Biomarkers in endometriosis: challenges and opportunities

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Endometriosis is a debilitating gynecologic disease affecting millions of women across the world, with symptoms including dysmenorrhea, chronic pelvic pain, and infertility. Theorized to stem from the phenomenon of retrograde menstruation, the diagnosis of endometriosis is typically delayed by 8–10 years owing to misinterpretation of symptoms as common menstrual cramps in adolescent girls and young women. With increased incidence of endometriosis in young girls correlated with earlier menarche, the development of diagnostic biomarkers is imperative for diagnosing and treating women afflicted with endometriosis as early as we can. In the past few years, multiple reviews highlighted the list of potential diagnostic candidates in peritoneal fluid, blood, urine, and endometrial biopsies from endometriosis patients in different stages of disease and menstrual cycle. In this review, we explore the opportunities and challenges facing the field of diagnostic biomarkers for endometriosis. We highlight the importance of eutopic endometrium as a source of potential diagnostic biomarkers by looking at the expression levels of noncoding RNA in tissue as well as in blood. Finally, we discuss some of the challenges that hinder our efforts in validating candidate diagnostic biomarkers for endometriosis. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, biomarkers, challenge

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Endometriosis is a debilitating gynecologic disease characterized by the presence of uterine epithelial and stromal tissues outside of the uterine cavity (1–5). Theorized to arise from the endometrial fragments escaping into the peritoneal cavity through the process of retrograde menstruation (6), endometriosis patients experience significant reduction in quality of life owing to increase in symptoms including nonmenstrual pelvic pain (38.7% vs. 14.3%), dyspareunia (29.5% vs. 13.4%), and infertility (11.6% vs. 3.4%) compared with women without endometriosis (7). Indeed, epidemiologic data show that in Canada, the inability of women to contribute to society because of disease amounts to the economic burden of $1.8 billion (8), which is increased to $18–22 billion in the United States (9, 10). Although decades of research into the pathogenesis of endometriosis have led to insightful elucidations into the hormonal and nonhormonal mechanisms involved in disease development and persistence, the therapeutic regimens to treat endometriosis and the methods for early diagnosis of endometriosis are still lacking.

The economic impact of endometriosis is compounded by the latency in the diagnosis of endometriosis, especially in young women that delay seeking treatment. Owing to the common misinterpretation of endometriosis-induced pain as menstrual-related abdominal pain, the diagnosis of endometriosis is typically delayed by 8–10 years: Adolescent girls who suffer from the symptoms of endometriosis delay seeking medical attention by 4.6 years, and by the time they seek medical attention, it takes another 4.7 years until diagnosis (11). In 2004, Ballweg reported an increase of endometriosis-like symptoms in girls before the age of 15 years as well as menarche occurring earlier (12), indicating the potential need to screen adolescent and younger girls as early as they display symptoms of endometriosis for confirmatory diagnosis. Laparoscopic surgery remains the current criterion-standard diagnostic tool; however, it is unlikely that women of reproductive age would subject themselves to such an invasive surgery when they can opt to temporarily diminish pain symptoms by means of other therapeutic mechanisms.

Indeed, to diminish the disease burden and minimize symptoms of pain, nonsteroidal antiinflammatory drugs, GnRH agonists, progestins, and oral contraceptive pills are mainstream therapeutic options (13). Because estrogen is the primary driver of endometriosis lesion development, most of the established therapeutics are targeted

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to create a hypoestrogenic state that offers temporary relief. One of the major disadvantages of all present drug treatments for endometriosis is they prevent women from pursuing pregnancy. Women are forced to decide whether to improve quality of life by diminishing pelvic pain symptoms, or forgo chances of pregnancy for the sake of minimizing endometriosis-associated pain.

Endometriosis is a complex disease with variable phenotypic and symptomatic presentation in women (14, 15). Aside from estrogen dependence, we know that immune dysfunction and inflammation play a role in its pathobiology (16–20). In addition, we are beginning to elucidate the genetic variants associated with endometriosis risk by means of genome-wide assessment studies (21–26), which have demonstrated that the endometrium of endometriosis patients displays aberrant molecular expression patterns that give it the ability to implant, invade, and develop into endometriotic foci (27–30). We are also beginning to map out the epigenetics of endometriosis by identifying aberrantly methylated genes (e.g., *TNFRSF1B*, *IGSF21*, and *TP73* [26]) involved in the pathogenesis of endometriosis (31). It is now well established that endometriosis thrives in an inflammatory environment. Researchers have documented elevated levels of proinflammatory cytokines in the peritoneal fluid, eutopic endometrium and ectopic lesion samples, and blood in women with endometriosis (32–36), which can decrease significantly on laparoscopic removal of the lesions (37). However, it remains unclear whether inflammation contributes to the pathogenesis of endometriotic lesion establishment or is a by-product of the process. Intriguingly, the only aspect of its pathogenesis targeted for therapeutic approach focus on the dependence of endometriosis on estrogen for growth, the suppression of which remains perhaps the single scientifically and clinically proven therapy with some success.

The hunt for a noninvasive biomarker for endometriosis has been an ongoing and challenging issue. The World Endometriosis Research Foundation, in a series of guidelines published in 2014 (38, 39), has prompted physicians, gynecologists, and researchers to standardize methods of sample collection and analysis of data, which in 2016 were further highlighted as research priorities for endometriosis (10). Indeed, the methods of tissue excision, body fluids collection, storage, and transportation need to be congruent in all facilities dedicated to endometriosis research to streamline data analysis. As a step forward, in 2016, an online multicenter documentation system across five hospitals and outpatient facilities was launched in Europe to optimize data collection and sharing and to improve molecular and clinical assessment of noninvasive biomarker discoveries (40). That study, however, did not specify whether the pain symptoms were included in patient characterization. Truly, the description of pain, including dyspareunia, chronic pelvic pain, and other related symptoms, needs to be systemically recorded to potentially identify biologic mechanisms underpinning the generation of pain (12). These potential confounding factors hinder our progress of noninvasive biomarker discovery because they affect the analysis of scientific findings. With improved characterization of patient history and standardized means of collection, storage, and interpretation of data, we will have the tools to identify noninvasive diagnostic biomarkers for endometriosis.

This review will elucidate the realistic opportunities and challenges in the verification of noninvasive diagnostic biomarkers for endometriosis. Potential biomarkers, including microRNA (miRNA) and long noncoding (lnc) RNA from blood, endometrial biopsies, and epigenetic markers of endometrium, will be evaluated. We will also assess their potentials as biomarkers in an attempt to guide our collective efforts into defining a noninvasive diagnostic biomarker or a group of markers that can precisely distinguish women with endometriosis from those without.

THE CHALLENGE

Heterogeneous Nature of Endometriosis and Endometriosis-Associated Symptoms

Endometriosis is often described as occurring in three areas in the pelvis—in the peritoneum, on the ovary, and in the rectovaginal pouch—which are referred to as peritoneal implants, ovarian endometrioma, and deep infiltrating endometriosis, respectively. However, endometriosis has been documented to manifest outside of the pelvic region (41). As such, the disease can be categorized into endopelvic and extrapelvic manifestations (42). The endopelvic manifestations include deep infiltrating endometriosis involving the posterior wall of the vagina and anterior wall of the rectum and ovarian endometrioma, which present as cystic growth on the surface of the ovary, where endometrial stroma and glands are found within the wall of the cyst (43). The extrapelvic manifestations include the typical peritoneal or abdominal wall endometriosis, including endometriosis found on surgical scars on the peritoneum (44, 45), the urinary and gastrointestinal tract, the thorax, and the nasal mucosa (42). Other extrapelvic locations of endometriosis include rare cases of vesical, or bladder, endometriosis, which occurs in 1% of women, especially during pregnancy (46). Twenty-two cases of hepatic endometriosis have also been cited in the medical literature (47). In addition, endometriotic foci have been found on the uterine ligaments, cervix, labia, and vagina (48). The widespread phenotype of endometriosis in patients is truly reminiscent of the metastatic characteristic of cancer, to which endometriosis is often compared. The ability of endometriosis to be found not only in the abdominopelvic cavity, but also in the thoracic cavity and even in nasal mucosa is an example that mechanisms aside from retrograde menstruation are involved in the pathogenesis of endometriosis. Such heterogeneity of disease phenotype also increases the potential of false-negative laparoscopic surgery in symptomatic women, wherein a “clean” pelvic cavity might not necessarily indicate the absence of endometriosis in other areas of the body (Table 1).

Unless accompanied by explorative laparoscopic surgery for complete diagnosis, accurate differential diagnosis in potential endometriosis patients based on symptoms proves to be a difficult challenge. In this regard, patients with

TABLE 1

Challenges of biomarker identification for endometriosis.

	Endometriosis	
Variable	**Endopelvic**	**Extrapelvic**
Phenotype of endometriosis	Ovaries Rectovaginal pouch Rectouterine pouch	Peritoneum Liver (hepatic) Urinary bladder Gastrointestinal tract Thorax Nasal mucosa
	Symptomatic	**Asymptomatic**
Endometriosis-associated symptoms	+ Infertility +/− Pelvic pain	+/− Infertility − Pelvic pain
Comorbidities[a]	Allergies Autoimmune disorders Migraines Gastrointestinal problems (e.g., bloating, irritable bowl disorder) Other uterine/pelvic disorders (adenomyosis)	

Note: Endometriosis is a heterogenous disease that can be phenotypically divided into endopelvic and extrapelvic manifestations. Each patient with endometriosis presents with variable pelvic pain symptoms and different reasons for infertility. Endometriosis can be accidentally discovered in women undergoing explorative laparoscopic surgery. Furthermore, comorbidities with similar inflammation and endocrine mechanisms can mask endometriosis symptomatology and can lead to false positive or false negative diagnosis of endometriosis.

[a] It is unknown whether asymptomatic endometriosis patients also have similar comorbidities as symptomatic patients.

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endometriosis can be broadly categorized into groups: with infertility and pelvic pain, with infertility but without pelvic pain, and without infertility but with pelvic pain. We can also categorize women as having symptomatic endometriosis and asymptomatic endometriosis. We can not forget odd cases of endometriosis being found beyond the pelvic cavity, and even cases reported in men (49). Physiologically, production of estrogen does not cease in the body after menopause. The distribution shifts from being systemic to local, to affect the area where it is produced. Not only has peritoneal endometriosis been documented in premenarche adolescent girls (50), but endometriosis has also been documented in 5% of postmenopausal women who underwent surgery for suspected endometriosis based on symptoms of pelvic pain (43). Punnonen et al. (43) attributed increased estrogenic activity due to obesity and age to the formation of ovarian endometriosis and adenomyosis in postmenopausal women, the latter of which was also a common finding in this cohort of women in the study. The conversion of androstenedione to estrone also increases in women with age. After menopause, the production of estrogen from extraovarian sites, typically by skin fibroblasts and adipose tissue, may be the mechanism behind recurrence or endometriosis or de novo formation of endometriotic foci (51, 52). To complicate our understanding of the pathogenesis, endometriosis has also been found in a nonobese postmenopausal woman without a history of receiving exogenous hormonal therapy and no history of endometriosis or infertility (53).

Case studies have shown that symptoms of pelvic pain are not a useful diagnostic parameter to differentiate women with or without endometriosis (54), owing to the similarity of symptoms with other pelvic pain pathologies, such as irritable bowel disease. Also, pelvic pain is not present in all endometriosis patients. Indeed, asymptomatic endometriosis (55, 56), which is the incidental finding of endometriotic foci during other laparoscopic surgeries, such as tubal ligation (46, 57), complicates our understanding of the relationship between the presence of endometriotic foci and incidence of pelvic pain in symptomatic women. Laux-Biehlmann et al. convincingly illustrated how menstrual debris in the peritoneal cavity can elicit pelvic pain symptoms through activation of mast cells and macrophages that in turn stimulate sensory nerve endings (58). Because endometriosis can be found in asymptomatic women, however, the biochemical pathway of nerve stimulation by the release of damage-associated molecular patterns and pathogen-associated molecular patterns by the menstrual debris (58) does not apply to the asymptomatic women, suggesting that the inflammation caused by "menstruating" ectopic foci and menstrual debris may not be the sole cause of pelvic pain.

Interestingly, functional neuroanatomic studies using magnetic resonance imaging have demonstrated that women with symptomatic endometriosis display hypersensitivity to pain owing to increased neuronal connectivity in areas of central pain perception (59). Furthermore, As-Sanie et al. demonstrated that increased concentration of glutamine and glutamate within the anterior insula and greater neuronal connectivity from the anterior insula to the medial prefrontal cortex positively correlated with not only pain intensity, but also clinical anxiety and depression in patients (60). These results suggest that women with incidental findings of endometriosis who do not suffer from typical symptoms of endometriosis perhaps have higher tolerance to pain. It is also likely that we do not understand the pathway to pain generation and perception in patients with endometriosis very well (61).

For decades, both basic and clinical research have been much focused on elucidating the pathogenesis of endometriosis, but they have lumped different phenotypes of the disease, including ovarian, peritoneal, and deep infiltrating endometriosis, as one entity. Back in 1997, Nisolle and Donnez (62) called for the recognition of peritoneal, ovarian and

rectovaginal endometriotic lesions as separate entities; however, almost 20 years on, current research does not reflect their insight. Furthermore, basic scientific research on the pathogenesis of extrapelvic endometriosis is limited. Indeed, it will be a daunting task to develop a specific set of diagnostic biomarkers that is capable in differentiating different types and degrees of endometriosis in suspected women based on symptomatology alone (14). It brings to light that we need to focus our research efforts on understanding the pathogenic mechanisms that drive the development of different phenotypes of endometriosis, and to redouble our effort to recognize them as separate entities of disease.

Comorbidities

Noninvasive biomarkers with high specificity and sensitivity will be a challenge for endometriosis, not only because of heterogeneity of disease characteristics, but also because of comorbidities suffered by endometriosis patients. The concentration of peripheral markers in blood and peritoneal fluid may be diluted with factors that are driving the development of conditions other than endometriosis in patients. For example, Sinaii et al. reported high incidence of autoimmune and endocrine disorders, as well as chronic fatigue syndrome, in endometriosis patients (63). Allergies were also common in adolescents and young women with endometriosis (12, 64). Furthermore, prevalence of endometriosis was reported to be higher in patients with migraines (65). Women with endometriosis, especially those diagnosed at an earlier age, displayed increased risk for ovarian cancer (66). Gastrointestinal-related abdominal pain, bloating, and constipation were also common in patients with endometriosis (67). In a recent review, Parazzini et al. amassed papers showing clear association between endometriosis and gynecologic cancers, gastrointestinal abnormalities, and immunologic diseases (68). These conditions may also have underlying immunologic and inflammatory pathologic mechanisms with peripheral increase in cytokines commonly associated with endometriosis, thereby potentially introducing bias in studies using peripheral cytokines as diagnostic indicators of endometriosis.

THE OPPORTUNITIES

Broadly, noninvasive diagnostic markers have been investigated in blood, tissue, and urine from endometriosis patients compared with menstrual-stage-matched control subjects: glycoproteins, cytokines, immune cell populations, miRNAs, transcriptome, proteome, metabolome, etc. In 2010 and 2011, May et al. published meta-analyses of peripheral and endometrial biomarkers in endometriosis (69, 70). Then in 2015, Fassbender et al. published the most up-to-date review of the biomarkers for endometriosis (71). Furthermore, from December 2015 to July 2016, Cochrane Database Systematic Reviews published meta-analyses primarily focused on noninvasive biomarkers of endometriosis from urine, blood, endometrial samples, and imaging tests (72–75). Most recently, Vicente-Monoz et al. expanded the list of noninvasive biomarkers of endometriosis by identifying elevated levels of certain metabolites—fucose and branched-chain amino acid—in the urine (76) and plasma (77) of endometriosis patients with the use of ^{1}H-nuclear magnetic resonance spectroscopy. Furthermore, Wessels et al. demonstrated the effectiveness of plasma brain-derived neurotropic factor as a noninvasive biomarker for detecting patients with stage I–II endometriosis with 91.7% sensitivity and 69.4% specificity (78). In addition, we (79) and others (80) documented elevated levels of synuclein-gamma (SNCG), a member of the synuclein family of neuronal proteins that is involved in cellular proliferation by means of interacting with the mitotic checkpoint kinase BubR1 in human endometriotic lesions. Indeed, treatment with the use of pthe eptide inhibitor of SNCG SP012 led to decreased growth and vascularization of endometriotic lesions in a mouse model of endometriosis, suggesting a potential role of SNCG in disease pathogenesis.

With the plethora of reviews assessing specificity and sensitivity of every possible potential invasive and noninvasive diagnostic biomarker reported to date, it is perhaps redundant to summarize similar findings in the present review, and readers are encouraged to revisit the earlier references (72–75). In this section, we will first assess the usefulness of endometrial biopsy as a minimally invasive means of obtaining potential diagnostic biomarkers for endometriosis. Then we will highlight the unique molecular differences with biomarker potential that are found in the eutopic endometrium. Finally, we will investigate why circulating and tissue miRNA and other noncoding RNA profilse might be better than using peripheral blood inflammatory cytokine levels as diagnostic biomarker candidates for endometriosis.

Assessing the Usefulness of Endometrial Biopsies

The method of obtaining endometrial sample by either pipelle or curette is minimally invasive; however, aside from minimal discomfort for the patient, biopsy can prove to be a useful not only to test endometrial receptivity in infertile women with or without endometriosis (81), but also as a diagnostic tool for endometriosis in an outpatient setting (82). According to the widely accepted theory of retrograde menstruation (6), the menstrual endometrium is the source of ectopic endometriotic foci. Instead of relying on peripheral blood or even urine for potential biomarkers, which may contain other pertinent information than to indicate the presence of endometriosis in women, using the direct source of the disease is perhaps logical in our quest to identify biomarkers for endometriosis.

It is important to emphasize that the endometrium of women with endometriosis has been documented to respond differently to ovarian hormones (83–86), and we can use this fact to discover new potential biomarkers through minimally invasive means (87). As thoroughly reviewed by Guo et al. (9), the eutopic endometrium displays aberrant molecular regulatory mechanisms due to epigenetic changes in steroid hormone–responsive genes. For example, Naqvi et al. (26) observed five hypermethylated (*MGMT*, *DUSP22*, *CDCA2*, *ID2*, and *RBBP7)* and five hypomethylated (*TNFRSF1B*, *MBPR1B*, *ZNF681*, *IGSF21*, and *TP73*) genes in eutopic

endometrium of patients compared with endometriosis-free control subjects. That study did not find significant differences in genes previously reported to be implicated in the pathogenesis of endometriosis, including *PR-B*, *CYP19A1* (aromatase), *SF1*, *COX2*, and *ER-β* (26). *HOXA10*, a gene implicated in the process of embryo implantation, was shown to be decreased in expression in the eutopic endometrium of baboons with endometriosis (84), owing to the hypermethylation of the promoter region of *HOXA10* gene (88, 89). Equally important is the characteristic progesterone resistance of eutopic endometrium in women with moderate to severe endometriosis, where the unresponsiveness to progesterone results in incomplete transition of the endometrium from proliferative to early secretory phase of uterine cycle (85). Knowing this, we may be able to deduce and potentially diagnose symptomatic endometriosis from endometrial biopsies by identifying dysregulation of steroid-dependent genes compared with a healthy control population.

A major limitation to using what we know to be molecularly different in endometrium of endometriosis patients as biomarkers is the overlap of similar findings in other gynecologic disorders. For example, Velasco et al. (90) did not find expression of aromP450 in the eutopic endometrium of endometriosis patients, whereas others (91) did find its expression. Furthermore, aberrant responsiveness to ovarian hormones is not a feature unique to endometriosis alone, but also shared with adenomyosis, a disease where endometrial tissue is found within the myometrium. Mehasseb et al. showed progesterone resistance in endometrium with adenomyosis, due to decreased progesterone receptor expression, which is similar to eutopic endometrium endometriosis patients (29). Not unlike the endometrium of endometriosis patients, proliferative-phase endometrium of women with diffuse adenomyosis also displays a molecularly distinct pattern of gene expression that is similar to the endometrium of women with endometriosis (92). Indeed, similar gene expression patterns between women with endometriosis and other pelvic/uterine pathology (adenomyosis, uterine prolapse, and uterine fibroids) were also observed by Tamaresis et al., which suggested that the gene expression pattern of endometrium might be similarly perturbed across women with different pelvic pathologies (93). Recognizing the significant overlap between molecular similarities between different uterine/pelvic pathologies, Tamaresis et al. voiced the need to properly define control groups in studies focusing on biomarkers for endometriosis (93).

To increase specificity of our diagnostic markers, we need to identify a pattern of gene expression from eutopic endometrium of potential patients that is specific for endometriosis to avoid false positives with other types of hormone-dependent inflammation-driven gynecologic diseases. Already, findings by Saare et al. discourage the use of endometrial DNA methylome as biomarkers for endometriosis, because significant methylation pattern differences in the endometrium were not found that could discriminate patients from healthy women with acceptable sensitivity and specificity (82). That study, however, did not compare findings based on stage of disease, which might have provided increased insight to their assessment in the biomarker potential of endometrium DNA methylome.

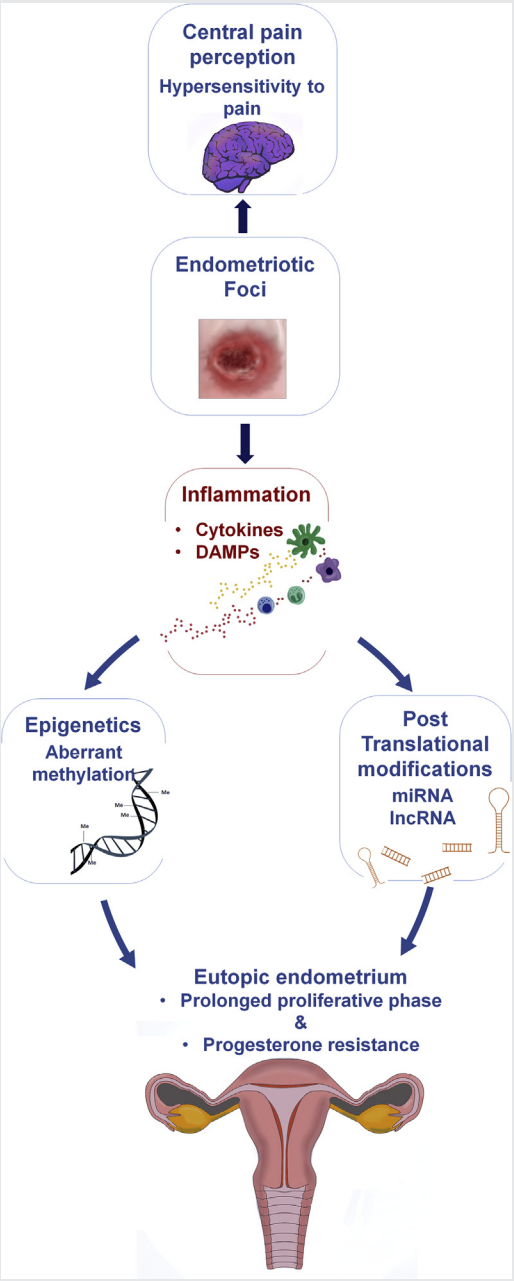
In Search of Molecular Patterns Unique to Endometriosis

Owing to ethical constraints, it is unlikely that we will be able to study the early stages of endometriosis development by injecting menstrual fragments into the peritoneal cavity of women. Therefore, we can not study the transient epigenetic transformation of endometrial fragments under the influence of inflammatory mediators and hypoxic peritoneal environment in humans. This is critical to elucidate why only 10% of women develop endometriosis although the phenomenon of retrograde menstruation occurs in 76%–90% of reproductive-age women (94–96). Using nonhuman primate models of endometriosis, it has been demonstrated that it is the presence of the menstrual fragments in the peritoneal cavity that directly influences gene expression pattern of the eutopic endometrium (97, 98). Those studies hint at the "cross-talk" between the ectopic endometriotic foci and the eutopic endometrium, wherein the presence of endometriosis likely programs hyperestrogen responsiveness and progesterone resistance into the eutopic endometrium as disease progresses (98) (Fig. 1). The study did not investigate whether altered eutopic endometrial response to steroid hormones was due to epigenetic changes. It is highly likely, however, that the altered endometrial response was due to transitory modification in the genome imposed by the external presence of ectopic foci (9).

Epigenetic modification of the genome by addition or removal of methyl groups is a reversible and dynamic process influenced by both the environment and lifestyle factors (99), and as such it may also be influenced by the level of hormones and inflammatory factors in the extracellular environment. Houshdaran et al. illustrated this by analyzing the DNA methylome of endometrium from patients in different phases of menstruation (100). In accordance with numerous studies demonstrating progesterone resistance in the endometrium of patients in secretory phase, they also found the greatest differences in methylation patterns between the endometrium of patients and from control subjects in the midsecretory phase of menstrual cycle (100), further confirming molecular abnormalities apparent in eutopic endometrium of women with endometriosis.

In a concerted effort, multiple genome-wide association studies (GWASs) identified single-nucleotide polymorphisms (SNPs) associated with disease risk in endometriosis patients (24, 101). A meta-analysis of eight GWASs published to date (24) demonstrated SNPs in six genetic loci, including 7p15.2, *WNT4*, *VEZT*, *CDKN2B-AS1*, *ID4*, and *GREB1* across European, American, and Japanese women with stage III–IV endometriosis. Pagliardini et al. conducted a replication study in an Italian cohort to confirm significant association of SNPs located on the locus of *VEZT* and *CDKN2B-AS1* with endometriosis compared with control (101). Holdsworth-Carson et al. then further identified, in an Australia/New Zealand endometriosis cohort, 11 coding variants of *VEZT* that may increase the risk for endometriosis by influencing the

FIGURE 1



Cross-talk between the ectopic foci and eutopic endometrium. Chronic inflammation induced by the ectopic presence of endometrial fragments (damage-associated molecular patterns [DAMPs]) lead to potential epigenetic modification programming within the eutopic endometrium. Aberrant regulation of gene transcription and post-translational regulatory mechanisms through noncoding RNAs likely contribute to development of progesterone resistance and heightened response to estrogen, which are two key characteristics of the eutopic endometrium of women with endometriosis. In addition, chronic inflammation leads to hypersensitization of nerve fiber endings characterized by enhanced neuronal connectivity in areas of pain perception. lncRNA = long noncoding RNA; miRNA = microRNA.

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expression of *VEZT* transcript and protein levels in the endometrium in a cycle stage– and cell type–specific manner (102). Even though GWASs have identified numerous SNPs associated with endometriosis risk across multiple populations, the strengths of the studies are affected by unstandardized methods of biologic sample collection, storage, and phenotyping of samples across studies, which may invariably lead to heterogeneous interpretations and findings (24).

Without a doubt, the concerted efforts of researchers have demonstrated the molecular differences apparent in the endometrium of endometriosis patients, including aberrant responsiveness to ovarian hormones and consequent display of proliferative phenotype (27) and decrease in endometrial receptivity (103). Currently, the use of DNA methylation pattern of the eutopic endometrium as a minimally invasive diagnostic biomarker for endometriosis seems to be, unfortunately, ineffective and not useful in predicting disease risk (101). For example, despite finding significant methylation differences in CpG islands in the secretory phase of the menstrual cycle when endometrium samples are compared by disease (with or without endometriosis), the methylation patterns between disease and control were not simply robust to be useful as diagnostic biomarkers independently from menstrual cycle (82, 100, 104). The studies, however, were beneficial in revealing the aberrant molecular expression pattern driving the pathophysiology of eutopic endometrium of women with endometriosis, and they provided the groundwork to pursue functional studies that can lead to new therapeutic interventions.

MicroRNA and Long Noncoding RNA

MicroRNA expression in normal endometrium exhibits dynamic changes across the menstrual cycle (105), where their involvement in nonpathologic mechanisms can be used to understand physiologic systems that have derailed into pathologic phenotypes. In endometriosis patients, the miRNA profile of eutopic endometrium and blood may provide useful information in confirming diagnoses of women with different stages of endometriosis. As a transcriptional regulator, it is likely that aberrant expression of miRNA translates to dysregulated gene expression in the eutopic endometrium of patients. Therefore, miRNA profiling of eutopic endometrium in endometriosis patients would provide pathophysiologic molecular fingerprints that we can use to further understand disease pathogenesis and as potential noninvasive biomarkers for endometriosis.

Potential use of circulating miRNA as a noninvasive biomarker for endometriosis is an ongoing area of research, and its therapeutic and diagnostic implications have been extensively reviewed (85, 87, 106, 107). Research groups investigating the functional role of miRNA are elucidating the implication of certain clusters of miRNAs in the pathogenesis of endometriosis, especially in the areas of dysregulation of endometrial function by ovarian hormones, regulation of apoptosis, cell adhesion, and proliferation.

Burney et al., based on their previous transcriptomics analysis (27) and with the use of computational analysis, discovered a potential functional relationship between decreased expression of miR-9 and miR-34 families in early secretory-phase eutopic endometrium of patients and their role in the delayed transition from proliferative to secretory phase in women with severe endometriosis (85). In addition, Joshi et al. recently demonstrated, in a nonhuman primate model of endometriosis, the functional implication of increased expression of miR-29c in endometrial progesterone resistance (108). Similarly, a number of studies have examined potential use of circulating miRNA as diagnostic marker for endometriosis (77, 78). Cho et al. showed that the circulating level of let-7b can be used as a reliable biomarker with 83.3% sensitivity and 100% specificity in diagnosis of endometriosis (109). Furthermore, Jia et al. demonstrated the applicability of the circulating level of miR-17-5p, miR-20a, and miR-22 in discriminating patients with severe endometriosis from control cohorts with an area under the curve value of 0.90 (110), further verifying the potential use of circulating miRNA as a diagnostic marker for endometriosis.

Indeed, there is merit for the circulating and tissue miRNA to be used as noninvasive or minimally invasive biomarkers for endometriosis, especially for severe cases of endometriosis (110). However, one limiting factor hindering the exploration of miRNA as biomarkers in clinical trials is the small sample sizes of patients and lack of representation of all stages of endometriosis, which are also stratified by menstrual stage (85, 109, 110). As cautioned by Hull and Nisenblat, and echoed by the conclusions from Cochrane Database of Systematic Reviews, the interpretation of data can not be hindered by the heterogeneity of study designs, where the results apply only to the cohort of patients and control subjects selected for that study (87). Furthermore, there seems to be a discrepancy between the level of miRNA expression in tissues (111) and in blood (109). Therefore, future investigations exploring miRNA as biomarkers for endometriosis need to account for such discrepancy between different types of biologic samples and for menstrual stage when necessary (107).

Relatively recent additions to the list of biomarkers are lncRNAs, which are a class of molecules with nucleotides >200 in length involved in the regulation of gene expression. Much like the family of miRNAs, lncRNAs are investigated for their involvement in a wide range of biologic processes (112). In the realm of endometriosis, there are very few reports published so far: one investigating tissue specific lncRNA from eutopic endometrium of patients and controls of Chinese descent in late secretory phase (113), and the other investigating the level of circulating lncRNA in serum as well as tissue samples (112). Using genome-wide microarray approach, Wang et al. in 2015 (113) found 1,277 lncRNAs that were dysregulated in the secretory-phase eutopic endometrium of patients compared with the menstrual-phase-matched endometrium from a control cohort. Wang et al. in 2016 (112), using a larger cohort of samples from serum and tissue, identified 1,682 and 1,435 lncRNAs with dysregulated expression in the serum and ectopic endometriotic tissues, respectively, of patients (112). They further identified 1,557 lncRNAs specific to the serum and 1,310 lncRNAs specific to the endometriotic tissues of endometriosis patients (112), demonstrating that the function of lncRNAs in serum may be different from that of lncRNAs found in tissue, echoing a similar pattern found in miRNA expression patterns in serum and tissue. Wang et al. (2016) further analyzed the serum samples accounting for different stages of endometriosis and found the expression level of one lncRNA—ENST00000482343—to be correlated with disease progression, suggesting the potential usefulness of the expression pattern of lncRNA in detecting the severity of disease (112). In addition, Wang et al. (2016) showed that a combination of five lncRNAs can differentiate patients from disease-free control subjects with 89.7% sensitivity and 73.2% specificity, further demonstrating the potential of circulating lncRNA as a noninvasive diagnostic biomarker for endometriosis (112).

Biomarkers That Were Not So Useful: Inflammatory Cytokines

The physiologic process of menstruation is an inflammatory event, as is the pathophysiologic process of endometriosis. Intuitively, with endometriosis being a chronic inflammatory disease, inflammatory cytokines are investigated as potential noninvasive biomarkers in the blood or peritoneal fluid of endometriosis patients. This was thoroughly reviewed by Fassbender et al. (71) and May et al. (69). The studies showed that a panel of cytokines, rather than a single biomarker, improve sensitivity and specificity values, with some panels showing the ability to discriminate between different stages of disease. Mihalyi et al. showed the possibility of diagnosing minimal to mild endometriosis with the use of a combination of cytokines (interleukin [IL] 6, IL-8, tumor necrosis factor [TNF] α, cancer antigen [CA] 125, CA-19-9, and high-sensitivity C-reactive protein) with 60%–71% specificity and 87%–92% sensitivity (114). With the use of a multivariate statistical approach, Vodolazkaia et al. investigated specificity and sensitivity of 28 biomarkers from plasma samples of women with and without endometriosis and identified a panel of biomarkers (vascular endothelial growth factor, annexin V, CA-125, glycodelin, and soluble intracellular adhesion molecule 1) that consistently showed 63%–81% specificity and 81%–90% sensitivity in its ability to diagnose women with ultrasound-negative endometriosis during the menstrual phase (115). Interestingly, though, they found increased levels of proinflammatory markers, including TNF-α, IL-6, and IL-1β, in the control group rather than the endometriosis group in the training data set, remarking on the possibility of nonendometriotic pelvic pathology contributing to the increase in the control group of women. This inappropriate selection of control led to their inability to discriminate endometriosis patients from others with nonendometriosis-related pelvic inflammation and pain (115), effectively demonstrating that proinflammatory cytokines may not be suitable candidates for a noninvasive biomarker for endometriosis, unless proper control subjects can be established.

CONCLUSION

Endometriosis is a complex gynecologic disease. For a disease with such high prevalence in women of reproductive age and with ongoing research to uncover its enigmatic etiology,

improvement in the areas of diagnosis and therapeutics has been lacking. In recent years, however, researchers have made significant strides in understanding the disease-specific molecular pathways governing the development of endometriosis in ectopic locations by studying the blood, peritoneal fluid, and eutopic endometrium of women with the disease. Of particular relevance is the significance of the fact that the initial presence of the ectopic foci can influence the gene expression pattern of the eutopic endometrium with disease progression (97). This shows that endometriosis is indeed a chronic inflammatory disease wherein the sterile inflammation induced by the cyclic deposition of menstrual fragments can lead to pathogenic epigenetic changes in the eutopic endometrium. Studying epigenetic modification of endometrial fragments influenced by the inflammatory and immune system, as well as investigating the cross-talk established by the lesion and the eutopic endometrium in future investigations will propel our understanding of pathophysiology of endometriosis, with the potential for new therapeutic and diagnostic interventions.

Along with the need to better understand the pathophysiology of endometriosis, the need for a diagnostic biomarker for endometriosis has been equally recognized. Owing to an increase in incidence of early menarche in young girls displaying endometriosis-related symptoms at an earlier age (12), the need to identify this cohort by means of a reliable diagnostic marker is imperative. It is more likely that instead of a single biomarker, a group of biomarkers will provide improved diagnostic performance and minimize false positives and negatives during differential diagnosis. With the advent in the field of GWAS and improved understanding between the functional significance of SNPs and genetic variants associated with endometriosis risk, it may be feasible to identify a panel of diagnostic biomarkers composed of endometrial methylome and expression patterns of circulating noncoding RNA (i.e., miRNA and lncRNA), or even from endometrial biopsies with reasonable specificity and sensitivity. Per the guidelines published by the World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project, we need concerted efforts from both clinicians and researchers toward standardization of patient information and biologic sample collection and storage across research centers (38, 39). Once achieved, we can undoubtedly discover reliable diagnostic biomarkers for young adolescents and women alike, offering them with the chance to live lives free from pain.

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