

Review

The Role of Long Noncoding RNAs in Endometriosis Progression

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Abstract

Endometriosis (EMs) is a common gynecological disease with an increasing incidence in recent years. Because of the lack of specific molecular biological indicators in clinical practice, diagnosis is often delayed and the quality of life of patients is seriously reduced. Therefore, the discovery of effective molecular biomarkers is crucial for the early diagnosis and treatment of EMs patients. With the development of high-throughput sequencing technology, the mechanism of lncRNAs in EMs has been increasingly confirmed experimentally. This article summarizes the biological characteristics and functions of EMs-related lncRNAs, and introduces the mechanisms of EMs-related lncRNAs in the context of ceRNAs, in exosomes, under hypoxic conditions, and related antisense RNAs. The mechanism of the most popular imprinted gene *H19* and metastasis-associated lung adenocarcinoma transcript 1 in EMs is then introduced. Finally, we explore the challenges of molecular biomarker EMs-related lncRNAs in the diagnosis and treatment of EMs, anticipating their potential value in clinical applications.

Keywords: endometriosis; long noncoding RNA; gene regulatory network; competing endogenous RNA

1. Introduction

Endometriosis (EMs) refers to the condition caused by the presence of endometrioid-like tissue with growth function outside the uterus. In recent years, the incidence has gradually increased, especially among young women [1], with approximately 10% of women of childbearing age suffering from EMs [2]. While its pathogenesis is still unclear, current hypotheses include retrograde menstruation, coelom metaplasia, and lymphatic and vascular metastasis [3]. Due to the high heterogeneity of EMs symptoms, diagnostic accuracy remains unsatisfactory and is only based on symptoms such as dysmenorrhea, chronic pelvic pain, and dyspareunia combined with biochemical and imaging examinations [4]; thus, the gold standard for diagnosis remains laparoscopy. However, due to its invasiveness and cost, EMs leads to a delay of 4–11 years from the first symptom onset to the definitive surgical diagnosis [5]. Studies have shown that a variety of long noncoding RNAs (lncRNAs) play important regulatory roles in the occurrence and development of EMs. Therefore, through early diagnosis and postoperative follow-up monitoring, certain biomarkers that can be used to detect EMs could improve diagnostic efficiency and help avoid treatment delays. Numerous studies on the correlation between lncRNAs and EMs have recently been carried out, providing new ideas for exploring the diagnosis of EMs. The research progress of lncRNAs in the etiology, diagnosis and treatment of EMs was reviewed in this article.

2. Biological Characteristics and Functions of lncRNAs

lncRNAs are functional RNA molecules longer than 200 nucleotides that cannot translate proteins. lncRNAs are transcribed by RNA polymerase II [6]. Unlike mRNA, lncRNAs can be transcribed and folded into thermodynamically stable secondary structures to bind to RNA, DNA or proteins respectively to regulate gene expression and protein synthesis, the basis for the important role of lncRNAs in biological activities such as epigenetic regulation [7]. At present, the GENCODE project has annotated more than 16,000 human lncRNAs, while other studies indicate that the total number may exceed 100,000. lncRNA can play its role in the nucleus or cytoplasm through a variety of mechanisms. In the nucleus, lncRNAs may target gene promoters by recruiting chromatin remodeling or modifying complexes, thus acting as epigenetic gene regulators. In other cases, lncRNAs may act as transcription regulators by competing with transcription factors to bind DNA and/or by binding to their DNA-binding domains. In the cytoplasm, lncRNAs act as sponges for related miRNAs to regulate translation or as scaffolds to facilitate interactions of related proteins, thus affecting the stability of transcripts [8]. Furthermore, lncRNAs can participate in the regulation of cellular signal pathways by binding to signal proteins and affecting their activation status. In research experiments, quantitative reverse transcription polymerase chain reaction (qRT-PCR) and other methods can be used to verify the expression of lncRNAs in EMs, and techniques



such as RNA interference (siRNA) can be used to study the biological effects of lncRNAs in endometrial stromal cells cultured *in vitro* [9].

With the development of high-throughput sequencing technology, more than 173,112 annotated lncRNAs have been confirmed to be transcribed from 96,411 genomic loci [10], while the action of lncRNAs in EMs is increasingly experimentally confirmed. Abnormal expression of lncRNAs in disease can lead to symptoms of EMs or infertility by affecting the proliferation, invasion, metastasis or epithelial-mesenchymal transition (EMT) of endometrial stromal cells (ESCs). These are closely related to the development of diseases such as EMs [11–13]. This article describes a key imprinted gene, *H19*, the metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*), and the urothelial carcinoma associated gene 1 (*UCA1*), three EM-associated lncRNAs. Furthermore, we also review the mechanism of EM-related lncRNAs in the context of ceRNAs, in exosomes, as antisense RNAs, and under hypoxia.

3. Mechanisms of LncRNAs in EMs

3.1 Mechanisms of EMs-Related lncRNAs in the Context of ceRNAs

Competing endogenous RNAs (ceRNAs) are transcripts that are mutually cross-regulated by competing shared microRNAs (miRNAs). A miRNA is a short-chain RNA approximately 22 nt in length that can lead to gene silencing by binding to mRNAs, while ceRNAs can competitively bind miRNA and act as a miRNA sponge in cells, thereby releasing target genes from its miRNA inhibition and increasing the expression level of target genes. This mechanism of action is called the ceRNA mechanism [14]. With the development of high-throughput sequencing, the regulatory mechanism of EMs-related lncRNAs in the context of ceRNAs has been proven by an increasing number of studies. Bai *et al.* [15] showed that a total of 4213 mRNAs, 1474 lncRNAs and 221 miRNAs were differentially expressed in ectopic endometrial (EC) and eutopic endometrial (EU) samples. By constructing a ceRNA network for differentially expressed RNAs and performing bioinformatics analysis, it was found that lncRNAs *H19*, *GS1-358P8.4* and *RP11-96D1.10* were closely related to EMs. Jiang *et al.* [16] constructed a ceRNA network based on differential miRNAs (DEmiRs), differential lncRNAs (DELs), and differential genes (DEGs), including 11 up-regulated and 16 down-regulated DEmiRs, 7 up- and 13 down-regulated DELs, and 48 upregulated and 46 down-regulated DEGs, and confirmed in validation analysis that this ceRNA network may mediate inflammatory responses through *LINC01018* and *SMIM25* as miR-182-5p sponges, thereby promoting the development of EMs. Wang *et al.* [17] identified a ceRNA network associated with EMs, including 45 pathways and 4 ceRNAs, demonstrating that differentially expressed RNAs and lower expression levels of

B-type progesterone receptors can reduce endometrial receptivity through a ceRNA mechanism, further leading to infertility.

3.2 Mechanisms of EMs-Related LncRNAs in Exosomes

Exosomes refer to nanoscale vesicles that can be secreted by various types of cells. As an important mediator of intercellular communication, they play an important regulatory role in tumor growth and migration [18]. Wu *et al.* [19] identified a total of 938 lncRNAs, 39 miRNAs, and 1449 mRNAs with overlapping differential expression in their study of EMs exosomal RNA and lncRNA-related networks. A total of 13 co-expression modules and 61 ceRNA networks were constructed, revealing a new molecular mechanism of lncRNA-mediated pathogenesis of EMs. The expression of exosomal lncRNA *CHL1-AS1* in peritoneal macrophages is upregulated. After being transported from peritoneal macrophages to ectopic ESCs, it acts as an endogenous competing RNA for miR-610, downregulating miR-610 and upregulating *MDM2*, thereby promoting the proliferation and migration of ESCs and inhibiting their apoptosis [20]. Antisense hypoxia-inducible factor (*aHIF*) is involved in the regulation of angiogenesis. Qiu *et al.* [21] found that *aHIF* was highly expressed in the ectopic endometrium and serum exosomes of patients with EMs, and the two expression levels were significantly correlated. Experiments have confirmed that *aHIF* is transferred from endometriotic cyst stromal cells to human umbilical vein endothelial cells through exosomes in an ectopic environment. It induces human umbilical vein endothelial cells to promote angiogenesis by activating vascular endothelial growth factors (VEGFs).

3.3 Mechanism of EMs-Related Antisense LncRNAs

Antisense lncRNA refers to a type of lncRNA that is transcribed from the antisense strand of a protein-coding gene and usually has sequence overlap with the mRNA of the gene. Studies have confirmed that antisense RNA can widely participate in the regulation of protein-coding gene expression through a “variety of mechanisms” [22]. This regulation can occur at the transcriptional, post-transcriptional and translational levels of protein-coding genes [23,24]. For example, antisense RNAs co-expressed at the transcriptional level can bind to their sense transcripts to form RNA hybrids or double-stranded structures that prevent the transcription of the sense transcript [25]. Regulation of gene expression by antisense RNAs can also occur in epigenetics. Antisense RNAs act as molecular scaffolds for protein-protein interactions or bind to chromatin-modifying complexes and can activate or repress target gene expression by mediating chromatin remodeling and recruiting enzymes involved in methylation and histone modification [23].

The abnormal expression of lncRNA actin filament-associated protein 1 antisense RNA1 (*AFAP1-AS1*) is

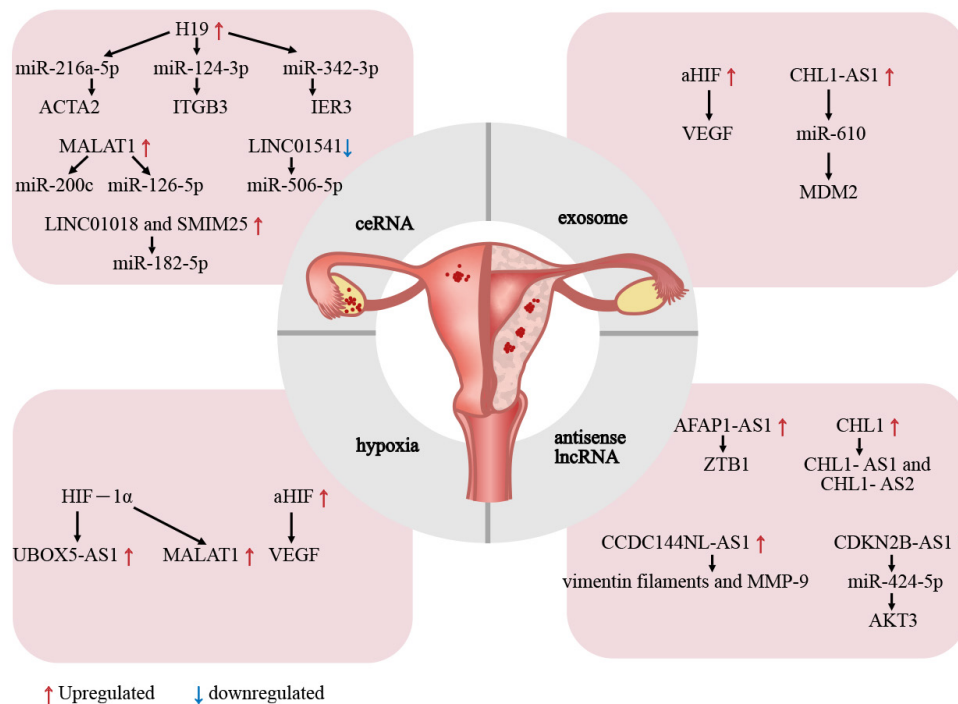


Fig. 1. The key mechanism of lncRNAs in EMs. lncRNAs can be transported through exosomes to promote cell growth and migration. Furthermore, lncRNAs can be used as miRNA sponges and antisense RNAs to promote the invasion and metastasis of ESCs and EMT. In addition, a hypoxic environment also induces the occurrence and development of EMs.

closely related to the occurrence and development of various tumors [26]. Lin *et al.* [27] found that lncRNA *AFAP1-AS1* was highly expressed in EC. lncRNA *AFAP1-AS1* attenuated the inhibition of the e2-induced transcription factor *ZBT1* promoter site pGL3-P886, thereby attenuating the inhibition of endometrial epithelial cell growth and leading to the occurrence of EMs by promoting EMT. lncRNA *CCDC144NL-AS1* is an antisense lncRNA located on human chromosome 17. The expression of *CCDC144NL-AS1* in EC tissues was higher than that in EU and normal endometrial (NE) tissues. Zhang *et al.* [28] found that elevated expression of lncRNA *CCDC144NL-AS1* may promote the migration and invasion of ESCs by regulating f-actin and vimentin, thereby promoting the development of EMs. lncRNA *CDKN2B-AS1* plays a key role in the occurrence and development of various cancers and nonmalignant diseases [29]. Wang *et al.* [30] found that the relative expression of *CDKN2B-AS1* was upregulated in both EC and EU. *CDKN2B-AS1* can act as a ceRNA that absorbs miR-424-5p and targets *AKT3* through sponging to affect cell proliferation, invasion and *AKT3* expression, which may be a potential target for EMs therapy. Close homolog of L1 (*CHL1*) is a member of the neuronal cell adhesion molecule (*NCAM*) L1 gene family and belongs to the immunoglobulin superfamily. Zhang *et al.* [31] confirmed that the expression level of *CHL1* in EC tissues is higher than that in EU tissues. They also found a significant correlation between *CHL1* and its antisense RNAs *CHL1-AS1*

and *CHL1-AS2*, which may be involved in the occurrence and development of EMs and may provide new targets that will be helpful for future research.

3.4 Mechanisms of EMs-Related lncRNAs under Hypoxia

Hypoxia is one of the characteristics of EMs. Hypoxic conditions can promote EMT and endometrial epithelial cell invasion through various mechanisms [32]. Liu *et al.* [33] confirmed that *HIF-1α* can induce upregulation of the expression of lncRNA *UBOX* antisense RNA 1 (*UBOX5-AS1*), promote EMT, and lead to the occurrence of EMs. Additionally, under the regulation of *HIF-1α*, the increased expression of *MALAT1* under hypoxia can promote cell migration and invasion by mediating pro-survival autophagy in ESCs, resulting in the occurrence of EMs [34]. *AHIF* is involved in the regulation of angiogenesis. Hypoxia induces an increase in the expression of *aHIF* in EC tissues, which is transferred to human umbilical vein endothelial cells through exosomes and then promotes angiogenesis by activating vascular endothelial growth factor and other factors, thereby promoting the occurrence of EMs [21]. The main mechanisms by which lncRNAs promote EMs are summarized in Fig. 1.

4. Abnormal Expressions of lncRNAs in EMs

In view of the mechanism of lncRNA in EMs, its abnormal expression is often associated with the pathogenesis

Table 1. Altered expression of lncRNAs in EMs.

LncRNA name	Upregulated/downregulated	Whether detected in body fluid	References
<i>SNHG4</i>	Upregulated	No	[35]
<i>aHIF</i>	Upregulated	No	[27]
<i>MALAT1</i>	Both	Yes	[27]
<i>CCDC144NL-AS1</i>	Upregulated	No	[31]
<i>LINC00261</i>	Downregulated	No	[36]
<i>CDKN2B-AS1</i>	Upregulated	No	[27]
<i>LINC01116</i>	Upregulated	No	[37]
<i>LINC01541</i>	Downregulated	No	[35]
<i>HOXA11-AS1</i>	Upregulated	No	[35]
<i>AFAP1-AS1</i>	Upregulated	No	[35]
<i>AC002454.1</i>	Upregulated	No	[35]
<i>TC0101441</i>	Upregulated	No	[35]
<i>CHL1-AS2</i>	Upregulated	No	[35]
<i>LINC01133</i>	Upregulated	No	[35]
<i>UCA1</i>	Both	Yes	[35]
<i>H19</i>	Both	Yes	[38]
<i>FTX</i>	Downregulated	No	[39]

of EMs. Recently, many studies have used whole genome high-throughput technology to identify the differentially expressed lncRNA in EMs (Table 1, Ref. [27,31,35–39]), which is not only helpful for its potential clinical application as a biomarker for disease diagnosis or prognosis, but also helpful for in-depth understanding of the pathogenesis of the disease. Among these differentially expressed lncRNAs, *H19*, *MALAT1* and *UCA1* have attracted much attention due to their roles in numerous aspects, such as EMs cell survival, angiogenesis, cell invasion, autophagy, oxidative stress, and endometrial receptivity.

4.1 Imprinted Gene *H19*

H19 is the first gene found to be associated with genetic imprinting, and its encoded lncRNA is one of the most abundant and conserved transcripts in mammalian development. The full-length gene is 2.5 kb and is located on human chromosome 11P15.5. The *H19* gene is highly active in various tissues during embryonic development, but its physiological function is unclear [40]. As an imprinted gene is usually expressed in the maternal allele, the *IGF2* gene paired with it is expressed only in the paternal line. However, in some cancers, this imprinting pattern is dysregulated, resulting in abnormal upregulation of *H19* in malignant tumor tissues; thus, *H19* is considered a cancer biomarker and a recognized therapeutic target [41]. Although EMs are considered benign inflammatory lesions, local invasion and resistance to apoptosis have tumor-like characteristics. Recent studies have shown that *H19* is involved in the pathogenesis of EMs, especially the recurrence mechanism, and is related to infertility, bilateral ovarian lesions, and elevated CA125 levels [38,42].

Studies have shown that *H19* expression is significantly reduced in the eutopic endometrium (EU) of women

with EMs compared with normal controls. The reduction in *H19* increases the activity of let-7, which in turn inhibits the expression of *Igf1r* at the post-transcriptional level, thereby reducing the proliferation of ESCs, which may cause symptoms such as infertility [11]. *H19* can also regulate the expression of *ACTA2* by competitively inhibiting the miR-216a-5p binding site, thereby promoting the invasion and migration of eutopic ESCs in EMs. Downregulation of *H19* or *ACTA2* inhibits eutopic ESCs invasion and migration [12]. The expression of *H19* was significantly elevated in ECs of women with EMs compared with normal controls. Elevation of *H19* can promote the proliferation and invasion of ESCs cells by regulating the downstream effector proteins miR-124-3p and *ITGB3*, and knockdown of *H19* can inhibit ESCs proliferation and invasion [43]. Elevated *H19* expression can also inhibit the proliferation of ESCs by regulating the expression of miR-342-3p and *IER3*, reducing the level of IL-17 and the percentage of Th17 cells/CD4⁺ T cells [44]. In the nude mouse EMs model, subcutaneous lesions and the *H19* levels in the lesions of *H19* knockout mice were higher than those of the negative control group. Furthermore, knocking out the *H19* gene suppressed EMs [45].

4.2 Metastasis-Associated Lung Adenocarcinoma Transcript 1

MALAT1 was initially found to be highly expressed in primary human non-small cell lung cancer [46]. Subsequent studies have shown that it is also highly expressed in many other cancers and can be involved in a variety of physiological processes such as epigenetic modification. *MALAT1* dysregulation can lead to abnormal cell proliferation, invasion and migration by affecting EMT, apoptosis and autophagy [13]. Liang et al. [47] found that

MALAT1 was upregulated in EC specimens and inhibited exogenous overexpression of miR-200c, thereby promoting the proliferation and migration of ESCs. Some studies have also found that *MALAT1* can also regulate the expression of MMP-9 through the NF-kappaB/INOS pathway [48] and the PI3K/AKT pathway mediated by the miR-126-5p/CREB1 axis [49] to regulate the apoptosis of ESCs, thereby mediating EMs onset. *MALAT1* can also participate in the survival autophagy of ESCs regulated by *HIF-1 α* under hypoxia conditions [34]. EMs can impair fertility and lead to infertility due to dysfunction of EMs granulosa cells (GCs). *MALAT1* is downregulated in GCs of patients with EMs. Via the ERK/MAPK pathway, *MALAT1* can regulate the proliferation of GCs by P21/p53-dependent cell cycle control, thereby inhibiting the normal growth and development of oocytes and affecting fertility [50]. Studies show that the expression of *MALAT1* is upregulated in GCs of EMs patients, and the decreased expression of 5'-AMP-activated protein kinase in patients mediated by the AMPK-MTOR pathway leads to increased cell proliferation and viability and decreased autophagy of GCs [51]. The differential expression of *MALAT1* in GCs of patients with EMs may be due to demographic differences or the effects of drugs used for surgical resection. Therefore, the mechanism of action of *MALAT1* in EMs needs to be further studied. However, it was found that *MALAT1* plays an important role in promoting EMs, which provides the possibility for its clinical application as a target in EMs.

4.3 Urothelial Carcinoma Associated Gene 1 (*UCA1*)

UCA1 is associated with the occurrence of various ovarian diseases [52,53]. *UCA1* is highly expressed in EMs patients. Increased *UCA1* expression can promote the proliferation of ESCs and inhibit autophagy and apoptosis. Knockout of *UCA1* *in vitro* can significantly inhibit the proliferation of ESCs and induce autophagy and apoptosis [9]. Zhang Yue *et al.* [54] showed that the expression of *UCA1* in the EU, proliferative endometrium, and secretory endometrium of patients with EMs was significantly higher than that in the normal control group during the same period. However, there was no significant difference in the expression of *UCA1* in the proliferative endometrium and the secretory endometrium between the two groups. It has been proven that the high expression of the *UCA1* gene in the eutopic endometrium may be involved in the occurrence and development of EMs and that the expression level is not affected by changes in the menstrual cycle [54]. Studies also found that EMs patients with higher *UCA1* are more likely to be infertile, and elevated *UCA1* may lead to infertility by affecting fatty acid metabolism in the reproductive system [55]. However, compared with the healthy controls, the level of serum levels of *UCA1* in EMs patients decreased, and the level of serum levels of *UCA1* increased after treatment. The recurrence rate was higher when the serum levels of *UCA1* was significantly lower on the day

of discharge [56].

4.4 Others

Long noncoding RNA five prime to Xist (lncRNA FTX) is considered a key lncRNA for normal uterine development as well as a key lncRNA for regulating cancer cell proliferation, migration and apoptosis [57–59]. The lower expression of lncRNA FTX in endometrial tissue and ESCs of patients with EMs leads to higher activity of the PI3K/AKT signaling pathway, which promotes the invasion, metastasis and EMT of ESCs [60].

lncRNA maternally expressed gene 3 (*MEG3*) is a tumor suppressor that is downregulated in various cancer cells and tissues and regulates various biological processes [61,62]. *MEG3-210* is lower in the endometrium of patients with EMs. Downregulation of *MEG3-210* promotes ESC migration, invasion and inhibition of apoptosis *in vitro* by interacting with Galectin-1 via the p38 MAPK and PKA/SERCA signaling pathways. This new regulatory mechanism may provide a novel target for EM drug therapy [63].

HOX transcript antisense RNA (*HOTAIR*) is involved in a variety of cell signal transduction pathways and is closely related to the occurrence and development of malignant tumors. Chang *et al.* [64] found that the functional axis of *HOTAIR/HOXD10* and *HOTAIR/HOXA5* may play an important role in regulating the progression of EMs. In ECs, *HOTAIR* mRNA levels were higher, and the downstream targets *HOXD10* and *HOXA5* were lower. Low expression of *HOXD10* and *HOXA5* reduces the negative regulation of cell proliferation, migration, and angiogenesis, which promotes lesion growth and spread and can lead to infertility. *HOTAIR* plays a prominent role in promoting EMs and can serve as a potential clinical target for the treatment of EMs.

A study by Mai *et al.* [65] showed that long intergenic nonprotein coding RNA 1541 (LINC01541) could mediate the occurrence of EMs by regulating the Wnt/ β -catenin pathway to inhibit the EMT process, ESC metastasis and VEGFA expression. Under normal circumstances, LINC01541 as a ceRNA can reduce its bioavailability by competitively binding to miR-506-5p. In ectopic tissues, the expression of LINC01541 was decreased, and the highly expressed miR-506-5p activated the Wnt/ β -catenin pathway by inhibiting the expression of WIF1, thereby inducing the proliferation, migration and invasion of ESCs and promoting EMT and the expression of VEGFA of ESCs [66].

Wang *et al.* [67] and Liu *et al.* [68] found that cyclin-dependent kinase-6 (CDK6) and AC002454.1 were highly expressed in both ectopic and eutopic endometrial tissues of patients with EMs. *In vitro* experiments proved that AC002454.1 may enhance the proliferation, migration and invasion of endometrial *in situ* cells by regulating the expression of CDK6, promoting the transformation of cells from G0/G1 phase to S phase. By inducing cell cycle dis-

order, it causes abnormal proliferation of endometrial tissue and promotes the development of EMs.

5. Challenges in Clinical Application

By September 2020, 11 kinds of RNA therapy products, including antisense oligonucleotide drugs and siRNA drugs, have been approved for marketing, indicating that RNA therapy has started clinical application. Many lncRNAs participate in gene transcription inhibition by acting as promoter related RNA or natural antisense transcripts, resulting in abnormal gene expression, which leads to diseases. Numerous studies have confirmed the important role of many lncRNAs in the pathogenesis of a variety of human diseases, including EMs. Developments in RNA therapy have raised the possibility of curing disease by targeting lncRNAs to inhibit abnormal gene expression. As of April 2022, 13 RNA therapeutic products have been approved for marketing, indicating that RNA therapies are beginning to be used clinically [39]. Four classes of drugs can be classified by mechanism of action: antisense oligonucleotides, small interfering RNAs, RNA aptamers, and messenger RNAs. Studies have shown that antisense oligonucleotides (ASOs) or siRNA therapy can reverse the negative regulation of lncRNAs on genes by targeting lncRNAs involved in gene transcriptional repression, thereby leading to transcriptional activation or unsilencing and inhibiting the occurrence and development of diseases [69]. This therapy has been applied to spinal muscular atrophy, acute hepatic porphyria and other rare diseases, cardiovascular diseases, and neurological diseases, but its application in EMs is still in the theoretical stage.

At the same time, there remain several technical bottlenecks in the research and development of RNA therapy. For example, the unstable degradation of RNA *in vivo* can easily lead to drug failure and even produce potential side effects and toxicity. Certain exogenous RNAs may modulate multiple mRNAs *in vivo*, resulting in off-target side effects and abnormal immune responses. Therefore, research institutions engaged in RNA therapy are currently limited, while research and development motivation is insufficient. If this therapy is to be used clinically on a large scale, more thorough research and large-scale cohort trials are needed [70]. However, the existing research findings of relevant biomarkers provide the possibility of improving the diagnostic efficiency and recurrence prediction of EMs, which is crucial.

6. Outlook

Changes in the expression of lncRNAs in EMs are involved in the regulation of numerous processes known to be related to the pathogenesis of the disease. These processes include angiogenesis, EMT, endometrial cell survival, endometrial cell desiccation, lesion establishment and growth, oxidative stress, proliferation and invasion, endometrial receptivity, and autophagy. The pathogenesis of

EMs is highly complex, while the clinical use of lncRNA biomarkers as indicators for early diagnosis and postoperative follow-up monitoring still faces great difficulties and challenges. The important regulatory role of lncRNAs in the occurrence and development of EMs is confirmed by an increasing number of studies, but most of the current experiments are only conducted *in vitro*, and there are few related studies *in vivo*. At the same time, most studies only point out that a variety of lncRNAs are highly correlated with EMs, but convenient and efficient detection methods in clinical practice are lacking. Therefore, more experimental studies are needed for clinical application. However, the proposal of multiple potential therapeutic targets provides the possibility of early diagnosis and targeted therapy of EMs for clinicians in the future, which is of great significance in improving the efficiency of diagnosis and the clinical prognosis of EMs.

Abbreviations

EMs, Endometriosis; lncRNAs, long noncoding RNAs; qRT-PCR, quantitative reverse transcription polymerase chain reaction; EMT, epithelial to mesenchymal transition; ESCs, endometrial stromal cells; *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; ceRNAs, competing endogenous RNAs; miRNAs, microRNAs; EC, ectopic endometrial/ectopic endometrium; EU, eutopic endometrial/eutopic endometrium; DE miRs, differential miRNAs; DELs, differential lncRNAs; DEGs, differential genes; *aHIF*, antisense hypoxia-inducible factor; VEGFs, vascular endothelial growth factors; *AFAP1-AS1*, actin filament-associated protein 1 antisense RNA1; NE, normal endometrial; *CHL1*, close homolog of L1; *NCAM*, neuronal cell adhesion molecule; *UBOX5-AS1*, *UBOX* antisense RNA 1; GCs, granulosa cells; lncRNA FTX, long noncoding RNA five prime to Xist; *UCA1*, urothelial carcinoma associated gene 1; *MEG3*, maternally expressed gene 3; *HOTAIR*, HOX transcript antisense RNA; LINC01541, long intergenic nonprotein coding RNA 1541; CDK6, cyclin-dependent kinase-6.

Author Contributions

YL designed, wrote and revised the manuscript, and WZ was a major contributor in writing the manuscript and prepared the figure. LJ and JR participated in the writing and improvement of the manuscript. DL and ZL made significant revisions and proofread the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Kim H, Lee M, Hwang H, Chung Y, Cho H, Yoon H, *et al.* The Estimated Prevalence and Incidence of Endometriosis With the Korean National Health Insurance Service-National Sample Cohort (NHIS-NSC): A National Population-Based Study. *Journal of Epidemiology*. 2021; 31: 593–600.
- [2] Zondervan KT, Becker CM, Missmer SA. Endometriosis. *The New England Journal of Medicine*. 2020; 382: 1244–1256.
- [3] Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nature Reviews. Endocrinology*. 2014; 10: 261–275.
- [4] Cai X, He ZQ, Zhang GM. Research Status of Long Non-Coding RNA Expression Patterns in Endometriosis. *Journal of International Obstetrics and Gynecology*. 2020; 47: 684–688. (In Chinese)
- [5] Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, *et al.* Clinical diagnosis of endometriosis: a call to action. *American Journal of Obstetrics and Gynecology*. 2019; 220: 354.e1–354.e12.
- [6] Aliperti V, Skonieczna J, Cerase A. Long Non-Coding RNA (lncRNA) Roles in Cell Biology, Neurodevelopment and Neurological Disorders. *Non-Coding RNA*. 2021; 7: 36.
- [7] Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. *Nature Structural & Molecular Biology*. 2013; 20: 300–307.
- [8] Yao R, Wang Y, Chen L. Cellular functions of long noncoding RNAs. *Nature Cell Biology*. 2019; 21: 542–551.
- [9] Jiang L, Wan Y, Feng Z, Liu D, Ouyang L, Li Y, *et al.* Long Noncoding RNA UCA1 Is Related to Autophagy and Apoptosis in Endometrial Stromal Cells. *Frontiers in Oncology*. 2021; 10: 618472.
- [10] Zhao L, Wang J, Li Y, Song T, Wu Y, Fang S, *et al.* NONCODEV6: an updated database dedicated to long non-coding RNA annotation in both animals and plants. *Nucleic Acids Research*. 2021; 49: D165–D171.
- [11] Ghazal S, McKinnon B, Zhou J, Mueller M, Men Y, Yang L, *et al.* H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis. *EMBO Molecular Medicine*. 2015; 7: 996–1003.
- [12] Xu Z, Zhang L, Yu Q, Zhang Y, Yan L, Chen Z. The estrogen-regulated lncRNA H19/miR-216a-5p axis alters stromal cell invasion and migration via ACTA2 in endometriosis. *Molecular Human Reproduction*. 2019; 25: 550–561.
- [13] Fu S, Wang Y, Li H, Chen L, Liu Q. Regulatory Networks of lncRNA MALAT-1 in Cancer. *Cancer Management and Research*. 2020; 12: 10181–10198.
- [14] Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. *Nature*. 2014; 505: 344–352.
- [15] Bai J, Wang B, Wang T, Ren W. Identification of Functional lncRNAs Associated With Ovarian Endometriosis Based on a ceRNA Network. *Frontiers in Genetics*. 2021; 12: 534054.
- [16] Jiang L, Zhang M, Wang S, Xiao Y, Wu J, Zhou Y, *et al.* LINC01018 and SMIM25 sponged miR-182-5p in endometriosis revealed by the ceRNA network construction. *International Journal of Immunopathology and Pharmacology*. 2020; 34: 2058738420976309.
- [17] Wang X, Yu Q. Endometriosis-related ceRNA network to identify predictive biomarkers of endometrial receptivity. *Epigenomics*. 2019; 11: 147–167.
- [18] EL Andaloussi S, Mäger I, Breakefield XO, Wood MJA. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nature Reviews. Drug Discovery*. 2013; 12: 347–357.
- [19] Wu J, Huang H, Huang W, Wang L, Xia X, Fang X. Analysis of exosomal lncRNA, miRNA and mRNA expression profiles and ceRNA network construction in endometriosis. *Epigenomics*. 2020; 12: 1193–1213.
- [20] Liu T, Liu M, Zheng C, Zhang D, Li M, Zhang L. Exosomal lncRNA CHL1-AS1 Derived from Peritoneal Macrophages Promotes the Progression of Endometriosis via the miR-610/MDM2 Axis. *International Journal of Nanomedicine*. 2021; 16: 5451–5464.
- [21] Qiu J, Lin X, Zheng T, Tang X, Zhang Y, Hua K. The Exosomal Long Noncoding RNA aHIF is Upregulated in Serum From Patients With Endometriosis and Promotes Angiogenesis in Endometriosis. *Reproductive Sciences*. 2019; 26: 1590–1602.
- [22] He Y, Vogelstein B, Velculescu VE, Papadopoulos N, Kinzler KW. The antisense transcriptomes of human cells. *Science*. 2008; 322: 1855–1857.
- [23] Najafi S, Tan SC, Raee P, Rahmati Y, Asemani Y, Lee EHC, *et al.* Gene regulation by antisense transcription: A focus on neurological and cancer diseases. *Biomedicine & Pharmacotherapy*. 2022; 145: 112265.
- [24] Goodrich JA, Kugel JF. Non-coding-RNA regulators of RNA polymerase II transcription. *Nature Reviews. Molecular Cell Biology*. 2006; 7: 612–616.
- [25] Cesareni G, Helmer-Citterich M, Castagnoli L. Control of ColE1 plasmid replication by antisense RNA. *Trends in Genetics*. 1991; 7: 230–235.
- [26] Zhang F, Li J, Xiao H, Zou Y, Liu Y, Huang W. AFAP1-AS1: A novel oncogenic long non-coding RNA in human cancers. *Cell Proliferation*. 2018; 51: e12397.
- [27] Lin D, Huang Q, Wu R, Dai S, Huang Z, Ren L, *et al.* Long non-coding RNA AFAP1-AS1 promoting epithelial-mesenchymal transition of endometriosis is correlated with transcription factor ZEB1. *American Journal of Reproductive Immunology*. 2019; 81: e13074.
- [28] Zhang C, Wu W, Zhu H, Yu X, Zhang Y, Ye X, *et al.* Knockdown of long noncoding RNA CCDC144NL-AS1 attenuates migration and invasion phenotypes in endometrial stromal cells from endometriosis. *Biology of Reproduction*. 2019; 100: 939–949.
- [29] Song C, Qi Y, Zhang J, Guo C, Yuan C. CDKN2B-AS1: An Indispensable Long Non-coding RNA in Multiple Diseases. *Current Pharmaceutical Design*. 2020; 26: 5335–5346.
- [30] Wang S, Yi M, Zhang X, Zhang T, Jiang L, Cao L, *et al.* Effects of CDKN2B-AS1 on cellular proliferation, invasion and AKT3 expression are attenuated by miR-424-5p in a model of ovarian endometriosis. *Reproductive Biomedicine Online*. 2021; 42: 1057–1066.
- [31] Zhang C, Wu W, Ye X, Ma R, Luo J, Zhu H, *et al.* Aberrant expression of CHL1 gene and long non-coding RNA CHL1-AS1, CHL1-AS2 in ovarian endometriosis. *European Journal of*

- Obstetrics, Gynecology, and Reproductive Biology. 2019; 236: 177–182.
- [32] Li Y, Lv X, Jiang M, Jin Z. Sitagliptin ameliorates hypoxia-induced damages in endometrial stromal cells: an implication in endometriosis. *Bioengineered*. 2022; 13: 800–809.
 - [33] Liu H, He H, Zhang Z, Wang L, Zhang L, Liu Y, *et al*. Upregulation of the long noncoding RNA UBOX5 antisense RNA 1 (UBOX5-AS1) under hypoxic conditions promotes epithelial-mesenchymal transition in endometriosis. *Annals of Translational Medicine*. 2021; 9: 790.
 - [34] Liu H, Zhang Z, Xiong W, Zhang L, Du Y, Liu Y, *et al*. Long non-coding RNA MALAT1 mediates hypoxia-induced pro-survival autophagy of endometrial stromal cells in endometriosis. *Journal of Cellular and Molecular Medicine*. 2019; 23: 439–452.
 - [35] Sun P, Jia S, Lin H, Leng J, Lang J. Genome-wide profiling of long noncoding ribonucleic acid expression patterns in ovarian endometriosis by microarray. *Fertility and Sterility*. 2014; 101: 1038–1046.e7.
 - [36] Bi J, Wang D, Cui L, Yang Q. RNA sequencing-based long non-coding RNA analysis and immunoassay in ovarian endometriosis. *American Journal of Reproductive Immunology*. 2021; 85: e13359.
 - [37] Cui L, Chen S, Wang D, Yang Q. LINC01116 promotes proliferation and migration of endometrial stromal cells by targeting FOXP1 via sponging miR-9-5p in endometriosis. *Journal of Cellular and Molecular Medicine*. 2021; 25: 2000–2012.
 - [38] Liu S, Xin W, Tang X, Qiu J, Zhang Y, Hua K. LncRNA H19 Overexpression in Endometriosis and its Utility as a Novel Biomarker for Predicting Recurrence. *Reproductive Sciences*. 2020; 27: 1687–1697.
 - [39] Kim Y. RNA therapy: rich history, various applications and unlimited future prospects. *Experimental & Molecular Medicine*. 2022; 54: 455–465.
 - [40] Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, *et al*. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nature Cell Biology*. 2012; 14: 659–665.
 - [41] Ghafouri-Fard S, Esmaeili M, Taheri M. H19 lncRNA: Roles in tumorigenesis. *Biomedicine & Pharmacotherapy*. 2020; 123: 109774.
 - [42] Korucuoglu U, Biri AA, Konac E, Alp E, Onen IH, Ilhan MN, *et al*. Expression of the imprinted IGF2 and H19 genes in the endometrium of cases with unexplained infertility. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 2010; 149: 77–81.
 - [43] Liu S, Qiu J, Tang X, Cui H, Zhang Q, Yang Q. LncRNA-H19 regulates cell proliferation and invasion of ectopic endometrium by targeting ITGB3 via modulating miR-124-3p. *Experimental Cell Research*. 2019; 381: 215–222.
 - [44] Liu Z, Liu L, Zhong Y, Cai M, Gao J, Tan C, *et al*. LncRNA H19 over-expression inhibited Th17 cell differentiation to relieve endometriosis through miR-342-3p/IER3 pathway. *Cell & Bioscience*. 2019; 9: 84.
 - [45] Liu S, Xin W, Lu Q, Tang X, Wang F, Shao W, *et al*. Knockdown of lncRNA H19 suppresses endometriosis in vivo. *Brazilian Journal of Medical and Biological Research*. 2021; 54: e10117.
 - [46] Paronetto MP, Dimauro I, Grazioli E, Palombo R, Guidotti F, Fantini C, *et al*. Exercise-mediated downregulation of MALAT1 expression and implications in primary and secondary cancer prevention. *Free Radical Biology & Medicine*. 2020; 160: 28–39.
 - [47] Liang Z, Chen Y, Zhao Y, Xu C, Zhang A, Zhang Q, *et al*. miR-200c suppresses endometriosis by targeting MALAT1 in vitro and in vivo. *Stem Cell Research & Therapy*. 2017; 8: 251.
 - [48] Yu J, Chen L, Zhang B, Zheng Q. The modulation of endometriosis by lncRNA MALAT1 via NF- κ B/iNOS. *European Review for Medical and Pharmacological Sciences*. 2019; 23: 4073–4080.
 - [49] Feng Y, Tan B. LncRNA MALAT1 inhibits apoptosis of endometrial stromal cells through miR-126-5p-CREB1 axis by activating PI3K-AKT pathway. *Molecular and Cellular Biochemistry*. 2020; 475: 185–194.
 - [50] Li Y, Liu Y, Chen S, Chen X, Ye D, Zhou X, *et al*. Down-regulation of long non-coding RNA MALAT1 inhibits granulosa cell proliferation in endometriosis by up-regulating P21 via activation of the ERK/MAPK pathway. *Molecular Human Reproduction*. 2019; 25: 17–29.
 - [51] Liu X, Zhang P, Li Y, Zhao N, Han H. The AMPK-mTOR axis requires increased MALAT1 expression for promoting granulosa cell proliferation in endometriosis. *Experimental and Therapeutic Medicine*. 2021; 21: 21.
 - [52] Qiu YR, Zhao MY, Sun L, Yang BC, Hei KW, Du X, *et al*. Expression of lncRNA UCA1 in ovarian cancer and its clinical significance. *European Journal of Gynaecological Oncology*. 2017; 38: 191–195.
 - [53] Zhang L, Cao X, Zhang L, Zhang X, Sheng H, Tao K. UCA1 overexpression predicts clinical outcome of patients with ovarian cancer receiving adjuvant chemotherapy. *Cancer Chemotherapy and Pharmacology*. 2016; 77: 629–634.
 - [54] Zhang Y, Liu KR, Tang TT, Li L. Expression and significance of urothelial carcinoma associated antigen 1 in eutopic endometrium of patients with endometriosis. *Maternal and Child Health Care of China*. 2017; 32: 5733–5735. (In Chinese)
 - [55] Chang CY, Yang L, Tse J, Lo L, Tseng C, Sun L, *et al*. Genetic variations in UCA1, a lncRNA functioning as a miRNA sponge, determine endometriosis development and the potential associated infertility via regulating lipogenesis. *PLoS ONE*. 2022; 17: e0271616.
 - [56] Huang H, Zhu Z, Song Y. Downregulation of lncrna uca1 as a diagnostic and prognostic biomarker for ovarian endometriosis. *Revista Da Associacao Medica Brasileira*. 2019; 65: 336–341.
 - [57] Huang S, Zhu X, Ke Y, Xiao D, Liang C, Chen J, *et al*. LncRNA FTX inhibition restrains osteosarcoma proliferation and migration via modulating miR-320a/TXNRD1. *Cancer Biology & Therapy*. 2020; 21: 379–387.
 - [58] Yang Y, Zhang J, Chen X, Xu X, Cao G, Li H, *et al*. LncRNA FTX sponges miR-215 and inhibits phosphorylation of vimentin for promoting colorectal cancer progression. *Gene Therapy*. 2018; 25: 321–330.
 - [59] Liu F, Yuan J, Huang J, Yang F, Wang T, Ma J, *et al*. Long noncoding RNA FTX inhibits hepatocellular carcinoma proliferation and metastasis by binding MCM2 and miR-374a. *Oncogene*. 2016; 35: 5422–5434.
 - [60] Wang H, Ni C, Xiao W, Wang S. Role of lncRNA FTX in invasion, metastasis, and epithelial-mesenchymal transition of endometrial stromal cells caused by endometriosis by regulating the PI3K/Akt signaling pathway. *Annals of Translational Medicine*. 2020; 8: 1504.
 - [61] Wei G, Wang X. LncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. *European Review for Medical and Pharmacological Sciences*. 2017; 21: 3850–3856.
 - [62] Cui X, Yi Q, Jing X, Huang Y, Tian J, Long C, *et al*. Mining Prognostic Significance of MEG3 in Human Breast Cancer Using Bioinformatics Analysis. *Cellular Physiology and Biochemistry*. 2018; 50: 41–51.
 - [63] Liu Y, Ma J, Cui D, Fei X, Lv Y, Lin J. LncRNA MEG3-210 regulates endometrial stromal cells migration, invasion and apoptosis through p38 MAPK and PKA/SERCA2 signalling via interaction with Galectin-1 in endometriosis. *Molecular and Cellular Endocrinology*. 2020; 513: 110870.
 - [64] Chang CY, Tseng C, Lai M, Chiang A, Lo L, Chen C, *et al*.

Genetic impacts on thermostability of onco-lncRNA HOTAIR during the development and progression of endometriosis. *PLoS ONE*. 2021; 16: e0248168.

- [65] Mai H, Wei Y, Yin Y, Huang S, Lin H, Liao Y, *et al*. LINC01541 overexpression attenuates the 17 β -Estradiol-induced migration and invasion capabilities of endometrial stromal cells. *Systems Biology in Reproductive Medicine*. 2019; 65: 214–222.
- [66] Mai H, Xu H, Lin H, Wei Y, Yin Y, Huang Y, *et al*. LINC01541 Functions as a ceRNA to Modulate the Wnt/ β -Catenin Pathway by Decoying miR-506-5p in Endometriosis. *Reproductive Sciences*. 2021; 28: 665–674.
- [67] Wang Y, Li Y, Yang Z, Liu K, Wang D. Genome-Wide Microarray Analysis of Long Non-Coding RNAs in Eutopic Secretory Endometrium with Endometriosis. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2015; 37: 2231–2245.
- [68] Liu J, Wang Y, Chen P, Ma Y, Wang S, Tian Y, *et al*. AC002454.1 and CDK6 synergistically promote endometrial cell migration and invasion in endometriosis. *Reproduction*. 2019; 157: 535–543.
- [69] Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. *Nature Reviews. Drug Discovery*. 2020; 19: 673–694.
- [70] Yang RN, Xu L, Xu P, Su Y. The Development Situation and Suggestions of RNA Therapy Industry. *China Biotechnology*. 2021; 41: 162–171. (In Chinese)