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Future Directions in Endometriosis Research and Therapeutics

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Abstract

Background: Endometriosis is a disease common among women of reproductive age characterized by pain, anxiety and infertility. Defined as the growth of endometrial tissue in ectopic locations, endometriosis remains an enigmatic disease for which current treatments are less than ideal. Much of these shortcomings to current therapy stem from our incomplete understanding on the pathogenesis of the disease. It is generally accepted that endometriosis is an estrogen-dependent disease and, as such, the majority of treatment approaches aim at reducing estrogen action and/or production. Unfortunately, this approach is not effective in all women with endometriosis and in those women where success is achieved with their use, there is potential for health-comprising side effects.

Objective: The objective of this review is to summarize current approaches for treatment of endometriosis, discuss their limitations and potential reasons for lack of progress towards better therapeutics for this disease.

Results: In this review we summarize the current approaches for treatment of endometriosis, discuss their limitations and potential reasons for lack of progress towards better therapeutics for this disease.

Conclusion: Based upon the current state of knowledge, there is a strong necessity for thorough assessment at the level of the genome, miRNAome and proteome as well as the importance of integrating clinically-relevant endpoints in future studies which evaluate potential endometriosis therapies in experimental models of endometriosis.

Keywords

endometriosis; estrogen-sparing; therapeutics; experimental models; microRNA

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⁶.CONFLICT OF INTEREST

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1. INTRODUCTION

Endometriosis is defined as the growth of endometrial tissue outside of the uterine cavity. The disease occurs primarily in women of reproductive age and is characterized by pain, anxiety and infertility as well as an overall reduction in quality of life [1]. Conventional therapies focus on reducing systemic levels of estrogen which results in cessation of endometriotic lesion survival and pain associated with the disease. However, these treatments are not effective in all women, are associated with unwanted and potentially harmful side effects often leading to patient non-compliance and offer no alternatives to women experiencing infertility. As such, there is considerable interest for identifying and developing novel, estrogen-sparing treatments for endometriosis which would not only be superior compared to current therapies with respect to pain relief and tolerability but also offer novel options to women seeking fertility. In this article we will review and discuss potential estrogen-sparing therapeutic targets as well as discuss future directions in endometriosis research which may lead to the development and implementation of more effective and suitable alternatives to conventional endometriosis therapies.

2. ENDOMETRIOSIS PATHOGENESIS AND TREATMENT APPROACHES

2.1 Pathogenesis

Endometriosis is defined as the presence of ectopic endometrial glands and stroma outside of the uterine cavity with development primary in the pelvic cavity [1]. Several theories and contributing factors have been proposed for endometriosis establishment and survival of the ectopic tissue. Some of these theories such as coelomic metaplasia [2] and endometrial stem cells [3] remain as just that lacking scientific support for these theories. Additional theories which have more definitive evidence include the theory of retrograde menstruation [4] and the embryological theory that claims a prenatal origin for this disease [5, 6].

Of these theories, perhaps the best-supported theory on endometriosis is that of retrograde menstruation [4] which suggests that ectopic endometrial lesions/endometriosis develop in the pelvic cavity due to the retrograde movement of endometrial contents into the peritoneal cavity allowing the establishment of ectopic lesions. While this theory would suggest that only women with retrograde menstruation develop endometriosis, the finding that essentially all women of reproductive age exhibit some degree of reverse menstruation [7] suggests that other contributing factors exist in women who develop the disease. These potential contributing factors include genetic components which may allow for alterations in cellular functions that increase attachment and survival of the ectopic tissue, as well as, immune dysfunction which may prevent elimination of refluxed menstrual debris by immune cells [8, 9]. Once established, additional alterations in the normal apoptotic process of the established lesions may allow for prolonged survival. Further contributing to the survival of endometriotic lesions is the recruitment of immune cells which produce cytokines which may stimulate lesion proliferation coupled with the well-established contribution of endogenous estrogen to lesion growth and survival. The pro-survival effect of estrogen, in conjunction with the progesterone-resistance of ectopic lesions further contributes to sustained lesion survival and persistence of the disease and its associated symptoms.

There is also support for the theory that endometriosis may arise from prenatal origin. In this theory it is proposed that perturbations may occur during the development of the female genital system which results in displacement of endometrial tissue and/or embryonic cell remnants outside the uterine cavity. This in turn leads to the development of ectopic lesions. Supportive evidence for this theory comes from the observation in female fetuses that endometriotic-like lesions (endometriotic glands) have been found upon autopsy [5, 6].

For more detailed information on the potential roles of stem cells, a dysfunctional immune response, genetic predisposition, and aberrant peritoneal environment in the pathogenesis of peritoneal lesions, the reader is referred to a recent review by Sourial and colleagues [8], while a review by Donnez and coworkers [9] discusses the contribution of Müllerian rest to nodular lesions.

2.2 Current approaches to endometriosis treatment

As emphasized in the preceding paragraph, endometriosis is an estrogen-dependent disease and much of our efforts to successfully treat the disease have focused on reducing the levels and/or action of this hormone. Current treatment regimes include oral contraceptives, progestin analogues, Dienogest, Danazol, gonadotropin-releasing hormone (GnRH) agonists and antagonists, levonorgestrel-releasing intrauterine device (IUD-LNG) as well as aromatase inhibitors.

Oral contraceptives, despite limited effectiveness, are often prescribed as a first line approach to treating endometriosis symptoms such as pain [10, 11]. These agents inhibit ovarian estrogen production as well as reduce the inflammatory milieu associated with the disease. Progestins such as medroxyprogesterone acetate (MPA) are also effective in controlling pain, but a drawback associated with its use is side effects such as menstrual irregularities and weight gain [12]. Levonorgestrel-releasing intrauterine device (IUD-LNG) has become a popular treatment option effective in reducing dysmenorrhea, pelvic pain, deep dyspareunia as well as reducing lesion burden [13, 14].

Testosterone derivatives such as norethisterone acetate have also been used as treatments for endometriosis. Norethisterone acetate induces a hypoestrogenic state by suppressing gonadotropin levels and has been shown to be effective in reducing pelvic pain [15]. Dienogest is a derivative of 19-nortestosterone which combines properties of natural progesterone derivatives which exhibits progestogenic action on endometrial tissue [16]. Dienogest is effective in reducing pelvic pain associated with endometriosis [17] and this effect is most likely mediated via complex mechanisms including anti-inflammatory activity [18]. Danazol is a synthetic androgen derivative which is effective in reducing endometriosis-associated pain [19] but is also associated with significant side effects such as weight gain, acne and other androgenic actions [20].

GnRH analogues suppress ovarian estrogen production leading to a hypo-estrogenic state which is detrimental to endometriotic lesion survival. GnRH analogs are often prescribed when oral contraceptives and/or progestin analogues fail to produce successful outcomes. These compounds are effective in some, but not all women with endometriosis [21] and their use is associated with significant side effects including altered lipid profile, hot flushes, loss

of libido and reduction in bone mass/bone health [22]. The loss of bone mass is over-come by estrogen add-back/hormone replacement therapy [23] but this must be balanced to avoid estrogen action upon lesion survival.

GnRH antagonists have also been used for treatment of endometriosis. Unlike GnRH analogues, they do not exhibit agonistic effects inducing estrogen “flare-up” prior to suppressing estrogen levels. Elagolix is the most recent GnRH antagonist evaluated in clinical trials. In a randomized double-blind study [24], elagolix, similar to subcutaneously administered DMPA, had minimal impact on bone mass density and demonstrated similar efficacy on endometriosis-associated pain. However, side effects and a similar discontinuation rate were noted with elagolix use compared to other treatment options [25]. Additionally, the high cost of elagolix is also a barrier to patient care.

The use of aromatase inhibitors in the treatment of endometriosis have gained attention based upon the observation that endometriotic lesions express aromatase and are able to synthesize their own estrogen [25]. While their use has exhibited beneficial effects in some women [26], these beneficial effects only occur with concurrent administration of additional hormonal treatments [27, 28] limitations to their use include recurrences after finishing treatment, severe side effects, and cost. It has been recommended that aromatase inhibitor use be associated with other hormonal treatments only in women in whom all surgical and medical treatments have failed [29].

In summary, the majority of currently prescribed endometriosis treatments still rely upon reduction of estrogen production and/or estrogen action. As emphasized above, while these treatments are effective in many women, they are not effective or well-tolerated in a large proportion of women suffering from endometriosis. These observations coupled with the limitations due to side-effects and potential health complications emphasize the need for the development of novel, estrogen-sparing endometriosis treatments.

2.3 Emerging therapeutic targets for endometriosis treatment

Outside of agents that block estrogen production or action, considerable effort has focused on inflammatory mediators and the potential to target them as a non-hormonal means of treating endometriosis. We have recently reviewed three emerging targets (Table 1) which may hold promise as potential therapeutics in endometriosis treatment [30].

In particular, macrophage migration inhibitory factor (MIF) and prostaglandin E2 (PGE2) have been recently examined as potential targets for endometriosis treatment. Much like earlier studies focusing on TNF- α , experimental model studies have yielded promising results on their ability not only to suppress lesion growth but also to reduce pelvic pain, both independently of reproductive cyclicity. As yet, confirmatory studies in human subjects remain to be initiated. In addition to MIF and PGE2, the estrogen receptor beta (ER- β) pathway has emerged as a potential target for endometriosis treatment. Of interest is the finding that the ER- β pathway appears to mediate many inflammatory mediators associated with endometriosis pathophysiology. While studies which have evaluated the potential utility of the MIF antagonist, ISO-1 for suppressing disease burden have provided encouraging

results [31, 32], these studies did not evaluate clinically-relevant endpoints such as pelvic pain.

More recently, Zhao and colleagues [33] utilized novel ER ligands chloroindazole and oxabicycloheptene sulfonate (which exhibit both anti-estrogenic and anti-inflammatory activity) to dissected the role of both ER- α and ER- β signaling in endometriosis. Both compounds induced lesion regression and suppressed inflammatory events associated with endometriosis without impacting fertility. Thus, the anti-estrogenic/antagonistic effect of these ligands suggest the involvement of the ER- β (and ER- α) pathway in the pathogenesis of endometriosis and that the effects of estrogen antagonism can be separated between those that impact inflammation and lesion regression and those that regulate reproductive cyclicity and fertility. Similarly, a recent study by Han and colleagues [34] suggested that inhibition of the ER- β pathway, which is responsible for inhibiting endometriotic cell apoptosis, and increasing cytokine production, may also be a viable target for endometriosis treatment. While these independent observations are exciting, and the fact that fertility was not compromised by the ER ligands utilized by Zhao and colleagues, neither study evaluated the impact on pain.

In opposition to the studies described above, Arosh and colleagues [35] evaluated the effect of selective inhibition of the PGE2 receptors, EP2/ EP4 on lesion dynamics as well as pain. Inhibition of PGE2 signaling decreased growth and survival, as well as, angiogenesis and innervation of ectopic lesions. Further, inhibition of PGE2 signaling was associated with suppression of pro-inflammatory state of dorsal root ganglia neurons and decreased pelvic pain, as well as a decrease in the pro-inflammatory, estrogen-dominant, and progesterone-resistant molecular environment of the eutopic endometrium and ectopic lesions. Thus, while there appears to be emerging targets for further development and examination, the critical component and perhaps missing link in identifying these agents, may lie in the examination of clinically relevant endpoints coupled with assessing lesions dynamics. This key concept is emphasized in the next section of this review on future directions in endometriosis research.

3. FUTURE DIRECTIONS IN ENDOMETRIOSIS RESEARCH

3.1 Assessment of transcriptome, proteome and miRNAome in endometriosis research

Our ability to successfully treat endometriosis is hindered by our incomplete understanding of the pathogenesis of the disease. As recently emphasized by the World Endometriosis Research Foundation [36], many uncertainties remain regarding the diverse clinical and molecular aspects of endometriosis likely due to inconsistencies in disease classification and variability among control populations. Much of our efforts in identifying potential therapeutic targets for endometriosis treatment are based upon discovery of factors which are mis-expressed in the disease tissue that potentially could play a role in the establishment, proliferation and survival of the ectopic tissue. As such, careful comparison must be made between the “endometriotic tissue” and “control endometrium” to which comparisons are being made. Both “endometriotic lesion” and “control endometrium” must be clearly defined. With respect to endometriotic lesions, ideally, the type of lesion (red, clear, black, etc.) as well as the location within the pelvic cavity from where it was removed (peritoneum,

ovary, ligament, etc.) should be clearly described. Secondly, “control endometrium” must be clearly defined indicating if this is eutopic endometrium from the same patient from which the lesion(s) were obtained, if this was endometrium from a separate individual and the medical diagnosis (fertile control, fibroids, etc.) of these patient.

An additional point to consider is that much of our understanding on altered gene expression between endometriotic lesion tissue and eutopic endometrium is based upon initial cDNA array assessments [37 – 39], or more recently RNA sequencing [40] analysis. While such studies provide a foundation for differential gene expression, changes in protein levels which are not reflected by changes in mRNA expression may be over-looked. This especially holds true with respect to potential post-translational gene regulation by microRNAs (miRNAs). miRNAs are small RNAs which are transcribed from the genome and regulate protein translation by either enhancing translation or repressing translation [41]. Repression of translation can either occur via direct mechanism by which transcript is prevented from entering the translation pathway or indirectly by degrading transcript thereby reducing template for protein translation (Figure 1).

The potential role of miRNAs in the pathogenesis has been an intense area of investigation over the last several years. We have recently reported a functional role of miRNAs on protein targets which have been proposed to play a role in the pathogenesis of endometriosis [42] as have several other groups [43 – 48]. Although the majority of these studies have demonstrated that altered protein expression is associated with altered transcript levels for the protein, the possibility that miRNAs may stabilize transcript levels (resulting in no change in mRNA levels) preventing protein translation (resulting in reduced protein levels) must be fully considered. This point is emphasized by recent findings that miRNAs may induce changes in protein expression levels without impacting mRNA expression. For example, Liu and colleagues [47] demonstrated that despite a lack of difference in transcript expression, altered expression of miR-126 was associated with altered levels of CRK protein. Similarly, Dai and coworkers reported that miR-199a overexpression reduced VEGF-A secretion, but did not affect the VEGF-A mRNA levels in endometrial stromal cells from women with endometriosis [48]. Thus, it is possible that by only assessing transcript levels, potential proteins which may be differentially expressed due to miRNAs, may be overlooked. If a future goal is to identify novel pathways and targets which could hold promise as endometriosis therapeutics, we must apply the most rigorous experimental design to more thoroughly understand the underlying pathophysiology of this truly complex disease.

3.2 Assessment of clinically-relevant endpoints in experimental animal models.

The majority of research with respect to therapeutics in experimental animal models has focused on lesion size which from treatment success standpoint may not be practical as changes in endometriosis stage or lesion regression as a result of treatment are often not evaluated; pain is the major endpoint for success or failure of chemical treatment. The majority of endometriosis research which evaluates potential therapeutic targets begins with rodent experimental models. Rats [49, 50] and mice [35] with experimentally-induced endometriosis exhibit pain, yet very few studies have concurrently assessed the impact of

potential endometriosis therapeutics on lesion survival and pain relief. Concurrent assessment of both endpoints may be critical in identifying potential future therapies.

Another key concept that must be taken into account when assessing both lesion survival and clinically relevant endpoints is the duration of treatment exposure. The majority of experimental animal studies are short term (weeks) and while this time-frame may be sufficient to detect an effect on lesion growth/size, it may not be sufficient to result in a reduction in clinically-relevant endpoints such as pain. We have evaluated lesion survival in an experimental mouse model for endometriosis and demonstrated that lesions can survive for months in this model [unpublished observation]. Thus, future evaluation of potential therapeutics should assure that sufficient time is allotted to detect both reduction in disease burden and relief of pain using appropriate experimental models.

4. CONCLUSION

In this review we have hoped to convey the need for the development of novel, estrogen-sparing treatments for endometriosis. The majority of current treatments still remain rooted in suppressing endogenous estrogen levels and/or action. While effective in many, but not all women, these shortcomings along with reported side effects call attention to the need for novel approaches to treating this disease. We feel that much of the inability to identify novel therapeutic targets stem from our still poor understanding on the pathophysiology, and the heterogeneity of the disease. Additionally, there needs to be a standardization of how endometriosis is studied with particular attention paid to experimental design, clearly defining endometriotic lesions, control populations including consistency among studies, and thorough evaluation of data. Second, when assessing potential novel therapeutic agents, there must be evaluation of clinically-relevant endpoints in addition to examining lesion dynamics. We hope that taking these recommendations into account will lead to the identification of the long-awaited estrogen-sparing targets which will yield beneficial outcomes for women with endometriosis.

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Figure 1. Simplified depiction of canonical miRNA biogenesis pathway.

miRNAs are transcribed from the genome first as pri-miRNAs which are processed to pre-miRNAs. Pre-miRNAs are transported from the nucleus to the cytoplasm where they are further processed to the mature miRNA (indicated by the solid black line). The mature miRNA is then incorporated as part of the RNA-induced silencing complex (RISC) where these small RNAs can either enhance translation or repress translation either directly or indirectly as a result of degraded transcript levels.

Table 1.

Estrogen-sparing endometriosis treatment targets.

Protein target	Endpoints assessed and result
MIF	Lesion growth (decreased) *
	Reproductive cycle (NE) **
ER β	lesion growth (decreased) \$
	Fertility (NE) \$
PGE2	Lesion growth (decreased) &
	Pain (decreased) &

* = reference 26, 27;

** = reference 27;

\$ = reference 28, 29;

& = reference 30.

NE = no effect on endpoint. Abbreviations used: MIF =macrophage migration inhibitory factor, ER β = estrogen receptor beta, PGE2 = prostaglandin E2.