Abstract. Endometriosis is defined by the presence and growth of functional endometrial tissue, outside the uterine cavity, primarily in the ovaries, pelvic peritoneum and rectovaginal septum. Although it is a benign disease, it presents with malignant characteristics, such as invasion to surrounding tissues, metastasis to distant locations and recurrence following treatment. Accumulating evidence suggests that various epigenetic aberrations may play an essential role in the pathogenesis of endometriosis. Aberrant DNA methylation represents a possible mechanism responsible for this disease, linking gene expression alterations observed in endometriosis with hormonal and environmental factors. Several lines of evidence indicate that endometriosis may partially be due to selective epigenetic deregulations influenced by extrinsic factors. Previous studies have shed light into the epigenetic component of endometriosis, reporting variations in the epigenetic patterns of genes known to be involved in the aberrant hormonal, immunologic and inflammatory status of endometriosis. Although recent studies, utilizing advanced molecular techniques, have allowed us to further elucidate the possible association of DNA methylation with altered gene expression, whether these molecular changes represent the cause or merely the consequence of the disease is a question which remains to be answered. This review provides an overview of the current literature on the role of DNA methylation in the pathophysiology and malignant evolution of endometriosis. We also provide insight into the mechanisms through which DNA methylation-modifying agents may be the next step in the research of the pharmaceutical treatment of endometriosis.

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1. Introduction

Endometriosis represents a common gynecological disease which affects >10% of women of reproductive age (1). Endometriosis is often characterized as an enigmatic condition, or ‘the disease of theories’, since it is still a diagnostic and therapeutic challenge despite decades of clinical experience and research (2). Although it is a benign disease, it presents with malignant characteristics, such as invasion to surrounding tissues, metastasis to distant locations and recurrence following treatment. The unique feature of endometriosis of being a benign metastasing disease and the multiple treatment options currently available, clearly indicate how difficult it can be to diagnose and effectively treat endometriosis based on our current understanding of the disease.

Endometriosis is classically defined by the presence and growth of functional endometrial tissue, outside the uterine cavity, primarily in the ovaries, pelvic peritoneum and rectovaginal septum (3). This ectopic endometrial tissue responds to hormones and drugs in a similar manner to the eutopic endometrium. The continued growth of endometriotic tissue, as with that of the endometrium, is dependent upon estrogen. Thus, endometriosis is prevalent in the reproductive years with a peak incidence between 30 and 45 years of age (4). The main pathological processes associated with the disease are peritoneal inflammation and fibrosis, and the formation of adhesions and ovarian cysts (5). Infertility and pelvic pain are the predominant symptoms that greatly affect the quality of life of women with endometriosis (6).

Various theories have been put forth in order to elucidate the possible mechanisms responsible for the development of endometriosis. While the etiology of the disease remains unclear, retrograde menstruation is the most widely accepted
mechanism of peritoneal endometriotic implants (7). The other traditionally suggested mechanisms include coelomic metaplasia, induction of the disease through immune system abnormalities, transformation of embryonic rests and vascular and lymphatic spread (3). No single theory however, can explain all the different manifestations of endometriosis. Although the transfer of endometrial cells in the peritoneal cavity is a realistic initiating factor of endometriosis, additional steps are necessary for the survival, implantation and growth of the ectopic endometrium. A defective immune clearance, attachment and invasion to the peritoneal epithelium, the establishment of local neoureovascularity and an altered hormonal milieu that will stimulate continuous growth, are necessary if endometriosis is to develop from retrograde menstruation (8-10). Recently, researchers have focused on several well supported hallmarks of the disease, such as genetic predisposition, altered hormonal dependence, inflammation and exposure to environmental toxins (11). The missing link in basic research to the pathophysiology of endometriosis may be a common denominator for a disease that influences its hormonal, immunological and genetic profile. Epigenetics has reformed the understanding of other multifactorial diseases, such as cancer, which shares many common characteristics with endometriosis; thus, epigenetic mechanisms may play a significant role in the origin and progression of endometriosis (12). The epigenome, the collection of DNA methylation and histone modifications can be influenced by environmental factors (13). It is reasonable to speculate that epigenetic alterations represent an attractive candidate, linking intrinsic molecular changes detected in endometriosis, to environmental and lifestyle influences.

2. Genetic and epigenetic basis of endometriosis

The contribution of genetic factors to the susceptibility to endometriosis is supported by a number of different studies (14-16). Higher rates of endometriosis are found among the relatives of patients with endometriosis compared with those of control subjects. The number of gene mapping studies for this disease has increased in recent years as the role of genetic factors has become more widely accepted. To date, many deregulated genes have been identified in endometriotic cells with a wide variety of functions, including apoptosis, vascularization, cell cycle regulation, DNA repair, encoding detoxification enzymes, immune system regulation and cell adhesion (17-19). Yet despite several publications, few reported data have been replicated by other investigators (20,21). In an earlier review by Falconer et al, the authors concluded that there is a strikingly large amount of conflicting results in the literature and that 'polymorphisms may have a limited value in assessing the possible development of endometriosis' (22).

Genetics and cell science have revealed in detail what cells in our body are composed of, but have not yet succeeded in explaining the diversity in morphology and function that derives from the same genetic material. Explaining the mechanisms that temporally regulate the expression of selected genes in guiding cell differentiation and function, for processes as divergent as placental development and schizophrenia, is the ground work of epigenetics. The word epigenetics is derived from the Greek word ‘epi’, for over or above, and genetics, or the science of heredity. Epigenetics provide a potential mechanism through which the genome can ‘capture’ the effects of environmental exposures and perpetuate their influence on cell function. Accumulating evidence on the other hand suggests that various epigenetic aberrations may play an essential role in the pathogenesis of endometriosis (23). Epigenetic alterations refer to stable alterations in gene expression with no underlying modifications in the genetic sequence itself (24). The field of epigenetics has rapidly evolved and has influenced research in different biological phenotypes, such as ageing, memory formation and embryological development (25,26). Epigenetics encompasses several different phenomena, such as DNA methylation, histone modifications, RNA interference and genomic imprinting. Epigenetic processes regulate gene expression and can alter malignancy-associated characteristics, such as growth, migration, invasion, or angiogenesis (27,28). Methylation represents one of the most important epigenetic functions which involves the addition of methyl groups on the cytosine residues of CG (also termed CpG) dinucleotides of DNA (29). Enzymes known as DNA methyltransferases (DNMTs) catalyse the addition of the methyl group to the cytosine ring to form methyl cytosine, using S-adenosylmethionine as a methyl donor. DNMT1 is the predominant mammalian DNA methylating enzyme responsible for the restoration of hemi-methylated sites to full methylation (maintenance methylation) which occurs after DNA replication (30). DNMT3A and DNMT3B are mainly involved in the methylation of new sites, known as de novo methylation (31). In humans and other mammals, DNA modification occurs predominantly on cytosines that precede a guanosine in the DNA sequence (32). These dinucleotides can be clustered in small stretches of DNA, termed CpG islands, which are often associated with promoter regions. In 98% of the genome, CpGs are present approximately once per 80 dinucleotides. By contrast, CpG islands, which comprise 1-2% of the genome, are approximately 200 base pairs to several kb in length and have a frequency of CpGs approximately 5-fold greater than the genome as a whole (33,34). The majority of CpG sites outside of CpG islands are methylated, suggesting a role in the global maintenance of the genome, while the majority of CpG islands in gene promoters are unmethylated, which allows active gene transcription (32,35). Once a CpG becomes methylated in a cell, it will remain methylated in all its descendants (36). Generally, when a given stretch of cytosines in a CpG island located in the promoter region of a gene is methylated, that gene will be silenced by methylation; such a CpG island would be termed ‘hypermethylated’. Conversely, when a given stretch of cytosines in a CpG island located in the promoter region of a gene is not methylated, that gene will not be silenced by methylation; the CpG island in this case would be ‘hypomethylated’ (37). The methylation of promoters inhibits their recognition by transcription factors and RNA polymerase, as methylated cytosines preferentially bind to a protein known as methyl cytosine binding protein (MeCP). When a promoter region normally recognized by an activating transcription factor, is methylated, its transcription will be inhibited (34).

Aberrant DNA methylation represents a possible mechanism, linking gene expression alterations observed in endometriosis with hormonal and environmental factors (38). It is difficult however, to determine whether aberrant methylation is the cause or the consequence of the disease. Given the theoretical multifactorial origin of endometriosis, a linear association that
comprises environmental factors, epigenetic alterations and disease, is difficult to established. Despite this fact, several lines of evidence indicate that endometriosis may partially be due to selective epigenetic deregulations influenced by extrinsic factors (39). DNA methylation is a delicate reversible event that is known to be influenced by environmental factors, such as exposure to xenobiotics, social behavior, metabolism and nutritional deficiencies that may exert their effects later in life, during critical periods of development, or may be transmitted transgenerationally to the offspring (40,41). Endometriosis has been frequently associated with exposure to toxins or synthetic compounds and dietary habits. The epigenome in women with endometriosis may be a reflection of the respective woman's age, reproductive history, body mass index and whether or not she was exposed to chemicals during her lifetime (42,43). Recent theories on fetal programming postulate that chronic adult onset diseases with an epigenetic component, originate in utero when the early embryo is exposed to factors that permanently shape its epigenetic mark (44,45).

Since eutopic and ectopic endometriotic stromal cells share the same genetic background, research has focused on mechanisms that may provoke different cellular responses, namely epigenetics. The silencing of progesterone and aromatase genes, which are essential elements in the development of endometriosis, by promoter hypermethylation, may be a strong contributing factor of the disease. It has been shown that a single endometriotic lesion originates form a single progenitor cell, forming a cellular lineage (46). This monoclonality of endometriotic lesions suggests that they may carry neoplastic potentials. This cellular lineage requires that cells transcribe, or it enables the transcription of specific genes, or regions of genes, whereas at the same time it suppresses others. To maintain cellular identity, the gene expression program must be maintained through cell divisions in a heritable manner through epigenetic processes.

3. Aberrant DNMT expression in endometriosis

It has been previously reported that DNMT1, DNMT3A and DNMT3B are overexpressed in the epithelial component of endometriotic implants as compared to normal controls or the in the eutopic endometrium of women with endometriosis. Moreover, the expression levels of DNMT1, DNMT3A and DNMT3B have been shown to positively correlate with each other (47). The upregulated expression of DNMTs in the endometriotic tissue, which leads to hypermethylation, has been confirmed in women with endometriosis only for the DNMT3A transcript and not for DNMT1 and DNMT3B (48). Conversely, significantly lower expression levels of DNMTs have been found by other studies where they compared endometriotic lesions to the eutopic endometrium of women with endometriosis and disease-free controls (49,50). In a previous study, the induction of hypoxia triggered global hypomethylation in ectopic stromal cells through the destabilization of DNMT1 mRNA, thus providing a plausible link of gene-environment interaction by means of DNA methylation (50). Unaltered DNMT expression in response to in vitro decidualization in endometriotic cells may also elucidate the aberrant epigenetic status that alters gene expression and contributes to the progesterone-resistant environment observed in endometriosis (51).

4. Estrogen and progesterone receptor genes

The uterine endometrium is targeted by the ovarian sex steroid hormones, estrogen and progesterone, which regulate the growth of endometrial tissue, basically by stimulating or inhibiting cell proliferation, respectively (52). Each hormone is estimated to regulate the expression of hundreds of genes during various phases of the menstrual cycle. Endometriotic tissue in ectopic locations, such as the peritoneum or ovaries, differs fundamentally from the eutopic endometrium within the uterus in terms of the production of prostaglandins (PGs) and cytokines, estrogen production and metabolism, as well as the clinical response to progestins. The ectopic endometrium is characterized by an imbalance in the function of estrogen and progesterone, namely estrogen dominance and progesterone resistance (53). Abundant quantities of estrogen are available in the endometriotic tissue via several mechanisms, including local aromatase expression. Estrogen and progesterone exert their functions by binding to their intracellular receptors, the estrogen receptor (ER) and progesterone receptor (PR), which are members of the steroid/nuclear receptor (SR) superfamily (54). The involvement of SRs with co-regulators and other recruited proteins in the transcriptional complex is essential for target gene regulation. The SRs interact with DNA-methylating/-demethylating enzymes in the transcriptional complex, and as a final point, histone modification along with DNA methylation both regulate gene expression (55,56). Thus, steroid hormone responsive tissues, such as endometriotic lesions, have epigenetic constituents which may be vulnerable to modifications.

The biologically active estrogen, estradiol (E2), enters cells and binds to the ER in both the eutopic and ectopic endometrial cells. There are two separate ER subtypes, ERα and ERβ, that appear to have overlapping, although different, tissue expression and localization profiles (57). ERα and ERβ are encoded by separate genes, ESR1 and ESR2, respectively, found at different chromosomes locations (58). The E2-receptor complex acts as a transcription factor that becomes associated with the promoters of E2-responsive genes via direct DNA binding or binding to other docking transcription factors, such as activator protein complex 1 (APC-1). Of note, while ERα and ERβ recognize the same estrogen responsive element, the two subtypes display different transactivational properties in a ligand-dependent manner when they are co-expressed (21). In addition, ERβ also has the capacity to regulate ERα (53). Recent studies using ERα, ERβ null mice with surgically induced endometriosis revealed that only ERα inhibits endometrial growth and leads to a decrease in estrogen target gene expression, whereas the deletion of ERβ does not affect the biological responses of the uterus to estrogen (59). Burns et al concluded that estrogen-regulated signaling responses are predominantly mediated by ERα in endometriosis-like lesions (60).

Progesterone has long been used for relieving endometriosis-induced pain, mainly by inducing pseudo-pregnancy, thus suppressing ovarian estrogen production, which in turn suppresses growth and inflammation in endometriosis. The uterine response to progesterone is dependent on PRs. The two predominant isoforms of PR, PRA and PRB, are both encoded by the same PR gene, but use alternative promoters and translation start sites (61).
The majority of altered expression endometriosis-associated genes, are downstream targets of ERs and PRs or overlap with genes known to be regulated by the SRs (62). The ER and PR levels differ markedly in the endometrium compared to endometriosis-derived stromal cells (63). Endometriotic lesions exhibit particularly higher ERβ and significantly lower ERα and PR levels compared to the eutopic endometrium (64, 65). The suppressed ERTα expression detected in the stromal cells of endometriosis may be a consequence of the strikingly high quantities of estradiol in addition to high ERβ levels produced via local aromatase activity (62). Although ERα seems to be the primary mediator of the estrogenic action, elevated ERβ levels and in particular increased ERβ/ERα ratio in endometriosis compared to that in endometrial tissues is associated with suppressed PR levels, contributing to the loss of progesterone signaling or progesterone resistance noted in endometriosis (53).

Xue et al identified a CpG island occupying the promoter region of the ERβ gene, which exhibited significantly higher methylation levels in endometrial cells versus endometriotic cells (66). Moreover, the activity of the ERβ promoter bearing the CpG island was strongly inactivated by in vitro methylation. The authors of that study concluded that the high ERβ mRNA and protein expression observed in endometriosis is mediated by an epigenetic defect involving the hypomethylation of the gene’s promoter. Another piece of evidence linking methylation to SR function is the high methylation levels of the PRβ promoter demonstrated in endometriosis (67). The hypermethylation of the PRβ promoter is in accordance to the already reported downregulation of PRβ in endometriosis, which in turn presents a plausible explanation to progesterone resistance in endometriotic tissues. PRα on the other hand, does not display similar epigenetic patterns. In contrast with the above observations, there are certain studies that report higher levels of PRβ in endometriotic tissues (68, 69). For instance, ovarian endometrioma samples have been shown to have significantly higher levels of PRβ mRNA when compared with the eutopic endometrium (68). The diversity of endometriosis is also prominent in studies, whereas different types of endometriosis exhibit diverse DNA methylation patterns of the SR genes. Intestinal endometriosis which is one of the most aggressive forms of the disease has demonstrated no differences in the DNA methylation patterns of the ESR1 and ESR2 genes compared to the eutopic endometrium obtained from the same patient. The methylation of the PGR gene was observed exclusively in a subset of the endometriotic samples which contributed to PGR gene suppression, which in turn was further confirmed by immunostaining of the PGR protein in the same samples (70).

5. Steroidogenic factor 1 (SF-1)

SF-1, also known as Ad4BP or NR5A1, is a member of the nuclear receptor superfamily and is encoded by the NR5A1 gene in humans (71, 72). SF-1 is a key transcription factor for steroid biosynthesis and is responsible for inducing the expression of steroidogenic acute regulatory protein (STAR) and cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19a1), which encode aromatase (73). Aromatase catalyses the final step of estrogen production through the conversion of C19 steroids to estrogens. SF-1 is also involved in the regulation of other adrenal and testicular steroidogenic genes, such as hydroxysteroid dehydrogenase genes (HSD3B and HSD11B2) and melanocortin 2 receptor (adrenocorticotropic hormone) (MC2R). The aberrant expression of steroidogenesis-related genes represents a possible pathogenetic mechanism of endometriosis, wherein estradiol synthesis is locally enhanced within endometriotic cells (74). It has already been reported that SF-1 is highly elevated in endometriotic tissues compared to the normal endometrium. Consistent with the mRNA levels of SF-1, the protein levels of SF-1 are also significantly elevated in the ectopic endometrium and this elevation corresponds to the severity of endometriosis (75).

In an earlier study, Xue et al reported that normal endometrial cells in which SF-1 transcriptional activity was completely suppressed, demonstrated aberrant methylation of the promoter and exon 1 region of the NR5A1 gene (76). On the contrary, endometriotic cells displayed higher SF-1 mRNA and protein levels along with reduced methylation levels at the SF-1 promoter region. Yamagata et al confirmed these results in a genome-wide methylation analysis, in cultured eutopic and ectopic cells. In NR5A1 and STAR, the CpG sites were hypomethylated in cultured cells from endometriotic cysts compared with those from eutopic endometrium (77). Taken together, these two studies have shown that methylation of the proximal promoter of the NR5A1 gene regulates SF-1 expression in endometriotic tissues, as well as in the normal endometrium. A few years later, however, Xue et al reported that hypermethylation of the CpG island that spans from exon 2 to intron 3 of the SF-1 gene activated mRNA expression in endometriotic cells (78). The authors of that study hypothesized that the hypermethylation of this particular region of the gene, distant to the promoter, encloses a silencer which, when hypermethylated, suppresses its silencer function, giving rise to increased SF-1 expression. Furthermore, in a similar study by the same team, the hypermethylation of a novel CpG island located downstream of intron 1 of the SF-1 gene was associated with a high expression of the gene (79). Although this observation is contradicted to the classical association of methylation to gene expression, it is consistent with a large body of literature, indicating that methylation outside of gene promoters leads to increased gene expression (80-83).

6. Homeobox A10 (HOXA10)

HOXA10 is a member of a family of homebox genes that serve as transcription factors which are expressed in the endometrium, where they are necessary for endometrial growth, differentiation and implantation (84). Its expression is regulated by estrogen and progesterone and markedly increases during the midsecretory phase which corresponds to the implantation window (85). Therefore, HOXA10 is considered essential in regulating endometrial development during menstrual cycle, thus facilitating conditions necessary for implantation. Increased HOXA10 levels remain elevated when successful implantation occurs, expressed by the developing decidua in early pregnancy. Both estrogen and progesterone individually stimulate the endometrial expression of HOXA10, and progesterone has additional stimulatory effects over estrogen (86). The first indication of the possible role of HOXA10 in endometriosis was postulated when a difference in the expression of the gene was noted in the endometrium of women with endometriosis (87). It has been reported that in patients with endometriosis there is
a decrease in HOXA10 expression during the secretory phase, resulting in decreased uterine receptivity and subsequent endometriosis-related infertility (88). Hypermethylation of the HOXA10 gene promoter provides a probable explanation for its reduced gene expression in the endometrium of women with endometriosis (89). The simultaneous occurrence of HOXA10 promoter hypermethylation and reduced HOXA10 expression has been demonstrated in induced endometriosis in baboons and in mice (90,91). It has been confirmed that DNA hypermethylation may be one of the potential molecular mechanisms silencing HOXA10 expression in the mid-luteal endometrium associated with infertility in women with endometriosis (88). In women with ovarian endometriomas, significantly higher HOXA10 promoter methylation levels have been documented during the mid-luteal phase (92).

Treatment of endometrial stromal cells from fertile women with endometriosis with 5-azacytidine, a demethylation agent, resulted in increased HOXA10 mRNA and protein levels, thus suggesting the regulatory role of methylation on gene expression in endometriosis (93). In another study, mice, prenatally exposed to diethylstilbestrol, exhibited the overexpression of both Dmnt1 and Dmnt3, along with Hoxa10 hypermethylation (94). Even though hypermethylation of the HOXA10 promoter is a constant finding in different studies on endometriosis, in a recent genome-wide methylation analysis, the epigenetic alteration of the HOXA10 gene was below the arbitrary threshold set by the authors, hence other epigenetically altered genes were considered more relevant to the pathophysiology of the disease (95).

7. Aromatase

Aromatase is the key enzyme in estrogen production which converts androgen to estrogen (96). Estrogen production in women with endometriosis is accomplished by de novo synthesis in the ovaries, by the conversion of circulating androstenedione to estradiol in adipose tissue, skin and skeletal muscle and lastly by a unique de novo system of local estrogen production which takes place in endometriotic lesions (97). Endometriotic stromal cell express the full complement of genes in the steroidogenic cascade, which is sufficient to convert cholesterol to estradiol. Aberrantly expressed aromatase in the endometriotic implants, is thought to be one of the major contributing factors in the development of the hyperestrogenic microenvironment of endometriosis (53). Aromatase expression has been reported to be absent in the eutopic endometrium of healthy women, whereas its mRNA levels are significantly increased in women with endometriosis (98). Moreover, aromatase expression is also increased in the eutopic endometrium of women with endometriosis.

Aromatase is encoded by a single gene, CYP19, which is located on chromosome 15q21. The aromatase gene has the unique characteristic of having multiple exons available for use which are flanked with unique promoters (99). The tissue-specific expression of aromatase is regulated by the alternative use of these exons. It has been shown that endometriotic cells use the same aromatase promoters, promoters II, I.3 and I.6, as endometrial cells (100). Since both the ectopic and eutopic endometrium share the same promoters, different gene expression relies on an epigenetic regulatory mechanism which switches off and on aromatase gene in healthy and diseased tissues, respectively. Izawa et al confirmed the above speculation by demonstrating a CpG island at approximately 20 kb upstream from the end of exon II which was hypomethylated in endometriotic and hypermethylated in endometrial cells (101).

8. Other genes

Cyclooxygenase-2 (COX-2) is the key enzyme in the conversion of arachidonic acid to PGs and has been mainly associated with the inflammatory response (102). The aberrant expression of COX-2, and thus the over-production of PGE2 has been shown to play critical roles in the development of endometriosis. The peritoneal microenvironment in the setting of endometriosis is notably rich in PGs, and these mediators likely play a central role in disease pathophysiology, as well as in the clinical sequelae of pain and infertility. COXA-2 overexpression has been observed in ectopic endometriotic lesions and has been correlated with the severity of endometriosis-associated pain and also the recurrence of the disease after surgery (103-105). The hypomethylation of the NF-IL6 site within the COX-2 promoter in the eutopic and ectopic endometrial tissues of women with endometriosis has been linked with the already reported increased COX-2 mRNA levels in the same tissues (106,107).

The E-cadherin (CDH1) gene encodes an epithelial cell-cell adhesion glycoprotein that modulates a wide variety of processes, including cell polarization, migration and cancer metastasis. The decreased expression of CDH1 in epithelial cells in peritoneal endometriosis has been reported in the advanced stages of endometriotic lesions (108). In two endometriotic cell lines, the E-cadherin gene has been found to be hypermethylated at the promoter region, and treatment with a histone deacetylase inhibitor, trichostatin A, induced expression (109).

Syncytin-1 plays a critical role in the maintenance of normal pregnancy. The hypomethylation and activation of the syncytin-1 gene has been found in placental trophoblast lineages and malignant cells. While the syncytin-1 gene is absent in the eutopic endometrium from patients with endometriosis, syncytin-1 mRNA and protein levels are detected in endometriotic lesions. The hypomethylation of the gene promoter in the eutopic lesions highlights the epigenetic regulation of the function of this gene in endometriosis (110).

9. Malignant transformation of endometriosis

The malignant transformation of endometriosis is believed to occur in approximately 1% of all cases (111). The most common site of malignant transformation of endometriosis is the ovaries. Ovarian endometrioid cancer (OEC) and ovarian clear cell cancer (OCCC) account for 76% of all endometriosis-associated ovarian cancer cases (112). The malignant transformation of endometriosis represents a newly launched, attractive research field in epigenetics. Endometriosis as a possible initiating factor in ovarian carcinogenesis, has gained attention since the recently proposed theory on ovarian cancer. Based on this theory, ovarian cancer originates from tissue implantation outside the ovaries. Since ovarian serous adenocarcinoma is thought to originate from tubal fimbria epithelial lesions, OEC and OCCC are likely to originate from endome-
Table I. Genes reported with aberrant methylation in the ectopic or eutopic endometrium.

| Genes      | Function                                                                 | Methylation                                      | (Refs.) |
|------------|--------------------------------------------------------------------------|--------------------------------------------------|---------|
| ERβ        | Estrogen nuclear receptor. Mediates estrogenic action                     | Hypomethylated in endometriotic cells            | (50)    |
| ERβ        | Estrogen nuclear receptor                                                 | Hypermethylated in endometrial cells of women with endometriosis | (50) |
| PRβ        | Progesterone nuclear receptor. Mediates progesterone action               | Hypermethylated in endometriotic cells           | (51)    |
| Eα, ERβ    | No difference in methylation levels of intestinal endometriosis compared to eutopic endometrium | (54)    |
| SF-1       | Key transcription factor for steroid biosynthesis                         | Hypomethylated in endometriotic cells            | (58,59) |
| SF-1       | Key transcription factor for steroid biosynthesis                         | Hypermethylated in endometriotic cells           | (60,61) |
| HOXA10     | Transcription factors necessary for endometrial growth, differentiation, and implantation | Hypermethylated in eutopic endometrium in women with endometriosis | (70-73) |
| Aromatase  | Key enzyme in estrogen production which converts androgen to estrogen     | Hypomethylated in endometrial tissues and hypermethylated in eutopic endometrium of women with endometriosis | (80) |
| COX-2      | Key enzyme in the conversion of arachidonic acid to prostaglandins       | Hypomethylated in both endometriotic and endometrial cells | (85,86) |
| E-Cadherin | Encodes an epithelial cell-cell adhesion glycoprotein that modulates cell polarization, migration and cancer metastasis | Hypermethylated in cultured endometriotic cells | (88) |
| Syncytin-1 | Human endogenous retroviral envelope gene (HERVW1) product is expressed in placental trophoblasts and mediates the formation of syncytiotrophoblasts | Hypomethylated in endometriotic lesions | (89) |

SF-1, steroidogenic factor 1; HOXA10, homeobox A10; COX-2, cyclooxygenase-2.

Figure 1. Schematic presentation of aberrant hypermethylation (upwards arrows) or hypomethylation (downwards arrows) in the eutopic endometrium (pink) or endometriotic lesions (brown). PR, progesterone receptor; ER, estrogen receptor; SF-1, steroidogenic factor 1; COX-2, cyclooxygenase-2.

triotic implants (113). The demethylation of known oncogenes and also epigenetic silencing through the hypermethylation of tumour suppressor genes might induce mechanisms such as uncontrolled cell division, the ability to infiltrate surrounding
tissues, avoiding apoptosis or sustaining angiogenesis (114). The hypermethylation of the *hMLH1* gene promoter, which represents one of the most important mismatch repair (MMR) genes, and the consequent decrease in gene expression has been associated with the malignant evolution of endometriosis (115). The hypomethylation of long interspersed element-1 (*LINE-1*) has also been described as an early molecular event involved in malignant progression of endometriosis (116). The runt-related transcription factor 3 gene (*RUNX3*) has been shown to be a tumour suppressor in a variety of cancers. Guo et al recently reported that the inactivation of the *RUNX3* gene by promoter hypermethylation plays a role in the malignant transformation of ovarian endometriosis and is closely related to estrogen metabolism (117). The tumour suppressor gene, Ras-association domain family member 2 (*RASSF2*), is inactivated by promoter hypermethylation in many types of cancer (118-120). A recent study confirmed that the epigenetic inactivation of *RASSF2* is associated with the malignant progression of ovarian endometriosis and that this epigenetic alteration may be an early event in ovarian tumourigenesis (121).

### 10. Genome-wide methylation in endometriosis

Genome-wide methylation analysis is an emerging technology which may be used to identify novel genes potentially involved in the development and pathogenesis of endometriosis. Analyses of the entire methylome are used to determine the unique epigenetic fingerprint of endometriosis. Recently, Borghese et al reported a whole-genome DNA methylation profiling in >25,000 promoters, using methylated DNA immunoprecipitation with hybridization to promoter microarrays (122). Consistent with the theory of the endometrial origin of endometriosis, the overall methylation profile was highly similar between the endometrium and the endometriotic lesions. Although there was no correlation between promoter methylation and the expression of nearby genes, 35 genes had both methylation and expression alterations in the lesions. In a more recent study which included 27,578 genes on the methylation array, 120 genes were significantly altered by ≥1.5-fold in the endometrial biopsies of women with endometriosis compared to those from healthy women (95). When comparing methylation profiles of the eutopic endometrium from women with or without endometriosis and ovarian endometriotic cysts, only a few genes were differentially methylated in the endometrium, whereas more hypermethylated and hypomethylated CpGs were detected in the endometrial ovarian cysts (77). In a previous study, in 42,248 differentially methylated CpGs that were investigated, 403 genes demonstrated significantly different methylation patterns. A disproportionately large number of transcription factors had different methylation profiles and many of these genes are already known to be involved in the process of decidualization and the pathophysiology of endometriosis (123).

### 11. Therapeutic implications

Unlike DNA mutations or copy number alterations, reversibility is an important characteristic of epigenetic aberrations, since it allows us to employ a number of epigenetic therapies which can potentially reverse the aberrant epigenetic patterns in affected tissues. Enzymes that regulate epigenetic alterations have been targets of research on pharmacological intervention in endometriosis. The target enzymes of epigenetic drugs include DNMTs, histone deacetylases, histone acetyltransferases, histone methyltransferases and histone demethylases (124). *In vitro* studies have demonstrated promising results with pharmaceutical agents that disrupt the methylation cascade (125). The selective inhibition of the PGE2 receptors, EP2 and EP4, has been shown to decrease the expression of *DNMT3a* and *DNMT3b*, but does not modulate the expression of *DNMT1* (126). The authors of that study postulated that targeting EP2 and EP4 receptors may emerge as long-term nonsteroidal therapy for the treatment of active endometriotic lesions in women (126). Treatment with a demethylating agent (DMA) has also been shown to significantly increase *ERβ* mRNA levels in endometrial cells and may indicate a possible epigenetic therapeutic target (127). Izawa et al also demonstrated that the treatment of endometrial stromal cells, which normally do not express aromatase, with a DMA, markedly increased the aromatase mRNA expression (101). Thus far, there are two classes of DNA DMAs: nucleoside and non-nucleoside DNMT inhibitors. They both inhibit DNMTs in the S phase of the cell cycle and consequently lower the overall DNA methylation in the target cells (101). Several practical considerations arise that influence the utilization of DMA in current therapeutic strategies of endometriosis. The administration of these drugs results in a long-lasting demethylating effect, even after a short period of treatment. Since endometriosis is a benign disease, prolonged cytopenia and gastrointestinal system toxicity are considered serious adverse effects in otherwise healthy patients. Moreover, many of the women with endometriosis may need repeated treatments during their reproductive lifespan, therefore safety issues should be the primary concern of future studies.

### 12. Conclusion

Endometriosis presents a diagnostic and therapeutic enigma for clinicians, but also an emerging field of research on how epigenetics intervene with the pathophysiology and progression of the disease. Previous studies have shed light into the epigenetic component of endometriosis, reporting variations in the epigenetic patterns of genes known to be involved in the aberrant hormonal, immunologic and inflammatory status of endometriosis (Table I and Fig. 1). Although recent studies, utilizing advanced molecular techniques, have allowed us to further elucidate the possible association of DNA methylation with altered gene expression, whether these molecular changes represent the cause or merely the consequence of the disease is a question which remains to be answered. *In vitro* studies have reported promising results on treatment with epigenetic modifying agents; however, we have a long way to go until we can employ these agents in current medical practice. Till then, endometriosis will be the ideal model on further research, thus being a benign disease with prominent malignant characteristics.

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