DNA methylation in endometriosis (Review)

OURANIA KOUKOURA[1], STAVROS SIFAKIS[2] and DEMETRIOS A. SPANDIDOS[3]

[1]Department of Obstetrics and Gynecology, University Hospital of Larissa, Larissa 41500;
[2]Department of Obstetrics and Gynecology, University Hospital of Heraklion, Heraklion 71003;
[3]Laboratory of Clinical Virology, University of Crete Medical School, Heraklion 71409, Greece

Received January 12, 2016; Accepted February 22, 2016

DOI: 10.3892/mmr.2016.4925

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 repsonsible 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.

Contents

Correspondence to: Dr Ourania Koukoura, Department of Obstetrics and Gynecology, University Hospital of Larissa, Mpatzelioti Street, Terpsithea, Larissa 41500, Greece
E-mail: okoukoura@yahoo.com

Key words: epigenetics, DNA methylation, endometriosis, pelvic pain

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 neoneurovascularity 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 suppesses 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 chromosomal 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 *ERα* 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 thye 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 *HSD11B*) 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 *Dnmt1* and *Dnmt3*, 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 microenviroment 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 ectopic 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 ectopic 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)
ERα, *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		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 endometriotic 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.

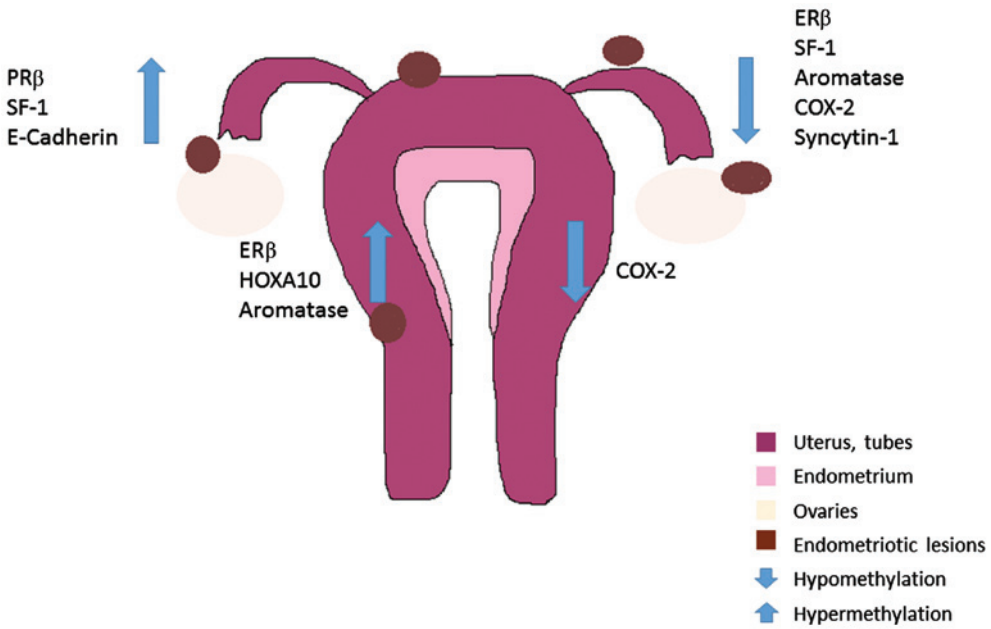

Figure 1. Schematic presentation of aberrant hypermethylation (upwards arrows) or hypomethylation (downwards arrows) in the eutopic endometrium (pink) or endometriotic lesions (brown). PR, progesterone receptor; ER, estrogen receptor; SF-1, steroidogenic factor 1; COX-2, cyclooxygenase-2.

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 expressional 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 endometrial 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 disproportionally 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 shnown to significantly increase *ER*β 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.

References

1. Viganò P, Parazzini F, Somigliana E and Vercellini P: Endometriosis: Epidemiology and aetiological factors. Best Pract Res Clin Obstet Gynaecol 18: 177-200, 2004.

2. Eskenazi B and Warner ML: Epidemiology of endometriosis. Obstet Gynecol Clin North Am 24: 235-258, 1997.
3. Seli E, Berkkanoglu M and Arici A: Pathogenesis of endometriosis. Obstet Gynecol Clin North Am 30: 41-61, 2003.
4. Houston DE: Evidence for the risk of pelvic endometriosis by age, race and socioeconomic status. Epidemiol Rev 6: 167-191, 1984.
5. Kobayashi H, Higashiura Y, Shigetomi H and Kajihara H: Pathogenesis of endometriosis: The role of initial infection and subsequent sterile inflammation (Review). Mol Med Rep 9: 9-15, 2014.
6. Hickey M, Ballard K and Farquhar C: Endometriosis. BMJ 348: g1752, 2014.
7. Sampson J: Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Obstet Gynecol 14: 422-469, 1927.
8. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R and Tayade C: Pathophysiology and Immune Dysfunction in Endometriosis. BioMed Res Int 2015: 795976, 2015.
9. Nisolle M, Casanas-Roux F, Anaf V, Mine JM and Donnez J: Morphometric study of the stromal vascularization in peritoneal endometriosis. Fertil Steril 59: 681-684, 1993.
10. Burney RO and Giudice LC: Pathogenesis and pathophysiology of endometriosis. Fertil Steril 98: 511-519, 2012.
11. Sanchez AM, Viganò P, Somigliana E, Cioffi R, Panina-Bordignon P and Candiani M: The endometriotic tissue lining the internal surface of endometrioma: hormonal, genetic, epigenetic status, and gene expression profile: Reprod Sci 22: 391-401, 2015.
12. Lopez J, Percharde M, Coley HM, Webb A and Crook T: The context and potential of epigenetics in oncology. Br J Cancer 100: 571-577, 2009.
13. Jaenisch R and Bird A: Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. Nat Genet 33 (Suppl): 245-254, 2003.
14. Bellelis P, Barbeiro DF, Rizzo LV, Baracat EC, Abrão MS and Podgaec S: Transcriptional changes in the expression of chemokines related to natural killer and T-regulatory cells in patients with deep infiltrative endometriosis. Fertil Steril 99: 1987-1993, 2013.
15. Hansen KA and Eyster KM: Genetics and genomics of endometriosis. Clin Obstet Gynecol 53: 403-412, 2010.
16. Baranov VS, Ivaschenko TE, Liehr T and Yarmolinskaya MI: Systems genetics view of endometriosis: A common complex disorder. Eur J Obstet Gynecol Reprod Biol 185: 59-65, 2015.
17. Campbell IG and Thomas EJ: Endometriosis: Candidate genes. Hum Reprod Update 7: 15-20, 2001.
18. Vigano P, Somigliana E, Vignali M, Busacca M and Blasio AM: Genetics of endometriosis: Current status and prospects. Front Biosci 12: 3247-3255, 2007.
19. Augoulea A, Alexandrou A, Creatsa M, Vrachnis N and Lambrinoudaki I: Pathogenesis of endometriosis: The role of genetics, inflammation and oxidative stress. Arch Gynecol Obstet 286: 99-103, 2012.
20. Altmüller J, Palmer LJ, Fischer G, Scherb H and Wjst M: Genomewide scans of complex human diseases: True linkage is hard to find. Am J Hum Genet 69: 936-950, 2001.
21. Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JN, Abecasis G, Altshuler D, Bailey-Wilson JE, *et al*; NCI-NHGRI Working Group on Replication in Association Studies: Replicating genotype-phenotype associations. Nature 447: 655-660, 2007.
22. Falconer H, D'Hooghe T and Fried G: Endometriosis and genetic polymorphisms. Obstet Gynecol Surv 62: 616-628, 2007.
23. Guo SW: Epigenetics of endometriosis. Mol Hum Reprod 15: 587-607, 2009.
24. Inbar-Feigenberg M, Choufani S, Butcher DT, Roifman M and Weksberg R: Basic concepts of epigenetics. Fertil Steril 99: 607-615, 2013.
25. Xu X: DNA methylation and cognitive aging. Oncotarget 6: 13922-13932, 2015.
26. Breiling A and Lyko F: Epigenetic regulatory functions of DNA modifications: 5-methylcytosine and beyond. Epigenetics Chromatin 8: 24, 2015.
27. Burggren WW and Crews D: Epigenetics in comparative biology: why we should pay attention. Integr Comp Biol 54: 7-20, 2014.
28. Koerner MV and Barlow DP: Genomic imprinting-an epigenetic gene-regulatory model. Curr Opin Genet Dev 20: 164-170, 2010.
29. Moore LD, Le T and Fan G: DNA methylation and its basic function. Neuropsychopharmacology 38: 23-38, 2013.
30. Xu F, Mao C, Ding Y, Rui C, Wu L, Shi A, Zhang H, Zhang L and Xu Z: Molecular and enzymatic profiles of mammalian DNA methyltransferases: Structures and targets for drugs. Curr Med Chem 17: 4052-4071, 2010.
31. Jeltsch A: Molecular enzymology of mammalian DNA methyltransferases. Curr Top Microbiol Immunol 301: 203-225, 2006.
32. Herman JG and Baylin SB: Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349: 2042-2054, 2003.
33. Bird AP: CpG-rich islands and the function of DNA methylation. Nature 321: 209-213, 1986.
34. Costello JF and Plass C: Methylation matters. J Med Genet 38: 285-303, 2001.
35. Weber M and Schübeler D: Genomic patterns of DNA methylation: Targets and function of an epigenetic mark. Curr Opin Cell Biol 19: 273-280, 2007.
36. Bird AP and Wolffe AP: Methylation-induced repression - belts, braces, and chromatin. Cell 99: 451-454, 1999.
37. Koukoura O, Sifakis S and Spandidos DA: DNA methylation in the human placenta and fetal growth (Review). Mol Med Rep 5: 883-889, 2012.
38. Romani M, Pistillo MP and Banelli B: Environmental Epigenetics: Crossroad between Public Health, Lifestyle, and Cancer Prevention. BioMed Res Int 2015: 587983, 2015.
39. Bruner-Tran KL, Resuehr D, Ding T, Lucas JA and Osteen KG: The role of endocrine disruptors in the epigenetics of reproductive disease and dysfunction: potential relevance to humans. Curr Obstet Gynecol Rep 1: 116-123, 2012.
40. Anway MD, Cupp AS, Uzumcu M and Skinner MK: Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308: 1466-1469, 2005.
41. Danchin É, Charmantier A, Champagne FA, Mesoudi A, Pujol B and Blanchet S: Beyond DNA: Integrating inclusive inheritance into an extended theory of evolution. Nat Rev Genet 12: 475-486, 2011.
42. Bulun SE, Zeitoun KM, Kilic G: Expression of dioxin-related transactivating factors and target genes in human eutopic endometrial and endometriotic tissues. Am J Obstet Gynecol 182: 767-775, 2000.
43. Chiaffarino F, Bravi F, Cipriani S, Parazzini F, Ricci E, Viganò P and La Vecchia C: Coffee and caffeine intake and risk of endometriosis: a meta-analysis. Eur J Nutr 53: 1573-1579, 2014.
44. Nugent BM and Bale TL: The omniscient placenta: Metabolic and epigenetic regulation of fetal programming. Front Neuroendocrinol 39: 28-37, 2015.
45. Smith CJ and Ryckman KK: Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome. Diabetes Metab Syndr Obes 8: 295-302, 2015.
46. Chan RW, Ng EH and Yeung WS: Identification of cells with colony-forming activity, self-renewal capacity, and multipotency in ovarian endometriosis. Am J Pathol 178: 2832-2844, 2011.
47. Wu Y, Strawn E, Basir Z, Halverson G and Guo SW: Aberrant expression of deoxyribonucleic acid methyltransferases DNMT1, DNMT3A, and DNMT3B in women with endometriosis. Fertil Steril 87: 24-32, 2007.
48. Szczepańska M, Wirstlein P, Skrzypczak J and Jagodziński PP: Expression of HOXA11 in the mid-luteal endometrium from women with endometriosis-associated infertility. Reprod Biol Endocrinol 10: 1, 2012.
49. van Kaam KJ, Delvoux B, Romano A, D'Hooghe T, Dunselman GA and Groothuis PG: Deoxyribonucleic acid methyltransferases and methyl-CpG-binding domain proteins in human endometrium and endometriosis. Fertil Steril 95: 1421-1427, 2011.
50. Hsiao KY, Wu MH, Chang N, Yang SH, Wu CW, Sun HS and Tsai SJ: Coordination of AUF1 and miR-148a destabilizes DNA methyltransferase 1 mRNA under hypoxia in endometriosis. Mol Hum Reprod 21: 894-904, 2015.
51. Dyson MT, Kakinuma T, Pavone ME, Monsivais D, Navarro A, Malpani SS, Ono M and Bulun SE: Aberrant expression and localization of deoxyribonucleic acid methyltransferase 3B in endometriotic stromal cells. Fertil Steril 104: 953-963.e2, 2015.
52. Critchley HO and Saunders PT: Hormone receptor dynamics in a receptive human endometrium. Reprod Sci 16: 191-199, 2009.
53. Shao R, Cao S, Wang X, Feng Y and Billig H: The elusive and controversial roles of estrogen and progesterone receptors in human endometriosis. Am J Transl Res 6: 104-113, 2014.
54. Tsai MJ and O'Malley BW: Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem 63: 451-486, 1994.

55. Fitzpatrick DR and Wilson CB: Methylation and demethylation in the regulation of genes, cells, and responses in the immune system. Clin Immunol 109: 37-45, 2003.
56. Fuks F: DNA methylation and histone modifications: Teaming up to silence genes. Curr Opin Genet Dev 15: 490-495, 2005.
57. Leung YK, Mak P, Hassan S and Ho SM: Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. Proc Natl Acad Sci USA 103: 13162-13167, 2006.
58. Herynk MH and Fuqua SA: Estrogen receptor mutations in human disease. Endocr Rev 25: 869-898, 2004.
59. Hewitt SC, Harrell JC and Korach KS: Lessons in estrogen biology from knockout and transgenic animals. Annu Rev Physiol 67: 285-308, 2005.
60. Burns KA, Rodriguez KF, Hewitt SC, Janardhan KS, Young SL and Korach KS: Role of estrogen receptor signaling required for endometriosis-like lesion establishment in a mouse model. Endocrinology 153: 3960-3971, 2012.
61. Graham JD and Clarke CL: Physiological action of progesterone in target tissues. Endocr Rev 18: 502-519, 1997.
62. Bulun SE, Monsivais D, Kakinuma T, Furukawa Y, Bernardi L, Pavone ME and Dyson M: Molecular biology of endometriosis: from aromatase to genomic abnormalities. Semin Reprod Med 33: 220-224, 2015.
63. Pellegrini C, Gori I, Achtari C, Hornung D, Chardonnens E, Wunder D, Fiche M and Canny GO: The expression of estrogen receptors as well as GREB1, c-MYC, and cyclin D1, estrogen-regulated genes implicated in proliferation, is increased in peritoneal endometriosis. Fertil Steril 98: 1200-1208, 2012.
64. Brandenberger AW, Lebovic DI, Tee MK, Ryan IP, Tseng JF, Jaffe RB and Taylor RN: Oestrogen receptor (ER)-alpha and ER-beta isoforms in normal endometrial and endometriosis-derived stromal cells. Mol Hum Reprod 5: 651-655, 1999.
65. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR and Bulun SE: Progesterone receptor isoform A but not B is expressed in endometriosis. J Clin Endocrinol Metab 85: 2897-2902, 2000.
66. Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, Milad MP, Confino E, Reierstad S, Innes J, *et al*: Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod 77: 681-687, 2007.
67. Wu Y, Strawn E, Basir Z, Halverson G and Guo SW: Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. Epigenetics 1: 106-111, 2006.
68. Misao R, Iwagaki S, Fujimoto J, Sun W and Tamaya T: Dominant expression of progesterone receptor form B mRNA in ovarian endometriosis. Horm Res 52: 30-34, 1999.
69. Bukulmez O, Hardy DB, Carr BR, Word RA and Mendelson CR: Inflammatory status influences aromatase and steroid receptor expression in endometriosis. Endocrinology 149: 1190-1204, 2008.
70. Meyer JL, Zimbardi D, Podgaec S, Amorim RL, Abrão MS and Rainho CA: DNA methylation patterns of steroid receptor genes *ESR1*, *ESR2* and *PGR* in deep endometriosis compromising the rectum. Int J Mol Med 33: 897-904, 2014.
71. Rice DA, Mouw AR, Bogerd AM and Parker KL: A shared promoter element regulates the expression of three steroidogenic enzymes. Mol Endocrinol 5: 1552-1561, 1991.
72. Morohashi K, Honda S, Inomata Y, Handa H and Omura T: A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. J Biol Chem 267: 17913-17919, 1992.
73. Zeitoun K, Takayama K, Michael MD and Bulun SE: Stimulation of aromatase P450 promoter (II) activity in endometriosis and its inhibition in endometrium are regulated by competitive binding of steroidogenic factor-1 and chicken ovalbumin upstream promoter transcription factor to the same cis-acting element. Mol Endocrinol 13: 239-253, 1999.
74. Kitawaki J, Kado N, Ishihara H, Koshiba H, Kitaoka Y and Honjo H: Endometriosis: the pathophysiology as an estrogen-dependent disease. J Steroid Biochem Mol Biol 83: 149-155, 2002.
75. Tian Y, Kong B, Zhu W, Su S and Kan Y: Expression of steroidogenic factor 1 (SF-1) and steroidogenic acute regulatory protein (StAR) in endometriosis is associated with endometriosis severity. J Int Med Res 37: 1389-1395, 2009.
76. Xue Q, Lin Z, Yin P, Milad MP, Cheng YH, Confino E, Reierstad S and Bulun SE: Transcriptional activation of steroidogenic factor-1 by hypomethylation of the 5' CpG island in endometriosis. J Clin Endocrinol Metab 92: 3261-3267, 2007.
77. Yamagata Y, Nishino K, Takaki E, Sato S, Maekawa R, Nakai A and Sugino N: Genome-wide DNA methylation profiling in cultured eutopic and ectopic endometrial stromal cells. PLoS One 9: e83612, 2014.
78. Xue Q, Zhou YF, Zhu SN and Bulun SE: Hypermethylation of the CpG island spanning from exon II to intron III is associated with steroidogenic factor 1 expression in stromal cells of endometriosis. Reprod Sci 18: 1080-1084, 2011.
79. Xue Q, Xu Y, Yang H, Zhang L, Shang J, Zeng C, Yin P and Bulun SE: Methylation of a novel CpG island of intron 1 is associated with steroidogenic factor 1 expression in endometriotic stromal cells. Reprod Sci 21: 395-400, 2014.
80. Hu M, Yao J, Cai L, Bachman KE, van den Brûle F, Velculescu V and Polyak K: Distinct epigenetic changes in the stromal cells of breast cancers. Nat Genet 37: 899-905, 2005.
81. Hoivik EA, Bjanesoy TE, Mai O, Okamoto S, Minokoshi Y, Shima Y, Morohashi K, Boehm U and Bakke M: DNA methylation of intronic enhancers directs tissue-specific expression of steroidogenic factor 1/adrenal 4 binding protein (SF-1/Ad4BP). Endocrinology 152: 2100-2112, 2011.
82. Feinberg AP and Tycko B: The history of cancer epigenetics. Nat Rev Cancer 4: 143-153, 2004.
83. Bell AC and Felsenfeld G: Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature 405: 482-485, 2000.
84. Zanatta A, Rocha AM, Carvalho FM, Pereira RM, Taylor HS, Motta EL, Baracat EC and Serafini PC: The role of the Hoxa10/HOXA10 gene in the etiology of endometriosis and its related infertility: A review. J Assist Reprod Genet 27: 701-710, 2010.
85. Eun Kwon H and Taylor HS: The role of HOX genes in human implantation. Ann N Y Acad Sci 1034: 1-18, 2004.
86. Taylor HS, Arici A, Olive D and Igarashi P: HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. J Clin Invest 101: 1379-1384, 1998.
87. Gui Y, Zhang J, Yuan L and Lessey BA: Regulation of HOXA-10 is and its expression in normal and abnormal endometrium. Mol Hum Reprod 5: 866-873, 1999.
88. Szczepańska M, Wirstlein P, Luczak M, Jagodziński PP and Skrzypczak J: Reduced expression of HOXA10 in the midluteal endometrium from infertile women with minimal endometriosis. Biomed Pharmacother 64: 697-705, 2010.
89. Wu Y, Halverson G, Basir Z, Strawn E, Yan P and Guo SW: Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am J Obstet Gynecol 193: 371-380, 2005.
90. Kim JJ, Taylor HS, Lu Z, Ladhani O, Hastings JM, Jackson KS, Wu Y, Guo SW and Fazleabas AT: Altered expression of HOXA10 in endometriosis: Potential role in decidualization. Mol Hum Reprod 13: 323-332, 2007.
91. Lee B, Du H and Taylor HS: Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biol Reprod 80: 79-85, 2009.
92. Fambrini M, Sorbi F, Bussani C, Cioni R, Sisti G and Andersson KL: Hypermethylation of HOXA10 gene in mid-luteal endometrium from women with ovarian endometriomas. Acta Obstet Gynecol Scand 92: 1331-1334, 2013.
93. Lu H, Yang X, Zhang Y, Lu R and Wang X: Epigenetic disorder may cause downregulation of HOXA10 in the eutopic endometrium of fertile women with endometriosis. Reprod Sci 20: 78-84, 2013.
94. Bromer JG, Wu J, Zhou Y and Taylor HS: Hypermethylation of homeobox A10 by in utero diethylstilbestrol exposure: An epigenetic mechanism for altered developmental programming. Endocrinology 150: 3376-3382, 2009.
95. Naqvi H, Ilagan Y, Krikun G and Taylor HS: Altered genome-wide methylation in endometriosis. Reprod Sci 21: 1237-1243, 2014.
96. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, *et al*: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev 15: 342-355, 1994.
97. Abu Hashim H: Potential role of aromatase inhibitors in the treatment of endometriosis. Int J Womens Health 6: 671-680, 2014.
98. Maia H Jr, Haddad C, Coelho G and Casoy J: Role of inflammation and aromatase expression in the eutopic endometrium and its relationship with the development of endometriosis. Womens Health (Lond Engl) 8: 647-658, 2012.
99. Bulun SE, Takayama K, Suzuki T, Sasano H, Yilmaz B and Sebastian S: Organization of the human aromatase p450 (CYP19) gene. Semin Reprod Med 22: 5-9, 2004.
100. Izawa M, Harada T, Taniguchi F, Ohama Y, Takenaka Y and Terakawa N: An epigenetic disorder may cause aberrant expression of aromatase gene in endometriotic stromal cells. Fertil Steril 89 (Suppl 5): 1390-1396, 2008.

101. Izawa M, Taniguchi F, Uegaki T, Takai E, Iwabe T, Terakawa N and Harada T: Demethylation of a nonpromoter cytosine-phosphate-guanine island in the aromatase gene may cause the aberrant up-regulation in endometriotic tissues. Fertil Steril 95: 33-39, 2011.
102. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB and Lipsky PE: Cyclooxygenase in biology and disease. FASEB J 12: 1063-1073, 1998.
103. Ota H, Igarashi S, Sasaki M and Tanaka T: Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. Hum Reprod 16: 561-566, 2001.
104. Matsuzaki S, Canis M, Pouly JL, Wattiez A, Okamura K and Mage G: Cyclooxygenase-2 expression in deep endometriosis and matched eutopic endometrium. Fertil Steril 82: 1309-1315, 2004.
105. Buchweitz O, Staebler A, Wülfing P, Hauzman E, Greb R and Kiesel L: COX-2 overexpression in peritoneal lesions is correlated with nonmenstrual chronic pelvic pain. Eur J Obstet Gynecol Reprod Biol 124: 216-221, 2006.
106. Zidan HE, Rezk NA, Alnemr AA and Abd El Ghany AM: COX-2 gene promoter DNA methylation status in eutopic and ectopic endometrium of Egyptian women with endometriosis. J Reprod Immunol 112: 63-67, 2015.
107. Wang D, Chen Q, Zhang C, Ren F and Li T: DNA hypomethylation of the COX-2 gene promoter is associated with up-regulation of its mRNA expression in eutopic endometrium of endometriosis. Eur J Med Res 17: 12, 2012.
108. Starzinski-Powitz A, Gaetje R, Zeitvogel A, Kotzian S, Handrow-Metzmacher H, Herrmann G, Fanning E and Baumann R: Tracing cellular and molecular mechanisms involved in endometriosis. Hum Reprod Update 4: 724-729, 1998.
109. Wu Y, Starzinski-Powitz A and Guo SW: Trichostatin A, a histone deacetylase inhibitor, attenuates invasiveness and reactivates E-cadherin expression in immortalized endometriotic cells. Reprod Sci 14: 374-382, 2007.
110. Zhou H, Li J, Podratz KC, Tipton T, Marzolf S, Chen HB and Jiang SW: Hypomethylation and activation of syncytin-1 gene in endometriotic tissue. Curr Pharm Des 20: 1786-1795, 2014.
111. Stern RC, Dash R, Bentley RC, Snyder MJ, Haney AF and Robboy SJ: Malignancy in endometriosis: Frequency and comparison of ovarian and extraovarian types. Int J Gynecol Pathol 20: 133-139, 2001.
112. Matalliotakis I, Mahutte NG, Koukoura O and Arici A: Endometriosis associated with Stage IA clear cell ovarian carcinoma in a woman with IVF-ET treatments in the Yale Series. Arch Gynecol Obstet 274: 184-186, 2006.
113. Shih IeM and Kurman RJ: Ovarian tumorigenesis: A proposed model based on morphological and molecular genetic analysis. Am J Pathol 164: 1511-1518, 2004.
114. Koukoura O, Spandidos DA, Daponte A and Sifakis S: DNA methylation profiles in ovarian cancer: Implication in diagnosis and therapy (Review). Mol Med Rep 10: 3-9, 2014.
115. Martini M, Ciccarone M, Garganese G, Maggiore C, Evangelista A, Rahimi S, Zannoni G, Vittori G and Larocca LM: Possible involvement of hMLH1, p16(INK4a) and PTEN in the malignant transformation of endometriosis. Int J Cancer 102: 398-406, 2002.
116. Senthong A, Kitkumthorn N, Rattanatanyong P, Khemapech N, Triratanachart S and Mutirangura A: Differences in LINE-1 methylation between endometriotic ovarian cyst and endometriosis-associated ovarian cancer. Int J Gynecol Cancer 24: 36-42, 2014.
117. Guo C, Ren F, Wang D, Li Y, Liu K, Liu S and Chen P: RUNX3 is inactivated by promoter hypermethylation in malignant transformation of ovarian endometriosis. Oncol Rep 32: 2580-2588, 2014.
118. Perez-Janices N, Blanco-Luquin I, Torrea N, Liechtenstein T, Escors D, Cordoba A, Vicente-Garcia F, Jauregui I, De La Cruz S, Illarramendi JJ *et al*: Differential involvement of RASSF2 hypermethylation in breast cancer subtypes and their prognosis. Oncotarget 6: 23944-23958, 2015.
119. Guerrero-Setas D, Pérez-Janices N, Blanco-Fernandez L, Ojer A, Cambra K, Berdasco M, Esteller M, Maria-Ruiz S, Torrea N and Guarch R: RASSF2 hypermethylation is present and related to shorter survival in squamous cervical cancer. Mod Pathol 26: 1111-1122, 2013.
120. Zhao L, Cui Q, Lu Z and Chen J: Aberrant methylation of RASSF2A in human pancreatic ductal adenocarcinoma and its relation to clinicopathologic features. Pancreas 41: 206-211, 2012.
121. Ren F, Wang DB, Li T, Chen YH and Li Y: Identification of differentially methylated genes in the malignant transformation of ovarian endometriosis. J Ovarian Res 7: 73, 2014.
122. Borghese B, Barbaux S, Mondon F, Santulli P, Pierre G, Vinci G, Chapron C and Vaiman D: Research resource: Genome-wide profiling of methylated promoters in endometriosis reveals a subtelomeric location of hypermethylation. Mol Endocrinol 24: 1872-1885, 2010.
123. Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, Kakinuma T, Ono M, Jafari N, Dai Y, *et al*: Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. PLoS Genet 10: e1004158, 2014.
124. Yoo CB and Jones PA: Epigenetic therapy of cancer: Past, present and future. Nat Rev Drug Discov 5: 37-50, 2006.
125. Jichan Nie, Xishi Liu and Guo SW: Promoter hypermethylation of progesterone receptor isoform B (PR-B) in adenomyosis and its rectification by a histone deacetylase inhibitor and a demethylation agent. Reprod Sci 17: 995-1005, 2010.
126. Arosh JA, Lee J, Starzinski-Powitz A and Banu SK: Selective inhibition of prostaglandin E2 receptors EP2 and EP4 modulates DNA methylation and histone modification machinery proteins in human endometriotic cells. Mol Cell Endocrinol 409: 51-58, 2015.
127. Bergman MD, Schachter BS, Karelus K, Combatsiaris EP, Garcia T and Nelson JF: Up-regulation of the uterine estrogen receptor and its messenger ribonucleic acid during the mouse estrous cycle: The role of estradiol. Endocrinology 130: 1923-1930, 1992.