Title: recent insights on the genetics and epigenetics of endometriosis

Bruno Borghese[1,4], Krina T. Zondervan[2], Mauricio S. Abrao[3], Charles Chapron[1,4] and Daniel Vaiman[1,4].

1Cochin Institute, U1016 INSERM, CNRS 8104, Université Paris Descartes, Paris, France.
2Nuffield Department of Obstetrics and Gynaecology, Endometriosis Care Centre, University of
Oxford, Oxford, United Kingdom.
3Endometriosis Division (Head), Ob/Gyn Department, Sao Paulo University, Sao Paulo, Brazil;
Reproductive Clinic, Sirio Libanes Hospital, Sao Paulo, Brazil.
4Department of Gynecology Obstetrics II and Reproductive Medicine, Faculté de Médecine, AP-HP, Groupe Hospitalier Ouest, Centre Hospitalier Universitaire Paris Centre, Paris, France.

Correspondence: daniel.vaiman@inserm.fr

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Abstract

Endometriosis is a gynecologic disease affecting up to 10% of the women and a major cause of pain and infertility. It is characterized by the implantation of functional endometrial tissue at ectopic positions generally within the peritoneum. This complex disease has an important genetic component with a heritability estimated at around 50%. This review aims at providing recent insights into the genetic bases of endometriosis, and present a detailed overview of evidence of epigenetic alterations specific to this disease. In the future, these alterations may constitute therapeutic targets for pharmacological compounds able to modify the epigenetic code.

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I. Endometriosis definition and clinical aspects

Endometriosis is defined by the presence of functional ectopic endometrial glands and stroma outside the uterine cavity affecting 6-10% of women of reproductive age (1) at several sites (intra-peritoneal or extra-peritoneal) including ovaries, peritoneum, uterosacral ligaments, posterior cul-de-sac, vagina, gastrointestinal tract, urinary bladder and ureters. The rectosigmoid colon represents the most common site of gastrointestinal endometriosis (2, 3). Extra pelvic involvement is rare (4).

Three main etiopathogenic hypotheses could contribute to endometriosis (Figure 1). The most widely accepted is based on retrograde shedding of endometrial cells into the abdominal cavity during menstruation (5), promoting a chronic inflammatory response (6, 7). In the coelomic metaplasia theory mesothelium differentiates *in situ* into endometrial tissue (8). Finally in the embryonic origin theory, lesions are present during all the development (9). In this complex and multifactorial disease, immune system and angiogenesis abnormalities, genetic causes, environmental and biochemical factors play relevant roles in specific biological pathways promoting the establishment and growth of the endometrial lesions (10-12). The presence of endometrial stem cells in the adult uterus, the menstrual fluid, and extra-uterine endometrial implants could be implicated as well in the pathogenesis of endometriosis (13).

According to the American Society for Reproductive Medicine, endometriosis exists in four stages: I (minimal disease), stage II (mild disease), stage III (moderate disease) and stage IV (severe disease) (ASRM, 1997). This surgical classification is based on the extent of adhesions and size of the lesions.

Considering the sites of the disease, endometriosis is classified into peritoneal, ovarian (endometriomas) and deep endometriosis, often associated with each other. By definition, deep endometriotic lesions infiltrate the vagina, bowel, bladder or ureters deeper than 5 mm under the peritoneum (14). In the case of bowel endometriosis, the infiltration of endometrial-like glands and stroma, is located at the bowel wall reaching the subserous fat tissue or adjacent subserous plexus (15).

Diagnosis of endometriosis is currently based on clinical manifestations, imaging techniques, and surgical approaches (16). Laparoscopic exploration and excision of all visible lesions allow histological confirmation of endometriosis and treatment of the disease (16). A diagnosis and treatment delay of 5 to 10 years is however common, due to a nonspecific symptomatology and a lack of noninvasive methods for detecting it (17).

Although a number of women presenting endometriosis may be asymptomatic, often patients manifest severe dysmenorrhea (6, 18-20), non-cyclical chronic pelvic pain, dysfunctional uterine bleeding, deep dyspareunia (6, 19), urinary tract symptoms, and gastrointestinal symptoms such as painful defecation (21). Infertility is also a prevalent clinical manifestation in up to 40% of women presenting endometriosis (22). Endocrine and ovulatory disorders are also observed in a smaller fraction of patients with endometriosis (6, 23). The clinical variability of symptoms is important even considering women with similar anatomical presentations (24).

II Genetics of endometriosis

Endometriosis is a complex disease, in which both genetic and environmental factors contribute to risk. The heritability of endometriosis has been estimated at around 50% based on large twin studies (25, 26). Although many candidate gene studies have been conducted to investigate hypotheses for the genetic basis of endometriosis, these have generally failed to produce replicable results (27, 28), an observation that is typical in the complex disease field. There are many reasons for this, including the validity of the biological hypothesis tested, the fact that typically only a few DNA variants in a handful of genes in a pathway are tested, and issues with heterogenous case definitions and inadequate study sample sizes.

The first attempt to understand the genetic basis of endometriosis through genome-wide, hypothesis-free, approaches was through collaborative family-based linkage studies conducted between 1995-2005 (29-31). These studies investigate the presence of major gene effects in families with multiple women affected with endometriosis. Two chromosomal areas of significant linkage were observed on 10q26 and 7p13-15 (harboring genes such as *CYP2C19*, *INHBA*, *SFRP4* and *HOXA10*) that were likely to harbor variants underlying endometriosis risk. However the LOD scores observed were barely above the threshold of 3, and not of the magnitude seen for monogenic traits. This suggested that there is unlikely to be a 'major gene' that accounts for the majority of familial endometriosis risk.

The advent of hypothesis-free genome-wide association studies (GWAS) in 2005 saw this study design also being adopted for endometriosis from 2010 onwards. GWAS studies are aimed at identifying common genetic variants (typically >1-5% population allele frequency) underlying complex disease. To date, five GWAS studies (32-36) on four independent datasets including women of Japanese and European ancestry have been conducted (for recent detailed reviews see (28, 37, 38)). These studies included discovery case sets from 696 to 4,604 cases, defined in a variety of ways, through surgical record confirmation, or a mixture of surgical and clinical confirmation. GWAS analyses have resulted in 10 genome-wide significant loci, which together explain ~4% of heritability. A meta-analysis published in 2014 of the four GWAS and four replication datasets based on >11,000 cases and > 30,000 controls (39) provided evidence for genome-wide significant association of six of the loci across datasets and populations (*rs7521902* near *WNT4*; *rs10859871* near *VEZT*; r*s12700667* on 7p15.2, *rs1537377* near *CDKN2B-AS1*, *rs7739264* near *ID4*, and *rs13394619* in *GREB1*). Further strong support ($p < 10^{-7}$) was provided for *rs1250248* in *FN1* and *rs4141819* on 2p14; a subsequent published analysis including a fifth replication dataset provided further evidence for a ninth locus, *rs6542095* at *IL1A* (40).

Endometriosis GWAS results clearly lie far behind other complex diseases in terms of number of discovered loci explaining heritability, for exemple when compared to – for example – breast cancer (>90 loci from 60,000 cases (41)) or Crohn's disease (>140 loci from 20,000 cases (42)). A new phase of endometriosis GWAS meta-analyses is currently ongoing, which will increase the discovery case set 4-5 fold in size and likely result in further loci implicated in its development.

The endometriosis GWAS results to date have offered a number of novel insights into its pathogenesis. Firstly, most studies that included surgically confirmed cases with rAFS-classified disease showed that effects were often much larger for, or limited to, stage III/IV disease than stage I/II (38). Stage III/IV disease is typically characterized by ovarian (cystic) or deep infiltrating disease including extensive adhesions, whereas stage I/II is by smaller

peritoneal lesions. Indeed, the largest GWAS including >3,000 surgically confirmed cases of European ancestry by the collaborative International Endogene Consortium (comprising datasets from Australia, the UK, and US) demonstrated that the disease variance due to common genetic variation assayed in GWAS was much larger for stage III/IV disease (31%) than for stage I/II (15%), suggesting distinct genetic origins of these disease subtypes.

Secondly, although much work remains to be done on understanding which and how biological pathways are perturbed by the implicated variants, the loci identified to date implicate estrogen-induced cell growth (*GREB1*), cell adhesion, migration, growth and differentiation (*VEZT*, *FN1*), inflammation (*IL1A*), but importantly also WNT/β-catenin signaling. The latter appears as a key pathway that has not been investigated extensively in relation to endometriosis – although it and *WNT4* therein has a known role in the development of the female reproductive tract (43). Intriguingly, one of the top GWAS loci, an intergenic signal on 7p15.2 marked by *rs12700667* was also found to be associated with fat distribution (waist-to-hip ratio adjusted for body mass index) in an independent GWAS (44). A subsequent enrichment analysis of the endometriosis and fat distribution GWAS results highlighted unexpected, statistically significant, sharing of genetic loci underlying both traits, which again implicated the WNT/β-catenin pathway (45).

As is typical for GWAS results, most of the loci reside in inter-genic regions for which the functionality remains to be uncovered. This requires the integrated analysis of genetic data with epigenomic and transcriptomic data for tissues relevant to the disease of interest - in this case: eutopic/ectopic endometrium and its constituting cells. However, large-scale molecular profiling initiatives aimed at allowing such functional follow-up work, e.g. the GTex project (GTex Consortium 2013) and the NIH Epigenome Roadmap (46), do not include endometrium. This substantially limits progress in the endometriosis field. In 2014, an international working group of 34 academic endometriosis research centers and 3 industry partners reached a consensus on the standardized collection of surgical and clinical data, as well as standard operating protocols (SOPs) for the collection of 11 biological sample types relevant to endometriosis, including endometrium: the WERF Endometriosis Phenome and Biobanking Harmonisation Project (EPHect). Data collection instruments and SOPs are freely available (37, 47-49). This initiative (endometriosisfoundation.org/ephect) now facilitates large-scale collaborative ‘omics’ analyses on deeply phenotyped endometrium and other tissues that is required for the translation of the genetic findings.

III Epigenetics of endometriosis

Epigenetics can be defined as the study of heritable changes in gene function that are not associated with changes in DNA sequence, inherited mitotically and/or meiotically. Epigenetics intervenes in every developmental, physiological or pathological mechanism. The impact of epigenetics in endometriosis has been thoroughly studied in the recent years (50).

III.1 DNA methylation

III.1.a) DNA methylation/demethylation in mammalian genomes

DNA methylation in mammalian cells occurs generally at the Cytosine residues of CG dinucleotides through the action of specific DNA methyltransferases. Some genomic regions are very rich in CG dinucleotides. Above 50% or when 1/10 of the C nucleotides are followed by a G, the region is called a 'CpG island'. Part of the methylation is labile and influenced by environmental conditions in particular through the action of *de novo* methyltransferases, DNMT3A and DNMT3B. Demethylation is also actively carried out by specific enzymes (such as TET, AID and GADD45 (51-53)). An increasing level of complexity has been the discovery of methylation intermediates, such as in particular hydroxymethylation, generated by oxidation of methyl-cytosines by the TET enzymes. Methylation and hydroxymethylation have a great relevance in terms of development and pathology (54-56) but hydroxymetyhylation has not yet been studied in the context of endometriosis.

III.1.b) DNA methylation in endometriosis

To the best of our knowledge, two articles compared global DNA methylation between eutopic endometrium and ectopic endometrial lesions. One made use of immunoprecipitation of DNA containing methylated cytosine, followed by microarray hybridization (MeDIP-chip technology – Methyl cytosine Immuno Precipitation followed by promoter microarray hybridization) (57). The second took advantage of the recently available Infinium Human Methylation 450 BeadChip, interrogating almost 500,000 CpG spread throughout the genome (58). The first study was focused on promoter regions and detected 37 promoters differentially methylated. The second tackled the genome extensively (in shores, islands, or open seas according to the distance from the CpG islands) as well as in various regions relative to the genes (gene body, first exon, 5' and 3' UTR, 200 nucleotides and 1500 nucleotides from the Transcription Start Site), revealed 42000 CpG differentially methylated. This study revealed that the GATA transcription factors, especially GATA6, as well as SF1 (Steroidogenic Factor 1), and ERβ, encoding estrogen receptor 2, are deregulated in association with strong alterations of the methylation in ectopic endometrial tissue compared with eutopic tissue (59). The overlap between the two studies is partial probably because of technical limitations of the MeDIP technology, nevertheless 21 promoters out of 37 were found consistent between the two studies (Table 2, $\chi 2$, $p = 6.98\ 10^{-25}$) . Among those, HOX genes appeared as major targets of abnormal DNA methylation, consistently with existing literature. *HOXA10* was discovered as hypermethylated in ectopic endometrial tissue, compared to eutopic in 2005 (60), and later confirmed in other studies (58, 61, 62). This was associated with differential gene expression between eutopic and ectopic uterine tissue in the Baboon model (63). In human ectopic endometrium samples, *HOXA10* and *HOXA11* are down-regulated (64), (65), (66). A systematic analysis of the literature linking DNA methylation and endometriosis is summarized in (67).

These observations on whole genome methylation are rather descriptive as for today. They are not connected to two major issues: (i) what is the origin of the anomalies in the ectopic tissue? And (ii) what are the consequences of these anomalies on pathophysiology? A well-spread dogma assimilates methylation to gene repression and demethylation to gene expression enhancement, but this appears far too simple (see for instance (68)).

III.2 The Histone code in endometriosis

III.2.a) A possible therapeutic avenue: modifying the histone code

The histone code is a complex epigenetic mode of gene regulation. More than 70 histone post-translational modifications (PTM) have been described (69). It is generally admitted that H3K4Me3, H3/H4Ac are associated to open and active chromatin, while H3K27Me3 and H3K9Me3 are associated to silenced chromatin (70). The histone code can be modified by drugs, such as HDAC inhibitors (HDACi) (71) that appear as promising candidates to improve endometriosis. HDACi are probably able to move the cells from a state of silenced chromatin, towards activation and differentiation, thus limiting proliferation. The HDACi suberoylanilide hydroxamic acid (SAHA or vorinostat) and romidepsin were able to reduce the expression of *GPER* (G-protein-coupled estrogen receptor) and then inhibit the proliferation of endometriotic cells (72). SAHA, trichostatin A (TSA) (73) or valproic acid (VPA), known from 1881, are known from a long time as epigenetic modulators. Romidepsin, more recently reported (1994) acts as a HDACi with a potential antitumor effect (74). Romidepsin interacts with a zinc-dependent pocket of histone deacetylases (similarly to SAHA), adverse effects being neurological, haematological, and metabolic. In 2011, Kawano and coworkers showed decreased levels of acetylated histones in endometriotic stromal cells (ESC) compared to normal stromal cells. HDACi treatment led to accumulating acetylated histones on the promoter of genes important for cell division and apoptosis (*p16*, *p21*, *Bcl2* and *BclX*), possibly contributing to their activation (75).

III.2.b) Endometriosis modifications of the histone code

Endometriosis induce the deposition of abnormal histone marks such as H3 and H4 acetylation on lysines in the lesions (76). This is associated with alterations of the expression of genes able to modify this code. In 2012, Colon-Diaz showed that HDAC1 and HDAC2 were up-regulated in ESC, at mRNA and protein levels (77). Samartzis showed in 2013 that HDAC1 only was increased in endometriosis (78), from the analysis of 74 pathological samples and 30 controls. The observation was not true for HDAC2/3. In another study, SIRT1 was found decreased in eutopic endometrium while HDAC1, SUV39H1, SUV39H2 and G9a were significantly downregulated in ectopic endometrium (79). Our own microarray analysis (80) showed that several isoforms of *HDAC5* were specifically increased, contrary to the other HDACs. Despite these contrasting results it appears that histone code modifications has important consequences on endometrial cells. Kawano and co-workers (81) treated ESC from endometriomas with VPA, and identified 5 genes highly modified by the treatment: *MT1G* (x 26.2), *IL8* (x 12), *CEBPA* (x 10), *PPP2R2B* (x 10) and *ABCB1* (x 5.9). The authors focused their work on *CEBPA*, encoding the transcription factor C/EBPα. The protein was decreased in endometriotic tissue, while VPA treatment induced the accumulation of ‘opened’ histone marks (H3/H4 acetylation) on its promoter, and its induction. C/EBPα is involved in proliferation, metabolism, differentiation, etc., but also in tumour suppression. By contrast, several transcriptomic analyses failed to reveal a deregulation of *CEBPA* in endometriosis (see for instance (66, 82)). These discrepancies may be linked to the ethnicity of the

population, as for *rs10965235* located in *CDKN2BAS* that is informative only in the Japanese population (33). Focusing on *CDKN2BAS*, Nakaoka showed a specific regulation by the non-coding RNA *ANRIL*, located nearby the susceptibility locus (9p21). The protective allele interacts more strongly with the *ANRIL* promoter as revealed by 3C (Chromosome Conformation Capture). This allele binds more strongly to *TCF7L2* and *EP300* and this was reinforced by analysis of H3K27Ac et RNA PolII (83). Several modifications of histone recruitment on promoters were identified for specific genes in endometriotic lesions, such as *SF1* (76), which together with the abnormal methylation (as previously described), posits this gene as a major regulator of abnormal steroidogenesis in endometriosis. In the same pathway of estrogen metabolism, abnormal histone marks were discovered at the promoter of *CYP19* (aromatase) (84), with potential consequences on endometriosis-associated infertility (85). These modifications of the histone code at several target genes could be initiated by *EP2* and *EP4* prostaglandin receptor deregulation. Indeed, pharmacological inhibition of these receptors led to strong deregulation of the epigenetic machinery in particular with abnormal histone marks (86). The *EP2* receptor normally transactivates the steroidogenic acute regulatory protein (StAR). StAR is a mitochondrial protein that regulates cholesterol transfer into mitochondria, a step necessary for the synthesis of steroid hormones. The authors showed that the HDACi TSA was also able to increase the transcription of *StAR* (87).

III.2.c) Experimental modifications of the histone code in the context of endometriosis

Cell models

In a preliminary study (88) with an immortalized cell line from endometrial stromal cells (89), Wu and Guo tested a panel of drugs (TSA, CDB, RU486, NAC and ICI 182780) and measured specific outcomes, such as cell proliferation and the expression of *PRA*, *PRB*, *AR*, *FasL* and *Fas*. All drugs, except ICI 182870, had various antiproliferative effects but the TSA effects were longer. The receptors expression (*PRA*, *PRB*, *AR)* was increased, possibly materializing an increased differentiation of the cells, associated with a decrease in proliferation. Later in two immortalized endometriotic cell lines (11Z, epithelial like and 22B, stromal like (90)), Wu and Guo demonstrated that TSA and VPA induce cell cycle arrest and reduce *p21* expression. TSA attenuates the invasion capacities of ESCs, with or without TNFα treatment (91), and suppresses *COX2* expression induced by IL1B (92).

In 2009, the Guo's team showed that the PPARG pathway (essential for lipid metabolism and inflammation) is altered by TSA treatment, in normal endometrial cells and endometriotic cell lines. By contrast, a RXR (Retinoid X Receptor) inhibitor, LG100268, had positive effects on endometriotic cell models, in terms of proliferation (93).

Transcription factors able to modulate chromatin are also possibly modified in endometriosis. For instance, Krüppel-Like Factor11 (KLF11) is decreased in endometriosis lesions. *KLF11* represses *COL1A1*, therefore, its inhibition can lead to increased fibrosis. KLF11 recruits SIN3A/HDAC; its function is mimicked by garcinol (HAT inhibitor), while HDACi generates the opposite effect in cell culture. KLF11 is also known to down-regulate various CYP enzymes of the endometrium (94). The authors showed an inverse expression between *KLF11* and *CYP3A4*. KLF11 binds to the promoter GC-rich elements, nearby the SIN3 co-repressor (a histone deacetylase) to repress the promoter. The data were also validated with a *KLF11* mutant and pharmacological treatment with the HDACi SAHA. Kai and coworkers (95) showed a silencing of the Death Receptor 6 encoding gene (*DR6*) – an inducer of apoptosis - by histone deacetylation of its promoter in isolated cells from endometriosis cysts stroma, compared to normal endometrial stromal cells. Treatment with

VPA led to the accumulation of acetylated histones H4 on the promoter of *DR6*. These data suggests that HDACi could contribute to control or treat endometriosis.

Animal models

Endometriotic lesions can be generated in rodents through autologous surgical transplantation of endometriotic explants into the peritoneum (96). TSA was able to reduce lesion growth *in vivo*. Endometriotic rats treated with the analgesic/sedative l-THP (levo-tetrahydropalmatine) in combination with VPA observed analgesic effect, while genes sensitive to HDAC2 treatment were decreased (97). In the same model, VPA alone was shown to be able to decrease pain in the rat models (98). Treatment slowed down lesion growth and improved specifically the sensitivity to nociceptive stimuli. The same team tested the effect of TSA, and 5-aza deoxy cytidine (an inhibitor of DNA methylation) on cell models derived from adenomyosis (the uterine form of endometriosis) focusing their analysis of the promoter of the progesterone receptor B (PRB). Treatments with both drugs reduced cell viability of the ectopic ESC. TSA decreased lesion size, sensitivity to pain and specific molecular markers of nociceptivity in a mouse model (99-102). Other modulators of the histone code can be envisaged as pharmacological tools to cure endometriosis. For instance, *LSD1* (KDM1A, Lysine-specific histone demethylase 1A) is overexpressed in endometrioma. Drugs targeting LSD1 (tranylcypromine) lead decreasing cell proliferation and invasiveness in ovarian endometriomas (103). This inhibitor improves the symptoms of endometriosis-induced mice (104).

III.3 Endometriosis and miRNA

MicroRNAs (miRNAs) are short single strand non-coding RNAs, containing on average 22 nucleotides. They constitute important regulatory molecules able to modify gene expression, by pairing with mRNA carrying a homologous sequence leading to inhibit its transcription/translation. miRNAs can also directly target DNA methyltransferases, or trigger histone modification, thereby modifying the epigenome. miRNAs are involved in most physiopathological processes, such as proliferation, differentiation, apoptosis, angiogenesis and matrix remodeling (105, 106). New therapies based on miRNAs are under development and seem promising.

In endometriosis, several studies have examined miRNA expression profiling by comparing endometriotic tissue to eutopic endometrium in endometriosis-afflicted women on the one hand and eutopic endometrium to normal endometrium of healthy women on the other (Table IA). Some miRNAs, such as miR-202-3p, miR-424-5p or miR-556-3p, may contribute to a reduction in the angiogenic activity observed in ovarian endometrioma or deep infiltrating nodules (106). Others, including miR-449b-3p and miR-29c-3p, may provide a low capability of remodeling the surrounding tissue in these lesions (Table IA). On the contrary, superficial peritoneal endometriotic implants show an inverse miRNAs expression profile, displaying a high potential of angiogenesis and invasion of extracellular matrix (106). The lack of consistency of the profiles between the different studies is however striking. Differences in miRNA expression do exist according to factors related to the patient (age, ethnicity) and to the disease (disease severity and classification) (105). miRNA expression may also vary across the menstrual cycle (107) and have a circadian rhythm (108, 109), with a potentially strong effect on expression (110). Yet, most of these confounding factors have not been addressed in the abovementioned publications.

Since there is a strong correlation between circulating and tissue levels of miRNAs (111), it has been proposed to use circulating miRNAs as biomarkers for diagnosing

endometriosis, monitoring the disease or early identifying a recurrence. To date, only few studies have been published addressing the presence and quantity of miRNA in serum and plasma (see Table IB) with a very limited consistency. In all cases, the results need to be replicated on large series of well-phenotyped patients and under stringent conditions of sampling taking into account the menstrual phase and the time of the day. None of miRNAs consistently met the full criteria for a replacement or triage diagnostic test.

Numerous studies have demonstrated the potential role of several miRNAs in the pathogenesis of endometriosis, by targeting inflammation through overexpression of COX-2 and PGE2 (miR-20a, see (112, 113)) estrogens synthesis through induction of SF-1 (miR-23a, miR-23b, see (114)), proliferation, angiogenesis and apoptosis (miR-145, miR-183, miR-196b, miR-199a-5p, (81, 115-117). Grechukhina and coworkers, reported a new polymorphism in the LCS6 let-7 miRNA binding site of the KRAS 30-UTR. This polymorphism found in 31% of all cases of endometriosis was significantly higher than the 5.8% incidence observed in the general population. In women harboring this KRAS variant, altered miRNA binding leads to increased proliferation of endometrial stroma cells, invasion, abnormal endometrial growth and finally endometriosis (118). However, these results were not replicated in a much larger study, without finding a large difference between cases and controls allele frequency (119). It was recently demonstrated that decreased H19 lncRNA in the endometrium of endometriotic women increases let-7 miRNA activity, which in turn inhibits Igf1r expression at the post-transcriptional level. This may lead to reduced proliferation of endometrial stromal cells, and finally contribute to impaired endometrial receptivity and infertility in women with endometriosis (120). Finally, modulation of miRNAs involved in the pathogenesis of endometriosis may have a potential therapeutic interest, as was recently reported for let-7 (121) or miR-210 (122).

Adenomyosis, the myometrial form of edometriosis, has also been analyzed in terms of miRNA expression (123). miR-9 -1, miR-139, miR-149, miR-197, miR-326, and miR-339 were upregulated, no miRNAs was downregulated. They may predispose to invasion and survival beyond the myometrial interface. Further studies are needed to assess miRNAs expression in the different types of adenomyosis, including focal adenomyosis, most frequently associated with deep endometriosis.

IV Conclusions

In this review, we give an update of genetics and epigenetic of endometriosis, focusing whenever possible on mechanistic insights and whole-genome approaches. We pinpoint some possible therapeutic tracks, in connection with epigenetic modulation (modification of the histone PTM landscape, or of the miRNA expression profile) that will affect specifically and topically pathological features of endometriosis (such as pain and inflammation or cell proliferation). These new compounds may help in the future since there is an urgent need of non-hormonal treatments in endometriosis that ideally would alleviate painful symptoms and preserve natural conception. In 2015, the FDA approved the first HDACi (Farydak) for treating patients with multiple myeloma (124). Drugs with acceptable side effects in cancer treatment may appear applicable in some cases of endometriosis in the future.

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Figure legend
The three theories of the origin of endometriosis lesions. See text for more details.

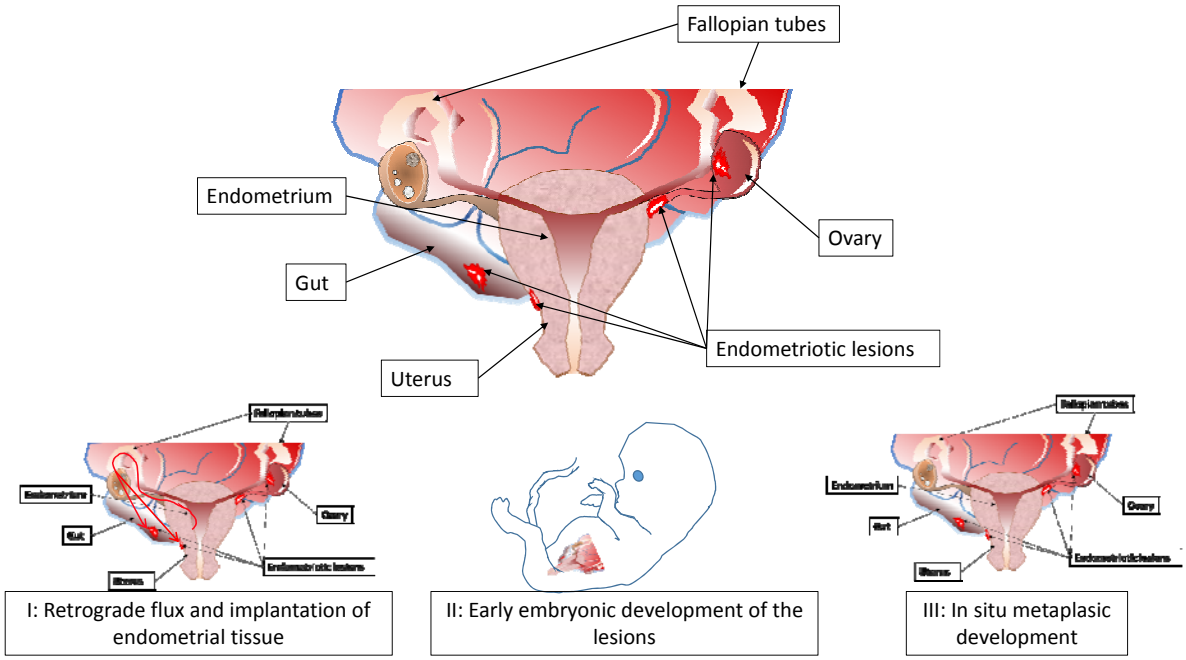

Table I – Published studies reporting miRNA expression in endometriotic lesions, plasma or serum fluid. Endo: endometriosis; SUP: superficial peritoneal endometriosis; OMA: ovarian endometrioma; DIE: deep infiltrating endometriosis; Endo stage refers to the revised American Society for Reproductive Medicine staging classification

A. miRNA in endometriotic tissue	***Tissues***	***Number of participants***	***Design***	***Downregulated***	***Upregulated***
Yang *Genet Mol Res* 2016	Ectopic vs. control	Endo stage III-IV (N=32) / controls (N=19)	array profiling	miR-200b, miR-15a-5p, miR-19b-1-5p, miR-146a-5p, miR-200c	miR-16-5p, miR-106b-5p, and miR-145-5p
Laudanski *Biomed Res Int* 2015	Eutopic vs. control	OMA (N=21) / controls (N=25)	array profiling	-	miR-5187-3p, miR-3152-5p, miR-30d-5p
Braza-Boils *Hum Reprod* 2014	Ectopic vs. eutopic vs. control	OMA (N=51), SUP (N=18), DIE (N=20) DIE) / controls (N=32)	array profiling	miR-202-3p, miR-424-5p, miR-556-3p, miR-449b-3p, (eutopic vs. control), miR-449b-3p (OMA vs. control), miR-556-3p (SUP vs control)	miR-202-3p, miR-29c-3p, miR-138-5p (ectopic vs. eutopic and vs. control)
Shi *Int J Mol Med* 2014	Ectopic vs. eutopic vs. control	Endo (N=20) / controls (N=20)	array profiling	miR-183, miR-215 and miR-363 (ectopic and eutopic vs. control)	-
Laudanski *Reprod Biol Endocrinol* 2013	Eutopic vs. control	OMA (N=21) / controls (N=25)	array profiling	miR-483-5p, miR-629*	-
Ramon *Hum Reprod* 2011	Ectopic vs. eutopic vs. control	OMA (N=41), SUP (N=24), DIE (N=13) / controls (N=38)	targeted	miR-15b, miR-17-5p, miR-20a (OMA vs. eutopic), miR-17-5p and miR-20a (OMA vs. control), miR-221 (SUP vs. OMA)	miR-21, miR-125 and miR-222 (OMA vs. eutopic), miR-21, miR-125a and miR-222 (OMA vs. control), miR-16, miR-20a, miR-21 and miR-222 (DIE vs. OMA)
Hawkins *Mol Endocrinol* 2011	Ectopic vs. eutopic	OMA (N=19)	array profiling	miR-504, miR-141, miR-429, miR-203, miR-10a, miR-200b, miR-873, miR-200c, miR-200a, miR-449b, miR-375, miR-34c-5p	miR-202, miR-193a-3p, miR-29c, miR-708, miR-509-3-5p, miR-574-3p, miR-193a-5p, miR-485-3p, miR-100, miR-720
Filigheddu *J Biomed Biotechnol* 2010	Ectopic vs. eutopic	OMA (N=16)	array profiling	miR-200a, miR-200b, miR-200c, miR-182	miR-202
Ohlsson Teague Mol Endocrinol 2009	Ectopic vs. eutopic	SUP (N=7)	array profiling	miR-200a, miR-141, miR-200b, miR-142-3p, miR-424, miR-34c, miR-20a and miR-196b	miR-145, miR-143, miR-99a, miR-99b, miR-126, miR-100, miR-125b, miR-150, miR-125a, miR-223, miR-194, miR-365, miR-29c and miR-1
Burney *Mol Hum Reprod* 2009	Eutopic vs. control	Endo (N=4) / controls (N=3)	array profiling	miR-9, miR-9*, miR-34b*, miR-34c-5p, miR-34c-3p	-
B. Circulating miRNA in endometriosis	*Biofluid*	*Number of participants*	*Design*	*Downregulated*	*Upregulated*
Wang *Reprod Sci* 2016	serum	Endo stage I-II (N=30) / controls (N=20)	deep sequencing	98 miRNAs (see article for detail), Only 21 of 98 were reported in published	miR-185-5p, miR-242-5p, miR-296-5p, miR-3127-5p, miR-424-3p, miR-4645-3p, miR-

				literatures	502-3p, miR-542-3p, miR-550a-3p, miR-636
Cosar *Fertil Steril* 2016	serum	Endo stage III-IV (N=24) / controls (N=24)	array profiling	miR-3613-5p, miR-6755-3p	miR-125b-5p, miR-150-5p, miR-342-3p, miR-143-3p, miR-145-5p, miR-500a-3p, miR-451a, miR-18a-5p
Cho *Fertil Steril* 2015	serum	Endo stage III-IV (N=24) / controls (N=24)	targeted	let7b, miR-125a	-
Rekker *Fertil Steril* 2015	plasma	Endo (N=61) / controls (N=35)	targeted	miR-200a-3p, miR-200b-3p, miR-141-3p	-
Hsu *J Pathol* 2014	serum	Endo (N=40) / controls (N=25)	array profiling	mir-199a-5p	-
Suryawanshi Clin *Cancer Res* 2013	plasma	Endo (N=33) / controls (N=20)	array profiling	-	miR-16, miR-191, miR-195
Wang *JCEM* 2013	serum	Endo (N=60) / controls (N=25)	array profiling	miR-145*, miR-141*, miR-542-3p, miR-9*	miR-199a, miR-122
Jia *Hum Reprod* 2013	plasma	Endo stage III-IV (N=23) / controls (N=23)	array profiling	miR-17-5p, miR-20a, miR-22	-

Table II – Genes abnormally methylated in both whole genome studies published today (57, 58). In the last column, when CpG statuses are discordant, the general trend is given. Inconsitencies between the two studies are in italics. They are mainly due to the interrogation of different regions of the gene, that were absent in the promoter microarray (57) or sometimes in the CpG coverage (58).

Gene symbol	**Chromosome**	**Methylation status of the promoters (Affymetrix active regions) in Endometriotic lesions vs Eutopic tissue (57)**	**Methylation in gene region (from 58)**
EDARADD	1	Hypermethylated	Hypermethylated (TSS)
ADAP1	7	Hypermethylated	Hypermethylated (gene body)
JAKMIP3	10	Hypermethylated	Hypermethylated (gene body)
SLC16A3	17	Hypermethylated	Hypermethylated (5' UTR)
FAIM3	*1*	*Hypomethylated*	*Hypermethylated (gene body)*
PIGR	1	Hypomethylated	Hypomethylated (TSS)
PLD2	*17*	*Hypomethylated*	*Hypermethylated (3' UTR)*
TNNI3K	*1*	*Hypermethylated*	*Hypomethylated (5'UTR and gene body)*
HOXD11	2	Hypermethylated	Hypermethylated (everywhere)
HOXD10	2	Hypermethylated	Hypermethylated (everywhere)
TBC1D2	9	Hypermethylated	Hypomethylated (gene body)
PIK3AP1	10	Hypermethylated	Hypermethylated (gene body)
DRD4	11	Hypermethylated	Hypermethylated (gene body)
ATP11A	13	Hypermethylated	Hypermethylated (everywhere)
DTNA	18	Hypermethylated	Hypermethylated (TSS and 5'UTR)
FXYD3	19	Hypermethylated	Hypermethylated (5'UTR and 1st exon)
AGPAT3	21	Hypermethylated	Hypermethylated (TSS and 5'UTR)
PRKAG2	7	Hypomethylated	Hypomethylated (TSS, 5'UTR and gene body)
PKP3	*11*	*Hypomethylated*	*Hypermethylated (everywhere)*
ANO1	11	Hypomethylated	Hypomethylated (gene body)
SCARB1	12	Hypomethylated	Hypomethylated