

Review Article

Epigenetics: an emerging research field of infertility associated with endometriosis

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Abstract: Endometriosis is a common gynecologic disease, which is characterized by the presence of uterine endometrial tissue outside the uterine cavity. It is associated with several complications, including pelvic pain, dysmenorrhea and infertility. Endometriosis has drawn wide attention due to its close involvement of infertility in various ways. There are several factors hindering gynecological doctors managing the disease, and therefore novel clues of diagnosis and treatment are largely demanded. Although several previous studies indicated that endometriosis was related with disorder of hormone, immunity, and genetics, and could be caused by overexposure to environmental toxins, and even it was a stem cell disease, our understanding of its etiopathogenesis, especially in infertility, is still inadequate. Endometriosis remains to be an enigmatic disease. Recently, there are an increasing number of studies focusing on epigenetics, a potential common denominator for hormonal and immunological aberrations in endometriosis. Moreover, epigenetics was indicated to be more creditable than genetics in explaining the etiopathogenesis by several studies. The present paper mainly focused on the latest insights into the present and potential contribution of epigenetics in the etiology of infertility associated with endometriosis, aiming to update the current knowledge and explore potential approaches for treatments of this enigmatic disease. All these data with clinical implications can provide new clues for therapeutic applications.

Keywords: Endometriosis, infertility, epigenetics, DNA methylation, histone modification, MicroRNAs

Introduction

As a common gynecologic disease, endometriosis is reported to be benign, estrogen-dependent and chronic inflammatory, which is characterized by the presence of uterine endometrial tissue outside the uterine cavity. It is a debilitating disease that affects approximately 10% of reproductive-aged women and leaving 20%-50% of them bearing with infertility [1]. It is the leading cause for disabling the women of reproductive age, producing inconstant symptoms ranging from no symptoms to severe chronic pelvic pain and infertility [2]. Endometriosis has aroused much concern because of its involvement of fertility in various ways. The association between endometriosis and infertility is generally accepted. However, the etiopathogenesis and pathophysiology of infertility in women with endometriosis are still elusive, and the precise mechanism by which infertility is induced still remains to be clarified.

It is widely accepted that endometriosis is a hormonal and an immunological disease [3]. The occurrence of endometriosis may be also related with some genetic factors and exposure to environmental contamination and toxins [4]. On the other hand, it was reported to be a stem cell disease [5]. It has been suggested that endometriosis-associated infertility may result from disorders of folliculogenesis [6], decreased fertilization, defective implantation, and reduced oocyte quality with low capacity of blastocyst implantation [7-9]. Estrogen and progesterone are responsible for the regulation of numerous gene expressions during the menstrual cycle [10, 11]. In endometriotic tissues, overexpression of estrogen was reported to be associated with the up-regulation of aromatase gene [12]. Accumulating evidence suggest that endometriosis is an epigenetic disease [13-15]. Currently epigenetics is attracting a multitude of attention in biomedical field, which may provide better illustrations for many diseases than

genetics. The present brief review mainly summarizes the up-to-date insights into the present and potential pathogenic role of epigenetics in the endometriosis-associated infertility, which was beneficial for providing novel clues for treatments of this enigmatic disease.

Epigenetics

In biology, the term of “epigenetics” refers to the study of mitotically and/or meiotically heritable changes in gene expression occurring without changes in the underlying DNA sequence or DNA content. The expression of genes may be inherited stably, and phenotypes of cells and organisms can be handed down between generations sometimes [13, 16]. Epigenetic changes in eukaryotic biology are most elegantly illustrated by cellular differentiation where pluripotent stem cells develop into various cell lines of embryo [17]. Epigenetic mechanisms involved in keeping the process stable may include silencing of some genes, removing of silencing marks on some other genes and permanently inactivating still other genes [13]. It involves nuclear processes such as DNA methylation, histone modifications (acetylation, deacetylation, methylation, phosphorylation, etc.), or MicroRNAs (miRNAs) [18], all of which function in a highly orchestrated manner. Enzymes involved in these processes include DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone acetylases (HATs), histone methyltransferases (HMTs), etc. [19]. Both genetic and environmental factors affect epigenetic marks, generating phenotypic variations that range from normal variation to human disease [20]. There are accumulating evidence that epigenetic mechanisms are involved in a large number of human diseases, such as cancers and infertility [17, 18].

Epigenetics for endometriosis-associated infertility

Since epigenetics has been largely investigated, numerous advances in investigating the genetic mechanism by which epigenetics contributes to causing endometriosis-associated infertility are obtained. Researches suggested that epigenetic regulation plays an important role in the establishment of endometrial receptivity and in subsequent embryo implantation.

DNA methylation is the best understood epigenetic alteration, which refers to the addition of a methyl group to the 5-carbon position of cytosines that precede a guanine in the DNA sequence, the CpG dinucleotide [21]. DNA methylation converts cytosine to 5-methylcytosine, which matches correctly with a complementary guanosine [22]. Unmethylated CpGs tend to be clustered as CpG islands, and are related to transcriptional starting sites in gene promoter regions. CpG islands are short stretches of DNA and located in 60% of all genes [23]. DNA methylation at CpG islands is relatively stable, yet reversible [24]. In the process of DNA replication, the maintenance of DNA methyltransferases (DNMTs), such as DNMT1, DNMT3A, and DNMT3B [25], ensures its epigenetic inheritance. Although most CpGs throughout the genome generally tend to be methylated, CpG sites in CpG islands, and especially those associated with gene promoters, are usually unmethylated. Unmethylated CpGs usually participate in active gene transcription [22]. Nowadays it is widely accepted that hypermethylation of promoter is typically associated with gene silencing [13, 26]. Recent years, researchers have discovered several genes hypermethylation associated with infertility in endometriosis patients.

Considerable researches have studied homeobox (HOX) genes, such as HOXA10 and HOXA11, recently. HOXA10 is one member of the homeobox gene family that plays a fundamental role in development [27, 28]. It was shown to be expressed in the eutopic endometrium. HOXA10-null mice are infertile due to the defect of stromal cell decidualization at the site of implantation [29]. HOXA10 and HOXA11 are homeobox genes that mediate embryonic development, including the development of reproductive tract [27, 30]. They are translated into transcription factors regulating downstream genes which are essential for embryonic development and differentiation [31]. Previously, researchers found that aberrant HOX gene expression could alter the development of endometrium at molecular level, which was implicated in the etiology of infertility [32]. The peak of HOXA10 expression occurs during the window of implantation, indicating its possible role in establishing uterine receptivity [33]. Recent human and animal researches have further indicated that reduced expression of HOX gen-

es, especially HOXA10 and HOXA11, in the implantation window of eutopic endometrium may facilitate infertility in women with endometriosis [34, 35]. Taylor et al. [33] certified the expression of HOXA10 to be significantly reduced in the eutopic endometrium of patients with endometriosis during the secretory phase, suggesting the presence of some functional defects in uterine receptivity.

Furthermore, researchers confirmed that significantly higher methylation levels of HOXA10 CpG islands may potentially contribute to silencing HOXA10 expression at molecular level in midluteal endometrium, which may facilitate infertility in women with endometriosis. Szczepanska et al. [36] once examined the HOXA10 transcript, protein and HOXA10 promoter methylation levels in midluteal endometrium from endometriosis-associated infertile and healthy fertile women. They revealed significantly lower levels of HOXA10 transcript, protein and significantly higher methylation levels of HOXA10 promoter in eutopic endometrium from infertile women as compared to healthy women. Later, they studied DNA sequencing of HOXA11 CpG islands, got a similar result which suggested that DNA methylation in HOXA11 CpG rich region II was significantly increased, and hence drew a conclusion that DNA hypermethylation might cause a decrease in HOXA11 expression contributing to endometriosis-associated infertility [35].

In addition, cyclooxygenase (COX) is the rate-limiting enzyme in the transformation of prostaglandins (PGs) from arachidonic acid [37]. There are two isoforms of COX: COX-1 and COX-2, which are constitutively expressed in many tissues, and induced by many factors (such as mitogen, growth factors, and cytokines) [38]. Cyclooxygenase is encoded by two separate genes, *Cox-1* and *Cox-2*. COX-2 is considered to be involved in endometrial cell proliferation, regeneration, and promotion of angiogenesis. It was reported that the increased expression of COX-2 in endometriosis can up-regulate the expression of PGs [39]. Researchers have verified that PGs may be involved in endometriosis-related severe dysmenorrhea and long-term inflammation [40], which further influence the endometrial receptivity and decrease the reproductive ability of women. Later, Haidy et al. [38] demonstrated that the frequencies of the methylation status of the NF-IL6 site within the

COX-2 promoter of the eutopic endometrium of the endometriosis group was significantly decreased compared with controls using methylation-specific PCR (MSP-PCR). Thus, DNA hypomethylation of the NF-IL6 site within the promoter of COX-2 gene could play a pivotal role in its elevated expression, as well as the up-regulation of PGs expression, in the eutopic tissues of endometriosis. This, however, may be one of the etiopathogenesis of endometriosis, resulting in diversified symptoms, especially chronic pelvic pain and infertility.

Moreover, it is reported that MicroRNAs (miRNAs) also plays a key role in regulating gene expression [15, 41]. They are a large class of endogenous, single-stranded, short, non-coding RNAs (ncRNAs) of approximately 22-nucleotides in length [42]. MicroRNAs comprise one of the abundant classes of gene regulatory molecules in related pathways, regulating output of many protein-coding genes through interaction with mRNA of protein-coding genes by either inhibiting mRNA translation or, less frequently, inducing mRNA degradation [15]. As with DNA methylation or histone modifications, miRNAs also regulate gene expression without any change of DNA sequences, they are components of epigenetics regulation [43]. Wakana et al. [44] identified that eight down-regulated miRNAs and four up-regulated miRNAs in endometriotic cyst stromal cells (ECSCs) by miRNA microarray analysis. And they focused on miR-196b (one of eight repressed miRNAs, the gene location of which is in the promoter region of HOXA10) and designed further experiments to investigate the functions of this miRNA, including regulations of the cell cycle, proliferation, differentiation, apoptosis, and cell motility, as well as functions of the anti-proliferation and pro-apoptosis. They found that there was a significant positive correlation between the HOXA10 and miR-196b expression in ECSCs and normal endometrial stromal cells (NESC). The miR-196b gene was hypermethylated in ECSCs compared with NESC. However, their research has some limitations. They failed to identify whether the miR-196b gene methylation in eutopic endometrium of endometriosis patients was higher than that of health people. If true, DNA hypermethylation of the miR-196b gene would partly clarify the mechanism of the decreased expression of HOX genes, which further explain the etiopathogenesis of endometriosis-associated

infertility. Therefore, more studies are needed to prove this hypothesis.

Except for DNA methylation and miRNAs, covalent histone modifications can also orchestrate DNA organization and gene expression. Histone modifications include lysine acetylation, lysine methylation, arginine methylation, phosphorylation, ubiquitination, sumoylation [45]. These modifications lead to subsequent changes in chromatin structure either accessible or inaccessible for gene transcription, thus influencing gene expression [43, 46]. Histone deacetylation, one of the best characterized histone modifications, is associated with gene silencing, however histone acetylation is associated with transcriptional activation [47]. Histone acetylation level depends on the activity of two families of enzymes, including HATs and HDACs.

Xia et al. [48] investigated the abnormal histone acetylation and expressions of chromatin modifier genes in endometriosis. Significant reduction of global histone H4 acetylation level was detected in eutopic endometrium of patients with endometriosis compared with controls. Their results suggested that histone modifications may play an important role in the pathogenesis of endometriosis. Elham et al. [49] studied the epigenetic mechanisms responsible for the aberrant aromatase expression (CYP19A1) in Cumulus Cells (CCs) of endometriosis-associated infertile patients. Their result revealed that, lower acetylation level of H3K9 at the promoter II region occurred in cumulus cells compared with controls. Besides, the hypoacetylation of promoter PII region in the endometriosis certified that the MeCP2 (a marker of DNA methylation) might inhibit gene expression by recruiting histone deacetylase to this area.

Histone modifications and miRNAs work in concert with DNA methylation, which control the chromatin structure and gene expression vigorously [50]. Increasing evidence suggests that an epigenetic cross-talk, namely interplay between DNA methylation and histone acetylation, may be involved in the process of gene transcription and aberrant gene silencing in tumors, so as to endometriosis [14]. However, to date, studies in the influence of histone modifications and miRNAs on fertility in endometriosis patients are relatively limited, and it is worthy of greater efforts to study in these fields.

Implications: novel therapies in the near future

Owing to lack of full understanding about the pathogenesis, the treatments of endometriosis are intractable. In a sense, a better comprehension of the pathogenesis of endometriosis could promote the development of treatment. Current medical treatments for endometriosis are based on methods which aimed lowering circulating oestradiol concentrations (contraceptive steroids, progestagens, and gonadotropin-releasing hormone agonists). However, it can only be used for a limited period because of unacceptable side-effects. While in infertile patients with endometriosis, these medicines are more essential to be carefully evaluated before used. Thus, new nonsteroidal medical therapy is urgent to be developed.

Emerging data have proved evidence that endometriosis is an epigenetic disease, and epigenetics plays an important role in delineating of its pathogenesis and pathophysiology. Epigenetics-based theories provide a powerful way not only for further detailed mechanistic investigation but also the ability to expand the range of and capability for the treatment of endometriosis. Where applicable, attempts are made not only to detail the role of epigenetics in the etiology, progression, diagnosis, and prognosis of endometriosis, but also to present novel epigenetic approaches to the treatment of this disease. The epigenetics-based perspective of aberrant DNA methylation, histone modifications, and miRNAs mechanism enlighten us potential approaches to therapy. For example, medicines for regulating methylation and miRNA expression in eutopic as well as ectopic endometrium have great potential in controlling endometriosis and dealing with endometriosis-associated infertility. Published literatures have shown that histone deacetylases inhibitors (HDACIs) are potential to become therapeutics for endometriosis [45]. Besides, DNA methylation based as well as miRNA-based biomarkers may serve as diagnostic makers or as indicators of therapeutic efficacy predicting recurrence risks. In light of their potentially important roles in endometriosis, miRNAs may well become promising therapeutic targets as their roles and mode of actions are unraveled in future studies. Janice et al. [51] described the histone code of lesions and endometrium from endometriosis patients, which provided support for

a possible role of histone modification in regulation of gene expression in endometriosis. This is, however, a sign for us that histone modification is a possible therapeutic target for endometriosis. Joe et al. [45] found that inhibition of prostaglandin E2 receptors EP2 and EP4 (PGE2-EP2/EP4) signaling could regulate DNA methylation, histone methylation and acetylation, and even epigenetic memory machinery proteins in human endometriotic cells. Therefore, targeting EP2 and EP4 receptors may emerge as a long-term nonsteroidal therapy for endometriotic lesions in women, which is suitable for infertile patients with endometriosis. Since endometriosis is thought to be a stem-cell disorder, epigenetic regulation of stem cells may open up new avenues for treatment of this enigmatic disease [52, 53]. However, these epigenetics-based theories for therapeutics are almost at experimental stage, more efforts are needed to make a breakthrough in this area.

Endometriosis is a recondite disease involving multi-discipline, a multidisciplinary, as well as multi-nation or multi-region efforts are necessary for its synthetic therapy. In short, we still have a long way to go.

Disclosure of conflict of interest

None.

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Epigenetics of infertility associated with endometriosis

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Epigenetics of infertility associated with endometriosis

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