Clinical Epigenetics

REVIEW

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Systematic review on the DNA methylation role in endometriosis: current evidence and perspectives

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Abstract

Background Endometriosis appears to have a multilayered etiology, with genetic and epigenetic factors each contributing half of the pathogenesis. The molecular processes that underlie the onset of endometriosis are yet unclear, but it is assumed that an important contributor in the etiopathology of the disease is DNA methylation.

Methods We conducted a systematic review of the literature regarding DNA methylation in endometriosis following PRISMA guidelines. Records were obtained from PubMed and Web of Science on May 31, 2024. Original research articles analyzing regional or genome-wide DNA methylation in patients with confirmed endometriosis (by surgery and/or histological examination) were given consideration for inclusion. Only human studies were included, and there were no restrictions on the types of tissue that was analyzed (i.e., endometrium, blood, or fetal tissue). The study selection process was run by two manual reviewers. In parallel, an adapted virtual artificial intelligence-powered reviewer operated study selection and results were compared with the manual reviewers' selection. Studies were divided into targeted (e.g., single gene or region level) and epigenome-wide association studies. For each, we extracted a list of genes studied with precise location of CpGs analyzed and the DNA methylation status according to the groups compared. Quality assessment of studies was performed following the Newcastle–Ottawa scale. Quality of evidence was graded following the Grading of Recommendations Assessment, Development and Evaluation.

Results A total of 955 studies were screened, and 70 were identified as relevant for systematic review. Our analyses displayed that endometriosis could be polyepigenetic and with alterations in specific genes implicated in major signaling pathways contributing to the disease etiopathology (cell proliferation, differentiation, and division [PI3K-Akt and Wnt-signaling pathway], cell division [MAPK pathway], cell adhesion, cell communication, developmental processes, response to hormone, apoptosis, immunity, neurogenesis, and cancer).

Conclusion Our systematic review indicates that endometriosis is associated with DNA methylation modifications at specific genes involved in key endometrial biological processes, particularly in the ectopic endometrium. As DNA methylation appears to be an integral component of the pathogenesis of endometriosis, the identification of DNA methylation biomarkers would likely help better understand its causes and aggravating factors as well as potentially facilitate its diagnosis and support the development of new therapeutic approaches.

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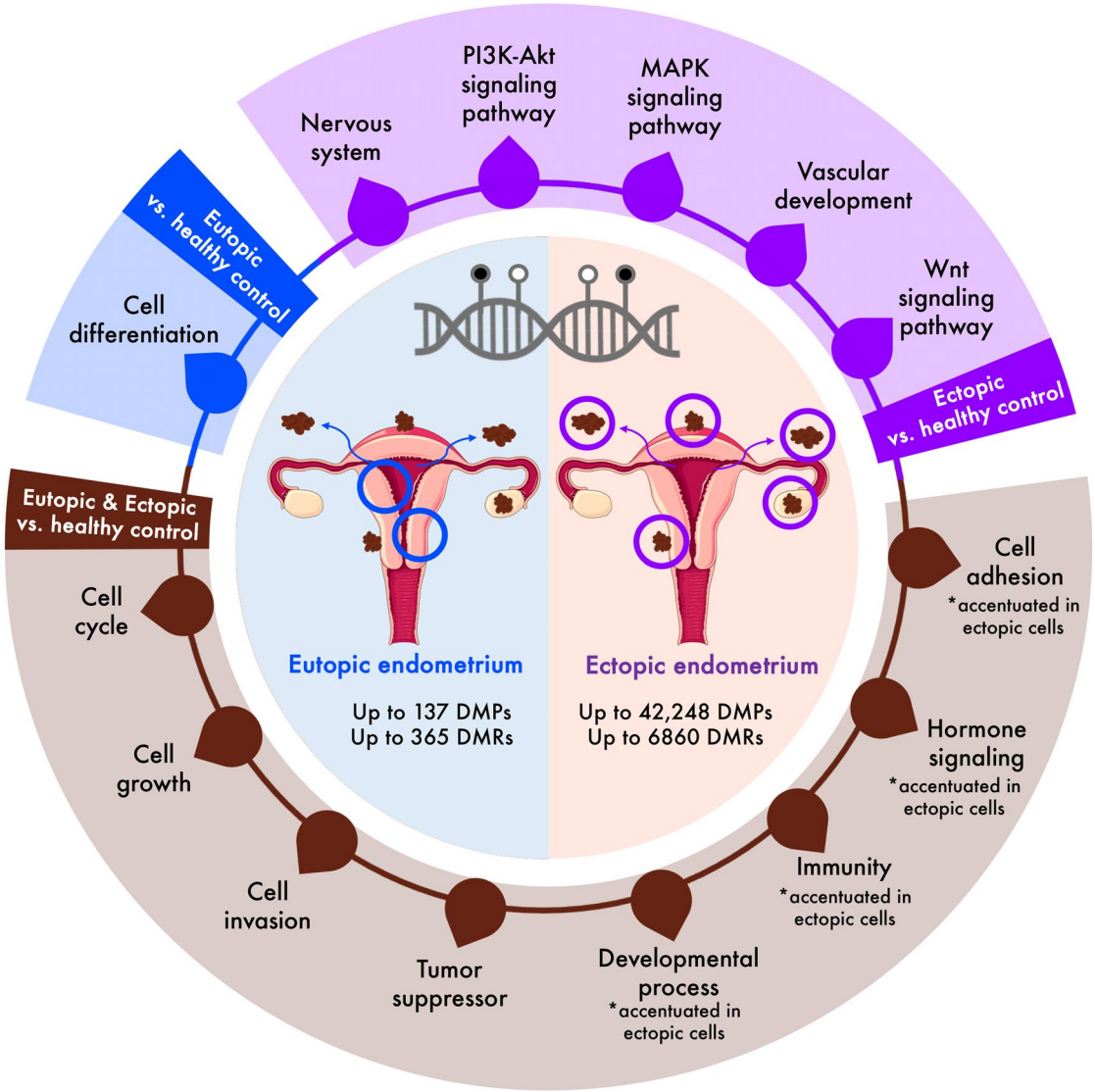
Keywords Biomarker, DNA methylation, Endometriosis, Endometrium, Epigenetics, Pathogenesis

Graphical abstract

DNA methylation modifications are observed in both eutopic and ectopic endometrium in endometriosis compared to the healthy endometrium, and their localization may influence important biological pathways of the female reproductive system. DMP: Differentially methylated position. DMR: Differentially methylated region.



Introduction

Endometriosis is a growing public health issue affecting up to 10–15% of women of reproductive age and has deleterious effects on their quality of life [1, 2]. From an anatomical point of view, endometriosis is characterized by the presence of endometrial-like tissues (glands and stroma) outside the uterine cavity with variable localization and degrees of severity [3]. Unfortunately, the molecular mechanisms underlying the development of endometriosis remain unresolved, and the multifaceted symptomatology of the disease makes its diagnosis and management complex [3]. Interestingly, the etiology of endometriosis seems multifactorial, with a genetic heritability estimated at around 50%, while the remaining 50% could be explained by environmental factors linked in part to epigenetic modifications [4–6].

The influence of the environment via epigenetic mechanisms, i.e., heritable modifications of gene expression that do not alter the DNA sequence, is suspected to have a central role in the etiopathology of endometriosis [7, 8]. Among epigenetic mechanisms, genomic DNA methylation is specifically believed to be involved in the onset of this disease. DNA methylation is characterized by the presence of methyl groups ($-CH_3$) at DNA cytosines, primarily in the cytosine–guanine (CpG) context [9]. When placed in regulatory, promoter, or enhancer regions, genomic DNA methylation is mostly associated with silent chromatin and a lack of transcription [10].

The epigenetic involvement in endometriosis is supported by many observations. Numerous expression changes of genes linked to steroid sex hormones, angiogenesis, cell proliferation, endometrial receptivity, immunity, and detoxification were found in endometriotic tissues [11–13]. For some of them such as *ESR1* and *ESR2*, their expression is controlled by epigenetic processes [12, 14]. Furthermore, there is a causal effect of oxidative stress on the progression of endometriosis, which is also mediated by epigenetic modifications [15]. Some authors also hypothesized that a single focus of an endometriotic lesion may have a single progenitor cell origin [16] meaning that the cell fate could have been influenced by DNA modifications or post-transcriptional regulations [7]. This cellular identity, heritable through successive divisions from the progenitor cell to the endometriotic foci, is thus likely to be maintained with epigenetic modifications, but this has yet to be fully demonstrated experimentally. As a result, the concept of genetic/epigenetic theory for the pathogenesis of endometriosis is gaining attention [17].

Meanwhile, the current diagnosis of endometriosis is performed via medical imaging and sometimes histological confirmation but identifying endometriotic lesions is taking a long period of errancy due to a non-specific symptomatology, with a mean diagnostic delay of 7 years in developed countries [3, 18]. This delay is causing unnecessary discomfort for patients seeking appropriate medical care and has deleterious effects on mental wellness, pain, and fertility [19]. As DNA methylation appears to be an integral component of the pathogenesis of endometriosis, the identification of DNA methylation biomarkers would likely help better understand its causes and aggravating factors as well as potentially facilitate its diagnosis and support the development of new therapeutic approaches.

To examine whether DNA methylation is a hallmark of endometriosis, we systematically reviewed the literature regarding DNA methylation in endometriosis. Study selection was performed by two independent reviewers and corroborated by a third virtual reviewer powered by artificial intelligence (AI). We applied rigorous quality evaluation of all studies, and we categorized them according to the tissues compared: healthy endometrium, eutopic (i.e., cells that have a normal location), and ectopic (i.e., cells in an abnormal location) endometrium in endometriotic patient, blood. We summarized the current evidence with reference to targeted gene and epigenome-wide association studies stratified by the level of evidence from high to very low. By applying cross-referencing methods after identifying the precise genomic regions analyzed in each study, we provide a deep investigation of genes and pathways that may be epigenetically regulated in endometriosis. Even if we cannot distinguish whether DNA methylation modifications are a cause or consequence of the disease, evidence suggests a possible epigenetic contribution in endometriosis etiopathology.

Materials and methods

Protocol and registration

This systematic review was prospectively registered in PROSPERO (CRD42022381625). Guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) were followed during the conception of this systematic review (Additional file 1).

Search strategy

We conducted a systematic search of the literature published in PubMed and Web of Science on May 31, 2024, with a large search strategy without year restriction that included all articles referencing "(endometriosis) AND (methylation OR "DNA methylation" OR methylcytosine OR hydroxymethylcytosine OR 5mc OR 5-mc OR 5hmc OR 5-hmc OR methyl*)." Records obtained for each database search were merged to remove duplicates. The

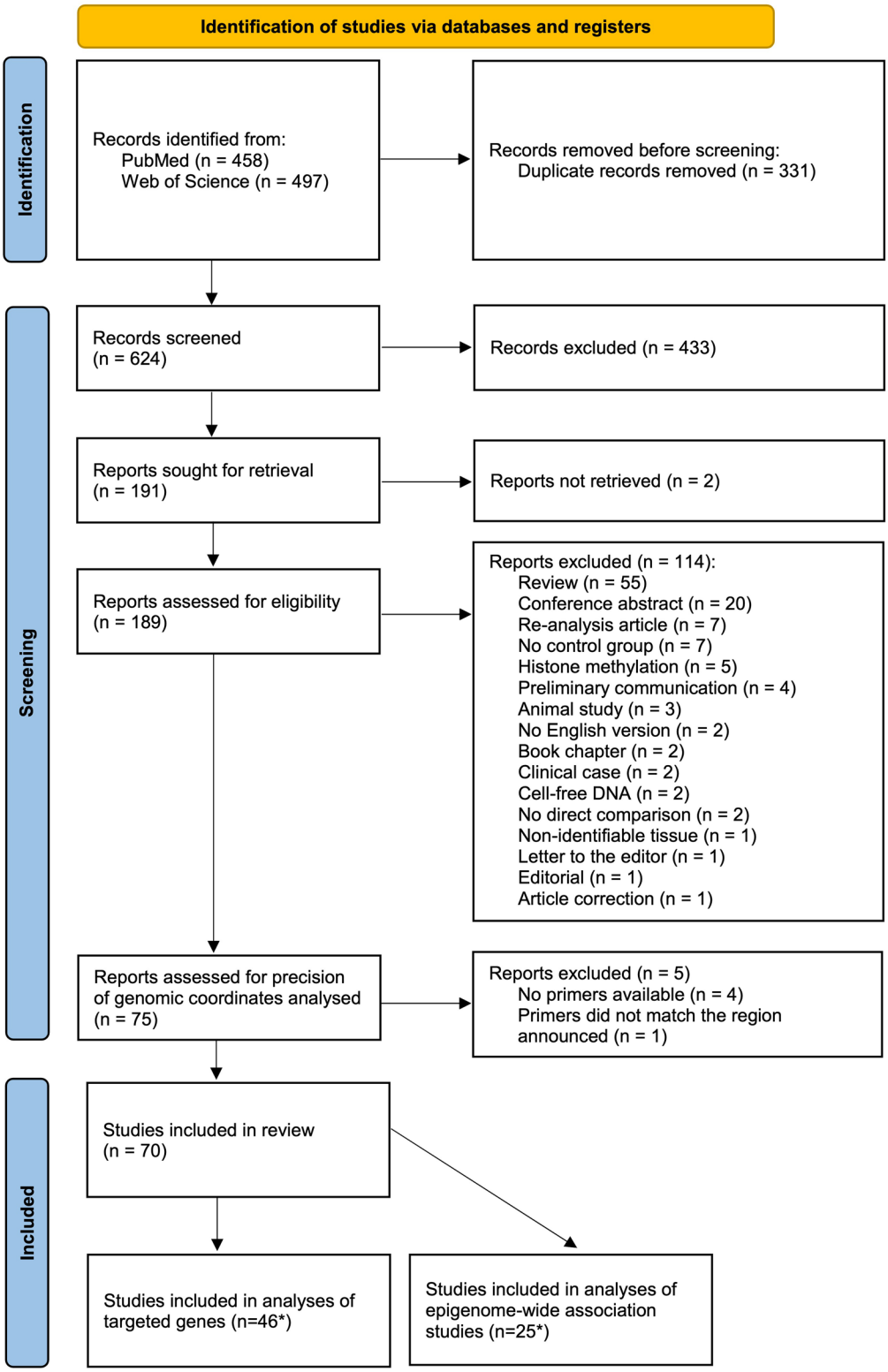


Fig. 1 Flow diagram of the study selection. We followed PRISMA 2020 guidelines throughout the study selection

complete flow diagram corresponding to the selection process is shown in Fig. 1.

Eligibility criteria and study selection

Eligible studies were selected according to the Patients, Interventions, Comparisons, and Outcomes model. Precisely, inclusion was considered for original research articles investigating genome-wide DNA methylation or at a regional scale in patients with clinically confirmed endometriosis (surgery and/or histological examination), whatever the disease severity (stages I–IV, ASRM classification [20]). There was no restriction on the type of tissue studied (i.e., endometrium, blood, and fetal tissue), but only human studies were included. The presence of a control group without endometriosis was necessary for inclusion except for studies comparing eutopic to ectopic tissues in endometriotic patients. Because the subject of the review is DNA methylation, studies investigating histone, RNA, or cell-free DNA methylation were excluded from the article selection.

During the initial screening of the studies, reviews, conference abstracts, book chapters, letters to editors, and preliminary communications were discarded from further analysis. Articles in any language other than English and articles with inaccessible full texts were excluded. Articles consisting of re-analyses of former datasets were also excluded (details in Fig. 1).

Two reviewers independently performed the literature search and selection according to the eligibility criteria. The study selection step was performed with Rayyan©. An independent validation of the selection was performed using AI-powered software ASReview (v1.5). We applied a supervised method adding prior knowledge of 20 random included and 20 random irrelevant references according to the first two reviewers. General ranking of studies was immediately collected, and the entire process was repeated 10 times. Ranking from top to bottom, studies were considered as included by the AI-powered method as long as less than 1% of consecutive studies (>=7) were previously considered irrelevant by the two manual reviewers. A reference was finally considered to be included by this method when it was found to be included in more than 6 out of 10 rankings (which is more than expected by chance).

Data collection

Data were collected according to a custom data extraction form (Additional file 2). Studies were divided into targeted (e.g., single gene or region level) and epigenome-wide association studies (EWAS). For all, general information was first recorded: first author, groups compared, sample size, population information (age, cycle phase, history of pathology, hormonal or medical treatment, type of endometriotic lesion, and fertility status), technique used for endometriosis diagnostic, disease stage, list of genes studied with precise location of CpGs analyzed or indication of genome-wide study, technique to assess DNA methylation, and statistical thresholds applied.

For targeted gene studies, information was recorded on whether the region was hypomethylated, hypermethylated, or did not show differences in the different comparisons. For the purposes of comparison between studies, the precise location of the region studied was recorded, or if not available, information on primer design was collected to retrieve genomic coordinates via in silico PCR [21]. At this step, additional studies were excluded from the systematic review because there were uncertainties regarding the precise region analyzed in the absence of primers or discrepancies between the regions supposedly analyzed and the primers attributed, or the reference paper mentioned for the primer (Fig. 1).

Quality assessment

The quality assessment of all included studies was performed independently by two reviewers, and any disagreement was resolved by a discussion. We used an adapted Newcastle–Ottawa scale for cohort studies, which evaluated the risk of bias out of a total of nine points according to three categories (selection, comparability, and outcome) (Additional file 3) [22]. The risk of bias was considered high (0–5), moderate (6–7), or low (8–9) depending on the grading score. The quality of the evidence was further evaluated using an adapted Grading of Recommendations Assessment, Development, and Evaluation (GRADE) model (Additional file 4) [23]. Briefly, the quality of the evidence from the studies was rated based on their risk of bias, consistency, directness, and precision. EWAS were considered as "inconsistent" if they lacked a replication or validation cohort [24], while targeted studies were downgraded for this category if their results were in contradiction with the literature. Studies were downgraded for indirectness if they lacked generalizability of the population (i.e., too restrictive population selected either in control or patient groups), if the cohort was not comprehensively described, if DNA methylation was not measured directly or was assessed for validation of expression changes. Studies were downgraded for imprecision according to their sample size, the absence of statistical analysis, or a qualitative measure of DNA methylation (Additional file 4). Because endometriosis is likely to be a complex interplay of alterations in several genes, publication bias was strongly suspected when less than three genes of the same biological pathway were investigated. Overall, studies with a score of 0 or more were assigned a "high" evidence

quality; − 1 were assigned "moderate"; − 2 were assigned "low"; and < = − 3 were assigned "very low."

Analysis

For each study and if accessible, lists of differentially methylated positions (DMP), regions (DMR), or genes (DMG) were collected and re-annotated using the same annotation reference. Gene symbols were harmonized using the UpdateSymbolList function from Seurat package [25]. Subsequent gene lists were cross-checked for any overlap and visualized using UpSet plots [26]. Results from biological pathways enrichment from EWAS were collected and compared to find similar terms/keywords between the different sources.

Results

Study selection

A total of 955 records were collected from two databases, 331 of which were duplicates. The two manual reviewers identified 191 references for full-text review while AI-powered review identified 208, 163 of which were in common. The 45 additional references suggested by AI reviewer proved to be irrelevant based on the abstract (no methylation, only expression, and no endometriosis) thus no further studies were included. After full-text review, 70 studies were considered for systematic review (Additional file 5). They were divided into 46 targeted gene studies and 25 EWAS for different analysis processes (one study was included in both groups). This selection process is presented in the PRISMA flowchart in Fig. 1.

Study characteristics

Characteristics from each study are shown in Additional file 6. The main tissue studied was endometrium (in 97% of the studies), followed by peripheral blood (4%), and some individual studies investigated menstrual blood, cumulus cells, abdominal fat, and choriodecidua (Fig. 2). The main comparison was ectopic endometriotic tissue versus healthy endometrium (60% of endometrium studies), followed by eutopic tissue in endometriosis versus healthy endometrium (57% of endometrium studies), and some studies compared eutopic versus ectopic tissue in endometriosis (37% of endometrium studies) (Fig. 2). Studies preferentially included a mix of samples collected in proliferative/secretory phase (34%), only samples collected during the secretory (21%) or proliferative (21%) phase, while this information was unknown for 24%.

Risk of bias and quality assessment

The ranking of the studies for their risk of bias and quality assessment is shown in Additional file 7. Six out of the 70 included studies are considered to have a high risk of bias. Overall, only two studies displayed a high quality of evidence, and 10 were in the moderate category. This is notably due to the very small sample size considering the difference tested, the high risk of bias, the absence of a replication cohort for EWAS, and the evaluation

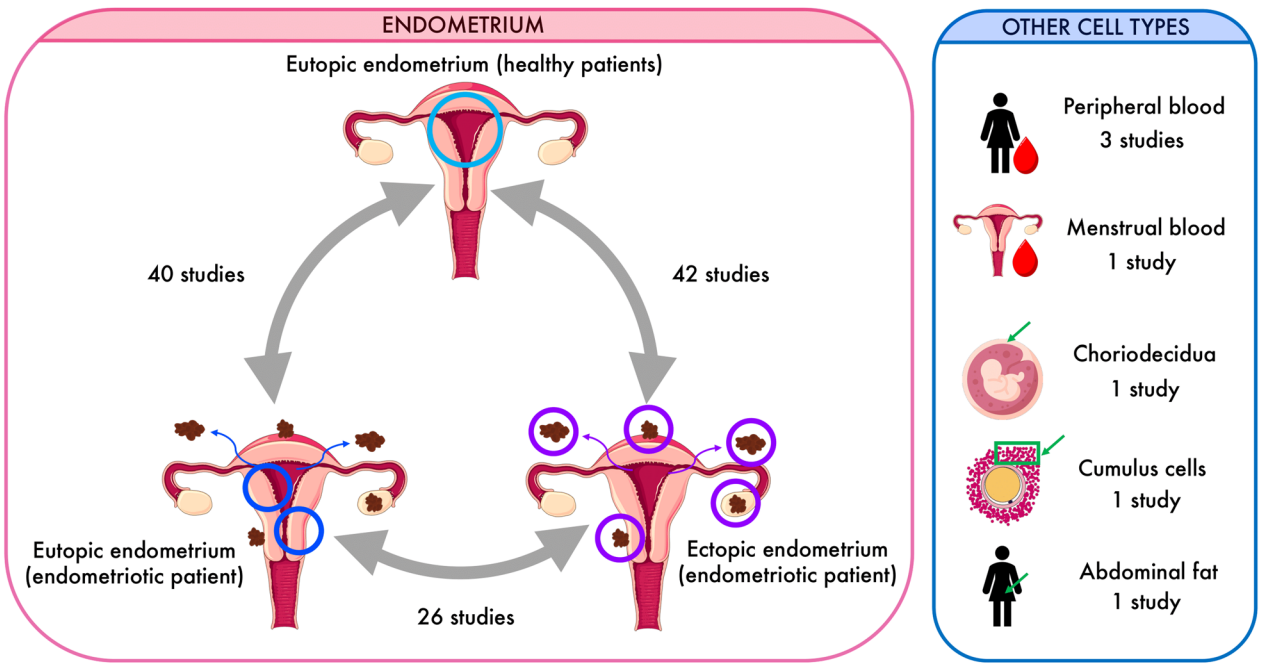


Fig. 2 Number and characteristics of studies included in the systematic review

of a single gene for targeted gene studies, whereas endometriosis is likely to be polyepigenetic.

Results from targeted genes studies

General characteristics

Most targeted gene studies investigated a unique candidate gene (78%), while only five out of 46 measured DNA methylation in at least three different genes. In total, the most studied genes across all targeted studies were *HOXA10* (six studies), *PGR* (five studies), *ESR1-ESR2-NR5A1* (three studies), and *CYP19A1-HOXA11-PTGS2-RUNX3-TWIST1* (two studies).

Considering the quality of the evidence from studies, only one has been rated as high quality, five as moderate, 14 as low, and 26 as very low quality (Additional file 7). One of the main issues was the mixing of samples originating from different phases of the menstrual cycle, which is a huge confounder of DNA methylation for endometrium-based studies [27]. In most cases, ectopic endometrium lesions were collected from ovarian endometrioma, but lesion biopsies from vaginal nodes, rectovaginal septum, muscle, peritoneal nodule, pouch of Douglas, uterovesical ligament, and ovarian fossa were also performed (Additional file 6). Cell type of eutopic endometrial biopsy was unknown in 67% of studies supposing a mix of all cell types, stromal cells were isolated in 27% and epithelial cells in the remaining 6%. For ectopic biopsies, stromal cells were isolated in 34% of studies, epithelial cells in 11% or cells were mixed for 55% (Additional file 6).

Candidate genes aberrantly methylated in endometriosis

- Eutopic endometrial tissue from endometriosis women versus non-affected counterparts

HOXA10 is the most studied gene to date (Table 1). The regulatory regions of *HOXA10* encompass three CpG islands regions (F1 located 50 bp upstream of exon I, F2 and F3 located in introns I–II). Endometriosis is associated with hypermethylation of F1, F2, and F3 in secretory eutopic endometrium, as indicated by the better-designed studies, but we still lack evidence for proliferative phase samples [28–30].

The second-most studied gene is *PGR* (Table 1). *PGR* has two isoforms, *PGRA* and *PGRB* encoded by two alternative promoters. DNA methylation studies in the *PGR* promoter have very low-quality evidence because qualitative techniques were applied (Additional file 7). However, for the *PGRA* promoter, studies agreed that in both control and endometriosis cases, this region was completely unmethylated [31, 32], although a study found cases with methylation [33]. For *PGRB*, the results were conflicting (hypermethylated, no difference, or no methylation in the region) [31–34].

In *HOXA11*, a moderate quality of evidence study shows hypermethylation in eutopic endometriosis samples in the first exon [35] and absence of DNA methylation in two other regions (upstream exon I, intron I) (Table 1). In a low-quality evidence study assessing the methylation status of the *HOX* family but with unknown localization of the probes, *HOXA11* was hypomethylated in eutopic endometriosis samples [36].

In *COX-2*, two studies with low quality of evidence agreed on hypomethylation of secretory eutopic endometrium with endometriosis [37, 38]. In the promoter of *TWIST1*, studies with low- and very low-quality evidence agreed on a hypomethylation in eutopic endometriosis tissue [39, 40]. For *ESR1*, the evidence remains scarce, as very low quality of evidence studies indicate that the AB promoter region is either unmethylated or methylated in eutopic samples from endometriosis patients [33, 41]. Finally, there may be no DNA methylation differences between eutopic endometriosis and control samples in *RUNX3* promoter, but hypermethylation could indicate transformation into ovarian carcinoma [42, 43].

In addition, about ten genes have been investigated to date only once and are shown in Table 2 with studies ranked from the highest quality evidence to the lowest, but results would need replication in the future.

- Ectopic endometrial tissue from endometriosis women versus non-affected counterparts

Only a few genes were investigated in the ectopic endometrium compared to the healthy eutopic one in more than one study. Wu et al. found hypermethylation of the *PGRB* promoter in ectopic samples compared to healthy endometrium, whereas Esfandiari et al. found no difference [32, 34]. No difference was found in the coding region of *PGR* (exon I) [14].

NR5A1 (encoding the protein SF-1) was investigated in three studies with a very low quality of evidence by the same team in three different regions [44–46]. They reported hypermethylation in intron I and exon II–intron III and hypomethylation in the promoter region/exon I in ectopic endometriosis tissue (ovarian endometrioma) compared to healthy samples, mixing proliferative and secretory phase samples.

In *ESR1,* a study with low quality of evidence [14] indicates that the AB and C promoters show no methylation differences between the ectopic and healthy endometrial tissues, but there may be an existing difference in the tissue-dependent DMR1 and DMR2, which are also regulatory regions of *ESR1* expression

Table 1 Targeted studies summary for genes studied at least twice

	Study	Year	Quality of evidence	Cycle	Region	Euto vs control	Ecto vs control	Ecto vs euto
HOXA10	Szczepanska et al.	2010	Moderate	Secretory	F1, F2, F3	↗		
	Yu et al.	2022	Moderate	Proliferative	F3	Not reported	Not reported	↗
	Andersson et al.	2014	Low	Secretory	F1	↗	Not reported	↘
	Ji et al.	2017	Low	Unknown	Promoter			↘
	Samadieh et al.	2018	Low	Proliferative and secretory	F1	↗ (secretory) — (proliferative)	↘ (secretory) ↘ (proliferative)	↘ (secretory) ↘ (proliferative)
	Wu et al.	2005	Very low	Proliferative and secretory	F1, F2, F3	↗		
PGR	Maekawa et al.	2019	Low	Proliferative	*PGR* Exon I		— (*PGR*)	
	Wu et al.	2006	Very low	Menses, proliferative and secretory	*PGRA* promoter *PGRB* promoter	Unmethylated (*PGRA*) — (*PGRB*)	Unmethylated (*PGRA*) ↗ (*PGRB*)	Not reported
	Meyer et al.	2014	Very low	Menses, proliferative (87%) and secretory	*PGRA* promoter *PGRB* promoter	Detection of methylation (PGRA) Unmethylated (PGRB)		Detection of methylation (*PGRA*) ↗ (*PGRB*) Unmethylated in eutopic (*PGRB*)
	Rocha-Junior et al.	2019	Very low	Secretory	*PGRA* promoter *PGRB* promoter	Unmethylated (*PGRA*) ↗ (*PGRB*)		
	Esfandiari et al.	2021	Very low	Proliferative and secretory	*PGRB* promoter	↗ (*PGRB*)	— (*PGRB*)	↘ (*PGRB*)
SF1	Xue et al.	2007	Very low	Proliferative and secretory	Exon I		↘	
	Xue et al.	2011	Very low	Proliferative and secretory	Exon II-intron III		↗	
	Xue et al.	2014	Very low	Proliferative and secretory	Intron I		↗	
ESR1	Maekawa et al.	2019	Low	Proliferative	AB promoter C promoter T-DMR1 T-DMR2		— (AB promoter) — (C promoter) ↗ (T-DMR1) ↗ (T-DMR2)	
	Meyer et al.	2014	Very low	Menses, proliferative (87%) and secretory	AB promoter C promoter			Methylated (AB) Methylated (C promoter)
	Toderow et al.	2017	Very low	Proliferative and secretory	AB promoter	Unmethylated	Unmethylated	
ESR2	Maekawa et al.	2019	Low	Proliferative	Promoter		—	
	Xue et al.	2007	Very low	Proliferative and secretory	Promoter		↘	
	Meyer et al.	2014	Very low	Menses, proliferative (87%) and secretory	Promoter (other region)			—
HOXA11	Szczepanska et al.	2012	Moderate	Secretory	Upstream of exon I, exon I, intron flanked by exons I and II	↗ (exon I) Unmethylated (upstream exon I, intron flanked exon I-II)		
	Esfandiari et al.	2021	Low	Proliferative	Unknown	↘	↘	
COX2	Wang et al.	2012	Low	Secretory	NF-IL6 site of the promoter	↘		
	Zidan et al.	2015	Low	Secretory	NF-IL6 site of the promoter	↘	↘	↘
RUNX3	Guo et al.	2014	Very low	Unknown	Promoter	↗	Not reported	Not reported
	Wang et al.	2022	Very low	Secretory	Promoter	—	—	—
TWIST	Juanqing et al.	2019	Low	Proliferative	Promoter	↘	↘	
	Yang et al.	2023	Very low	Unknown	Promoter	↘		

From top to bottom, genes are first ranked by number of studies, and secondly, studies are ranked by quality of evidence. An upward arrow indicates hypermethylation in the group compared with the reference, and conversely, a downward arrow indicates hypomethylation (group vs. reference). A dash indicates that there is no difference between the groups being compared. T-DMR: tissue-specific DMR

Table 2 Targeted studies summary for genes studied once

	Study	Year	Quality of evidence	Cycle	Region	Euto vs control	Ecto vs control	Ecto vs euto
MMP1, MMP2, MMP3, MMP7, MMP9, TIMP1, TIMP2, TIMP3, TIMP4	Tang et al.	2017	1	Proliferative	Promoter		↗ (MMP3, MMP7, TIMP3) ↘ (MMP2, TIMP4) — (MMP1, MMP9, TIMP1, TIMP2)	
GSTM1	Zhao et al.	2019	−1	Secretory	Promoter	↘	↘	↘
GRHL2	Hao et al.	2022	−1	Secretory	Promoter		↗	
H19-DMR	Kamrani et al.	2022	−2	Proliferative	Region I (CTCF6) and II	↘ (region II) — (region I)	↘ (region II) — (region I)	—
HOXA11, HOXA2, HOXA4, HOXA7 HOXA1, HOXA3, HOXA5, HOXA9 HOXB1, HOXB3, HOXB9 HOXB2, HOXB7, HOXB8 HOXC12, HOXC13, HOXC8, HOXC10 HOXD1, HOXD11, HOXD12, HOXD3 HOXD4, HOXD13, HOXD9 HOX cofactors (57 genes)	Esfandiari et al.	2021	−2	Proliferative				
IL-12B	Zhao et al.	2019	−2	Secretory	Site 1 (95bp upstream TSS) Site 2 (1444-1499bp upstream TSS)	↘ (site 1) — (site 2)	↘ (site 1) — (site 2)	↘ (site 1) — (site 2)
RASSF1A	Wu et al.	2016	−2	Proliferative and secretory	Promoter	↗	↗	
COMT	Ji et al.	2017	−2	Unknown	Promoter			↗
G2/M checkpoint regulators (ATM, ATR, p53, p21, Chk1, Chk2)	Hirakawa et al.	2019	−2	Proliferative	Promoter		↗ (ATM) — (ATR, p53, p21, Chk1, Chk2)	
ENPP3	Qin et al.	2024	−2	Secretory	Promoter		↘	
miR-503 gene	Hirakawa et al.	2016	−3	Proliferative	Promoter		↗	
Let7-b	Meixell et al.	2022	−3	Unknown	4 regulatory regions (promoter, enhancer, CTCF, TFBS) upstream coding region			↗ (region 2) — (other regions)
CYP19A1	Izawa et al.	2011	−3	Proliferative and secretory	CpG island 20 kb before exon II		↘	
MLH1	Ren et al.	2012	−3	Unknown	Promoter	—	—	—
CDH1	Li et al.	2017	−3	Proliferative and secretory	Promoter	↗	↗	—
PAX2	de Graaff et al.	2012	−3	Proliferative	Promoter	Unmethylated	Unmethylated	Unmethylated
TSLP	Habibi et al.	2022	−3	Proliferative and secretory	Promoter	—	—	—
RASSF2	Ren et al.	2014	−3	Unknown	Promoter	—	—	—
HAND2	Liu et al.	2023	−3	Secretory	Promoter	↗	↗	↗
CXCL16	Febriyeni et al.	2024	−3	Unknown	Promoter	— (↘ in menstrual blood)		
miR-196b	Abe et al.	2013	−4	Proliferative			↗	
HERV-W	Zhou et al.	2014	−4	Unknown				↘
SFRP2	Yang et al.	2023	−4	Secretory	Promoter		↘	

Studies are ranked by quality of evidence. An upward arrow indicates hypermethylation in the group compared with the reference, and conversely, a downward arrow indicates hypomethylation (group vs. reference). A dash indicates that there is no difference between the groups being compared

upstream of the promoter region. Likewise, in *ESR2*, Maekawa et al. showed no differences in the same samples, whereas Xue et al. (very low quality of evidence) identified hypomethylation in ectopic endometrium samples [12, 14].

Remaining list of genes investigated only once with their level of proof and associated results are shown in Tables 1 and 2. Remarkably, a very high-quality study reported alteration in the methylation status of metalloproteinases *MMP2*, *MMP3*, and *MMP7* (hypomethylation for the first one, and hypermethylation with endometriosis for the other two) and their inhibitors *TIMP3* and *TIMP4* (respectively, hypermethylation and hypomethylation) in proliferative ovarian endometrial cysts [47].

- Ectopic versus eutopic endometrium in endometriosis

Among 19 studies comparing ectopic and eutopic samples in endometriosis, 12 assessed DNA methylation in both tissue types for the same patient, whereas for 2, tissues originated from different patients or the information was unclear for 5. Only *HOXA10* and *PGR* were investigated twice. In *HOXA10* in moderate and low-quality evidence studies, compared with eutopic endometrium in the same patient, F1 was hypomethylated in secretory ectopic endometrium [28, 29]. Very low evidence comes from the Meyer et al. and Esfandiari et al. studies, which found contradicting results regarding hypomethylation or hypermethylation in the ectopic endometrium, probably due to differences in methodology [33, 34]. Other genes were only investigated once and would need further validation (Tables 1 and 2).

Results from global methylation and epigenome-wide association studies

General characteristics

Among the 25 studies included in this section, 18 assessed DNA methylation without region restriction across the genome, two examined promoter regions only, two measured LINE-1 methylation, one measured DNA hydroxymethylation, and two calculated global average DNA methylation. The quality of the evidence was high in one study, moderate in five studies, low in eight, and very low in the remaining 11 (Additional file 7). The main tissue studied was endometrium (in 92% of studies), followed by peripheral blood (12%) and choriodecidua (4%). The main comparisons were between eutopic endometrium in endometriosis versus healthy eutopic endometrium (14 studies), followed by ectopic endometrium in endometriosis versus healthy eutopic endometrium (nine studies) and eutopic versus ectopic samples from endometriotic patients (nine studies). Stromal cells were isolated from eutopic endometrium for 32% of studies or included presumably a mix of cell types for the remaining 68%. For 47% of ectopic studies, stromal cells were isolated, the remaining 53% assumed to mix all cell types without distinction.

Regional analysis of global methylation

The methylation status of specific or entire regions of the genome often reflects the pathogenetic state of a tissue. In the same patients, a global hypomethylation of the ectopic endometrium compared to the eutopic endometrium was observed using by ELISA for 5-methylcytosine ($n=8$), and this was associated with a decreased expression of *DNMT1*, which is responsible for maintaining DNA methylation [48]. LINE-1 are transposable elements found in many loci distributed across the genome, and their methylation status is a good surrogate for global DNA methylation. Hypomethylation is observed in many diseases, especially cancers. In endometriosis, one study found LINE-1 hypomethylation of ovarian cysts compared to the eutopic endometrium of healthy controls with COBRA [49]. In eutopic and ectopic tissues from the same patients, there was no difference in LINE-1 methylation in another study with the same technique but with very low quality of evidence [50].

The evaluation of DNA methylation in promoters could indicate genes likely to be activated or repressed with endometriosis. Using MeDIP combined with microarray hybridization, Borghese et al. (2010) observed methylation patterns specific to endometriosis subtypes (superficial endometriosis, endometriomas, and deep infiltrating endometriosis) between eutopic and ectopic luteal tissues from the same patients, with 20 promoters common to all three subtypes differentially methylated [51]. Linking expression data to methylation data revealed a low correlation between expression and methylation levels [51]. In small groups of patients with high or low integrin avb3 levels, 1304 and 1325 DMRs, respectively, were found in promoter regions in the eutopic secretory endometrium [52]. In patients with low integrin levels, 14 transcripts showed an inverse relationship between methylation and expression status, but the sample size was limited ($n=4$ patients).

DNA hydroxymethylation of 5′-methylcytosine (5-hMC) is an intermediate of DNA demethylation mediated by TET enzymes. DNA hydroxymethylation is structurally similar to DNA methylation, but they imply different biological regulation mechanisms. Ectopic endometrium from mixed menstrual phases displayed abnormally high levels of 5-hMC compared

to healthy endometrium, while blood showed low hypohydroxymethylation in endometriosis [53]. In addition, in another study using a fluorescence-based ELISA kit, ectopic tissues during proliferative phase were globally hypermethylated compared to paired eutopic tissues [54]. On the contrary, a third study with higher quality evidence found lower 5-hMC levels in epithelial endometriotic lesions (in mixed secretory and proliferative phases) compared to healthy endometrium epithelial cells [55].

Evidence from epigenome-wide association studies

It is interesting to qualitatively decipher common findings from EWAS, especially specific genes and pathways that might suffer from epigenetic dysregulation in multiple studies (Additional files 8 and 9). We indexed these findings from the highest to the lowest quality of evidence studies in the following sections.

- Eutopic endometrial tissue from endometriosis women versus non-affected counterparts

A unique recent study reached the top quality of evidence category, using EPIC beadchips to compare the eutopic endometrium from 637 endometriosis cases and 347 controls [56]. Single CpG or regional analysis yielded no difference in DNA methylation, but sub-analysis restricted to patients with moderate or severe endometriosis showed hypermethylation at two CpG sites in this group (located in 5'UTR of *ELAVL4* and promoter of *TNPO2*). Authors also applied weighted gene correlation network analysis and identified four clusters of CpG sites associated with endometriosis which led to the assumption that genes related to WNT-signaling, MAPK-signaling, adhesion, cancer pathways, and cellular proliferation show methylation changes with endometriosis (Additional file 9). Other studies were classified with reduced quality of evidence, particularly due to the small sample size or absence of validation via independent cohorts or techniques and, in some cases, the absence of adjustments for menstrual phase/age. The three studies with moderate quality of evidence with the same technique (450 k array) yielded either no differentially methylated positions (DMPs), few DMPs (15), or a small number (28) of differentially methylated regions (DMRs) with low divergence [27, 57, 58]. Studies with low quality of evidence and using the same technique were consistent with the absence of aberrant methylation patterns in the eutopic endometrium of endometriotic patients by finding 0, 1, and 32 DMPs, respectively [59–61]. Some further differences were identified in studies with very low quality of evidence, but the lack of adjustments for age, menstrual phase, and patient description is a major limitation for the interpretation of results: 365 DMRs with MBD-seq, between 39 and 137 DMPs depending on menstrual phase with 27 k array, "*Numerous aberrantly differentially methylated loci*" with 450 k, 120 differentially methylated genes (DMGs) with 27 k array, or 15 DMPs again with 27 k array [62–66].

When available, we cross-referenced genes found epigenetically modified in different cohorts. This has resulted in very few genes whose DNA methylation is repeatedly altered in different studies (Fig. 3A). Apart from Houshdaran's study, where some twenty genes overlapped according to menstrual cycle groups, a single gene was found to be common to two studies (*GLRX3*). Only four out of 12 genes previously found with hyper- or hypo-methylation in targeted approaches were confirmed once in the 13 corresponding studies (*HOXA10*, *GSTM1*, *HAND2*, *H19*) (Fig. 3A). Comparison of functional analysis of genes likely to be epigenetically modified across studies yielded some redundant biological and signaling pathways such as immunity, Wnt-signaling, kinase activity, cell adhesion, and hormone response (Additional file 9).

- Ectopic endometrial tissue from endometriosis women versus non-affected counterparts

Compared to eutopic healthy endometrium, the number of methylation differences in ectopic tissue is much higher than in eutopic tissue in endometriosis (Additional file 6). One study with moderate quality of evidence found 42,248 DMPs in approximately 450,000 tested, mainly located in transcription factors, *GATA*- and *HOX*-family genes [67]. Other studies on 450 k arrays with low quality of evidence identified, respectively, 1753 DMRs (related to developmental processes and regulation of signaling), 12,378 DMPs (related to embryonic skeletal system development, MAPK activity activation, response to hormone stimulus, regulation of cell adhesion, and apoptosis) and 6360 DMRs, and 68 DMPs (related to cell proliferation, nervous system development, and immunity) [55, 59, 61]. Finally, a study with very low quality of evidence due to the absence of control for menstrual phase and age identified 1811 DMPs in endometriotic cyst stromal cells using a 450 k array, many which were located in the *GATA6* gene and showing a huge DNA methylation difference (> 90%) [68].

A gene cross-referencing analysis was complicated by the large number of deregulations found in the Dyson et al. study and the incompleteness of available data for the others. However, some genes were common to three studies (*DIP2C*, *EMX2*, *FOXL1*, *FOXP1*, *NID2*, *PRDM16*, *RUNX2*, and *ZNF423*) (Fig. 3B). Results from targeted genes studies were not confirmed, except in

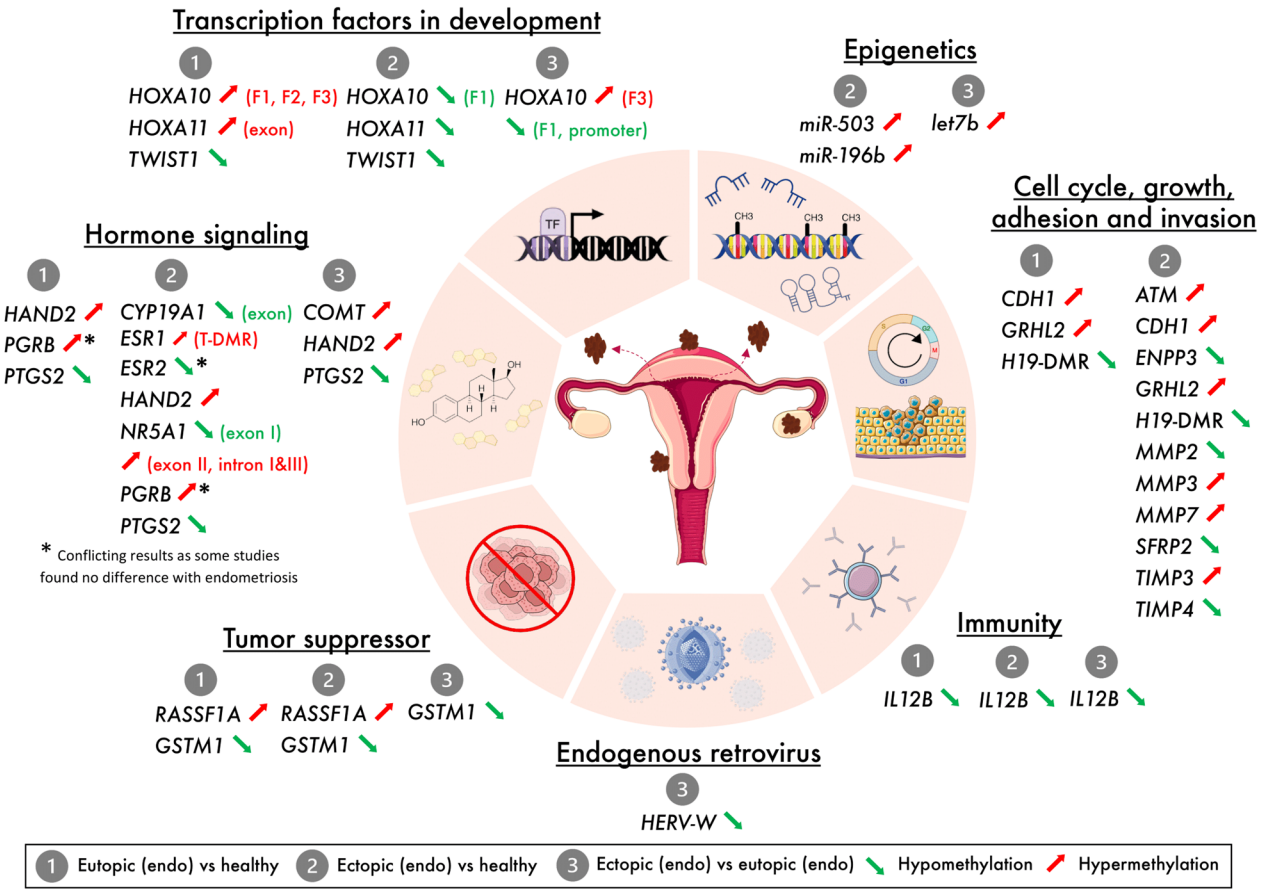


Fig. 3 Comparative analysis of methylome studies main findings with Upset plots. Gene lists include either DMG as defined in corresponding study or genes with DMP or DMR. Studies are grouped according to the number of DMG reported (0 or more), the completeness of data available (complete, partial, or absent), and the menstrual phase of tissues collected (proliferative, secretory, mixed, or unknown). Results from targeted studies are indicated for comparison purpose. Genes overlapping multiple studies are given in the upper part of the plot. Genes in red highlight concordance between EWAS and targeted studies. **A** For the analysis of eutopic endometrium from endometriotic patients versus eutopic healthy endometrium. **B** For the analysis of ectopic endometriotic endometrium versus eutopic healthy endometrium. Because Dyson et al. study identified a large proportion of genes in the genome (> 10,000), only DMG common to at least three studies were inscribed. **C** Comparative analysis of methylome studies main findings for the analysis of eutopic versus ectopic endometrium for endometriotic patients

Dyson et al. where 18 genes were retrieved. Functional analysis revealed common biological processes and pathways which may be modified in relation to ectopic cells, namely, cell adhesion and differentiation, hormone regulation/response, and developmental processes (Additional file 9).

- Ectopic versus eutopic endometrium in endometriosis

Four studies with moderate quality of evidence using 450 k arrays found numerous differences in the methylation profiles of eutopic and ectopic endometriotic cells from the same patients. Barjaste et al. counted 2108 DMRs that preferentially overlap with genes involved in biological adhesion, multicellular organismal processes, response to stimulus, and cell proliferation [59]. Rahmioglu et al. detected a number of 3915 DMPs in paired eutopic and ectopic samples (peritoneal and/or endometrioma) enriched in genes related to WNT and CCKR signaling and the gonadotropin-releasing hormone-receptor pathway [60]. In Wang et al., there were still a high number of differences, namely, 12,159 DMPs (related to embryonic skeletal system development, MAPK activity activation, response to hormone stimulus, regulation of cell adhesion, and apoptosis) and 6142 DMRs [61]. Within the same range of differences, 17,551 DMPs were observed in another recent study in proliferative matched eutopic and ectopic tissues from

endometriotic patient with ovarian endometrioma [69]. Authors identified ECM-receptor, focal adhesion, HPV-infection and PI3K-Akt, Wnt, Hippo, and estrogen signaling pathways as enriched in differentially methylated genes. Furthermore, the study by Yamagata et al. found 883 DMPs (very low quality of evidence) located in genes related to signal transduction, development, and cell adhesion but samples were not originating from same patients [66].

The main limits to a qualitative analysis of genes with abnormal DNA methylation between eutopic and ectopic samples in endometriosis are the incompleteness of available data and the few overlaps between different studies (12 genes) (Fig. 3C). Functional analysis comparisons revealed common biological pathways related to genes with DNA methylation modifications in ectopic versus eutopic endometriosis tissues such as cell adhesion, development, PI3K-Akt and Wnt-signaling, neurogenesis, hormone signaling, and immunity (Additional file 9). Targeted studies observations were not confirmed in any of the study included in this EWAS analysis (Fig. 3C).

- Peripheral blood

Two studies investigated genome-wide DNA methylation markers for endometriosis in peripheral blood. Study with the highest quality of evidence from Mortlock et al. found no DMP with a 450 k array, but the authors identified five genetic variants associated with endometriosis that could affect DNA methylation levels at specific CpG sites (mQTL) [57]. Confirming previous observations, another study using the EPIC array found no DMP in a large cohort [70]. However, the quality of the evidence was classified as moderate due to the lack of generalizability of the included participants, who are likely to have been exposed to polybrominated biphenyl (PBB) during their lives.

- Fetal tissues

After observing endometriotic-like lesions within the fetal membranes of women with deeply infiltrating endometriosis, Marcellin et al. performed for the first time a differential methylation analysis to better characterize the molecular mechanisms that lead to the development of endometriotic lesions in semiallogenous fetal membranes [71]. They captured 5999 DMRs using 450 k arrays, the vast majority of which were hypermethylated and located in genes related to neurons, neoplasms, receptor activity, and immunity.

Discussion

Our systematic review supports the notion that endometriosis is partly an epigenetic disease. The presence of repeatedly observed changes in different cohorts and populations using different techniques and methods is one element that supports the partial epigenetic contribution in endometriosis etiopathology. Our results substantiate the importance of DNA methylation dysregulations focused on critical signaling pathways closely associated with the pathogenesis of endometriosis.

Current evidence from targeted gene studies

The various targeted gene studies that examined DNA methylation modifications in the context of endometriosis provided insight into signaling pathways that could contribute to the development of the disease (Fig. 4). This likely supports the notion that endometriosis is caused in part by epigenetic dysregulations of multiple genes and reflects the complexity of its etiopathology. In the following sections, we clarify the results obtained for genes multiple times assessed as well as for genes studied once but restricted to high to low quality of evidence studies (Tables 1 and 2). It is possible that the alterations we will discuss below could be merely consequences of overarching dysregulations in key epigenetic processes.

Transcriptional factors involved in development

The gene that attracted the most attention in the scientific literature was *HOXA10*. *HOX* genes encode a series of **transcription factors** that play a key role in embryonic development and are expressed particularly in the endometrial glands and stroma. *HOXA10* specifically controls **uterine development and endometrial differentiation** [72]. *HOXA10* shows a cyclic pattern of expression throughout the complete menstrual cycle, being upregulated during the mid-luteal phase, which is the framework of implantation [73]. Endometriosis patients do not exhibit this *HOXA10* expressional cyclic pattern, which may be a triggering factor for pathogenesis [74]. Treatment with 5-aza-dC suggests that *HOXA10* is epigenetically regulated by DNA methylation [75], and silencing of *HOXA10* in endometriotic tissues could occur by hypermethylation of its promoter region. The F1 region has been the best studied to date, and studies agree that endometriosis may be associated with its hypermethylation in eutopic tissues during the secretory phase [28–30, 76]. This is consistent with the theory of lack of cyclic expression of *HOXA10* in the mid-luteal phase, as hypermethylation of the *HOXA10* promoter could suppress its expression. It is likely that alteration of *HOXA10* expression via inappropriate promoter

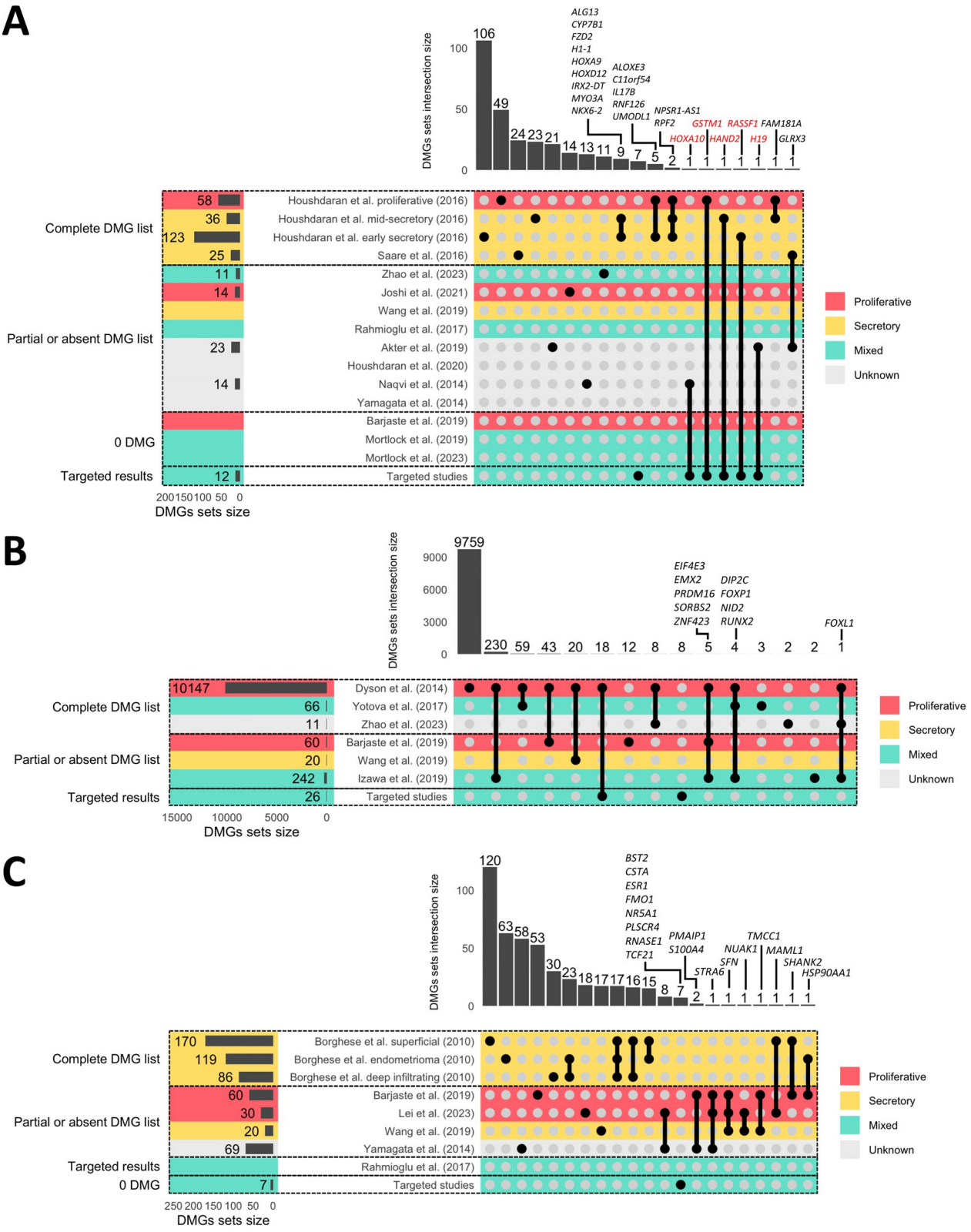


Fig. 4 Summary of epigenetically altered pathways in endometriosis according to studies of targeted genes. When region is not specified (exon, DMR), the modifications concern the promoter region of the genes listed. Red arrows are represented to signify hypermethylation and green arrows hypomethylation, respectively, for the comparison detailed in caption (1, 2, or 3). T-DMR: tissue-specific DMR

DNA methylation has an implication in endometriosis-associated infertility via increased resistance to progesterone and reduced endometrial receptivity during decidualization and implantation [77, 78]. More generally, it is the majority of *HOX* genes in clusters (from A to D) and HOX cofactors that exhibit differential DNA methylation in eutopic and ectopic endometrial samples [36]. It is hypothesized that *HOXA10* could drive the misplacing of endometrial cells during embryonic life and causing endometriosis, but there is currently no in vivo proof that *HOXA10* dysregulation during uterine development may be a possible contributor to the etiology of endometriosis later in life [79, 80].

TWIST1 is another transcription factor involved in cell differentiation during embryogenesis, and promoter hypomethylation has been observed in the ectopic endometrium, suggesting an additional role for key transcription factors in endometriosis [39, 40].

Hormone signaling

Local estrogen production may play a pathophysiological role in endometriosis by enhancing the inflammatory status of the endometrium [81]. In ectopic endometrial cells, *NR5A1* (encoding the steroidogenic factor-1 (SF-1)) showed hypomethylation of its promoter and first exon and hypermethylation in the first and the second intron, and second and third exon which are associated with higher expression of SF-1 [44–46]. Since SF-1 binds to the promoter of steroidogenic genes such as *CYP19A1* (encoding the aromatase), it could contribute to increased estradiol biosynthesis and subsequent inflammation in endometriosis [82].

Further involved in **estrogen activity**, *ESR1* and *ESR2* encode estrogen receptor alpha (ERalpha) and beta (ERbeta). ERbeta is overexpressed in endometriotic tissues, leading to suppression of ERalpha via complex mechanisms [83]. Consequently, it can down-regulate progesterone expression and promote proliferation and inflammation [84]. The genomic cause of ERbeta overexpression in endometriosis may originate from DNA hypomethylation of the *ESR2* promoter, but the evidence is conflicting [12, 14]. The *ESR1* AB and C promoters were not differentially methylated in endometriotic tissues, suggesting that *ESR1* is mainly regulated by *ESR2* rather than epigenetic mechanisms [14, 33, 41]. Additionally, *COMT*, the methyltransferase responsible for inactivating catechol estrogen through DNA methylation, exhibits promoter hypermethylation in ectopic endometriotic samples, possibly reflecting an alteration of estrogen metabolism functions [85].

Furthermore, **progesterone** is critical for endometrial receptivity, and its action is mediated by *PGRA* and *PGRB*. In patients with endometriosis, progesterone resistance is observed, possibly related to a decrease in PRB levels [86], which tends to inactivate progesterone target genes, leading to inflammation and deficient estrogen metabolism [87]. The *PGRA* promoter region is found unmethylated in both eutopic and ectopic endometrium from endometriotic women and also in healthy endometrium [31, 32]. In contrast, the *PGRB* promoter can be hypermethylated in endometriosis, providing a possible mechanism to explain the decrease in *PGRB* expression [31–34]. Alterations in progesterone receptors within the endometrium may be responsible for impaired endometrial receptivity, contributing to the infertility observed in patients with endometriosis.

Cyclooxygenase-2 (COX-2) is an essential enzyme of **prostaglandin synthesis** encoded by the *PTGS2* gene [88]. Prostaglandin regulates proliferation, apoptosis, inflammation, and aromatase expression [38]. Two studies have found hypomethylation of the NF-IL6 site in the *PTGS2* promoter in endometriosis, that could be associated with the upregulation of COX-2 and increased levels of prostaglandin E2 [37, 38].

Tumor suppressor genes

Tumor suppressor genes may contribute to the nonmalignant behavior of ectopic endometrial tissue overgrowth and proliferation [89]. *RASSF1* is a **tumor suppressor gene** where hypermethylation of the promoter in eutopic and ectopic tissue with endometriosis could mediate *RASSF1* downregulation and cell proliferation/apoptosis [90, 91]. Glutathione S-transferase M1 (*GSTM1*) is also a **tumor suppressor** regulating apoptosis signaling [92, 93]. The *GSTM1* promoter region was hypomethylated in the eutopic and ectopic endometrium of endometriosis patients compared to a healthy cohort, which correlated with higher expression of *GSTM1* [94].

Cell adhesion, invasion, and cell cycle

The conditions required for the development of endometriosis include the detachment of endometrial epithelial cells from their primary site, the adhesion of endometrial cells outside the uterine cavity in a host tissue, their invasion, and their proliferation. *CDH1* encodes E-cadherin, a transmembrane glycoprotein involved in **cell–cell adhesion** which is regulated by the key transcription factor GRHL2 [95–97]. The *GRHL2* promoter is significantly hypermethylated in the ectopic endometrium compared to the healthy endometrium, correlated with reduced expression of the GRLH2 protein [98]. In addition, *GRHL2* knockdown increases endometrial epithelial cell invasion and migration, providing a direct mechanism between DNA

hypermethylation and endometriosis-exacerbating factors.

H19-DMR is an imprinted region that coordinately controls the expression of important regulators of **cell growth** and differentiation *H19* and *IGF2* genes. Imprinting loss is found in the eutopic endometrium of endometriotic patients compared to matched control samples [99]. This hypomethylation may be accompanied by a decrease in *IGF2* expression, disrupting important processes of cell proliferation.

Matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are key regulators of endometrial **tissue remodeling and invasion**, abnormally expressed in endometriotic patients [100–102]. The promoter regions of *MMP2*, *MMP3*, *MMP7*, *TIMP3,* and *TIMP4* exhibit a different DNA methylation pattern in endometriotic lesions compared to normal endometrium in a well-designed study [47]. Ectonucleotide pyrophosphatase–phosphodiesterase 3 (ENPP3) is expressed in the endometrium and hypomethylation of *ENPP3* promoter observed in ectopic tissues might be a driver of endometrial migration and invasion of endometrial stromal cells [103].

In addition, Hirakawa et al. examined the promoter methylation status of a bunch of genes involved in the **G2/M checkpoint** and thus controlling cell proliferation, apoptosis, and cell cycle progression in ectopic endometrial cells [104]. The results suggest that the *ATM* promoter is hypermethylated in endometriosis, and treatment with 5-aza-dC confirms that it leads to repression of *ATM* expression. As a result, endometriotic cells could bypass the G2/M checkpoint and escape cell cycle arrest, promoting tumorigenesis and acquiring features of endometriosis progression.

Immunity

Concomitantly with the observation of increased immune cell infiltration in the peritoneal fluid and the eutopic endometrium of endometriotic patients, key **immunity genes** could be involved in the etiology of endometriosis [105–108]. This is the case for *IL-12* which plays a defensive role against endometrial lesions by activating NK cells [109]. DNA methylation in the promoter region of *IL12B*, which encodes a subunit of IL-12 (IL-12p40), showed hypomethylation in both ectopic and eutopic endometrial tissues compared to normal endometrium [110]. In parallel, *IL12B* expression was higher in both endometriosis tissues.

Other biological processes

Other studies with very low quality of evidence suggest implication of other biological pathways in endometriosis development, such as **microRNAs**. Two of them are particularly dysregulated and may play a biological role in the disease, let7b and miR-503, but their DNA methylation regulation remains to be confirmed. The implication of endogenous retrovirus is also an area worth exploring, notably for syncytin-[111, 112].

Genome-wide analysis of DNA methylation: are there universal biomarkers for endometriosis?

The conclusion of targeted genes studies is the plurality of dysregulated mechanisms as a cause or consequence of epigenetic modifications. An unfavorable epigenetic landscape might predispose women to develop endometriosis, and it is of great interest to perform genome-wide methylation association studies to fully understand the epigenetic roots of endometriosis and identify methylated sequences that may serve as a diagnostic. The first conclusion of our qualitative analysis is the probable absence of large DNA methylation modifications in the eutopic endometrium of endometriotic patients. Indeed, better-designed studies including large sample size indicate a drastic absence of differentially methylated positions across the genome. This result may be dependent on the stage of the disease because the largest study still found differences in the eutopic endometrium when restricting to patients with most severe forms. Thus, the epigenetic dysregulation associated with endometriosis may preferentially be located in the ectopic endometrium, which shows a large amount of methylation differences compared either with the eutopic endometrium from healthy women or from the same patient. As regards the data available for each study to date, no solid candidate DNA methylation biomarker has emerged.

The most striking conclusion from genome-wide studies is the discrepancy with targeted genes studies. Genes that showed consistent DNA methylation alterations with endometriosis such as *HOXA10* or *PGR* were partially reported when spanning the entire genome. This could, however, be explained by the preferred use of microarrays which cover only a subset of the total number of CpGs in the whole genome (between 0.1 and 3%). Important genes in the pathogenesis of endometriosis may not be adequately covered to fully investigate the epigenetic roots of endometriosis. For example, *HOXA10*, which appears to play a key role in the infertility phenotype, contains 33 CpGs in the fragment F1 promoter region, whereas only 8 CpGs are present on the 450 k array.

Interestingly, we could note a recurrence of pathways dysregulated caused by DNA methylation modifications (Fig. 5). Biological processes implicated in endometriosis may involve epigenetic modifications in genes related to the Wnt-, PI3K-Akt, and MAPK-signaling pathway,

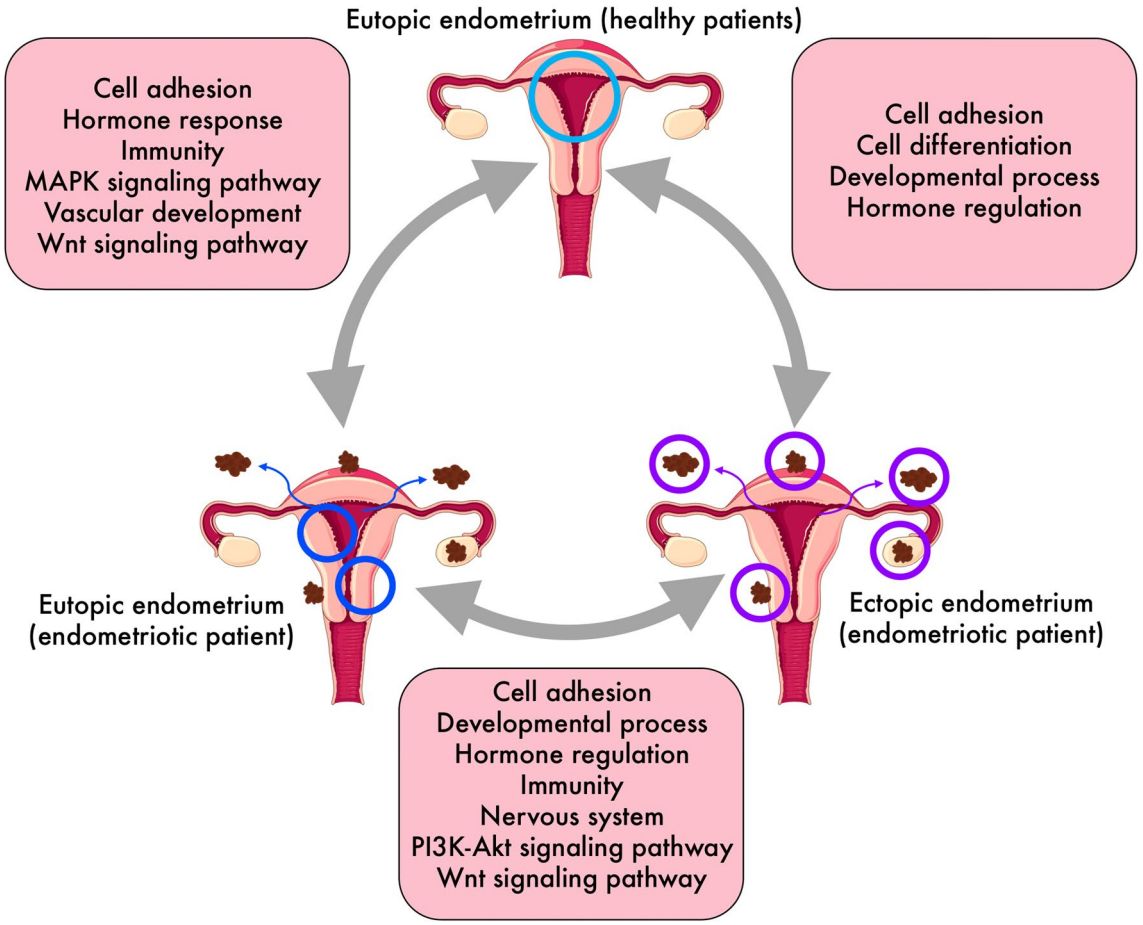


Fig. 5 Summary of epigenetically altered pathways in endometriosis according to EWAS. This diagram depicts common biological processes enriched for DMG in the different functional analyses performed in each EWAS. Complete list of pathways recorded is available in Additional file 7

cell adhesion, cell proliferation, cell differentiation, cell communication, developmental processes, response to hormone, apoptosis, immunity, neurogenesis, and cancer. Mechanisms by which cells invade ectopic tissues could be revealed in particular by comparing eutopic and ectopic endometrial cells from the same women with endometriosis in larger cohorts.

The complex interplay between genetic background, epigenetic factors, and environmental exposure makes it hard to decipher endometriosis physiology and find appropriate therapeutics. However, DNA methylation is a dynamic and reversible process that opens promise for the development of research models to understand disease mechanisms, notably endometriotic cell progression or suppression, and to develop therapies that target affected pathways, potentially through existing (de)methylating enzymes. Drugs that target genes like *PTGS2* that are epigenetically dysregulated in endometriosis have shown promising results in clinical trials [88]. The overarching mechanisms leading to global alterations in DNA methylation may stem from the dysregulation of enzymes responsible for the establishment and maintenance of DNA methylation, the methyltransferases. Even if results are still inconsistent regarding a down- or up-regulation, an aberrant expression of *DNMT1*, *DNMT3A*, and *DNMT3B* has been observed in endometriotic lesions [35, 61, 113]. DNA hydroxymethylation also remains a poorly explored field.

Strengths and limitations

This systematic review has several strengths in its methodology that enable us to assess the existence of disease biomarkers and the biological processes most likely to be affected by epigenetic deregulation. Criteria to select studies were very strict. We also adapted and validated an AI-powered method to select the studies in this systematic review, method which represents a real benefit for the scientific and medical community as it is an efficient, quicker, and

easier method of selection than the "manual" method for large bibliographies. In addition, we applied a complete exclusion when precise coordinates of the genomic region analyzed were not available. Performing in silico PCR, we were able to assess whether regions analyzed were in the same genomic position and discuss the results accordingly. After applying rigorous risk of bias and quality of evidence grading, studies were stratified by evidence to give better insight on the level of proof for each gene. Our EWAS qualitative analysis was hampered by the lack of complete data available for each publication. In the future, a quantitative analysis such as a meta-analysis approach could identify more putative DNA methylation biomarkers of endometriosis.

Different cell mixtures from the same tissues may hide critical DNA methylation differences between cells of the same type among distinct biological groups. The endometrium is structured into two layers of cells, mesodermal-derived glandular and luminal epithelium over a connective tissue specified as stroma. Recently, single-cell RNA-seq analysis of > 108,000 cells highlighted three more cell types in endometrial biopsies, namely, endothelial cells, lymphocytes, and myeloid cells [114]. Interestingly, cell types proportion is variable between eutopic endometrium from controls and endometriotic patients, with a preponderance of epithelial cells in controls and stromal cells with endometriosis. In addition, different types of endometriotic lesions are heterogeneously composed with more or less endometrium-specific cells and surrounding tissues [115]. Ectopic ovarian tissue displays a highly specific profile compared to ectopic peritoneal tissue, being drastically composed of stromal cells (70% vs. 32%) and fewer myeloid and lymphocyte cells (16% vs. 46%). Eutopic versus ectopic comparison often led to a high number of DMPs/DMRs which may be skewed by cell-type proportions. The best solution would be to use purify cell types, but this could be very laborious. Saare et al. proposed several ways to reduce the tissue heterogeneity concern in DNA methylation endometrial studies including cell isolation by laser capture microdissection or fluorescence activated cell sorting [115]. In the future, the single-cell methylation approach would benefit from verifying the hypothesis of the presence of progenitor cells of endometriotic foci inside the endometrium. Deconvoluting methods could also be generalized but there are few amounts of genome-wide data accessible for endometrial stromal cells and no reference for epithelial cells, thus reference-free deconvolution methods should be advantageous in endometrial studies [116].

Conclusion

Mining the scientific literature regarding the epigenetics of endometriosis has led us to the conclusion that endometriosis could partially be an epigenetic-related disorder. However, at this time, we cannot discriminate whether the epigenetic modifications are the cause or the consequence of the disease. Each biological process presented in this systematic review that is epigenetically regulated by DNA methylation may have great significance in endometriosis onset, but individually, they may not be sufficient to explain the development and/or progression of the disease. Endometriosis is likely to affect women with an unfavorable environment in the interplay of a disadvantageous genetic background, adverse epigenetic modifications triggered by factors that remain to be precisely identified (developmental, immunological, hormonal, chemical, and microbiomal factors). Although the exact epigenetic mechanisms directly involved in the pathogenesis of endometriosis are not yet known, evidence suggests that endometriosis could be associated with polyepigenetic alterations involving major signaling pathways (steroid hormone signaling, development, immunity, cell adhesion, Wnt- and MAPK-signaling, etc.).

DNA methylation could play a dual role by reducing diagnosis delays and contributing to epigenetic drug development. Finding biomarkers may help create diagnostic tests that can be performed as soon as symptoms start to show up. These tests may even be applied to menstrual blood or saliva to avoid invasive procedures. In this regard, efforts are needed to establish large datasets and sample collections of eutopic and ectopic tissues from endometriotic patients highly characterized (including patients' characteristics, localization, and degrees of severity of endometriosis), which will certainly help to identify the epigenetic roots of endometriosis.

Abbreviations

AI	Artificial intelligence
CpG	Cytosine–phosphate–guanine
DMG	Differentially methylated gene
DMP	Differentially methylated position
DMR	Differentially methylated region
EWAS	Epigenome-wide association study
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01828-w.

Additional file 1 PRISMA checklist.

Additional file 2 Data extraction form.

Additional file 3 Quality assessment of cohort studies by the Newcastle–Ottawa scale.

Additional file 4 Grading of studies by the GRADE scale.

Additional file 5 Included and excluded studies.

Additional file 6 Characteristics of included studies.

Additional file 7 Newcastle–Ottawa and GRADE scores of each included study.

Additional file 8 Complete sets of DMP/DMR/DMG recorded in all studies.

Additional file 9 Functional analysis results available for studies included.

Author contributions
BD and PF conceived the study. BD and PF performed the study selection process, data extraction, and study evaluation. BD performed data analysis. BD and PF drafted the manuscript. CC, CP, DV, JF, LF, LM, GP, MB, and PS provided critical revision and edited the article. All the authors approved the final version of the article.

Funding
No funding.

Availability of data and materials
The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 28 October 2024 Accepted: 30 January 2025
Published online: 21 February 2025

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