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REVIEW ARTICLE

Could DNA hydroxymethylation be crucial in influencing steroid hormone signaling in endometrial biology and endometriosis?

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Abstract

Endometriosis affects 10% of reproductive-aged women. It is characterized by the growth of the endometrium, outside the uterus and is associated with infertility and chronic abdominal pain. Lack of noninvasive diagnostic tools and early screening tests results in delayed treatment and subsequently increased disease severity. Endometriosis is a disease associated with a deregulated hormonal response, therefore, understanding the molecular mechanisms that govern this hormonal interplay is of paramount importance. DNA methylation is an epigenetic mark that regulates gene expression and is often associated with genes that code for steroid receptors and enzymes associated with estrogen synthesis and metabolism in endometriosis. DNA hydroxymethylation, which is structurally similar to methylation but functionally different, is a biologically critical mechanism that is also known to regulate gene expression. Ten Eleven Translocation (TET) proteins mediate hydroxymethylation. However, the role of DNA hydroxymethylation or TETs in the endometrium remains relatively unexplored. Currently, the "gold standard" technique used to study methylation patterns is bisulfite genomic sequencing. This technique also detects hydroxymethylation but fails to distinguish between the two, thereby limiting our understanding of these two processes. The presence of TETs in the male and female reproductive tract and its contribution to endometrial cancer makes it an important factor to study in endometriosis. This review summarizes the role of DNA methylation in aberrant steroid hormone signaling and hypothesizes that hydroxymethylation could be a factor influencing hormonal instability seen in endometriosis.

KEYWORDS

DNA hydroxymethylation, DNA methylation, endometriosis, endometrium, steroid hormones

1 | BACKGROUND

Endometriosis is one of the most common gynecological disorders that is known to reduce health-related quality of life and affects every 1 in 10 women of reproductive age (Eskenazi & Warner, 1997; Hogg & Vyas, 2015; Kitawaki et al., 2002). Due to the societal stigma associated with period pain and lack of noninvasive diagnostic

Abbreviations: 5ac, 5-carboxylcytosine; 5hmC, 5 hydroxymethylcytosine; 5mC, 5-methylcytosine; 5fc, 5-formylcytosine; COX2, cyclooxygenase 2; DHT, dihydrotestosterone; DNMT, DNA methyltransferases; ERα, estrogen receptor α; ERβ, estrogen receptor β; GADD45, DNA damage inducible protein; HOXA10, homeobox A10; HPG, hypothalamus pituitary gonadal axis; PRA, progesterone receptor-A; PRB, progesterone receptor-B; SF-1, steroidogenic factor 1; TET, ten eleven translocation proteins.

techniques, the magnitude of this disease remains unknown and the actual prevalence is presumed to be much higher (Meuleman et al., 2009). The symptoms associated with endometriosis are commonly seen in other disorders such as irritable bowel syndrome or adenomyosis (Sinaii, Cleary, Ballweg, Nieman, & Stratton, 2002). Additionally, some women may also be asymptomatic, which makes diagnosing the disease an even bigger challenge (Hogg & Vyas, 2015). A combination of all of these factors can lead to a staggering 8–11-year delay in accurate diagnosis. Endometriosis is an inflammatory disease that is characterized by the growth or presence of endometrial tissue outside the uterine cavity. These tissues are often found in locations such as the ovaries, fallopian tubes, or the pelvic sidewall (Burney & Giudice, 2012; Eskenazi & Warner, 1997; Giudice & Kao, 2004; Sampson, 1921). This ectopic tissue responds to the hormonal stimuli in a cyclic manner, mimicking the normal endometrial tissue and can subsequently result in abdominal bleeding, inflammation, development of scar tissue, and/or endometrioid cysts (Farquhar, 2000). Furthermore, 30–50% of women who are infertile and/or have chronic pelvic pain are subsequently diagnosed with endometriosis (Burney & Giudice, 2012; Eskenazi & Warner, 1997; Hogg & Vyas, 2015; Meuleman et al., 2009). Hormonal supplements are commonly used to manage endometriosis, however, they do have limitations and can lead to women developing resistance (Rafique & Decherney, 2017). This highlights the need for better therapeutic interventions that can be used to control endometriosis.

Endometriosis is regarded as an estrogen-dependent disease, because women with the condition, display an increased estrogenic expression and activity (Bulun et al., 2002; Burney & Giudice, 2012; Sampson, 1921). Steroid hormones mediate their action through steroid receptors in the endometrium. The expression of these receptors in the endometrium varies with the plasma hormonal levels throughout the menstrual cycle (Hewitt & Korach, 2003; Mertens, Heineman, Theunissen, de Jong, & Evers, 2001; Snijders et al., 1992). Aberrations in the molecular pathways hinder with this hormonal regulation favoring an overproduction of estrogen, prostaglandins, and cytokines which could potentially lead to the onset of endometriosis (Bulun, 2009; Bulun et al., 2002; Tseng et al., 1996; Wu, Strawn, Basir, Halverson, & Guo, 2006). This paper aims to review the currently available literature on the possible role of epigenetic deregulation in endometriosis, mainly focusing on two epigenetic modifications—DNA methylation and the newly discovered DNA hydroxymethylation and their potential influence on steroid hormone signaling.

2 | DNA METHYLATION AND HYDROXYMETHYLATION IN THE ENDOMETRIUM

The endometrium is a dynamic tissue that is sensitive to hormonal influences. The menstrual cycle involves a synchronous interplay of steroid hormones namely estrogen and progesterone, allowing the endometrium to undergo molecular and morphological changes of proliferation, differentiation, degeneration, and regeneration every month (Barbieri, 2014). Several mechanisms regulate the menstrual cycle to maintain a healthy endometrium and one such mechanism is epigenetics, which refers to the phenomenon influencing gene expression without altering the underlying DNA sequence (Moore, Le, & Fan, 2013). DNA methylation is a widely studied epigenetic process where a methyl group is added to cytosine to form 5 methylcytosine (5mC) and is most often associated with repressing gene activity. The role of DNA methylation in endometrial biology is directly correlated with the expression of many implantation related and progesterone regulated genes as described by Guo (2009a); Houshdaran et al. (2016); Koukoura, Sifakis, and Spandidos (2016); Naqvi, Ilagan, Krikun, and Taylor (2014). Similarly, the aberrant establishment of methylation patterns have been linked to several endometrial abnormalities such as endometrial cancer, implantation failure, deviant endometrial pathologies, adenomyosis, and endometriosis (Guo, 2009a, 2009b; Ma & Gao, 2014; Tao & Freudenheim, 2010). Endometriosis is associated with significant estrogen and progesterone imbalance. Many genes, including hormonal receptor genes that are responsible for regulating the effects of estrogen and progesterone, are under epigenetic control in the endometrial tissue (Dyson et al., 2014; Hsiao, Wu, & Tsai, 2017; Munro, Farquhar, Mitchell, & Ponnampalam, 2010). Promoters of genes associated with estrogen-metabolism are hypermethylated in endometriotic tissues, correlating with their significant downregulation in endometriosis (Table 1; Wu et al., 2005; Wu, Strawn, et al., 2006; Zanatta et al., 2010). On the other hand, the promoters of genes associated with estrogen biosynthesis are hypomethylated, associated with their upregulation in endometriosis (Table 1; Buchweitz et al., 2006; Izawa et al., 2011; Ota, Igarashi, Sasaki, & Tanaka, 2001; Wang, Chen, Zhang, Ren, & Li, 2012; Xue, Lin, Yin, et al., 2007; Yamagata, Nishino, et al., 2014). In addition, Dyson et al. (2014) report a significant difference in methylation patterns of several genes as well as transcriptional regulators, associated with the pathology of endometriosis and decidualisation. A possible correlation between altered methylation patterns in GATA2 and GATA6 of the GATA family of transcription factors is also suggested to control the progression of endometriosis. Estrogen and progesterone receptors modulate successful hormonal action during the normal menstrual cycle. During the proliferative phase, estrogen significantly upregulates the steroid hormone receptor levels priming and preparing it for subsequent progesterone action (Barile, Sica, Montemurro, Iacobelli, & Corradini, 1979).

Data suggest a discrepancy in methylation patterns during the different menstrual cycle phases. Ghabreau et al. (2004) reported higher DNA methylation during the proliferative phase, while Saare et al. (2016), suggested maximum levels of fluctuations in the endometrial methylome during mid to late secretory phase. These studies highlight the importance of understanding DNA methylation in maintaining a hormonal equilibrium in the endometrium and indicate a potential correlation between aberrant methylation status, menstrual cycle, and endometrial pathologies. In 2009, 5-hydroxymethylcytosine (5hmC) was identified as another important epigenetic modification that is related to

TABLE 1 List of genes that are differentially methylated and are associated with hormone imbalance seen in endometriosis

Genes	Function	Methylation pattern in endometriosis	Reference
ESR2	Estrogen receptor	Hypomethylated/active	Xue, Lin, Cheng, et al. (2007)
ESR1	Estrogen receptor	Hypermethylation/inactive	Dyson et al. (2014)
CYP19A1 (Aromatase)	Estrogen synthesis	Hypomethylated/active	Izawa et al., (2011)
COX2	Estrogen synthesis	Hypomethylated/active	Buchweitz et al. (2006); Wang et al. (2012)
SF-1	Estrogen synthesis	Hypomethylated/active	Xue, Lin, Yin, et al. (2007); Yamagata et al. (2014)
HOXA10	Estrogen metabolism	Hypermethylated/inactive	Lee, Du, and Taylor (2009); Szczepańska, Wirstlein, Łuczak, Jagodziński, and Skrzypczak (2010); Wu et al. (2005)
HSD17B2	Estrogen metabolism	Hypermethylation/inactive	Yamagata et al. (2014)
PRB	Estrogen metabolism	Hypermethylation/inactive	Wu, Strawn, et al. (2006)

DNA methylation but is associated with gene activation (Richa & Sinha, 2014; Shukla, Sehgal, & Singh, 2015; Tahiliani et al., 2009). Hydroxymethylated 5mC is an intermediate of the DNA demethylation cascade, its presence in mammalian stem cells and differentiated tissues make it an important developmental aspect to study. Studies that aimed to look at DNA methyltransferases (DNMTs), enzymes that facilitate DNA methylation, reported higher mRNA levels of DNMTs upon estrogen treatment and lower mRNA levels during progesterone treatment or a combined estrogen and progesterone treatment (Vincent, Farquhar, Mitchell, & Ponnampalam, 2011; Yamagata, Asada, et al., 2009; Zelenko, Aghajanova, Irwin, & Giudice, 2012). As far as we know, there are no studies reporting the role of DNA demethylation in endometrial biology. However, DNA damage-inducible protein (GADD45), a gene that is involved in DNA demethylation is reported to be upregulated during the midsecretory phase (Aghajanova, Hamilton, & Giudice, 2008; Vincent et al., 2011). The downregulation of DNMT mRNA levels and the upregulation of GADD45 during the secretory phase could be suggestive of a potential role that DNA demethylation may play in regulating genes that maintain the successful function of the endometrium.

Ten Eleven Translocation (TET) enzymes facilitate the conversion of 5mC to 5hmC and subsequently to 5-formylcytosine (5fc) and 5-carboxylcytosine (5ac), that make up the demethylation cascade (He et al., 2011; Ito et al., 2010; Tahiliani et al., 2009). TETs have been previously implicated in breast and endometrial cancer as well as in endometriosis (Ciesielski et al., 2017; Delhommeau et al., 2009; R. Li et al., 2018; Morlans, 2015; Roca, Loomans, Wittman, Creighton, & Hawkins, 2016; Wielscher et al., 2013). Nonetheless, the cellular distribution of TETs in the endometrium is uncharacterized and the contribution of DNA hydroxymethylation in activating genes associated with hormonal regulation in the endometrium remains undetermined.

3 | THE ROLE OF METHYLATION IN STEROID HORMONE RECEPTOR DISTRIBUTION IN ENDOMETRIOSIS

There are two main types of estrogen receptors, which predominantly act as transcription factors: estrogen receptor α (ERα) and estrogen receptor β (ERβ). They are encoded by two different genes, *ESR1* and *ESR2*, respectively (Greene & Press, 1986; Herynk & Fuqua, 2004). Progesterone receptor has two protein isoforms that are translated from a single gene: Progesterone receptor-A (PRA) and progesterone receptor-B (PRB), both are expressed in the endometrium and despite originating from a single gene, display distinctive transcriptional activities (Conneely, Mulac-Jericevic, & Lydon, 2003). Women with endometriosis show an increased expression of ERβ and a decreased expression of ERα in the eutopic endometrium and primary stromal cells (Brandenberger et al., 1999; Bulun et al., 2006; Fujimoto, Hirose, Sakaguchi, & Tamaya, 1999). It is noted that in endometriosis, the absence of PRB contributes to overall decreased mRNA levels of progesterone receptors (Attia et al., 2000). Several other studies also report discrepancies in the PR-isoforms in endometriosis, suggesting untraceable levels of PRB in stromal cells of the eutopic endometrium (Attia et al., 2000; S. Bulun et al., 2010; Izawa, Taniguchi, Terakawa, & Harada, 2013). Women with endometriosis often present with progesterone resistance (Kim, Kurita, & Bulun, 2013). The exact cause of this resistance is not entirely known, however, high expression of ERβ and a lower concentration of ERα could be responsible for an inadequate estrogen-priming, contributing to the suppression of progesterone receptors and thus progesterone resistance in women with endometriosis (Bergman et al., 1992). Another theory suggests that high levels of ERβ suppresses ERα and is involved in the regulation of cell cycle progression thereby contributing to ectopic endometrial tissue proliferation (S. Bulun et al., 2010). Since estrogen and its receptors are imperative for mediating estrogen-induced progesterone receptors, understanding the various factors that are involved in its regulation and expression in endometriosis is necessary.

Evidence supports that the failure to achieve an optimal estrogen-primed endometrium in the proliferative phase could potentially result in aberrant steroid receptor distribution and lead to subsequent progesterone resistance in endometriosis. This makes steroid hormone regulation in endometriosis extremely crucial to expand our understanding of the disease. Several genes including genes coding for ERβ, PRB, and aromatase are under epigenetic control in the endometrial tissue (Hsiao et al., 2017; Izawa et al., 2011; Munro et al., 2010; Wu, Strawn, et al., 2006; Xue, Lin, Cheng

et al., 2007). Additionally, as shown in Table 1, aberrant methylation patterns of genes that regulate estrogen production and metabolism in the endometrium such as *Steroidogenic Factor-1 (SF-1)*, *Homeobox A10 (HOXA10)*, and *Cyclooxygenase2 (COX2)*, have been reported (Buchweitz et al., 2006; Ota et al., 2001; Wang et al., 2012; Wu et al., 2005; Yamagata, Nishino, et al., 2014; Zanatta et al., 2010). The DNA methylome varies the most during secretory to midsecretory phase in endometriosis which corresponds to an anomalous progesterone response (Houshdaran et al., 2016). Existing data imply that the two-way communication between epigenetic modulators and steroid hormone receptors is disrupted in endometriosis and that generally leads to progesterone resistance and thus contributes to the development of resistance to hormone treatments.

These studies, however, come with significant limitations since most of them do not consider differences at the cellular and cyclic levels. Endometrial tissues comprise of two major cell types (luminal and glandular epithelial and stromal cells) which are likely to have their own cell-specific methylation patterns, making it important to study these individually as well as in conjunction (Logan, Yango, & Tran, 2018; Saare et al., 2018). A recent study revealed that in endometriosis, epithelial and stromal cells in the ectopic tissue developed independently of each other, containing distinct clones which makes them functionally different (Noë, Ayhan, Wang, & Shih, 2018). Several studies so far, that aimed to look at the methylation status of genes that are associated with endometriosis consider either the endometrial tissue as a whole or one of the two cell types which could provide bias results (Buchweitz et al., 2006; Izawa et al., 2011; Roca et al., 2016; Van Kaam et al., 2011; Wang et al., 2012; Wu et al., 2005; Wu, Kajdacsy-Balla, et al., 2006; Xue, Lin, Yin, et al., 2007; Yamagata et al., 2014a). Furthermore, It has been suggested that epigenetic patterns vary throughout the menstrual cycle making it an essential covariate of methylation studies in the endometrium (Houshdaran et al., 2016; Munro et al., 2010; Rahmioglu et al., 2017; Saare et al., 2016). Lastly, aberrant hydroxymethylation patterns could also potentially be contributing to the deregulation of genes in disease states, making it important to understand its role and correlation to progesterone resistance in endometriosis. As of now, our understanding of DNA hydroxymethylation patterns or TET gene expression in endometriosis is very limited.

4 | HYDROXYMETHYLATION AND TEN ELEVEN TRANSLOCATION PROTEINS

DNA Methylation and hydroxymethylation are known to perform contradictory functions whereas the former are involved in gene silencing; the latter is associated with gene activation (Spruijt & Vermeulen, 2014; Wu & Zhang, 2011). Currently, three types of TETs are known to be expressed in mammalian cells which display distinct as well as collaborative function: TET1, TET2, and TET3. Recent studies suggest the involvement of TETs in the male as well as female reproductive tracts (Kurian et al., 2016; Yosefzon et al., 2017). This process is still not clearly understood and its potential role in development, progression, and other abnormalities is yet to be explored. Knockout studies have been widely used to provide insight into the characteristics of these enzymes. Mice that lacked TET1, displayed an abnormal follicular development and consequently impaired fertility. Additionally, an abnormal TET1 isoform suppressed the luteinizing hormone gene, suggestive of a critical role that these proteins play in the female reproductive tract (Yosefzon et al., 2017). Similarly, young adult male mice lacking TET2 in the neurons that secrete gonadotropin-releasing Hormone, displayed decreased levels of plasma luteinizing hormone along with compromised fertility, highlighting the involvement of TET2 in the neuroendocrine regulation of reproduction in males (Kurian et al., 2016). The hypothalamus–pituitarygonadal (HPG) Axis is pivotal in regulating the production of estrogen and progesterone from the ovaries in women (Barbieri, 2014). These studies demonstrate the ability of TET1 and TET2 to disrupt the HPG axis, which could also potentially be linked to the hormonal instability seen in endometriosis. Further studies that examine the role of TETs in establishing a successful HPG feedback loop and regulating steroid hormone production are needed to understand its influence in endometriosis.

It was also noted that mice lacking either TET1 or TET2 were viable, suggesting that the genes function in association and the absence of one may be compensated by the other (Dawlaty et al., 2011, 2013; Moran-Crusio et al., 2011; Quivoron et al., 2011). Studies on the combined loss of TET1 and TET2 in mice models revealed that while males had normal gonads and were fertile, females displayed phenotypically smaller ovaries and were subfertile (Dawlaty et al., 2011, 2013). Moreover, these studies propose an overall increased level of 5mC and a decreased level of 5hmC, indicating that lower hydroxymethylation contributes to global hypermethylation (Dawlaty et al., 2013). TET1 and TET2 combined loss not only reflected on the global hydroxymethylation patterns but also showed reduced levels of hydroxymethylated 5mC in specific tissue types such as adult tissues and a complete loss in embryonic stem cells and germ cells (Dawlaty et al., 2013). Endometriosis is a disease of multifactorial origin however, studies such as Bouquet De Jolinière et al. (2012) and Signorile et al. (2012), suggest an embryologic origin of endometriosis. Failure of establishing successful hydroxymethylation patterns during critical periods of embryologic development could also be potentially associated with the pathogenesis of endometriosis. Further studies assessing the correlation between DNA hydroxymethylation patterns during embryo development and its effect on the pathogenesis of endometriosis are needed.

The role of TETs is not only suggested in reproductive tracts but is indicated in several other biological processes. TETs execute their function through differential expression and in a cell-specific manner. TET1 is shown to be highly and specifically expressed in embryonic stem cells (Ito et al., 2010; Koh et al., 2011). In addition, these enzymes are also expressed in differentiated tissues of the brain and central nervous system in adults (Kriaucionis & Heintz, 2009). TET2 mutations lead to abnormal methylation patterns and, loss of TET2 in bone marrow mesenchymal stromal cells increases malignancies (Delhommeau et al., 2009; R. Li et al., 2018). In comparison to TET1 and TET3, TET2 is reported to be significantly overexpressed in

breast cancer cells. It was also proposed that the absence of TET2 from breast cancer cells, critically altered the expression of several progesterone-responsive genes which indicates the potential role of TET2 in progesterone-mediated gene expression (Morlans, 2015). Concomitantly, another study stated lower levels of 5hmC in a tumor suppressor gene (*leucine zipper, putative tumor suppressor 1*), associated with metastasis in breast cancer. This finding was further correlated with reduced expression of TET1 in breast cancer samples suggesting its potential involvement in cancer progression (Wielscher et al., 2013).

A genome-wide analysis of individual TET deletion in an embryonic carcinoma cell model by Putiri et al. (2014), revealed that only the loss of TET1 leads to a widespread reduction of 5hmC. Meanwhile, it was noted that while TETs work in a co-dependent manner to establish the successful conversion of 5mC to 5hmC, only TET2 and TET3 are responsible for its removal during demethylation. Similarly, Gu et al. (2011) reported that hydroxymethylation in mice zygotes is seen in the paternal genome where the male pronucleus predominantly hosts TET3 proteins, mediating global erasure of 5mC. Furthermore, knocking out TET3 in male zygotes failed to achieve the 5mC to 5hmC conversion. In female mice, TET3 deficiency in the germline reduced fertility. Additionally, the mutant offspring, lacking maternal TET3, also showed the increased developmental failure of the embryo suggesting a crucial role of TET3 in the maternal germline (Gu et al., 2011). All the previously mentioned studies highlight the involvement and varied functions of the TET family members and the increasing need to understand its contribution individually as well as in conjunction with other factors in endometrial biology.

To our knowledge, there are three studies that have investigated TET expression in endometrial pathologies (Ciesielski et al., 2017; Roca et al., 2016; Szczepańska, Wirstlein, Zawadzka, Wender-Ożegowska, & Jagodziński, 2018). Ciesielski et al. (2017), suggest a discrepancy in TET mRNA levels in endometrial cancer, when compared to normal endometrium. They also suggest a correlation between lower TET1 and TET2 mRNA expression with reduced global 5hmC levels. This data in agreement with other studies, confirming an association between global 5hmC levels and TET expression (Ciesielski et al., 2017; Du et al., 2015; Murata et al., 2015; Putiri et al., 2014). Other recent studies looking at the involvement of TETs in endometriosis, reported TETs to be down-regulated in an ectopic (Roca et al., 2016) and eutopic (Szczepańska et al., 2018) endometrial tissues. Despite having a lower TET gene expression, Roca et al. (2016) reported high global hydroxymethylation levels in the ectopic endometrium. Roca et al. (2016) aimed to study TET gene expression in the whole tissue and further, in vitro decidualized stromal fibroblast cells but do not report other differences at the cellular level. Additionally, this study only aimed to look at decidualized cells and does not factor in other menstrual cycle phases, limiting a comprehensive understanding of the nature of TETs in the endometrium and endometriosis. The above-discussed studies demonstrate the importance of understanding TET proteins in the endometrium. However, further studies assessing the contribution and association of TETs in hormonal deregulation seen in endometriosis are required.

Apart from being involved in the DNA demethylation process and in catalysing hydroxymethylation, TET family proteins also display noncatalytic functions (Gao et al., 2016; Lian, Li, & Jin, 2016; Montagner et al., 2016; Tsai et al., 2014; Xu et al., 2012; Zhang et al., 2015). Their ability as transcriptional coactivators/corepressors, allows them to form complexes to regulate important developmental processes. The involvement of TET2 in regulating mast cell differentiation and proliferation as highlighted by Montagner et al. (2016), and its ability to regulate inflammation through HDAC2-mediated IL-6 inhibition, reported by Zhang et al. (2015), suggested that TET2 exerts relevant noncatalytic functions. TET1 is reported to negatively regulate Neuro2a (mouse neural crest-derived cell line) cells, independent of its enzymatic activity, contributing to neuronal differentiation (Gao et al., 2016). Cell proliferation and inflammation are both important aspects of endometriosis. Along with its catalytic functions, understanding the noncatalytic involvement of TETs in inflammation and cell proliferation could help identify prominent targets for clinical intervention of endometriosis.

5 | METHYLATION VERSUS HYDROXYMETHYLYATION: AN OVERLAP

While quantifying the amount of methylation and hydroxymethylation in murine embryonic stem cells, Tahiliani et al. (2009), discovered the presence of about 55–60% of 5mC and 4–6% of 5hmC. Additionally, 5hmC makes up for 0.6% and 0.2% of total nucleotides in Purkinje cells and granule neurons, respectively, in the mouse genome (Kriaucionis & Heintz, 2009; Tahiliani et al., 2009). However, in mouse embryonic stem cells, global level of methylation increases and hydroxymethylation decreases dynamically during development, which corresponds to the rise in DNA methyltransferases (DNMT)-DNMT3a and DNMT3b expression and a decline in TET1 expression (Kinney et al., 2011). Methylation and hydroxymethylation are functionally distinct however, an overlap between the two has been proposed (J. Li et al., 2018; Putiri et al., 2014). It was also noted that in cancer, TET-mediated hydroxymethylation sites coincided with those that were aberrantly methylated (Putiri et al., 2014). A close association between regions that exhibit higher 5hmC and lower 5mC is also suggested by J. Li et al. (2018). These findings further highlight a structural similarity but a functional disparity between the two epigenetic modifications suggesting that the data in the current literature that aims to look at global methylation patterns in association to diseases could in fact, be a combination of both. This makes it increasingly important to conduct hydroxymethylation studies to understand its association with methylation and to determine how much they respectively influence disease pathogenesis.

Most studies that have aimed to study methylation patterns use the “gold standard” bisulfite genomic sequencing technique

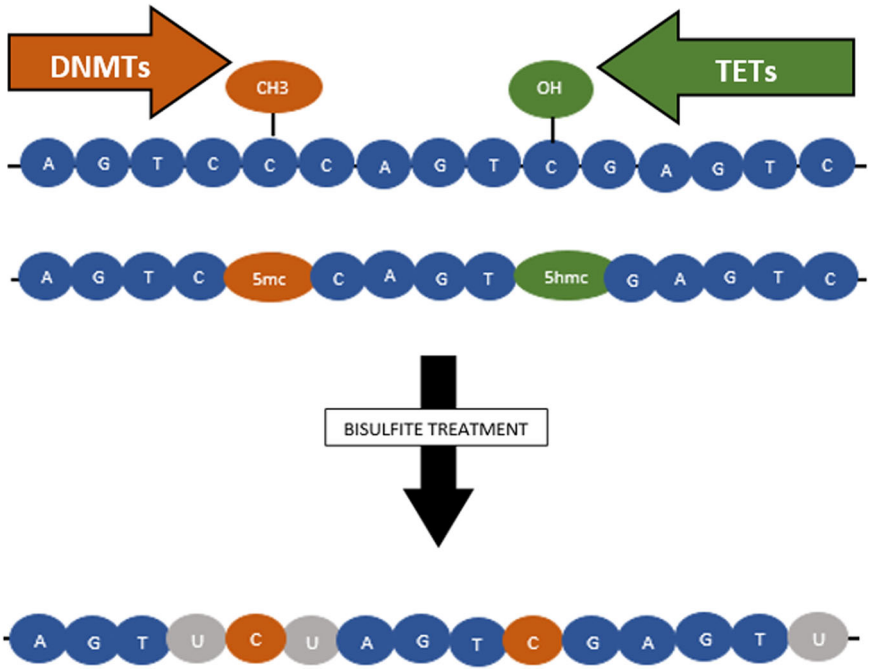

FIGURE 1 The "gold standard" identification technique used to detect methylation patterns. The first set of sequence represents a DNA strand comprising of 4 bases (A: Adenine; C: Cytosine; T: Thymine; G: Guanine). DNMT-mediated methylation and TET-mediated hydroxymethylation are two epigenetic modifications that execute their functions by tagging the sequence with a methyl group and a hydroxyl group, respectively. Bisulfite treatment allows to distinguish between methylated and unmethylated sites by converting the unmethylated cytosines to a different base (U: Uracil) and retaining all methylated cytosines as cytosines. This technique also picks up 5hmc (a mark for hydroxymethylation) as 5mc (a mark for methylation), thereby failing to distinguish between the two [Color figure can be viewed at wileyonlinelibrary.com]

(Chen et al., 2017; Darst, Pardo, Ai, Brown, & Kladde, 2010; Frommer et al., 1992; Y. Li & Tollefsbol, 2011). This technique serves as a quantitatively and qualitatively efficient method of assessing methylation patterns (Figure 1). As shown in Figure 1, the traditional technique involves treating the single stranded DNA with sodium bisulfite which yields a sequence that allows to distinguish between methylated and unmethylated cytosine (Y. Li & Tollefsbol, 2011). However one of the major limitations of this technique is that it does not differentiate between hydroxymethylation and methylation (Hayatsu & Shiragami, 1979; Huang et al., 2010). Since methylation and hydroxymethylation influence gene expression in opposing ways, an inability to distinguish between these two marks restricts our understanding of these processes resulting in an inaccurate measure of which epigenetic mark is truly responsible for a particular epigenetic alteration.

6 | FUTURE DIRECTIONS

The changes that the endometrium undergoes are orchestrated by estrogen and progesterone in a cyclic and synchronous manner. It has been suggested that epigenetic patterns vary throughout the menstrual cycle making it an essential covariate of methylation studies in the endometrium (Houshdaran et al., 2016; Munro et al., 2010; Rahmioglu et al., 2017; Saare et al., 2016). Moreover, significant changes in the expression of endometrial DNMTs during the menstrual cycle have been reported (Liao et al., 2008; Vincent et al., 2011; Yamagata, Asada, et al., 2009). Some studies revealed that DNMT 1 and 3 are significantly downregulated in the secretory phase than in the proliferative phase (Vincent et al., 2011; Yamagata, Asada, et al., 2009; Zelenko et al., 2012). Conversely, another study highlighted that DNMT1 expression was higher in the secretory phase (van Kaam et al., 2011). Previous data by our lab depict that DNMT3a expression was significantly downregulated in the early and late secretory phases, and DNMT3b transcription was significantly less abundant during the early, middle, and late secretory phases when compared with the proliferative phase in human endometrium (Vincent et al., 2011). Transcriptions of all three DNMTs were significantly repressed in endometrial explants of proliferative phase after a 48 hr combined treatment with estrogen and progesterone. Similarly, progesterone treatment alone led to a significant down-regulation of DNMT1 mRNA after 48 hr, and DNMT3b mRNA was significantly downregulated after 48 hr of estrogen treatment (Vincent et al., 2011). There has been conflicting data on the expression and regulation of DNMTs in the endometrium, which implies the need for additional studies. As mentioned previously, studies by Ciesielski et al. (2017), Roca et al. (2016), and Szczepanska et al. (2018), suggest the involvement of TETs in the onset of endometrial pathologies. However, these are the only three studies currently, that are indicative of a potential role of TETs in the endometrium and warrants further investigation on its role in endometriosis. Given the dynamic nature of this tissue, it is important

to understand the role of TET-mediated hydroxymethylation and DNMT-mediated methylation patterns in a cell-specific as well as cyclic manner which may help explaining complex diseases such as endometriosis and help in the development of targeted epigenetic therapies.

7 | CONCLUSION

To conclude, it is known that aberrant DNMT distribution and methylation patterns contribute to abnormal endometrial health. The discovery of TET-mediated hydroxymethylation as an epigenetic mark opened new horizons for molecular research. The presence of TETs in germ cells and its suggested role in the male and female reproductive tract as well as endometrial diseases, imply its importance in reproductive and endometrial biology. DNA hydroxymethylation is an epigenetic mark that works in close association with methylation, possibly regulating it. The various aspects through which it executes its function needs to be assessed to understand its role in endometrial biology. We speculate that abnormal methylation-mediated silencing of genes or/and abnormal hydroxymethylation-mediated activation of genes could be contributing to the hormonal instability seen in endometriosis. Epigenetic alterations have been used for targeted therapeutic interventions in diseases such as cancer and if its role in endometriosis is established, it will help in the development of better and effective treatment options.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

V. M. was the primary and major contributor in writing the manuscript. A. P. conceived the idea, provided insightful comments on drafts and approved the content of the manuscript. All authors read and approved the final manuscript.

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