endometriosis. Because they undergo menstruation, they provide

a phylogenetically similar model organism to humans. Their identical endometrial morphology, physiology, and menstrual cycle nearly identical to those of women (Dick et al., 2003). Baboons can also develop spontaneous endometriosis, which makes them one of the most suited and relevant models for investigating this disease (Nair et al., 2016).....
.....The cynomolgus monkey (71,72) has been described, with the limitations that deep lesions were difficult to diagnose and time course changes in the condition were not investigated. (Add the following details after this sentence). Two types of endometriotic models have been established in baboons: spontaneous (Fazleabas et al., 2002) and experimentally generated endometriosis via autologous endometrial transplantation (Afshar et al., 2013, Slayden, O. D. 2013) Moreover, induced endometriosis in nonhuman primates demonstrated has been shown to closely resemble spontaneous endometriosis developing in women (D'Hooghe et al., 1995). It was also claimed that iatrogenically induced retrograde menstruation might lead to the onset of endometriosis validating the concept of Sampson. In fact, endometriosis was experimentally generated in rhesus macaques via surgical diversion of cervix into the abdomen. Yet, endometriosis was identified in only 50% of the animals (Kennedy et al., 2019). The first baboon experimental model of nodular endometriosis was established in by Donnez et al., 2023 for the exploration of deeper nodular lesions as well as invasion events connected with nodular lesions (Donnez et al., 2013). Frequent surgical interventions, however, are shown to provoke the spontaneous growth of endometriotic lesions and could possibly modify the functionality of the endometrium (Harirchian et al., 2012).
Regarding the fibrosis aspect of the disease, According to Zhang et al., a baboon endometriosis model demonstrated the progressive nature of EMT, FMT, and fibrosis. This led to the expansion of fibrosis from a minor fibrosis at three months to a highly fibrotic lesion at twelve months after endometriosis induction. This strongly suggests the progressive nature of the disease (Zhang et al., 2016b). Additionally, histological analyses reveal that fibrosis in baboon endometriosis closely mirrors that seen in human cases, making it an appropriate model for investigating disease progression and treatment influences on fibrosis (Giudice et al., 2012). Donnez et al., discovered altered morphology, elevated mitotic activity, and fewer adhesion molecules in invasive glands associated with induced nodular endometriosis implying that cell migration is involved in the process of invasion of deep fibrotic endometriotic lesions generated in a baboon model (Donnez et al., 2015). A model of iatrogenic deep nodular endometriotic lesions was developed in order to build an experimental model of replicating human deep nodular fibrotic lesions (Donnez et al., 2013). Deep nodular endometriotic lesions created in the baboon were shown to closely mirror spontaneous deep-infiltrating nodules in invasive and non-invasive lesions (Donnez et al., 2013, Orellana et al., 2017). A recent investigation in baboon models has increased the understanding of fibrosis in endometriosis. The study indicated the overexpression by IL-6 enhance the expression of fibrotic factors, inducing fibrosis via the TGF-β signalling pathway. These findings in baboons, which closely match human endometriosis, reinforce the concept that fibrosis is a critical component of the disease's course (Ochoa Bernal et al., 2024).
Limitations of non-human primate models in endometriosis research
The use non-human primates in endometriosis research has potential drawbacks or limitations. First. Firstly, the low incidence rate i.e., 4.8% and 20.7% of spontaneous and induced endometriosis, respectively demonstrating that baboons are able to cleanse and regenerate their peritoneum which may downgrade the significance of model (Dehoux et

al., 2011). Other challenges include relatively small cohort of endometriotic animals for experimentation, difficulty of dealing with conscious baboons, and the high cost of experimentation and maintenance which require larger doses of medications, specialized infrastructure, logistics, and special training for handling these animals. It is also perceived to be ethically sensitive and expensive (Grummer, R. 2006, Slayden, O. D. 2013). Consequently, rodent models are commonly used for preclinical efficacy testing for therapeutic interventions due to their reduced costs and ease of handling.

Reviewer comment:

1. Although the mouse model does not fully capture the fibrosis characteristics seen in human endometriotic lesions, it remains essential for exploring fibrosis' mechanisms and therapeutic approaches. This model has been valuable for studying the role of inflammation in fibrosis development and developing the methods that detect endometriotic lesions. A more detailed classification of endometriotic fibrotic rodent models in Table 1 would also strengthen this discussion.

Author response:

(Major changes done in this section).

Preclinical modeling is crucial for investigations of disease pathogenesis, biomarker development, and preventative and therapeutic discovery. This is particularly true for complex conditions, such as endometriosis where non-surgical diagnostic techniques to allow longitudinal clinical study designs remain unavailable. Rodents are frequently employed as a preclinical model in biomedical research since they are a molecularly well-annotated species. This permits researchers to utilize different interrogative strategies to dissect multifactorial disorders. Their usefulness for examining the molecular foundations of disease pathogenesis lies in the simplicity of genetic modifications and their ability to target potential genes for specialized study (70). Additionally, given the lack of accessibility and high costs related to non-human primates, rodents offer as a convenient and inexpensive alternative for researching the origins and course of disorders like endometriosis. However, because research facilities for primates/non-human are constrained, non-primate experimental animal species, such as mice or rats, are regarded suitable first-line tools for researching the origin of this puzzling disease. Endometriosis is characterized by the recurrent development of new lesions with each menstrual cycle and the advancement of preexisting lesions. Therefore, additional research is needed to understand the natural course and gradual development of endometriosis lesions (71). There is evidence of gradual lesion clearing, but only a small number of studies using mouse models of endometriosis have investigated disease induction and regression (71,72). While rodent models have been valuable for researching the disease, especially its pathophysiological and molecular underpinnings, gaps exist in understanding fibrotic lesion progression. Most importantly, due to the ethical limits of frequent laparoscopic screening of endometriotic patients, rodent models provide essential longitudinal investigations to boost the translational value of preclinical findings (71).

Mice are the most popular experimental animal models because of their ease of gene manipulation, availability, easy handling, tissue similarity in vivo, small size and large litter, which make them cost-effective, and their relatively short gestation, which allows transgenerational examination (22). Based on the available research publications, two types of mouse models have been successfully used to implant endometriotic lesions. The first approach involves suturing, where human endometriotic implants are surgically auto-

transplanted into the peritoneum of immunocompromised mice (73–75). The second approach involves the intraperitoneal or subcutaneous implantation of autologous uterine segments into the peritoneum of recipient mice from a syngeneic donor (76–78). The mouse models have aided in investigating several aspects of the disorder, such as early disease phases (79) steroid hormone involvement (80), host inflammatory mechanisms (81,82), oxidative stress (83,84), neuroangiogenesis (76), and infertility (85).While these methods have enhanced our understanding of disease pathways, challenges persist. For example, Immunocompetence is a difficulty when employing human uterine tissue or human endometriotic tissue in a mouse model. Immunocompromised mice may not reflect the environment within the human peritoneal cavity, and the outcomes of the experiment may not correctly reflect disease onset (86). In ovariectomized mice models using exogenous estrogen, it was proven that estrogen reliance drives lesion progression in endometriosis; However, these models added surgical factors and off-target effects. Because endometriosis mirror natural hormonal cycles, hormonally intact mice offer a more realistic representation (75). However, mouse like other members of the rodent family, is typically do not menstruate and hence does not develop endometriosis spontaneously. They also have a closed reproductive system and are highly fragile with dietary needs. Consequently, earlier studies modeling endometriosis utilizing mice required stimulation of menstruation or endometrium transplantation for the development of endometriotic lesions (70). Hence, there are publications which claim that these lesions do not adequately mirror real endometriosis as they lack features such as persistent fibrosis (87).

On the other hand, rats can only produce superficial lesions, which are the most fundamental and possibly least clinically significant types of lesions. Many studies using rodents as a model for endometriosis have investigated the gene expression patterns of ectopic tissue deposits in rats in an attempt to correlate them with human endometriotic lesions. Chronic inflammation, angiogenesis, and extracellular matrix remodeling are common pathways (86–88). While some aspects of the disease are replicated in the rodent model, all the modifications involve suturing uterine fragments (endometrium plus myometrium) to different sites, which does not accurately represent the formation of lesions from those shed endometrial tissue or the dissemination of menstrual tissue into the peritoneum. Notably, particularly in terms of understanding its pathophysiology and treatment options, the current rodent models have not been successful in yielding findings that apply to human endometriosis. The inability of any study to recreate fibrotic endometriotic lesions may account for the failure of rat models to yield data relevant to the pathophysiology and treatment of human endometriosis. This situation demonstrates that the preclinical animal studies that have been established are not transferable (91).Therefore, fibrosis, a mostly disregarded component of human endometriosis, should be taken into consideration (90, 91). We reviewed the existing mouse models in the context of optimal parameters found on well-evidenced pathophysiologic aspects identified in endometriosis (Table 1). Collectively, these models have yielded critical insights and sustained advancement toward replicating the molecular characteristics of this disease. With completing knowledge gaps such as the modeling of chronic fibrosis, mouse models constitute a powerful resource for translational research in endometriosis. Therefore, developing novel rodent models that mirror the continuous fibrotic process observed in endometriotic patients is essential for improving our understanding of this disease. Emerging research has recently focused on the role that fibrosis plays in clinical-grade endometriosis. On the other hand, little is known about fibrosis treatment strategies.

Therefore, developing a fibrotic mouse model of endometriosis, elucidating the regulatory processes underlying fibrosis in endometriosis, and identifying more precise specific biomarkers for this disease are critical. These markers can also be utilized to find effective therapeutic targets and identify endometriosis in its early phases. The successful translation of potential discoveries obtained in a preclinical model to human is primarily dependent on model fidelity. To mimic the fibrotic scarring observed in endometriosis, many endometriotic fibrotic animal models have been developed (Table 1).

(Deleted this). To mimic the fibrotic scarring observed in endometriosis, many endometriotic fibrotic animal models have been developed (Table 1). Furthermore, new in vivo models that use stromal cells generated from menstrual blood have been created to study endometriosis; these models show enhanced endometriotic cell migration and proliferation. 58 Many cues, including estrogen stimulation, may trigger EMT. 88 Furthermore, estrogen-induced EMT in Ishikawa cells promotes adenomyosis. 89 However, how estrogen causes EMT in endometriosis at the molecular level remains unknown. To prevent fluctuations in mouse estradiol levels during the estrous cycle, the majority of established mouse models use ovariectomized mice. 90– 93 As a result, the steady availability of estradiol in the circulation may help promote lesion establishment and growth. However, research on how estrogen-induced EMT in endometriosis affects fertility, such as in women with normal circulating estrogen, is impossible. Therefore, studies of endometriosis produced in intact mice call for more research on the connection between ectopic tissue and fertility.
These findings suggest potential targets for treatment to mitigate fibrosis. (Change this paragraph). Many of the current animal models of endometriosis can be further enhanced by altering them to allow non-invasive in vivo monitoring of lesion size as it is desirable for preclinical models of endometriosis. Although, studies have given insight on identifying genes that contribute to fibrosis in endometriosis, more exploration of the complicated signaling networks underlying the disease remains important. This gap points out the necessity for future investigations employing advanced methodologies such as knockout animal models, high-throughput RNA sequencing, and omics techniques. These techniques provide greater insights into the mechanisms of fibrotic markers and assist in confirming their function in endometriosis growth, providing strong evidence for the creation of medications that delay, terminate, and reverse fibrosis advancement and benefit endometriotic patients.

Table and the legend has been changed.
Table 1: Summary of available mice models of endometriosis, demonstrating the presence of fibrotic markers. The table includes details on the type of model, approach used for the model development, and the specific fibrotic markers and the pathways explored. This analysis emphasizes the heterogeneity in fibrotic marker expression across different models and provides insights into their relevance for researching the fibrotic elements of endometriosis. Alpha-smooth muscle actin (α-SMA), connective tissue growth factor (CTGF), Fibronectin (FN), Transforming Growth Factor Beta (TGF-β), COL1A1 and A3 (Collagen type 1 and 3), epithelial-to-mesenchymal transition (EMT), fibroblast-to-myofibroblast transdifferentiation (FMT), and smooth muscle metaplasia (SMM), tumor necrosis factor α (TNFα) and the monocyte chemoattractant proteins chemokine ligands 2 and 5, Fibroblast-Specific Protein 1 (FSP1), S100 Calcium Binding Protein A4 (S100A4), TNF-α (Tumor Necrosis

Factor-alpha) and IL-6 (Interleukin-6), Mesothelial-mesenchymal transition (MMT), and Endothelial-mesenchymal transition (EndoMT), Endometrium-derived mesenchymal stem cells (eMSCs), mammalian target of rapamycin (mTOR)

Added limitations

Limitations of rodent models in endometriosis research

Endometriosis is termed the 'missing disease' because of its ambiguous etiology and discrepancies in its origin, diagnosis and treatment (103). Despite a recent surge in endometriosis research, the underlying pathobiology of the disease remains poorly known, implying that animal models of the disorder are crucial for future studies in this field. This ambiguity highlights the need for animal models that precisely mimic human endometriosis and elucidate its conditions, which can provide a basis for subsequent research (104). One of the most significant obstacles in endometriosis research is the lack of reliable mouse models that characterize the manifestations of this condition in humans (105). Ideally, a disease model should mirror human disease while also allowing researchers to investigate the effects of intrinsic (e.g., genes) and extrinsic (e.g., environment) factors on disease progression. Many previous studies linked fibrosis secondary to the development of endometriosis, and there has not been much research on fibrosis itself (15,106). Research from animal models clearly revealed that a percentage of women receiving hormone therapy in human trials do not respond to these drugs (106) and require surgical lesion removal to alleviate symptoms. Women may have endometriotic lesions that have progressed to a fibrotic state by the time they seek medical attention, rendering treatment ineffective. This highlights the urgent need for the establishment of an in vivo model that can effectively mimic the development and characteristics of human endometriosis, opening avenues for more effective treatments and a deeper understanding of this disease. These findings will also facilitate the understanding of the connection between the origin of fibrosis in endometriosis, existing medical care, and potential targets for therapy. In conclusion, although literature emphasizes the significance of fibrosis in the course of endometriosis, there remain gaps in understanding the underlying genes and pathways related to the fibrotic aspect of the disease. While the existing rodent models highlight certain factors such as inflammation and immune dysregulation, they often overlook fibrosis, thus poorly reflecting the complexity of the disease. In addition, these models insufficiently depict the degree of severity, traits, and drivers of fibrosis in clinical human endometriosis. Also, the complex interplay of signalling mechanisms that promote lesion formation in a fibrotic milieu remain inadequately studied. These limitations highlight the demand for improved fibrotic based animal models that accurately replicate the disease which offer an in-depth investigation of fibrotic pathways. Although, studies have given insight on identifying genes that contribute to fibrosis in endometriosis, more exploration of the complicated signaling networks underlying the disease remains important. This gap points out the necessity for future investigations employing advanced methodologies such as knockout animal models, high-throughput RNA sequencing, and omics techniques. These techniques provide greater insights into the mechanisms of fibrotic markers and assist in confirming their function in endometriosis growth, providing strong evidence for the creation of medications that delay, terminate, and reverse fibrosis advancement and benefit endometriotic patients. Also, many of the current animal models of endometriosis can be further enhanced by altering them to allow non-invasive in vivo monitoring of lesion size as it is desirable for preclinical models of endometriosis.

Reviewer comment:

1. Section “Human Experiment Details”: The subtitle “Human Experiment Details” is misleading, as this section includes not only human studies but also rodent and non-human primate studies. Consider combining this section with “Interplay of EMT and MMPs in Endometriosis” into a summary of mechanisms contributing to fibrosis in endometriosis. While this section discusses several genes and pathways associated with fibrosis, a more in depth discussion of mechanisms, with specific examples of pathway interactions that promote fibrosis, would enhance the context of the manuscript.

Author response:

The content under human experiment details is completely changed.

Except for several non-human primates, animals do not develop endometriosis spontaneously and hence in vitro models employing human tissues have been employed to research the pathophysiology of this medical condition (Table 2). The majority of currently known in vitro models utilize a number of cell or tissues types, including endometriotic cell lines as the monolayer culture model, human primary endometrial epithelial and stromal cells, endometrial stem cells, endometrial explant culture, co-culture models with peritoneal cells and immune cells (107–109). Each model exhibits unique characteristics and functions and were able to illustrate one or more components of the process of endometriosis. These models are helpful and can be used to explore the origin of endometriosis and the underlying mechanisms of this condition in depth, and assist investigators select relevant models for their research (21). In recent years, researchers developed different in vitro models of varying complexity that provide helpful tools to unravel processes involved in the etiology of endometriosis. Most cell culture methods are maintained in 2D settings; however, more advanced 3D models are becoming more prevalent to better the specific endometriosis milieu. They offer the chance to examine endometriotic cell connection with surrounding cells and analyze unique cross-talks between cells (107). The patient obtained tissues of ectopic and eutopic endometrium or biopsy samples from the endometriotic cysts and fluids from women with and without endometriosis undergoing laparoscopy for diverse research goals are being used. However, protocol variation employed for collecting, processing, and storing samples certainly restricts the compilation and repeatability of data produced in different research institutions

According to Fan 2020, except for studying the origin and mechanisms behind fibrosis in endometriosis, in vitro models are a viable tool to investigate therapeutic innovations for the management of endometriosis (21). The idea that endometriosis is a fibrotic disease has prompted studies to look into how myofibroblasts differentiation and how fibrosis develops in endometriotic lesions. This will lead to the development of new models that can be used to study endometriotic fibrosis. Thus, future studies should concentrate on the myofibroblasts differentiation and activity in endometriotic lesions. Advances in modeling in vitro technology could potentially revolutionize the study of endometriosis pathophysiology and allow the discovery of new targets to develop effective treatment approaches.

New table has been added and legend has been changed

Table 2: Overview of in vitro studies on endometriosis tissues demonstrating the presence of fibrotic markers. The table outlines the type of endometriosis tissue used, specific fibrotic markers evaluated, and key pathways. This compilation highlights the contributions of in vitro systems in unraveling the molecular mechanisms underlying fibrosis in endometriosis. OE- Ovarian endometrioma, DIE-Deep infiltrating endometriosis, Transforming Growth

Factor β1 (TGF-β1) Pathway, Platelet-Derived Growth Factor (PDGF) Pathway, Wnt/β-catenin Pathway, α-SMA (alpha-Smooth Muscle Actin), COL1A1 (Collagen Type I Alpha 1 Chain), CTGF (Connective Tissue Growth Factor), FN (Fibronectin), rapamycin (mTOR) signaling, GLI3: GLI Family Zinc Finger 3, HOXC8, HOXA9 and A10: Homeobox C8 and A10, MAPK8: Mitogen-Activated Protein Kinase 8 (also known as JNK1), ETS2: ETS Proto-Oncogene 2, Transcription Factor, GATA2: GATA Binding Protein 2, FAK (Focal Adhesion Kinase), TFAP2C: Transcription Factor AP-2 Gamma, PRDM1: PR/SET Domain 1 (also known as BLIMP-1)

Reviewer comment:

1. The manuscript would benefit from a more detailed comparison of different animal models (non-human primates, mice, rats) and their individual strengths and weaknesses concerning fibrosis research in endometriosis. A table or figure summarizing this information could help readers better understand which models are suitable for addressing specific questions related to this pathology.

Author response:
Limitations was discussed in corresponding section
Figure 2 has been added for the comparison of primate, rodent and in vitro as suggested
Figure 2: Schematic representation displaying the endometriotic lesion microenvironment and a comparative analysis of non-primate and non-human primate models, emphasizing their advantages and disadvantages in investigating endometriosis. This image shows the importance of selecting appropriate models based on unique research objectives (Created with Biorender.com).

Reviewer comment:

1. Table 1 References: Ensure that all references in Table 1 are accurate; for instance, Reference 99 does not involve any animal model.

Author response:
Changed Table 1 with new references

Reviewer comment:

1. The quality of Figure 2 is not good, and abbreviations should be written out in full in the figure legend for better readability.

Author response:
Changed Figure 2

Competing Interests: We have no competing interests to disclose

Reviewer Report 12 October 2024

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The authors have adequately addressed my concerns and the paper is improved accordingly.

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Partly

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endometriosis; Translational study; Preclinical animal model

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 12 Oct 2024
Rahul Dutta

We are thankful to the reviewers for their constructive input. We thank the reviewers for approving the revised version of the manuscript.

Competing Interests: We have no non-financial or financial competing interests to disclose

Version 1

Reviewer Report 22 August 2024

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Overall Rating: Average
Reviewer Opinion: Major Revision

The manuscript effectively emphasizes the significance of understanding endometriosis-related fibrosis and the limitations of current treatments. It underscores the need for better models and addresses the challenges in understanding the disease's pathogenesis. However, the presentation would benefit from a more structured approach and a clearer focus on specific research gaps and proposed solutions. Adding details on how the review addresses these gaps and improves therapeutic strategies would enhance its impact. While the review offers valuable insights into fibrotic models and treatments, greater clarity on its specific contributions is needed.

- Abstract: The abstract effectively outlines the importance of fibrosis research in endometriosis. Restructuring for clarity and adding specific research gaps and proposed solutions would strengthen overall outlook.
- Introduction:
  - Link to Fibrosis: The introduction does not clearly connect endometriosis issues to fibrosis. Adding specifics on how the review addresses this would improve its rationale.
  - Redundancy: The introduction repeats information on endometriosis mechanisms and diagnostic challenges. Reducing repetition would improve clarity.
- Literature Review:
  - Fibrotic Endometriosis Overview: The discussion on animal model limitations could be more focused. Highlighting specific shortcomings, especially in fibrosis and EMT representation, would provide clearer insights. There is a mouse model for endometriosis and fibrosis (PMID: 30626716).
  - Endometriotic Models: While the challenges of developing accurate models are discussed, more specific examples would be beneficial. Consider adding key limitations of rodent models and clarifying how estrogen-induced EMT impacts translation to human disease.
  - EMT and MMPs in Endometriosis: The discussion on MMPs and EMT lacks detailed mechanisms and direct evidence. Emphasizing experimental findings and strengthening the link between MMPs, EMT, and disease progression would improve this section.
- Discussion: While acknowledging the importance of translating research into clinical care, the section lacks concrete examples of how this might occur or what specific therapeutic advancements are expected.
- Conclusion: The call for longitudinal studies is important, but the section does not address

the obstacles in conducting these studies.

Minor
Abstract: the first sentence: “estrogen-dependent” repeated word
mesenchymal to epithelial transition (FMT) change to “MET”

Is the topic of the review discussed comprehensively in the context of the current literature?
Partly

Are all factual statements correct and adequately supported by citations?
Partly

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Endometriosis; Translational study; Preclinical animal model

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 16 Sep 2024

Rahul Dutta

Dear Mr. Reviewer,
We are thankful to you for the constructive feedback. We have incorporated the suggested improvement into the revised manuscript.

Here are the specific responses to the comments/suggestions-

Abstract: The abstract effectively outlines the importance of fibrosis research in endometriosis. Restructuring for clarity and adding specific research gaps and proposed solutions would strengthen overall outlook.
The abstract has been modified as advised

Introduction:
Link to Fibrosis: The introduction does not clearly connect endometriosis issues to fibrosis. Adding specifics on how the review addresses this would improve its rationale.-
The introduction has been modified as advised

Redundancy: The introduction repeats information on endometriosis mechanisms and diagnostic challenges. Reducing repetition would improve clarity.
The redundant portion has been edited as advised

Minor Abstract: the first sentence: "estrogen-dependent" repeated word- *Removed*
mesenchymal to epithelial transition (FMT) change to "MET"- *Changed*
Literature Review:
Fibrotic Endometriosis Overview: The discussion on animal model limitations could be more focused. Highlighting specific shortcomings, especially in fibrosis and EMT representation, would provide clearer insights.
The overview has been modified as advised
There is a mouse model for endometriosis and fibrosis (PMID: 30626716).
But it is developed in Baboon, what we are trying to discuss here is the fibrotic mice model
Endometriotic Models: While the challenges of developing accurate models are discussed, more specific examples would be beneficial. Consider adding key limitations of rodent models and clarifying how estrogen-induced EMT impacts translation to human disease.
Modified as advised
EMT and MMPs in Endometriosis: The discussion on MMPs and EMT lacks detailed mechanisms and direct evidence. Emphasizing experimental findings and strengthening the link between MMPs, EMT, and disease progression would improve this section.
Modified as advised

We extend our heartfelt gratitude for the feedback.

Competing Interests: None