Comprehensive characterization of endometrial competing endogenous RNA network in infertile women of childbearing age

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ABSTRACT

Endometriosis is widely associated with infertility in women of childbearing age, for which there have been no effective treatments. Recent studies suggest that the dysregulation of RNAs contributes to the pathogenesis of endometriosis, so we conduct the case-control genetic analysis to characterize the expression and interaction of different subtypes of RNAs in infertile women with endometriosis. The ectopic and eutopic endometrium of patients undergoing infertility treatment were collected and subjected to high throughput sequencing, and bioinformatics analysis was conducted to construct the competing endogenous RNA (ceRNA) network. As a result, the RNA interactive network was constructed in endometriosis, and a set of mRNAs such as cyclin-dependent kinase 1 (CDK1) and proliferating cell nuclear antigen (PCNA) along with their corresponding miRNAs and lncRNAs were found to promote the growth and death of endometrial stromal cells, which was essential for the pathogenesis of endometriosis. These data suggest that RNA crosstalk is a crucial segment in the development of endometriosis, where CDK1 and PCNA may serve as emerging targets for the treatment of endometriosis-related infertility in women of childbearing age.

INTRODUCTION

Endometriosis is characterized by the uterine tissue growing outside of the uterus, chronic inflammation of the endometrium, tissue-specific excess production of estrogen, and resistance to progesterone [1]. Worldwide, endometriosis affects 5-10% of women who are of reproductive age [2], and large-scale studies suggest a prevalence of 25 - 40% in infertile women, which is 5-8 times higher than that in fertile women [3]. For example, D'Hooghe and colleagues found that the morbidity of endometriosis was significantly higher in infertile women

than that in fertile women, and infertile women were more likely to be diagnosed with advanced stages of endometriosis [4]. In particular cases, endometriosis is tightly associated with gynecologic cancer. Evidenced by previous studies, endometriosis increased the risk of ovarian cancer (standardized incidence ratio of 1.76 [95% confidence interval 1.47-4.38]) [5]. For a long time, various therapeutic strategies have been applied to endometriosis, but the expected yield was not achieved [6]. Among the most current therapeutic opinions for endometriosis-associated infertility, in vitro fertilization (IVF) is recommended with relatively high priority [7].

The pathogenesis of endometriosis is a complex biological process that is regulated by large amounts of molecules, in which messenger RNAs (mRNAs), microRNAs (miRNAs), and long noncoding RNAs (lncRNAs) are critical candidates. For mRNAs, anthrax toxin receptor 2 (ATR2) has been detected to be upregulated in endometriotic specimens, and elevation of ATR2 promotes endometriotic cell adhesion and angiogenesis, which ultimately leads to endometriosis [8]. Ubiquitin-specific protease 10 (USP10) acts similarly with ATR2, which promotes proliferation and migration of endometrial stromal cells via activating the Raf-1/MEK/ERK pathway [9]. miRNAs are dozens of highly conservative noncoding small RNAs that regulate mRNA translation and play essential roles in the pathogenesis of endometriosis. A meta-analysis based on 12 studies reported that 134 dysregulated miRNAs were potential biomarkers of endometriosis [10]. Specially, let-7 families were detected to be repressed in endometriosis, and loss of let-7 contributes to the development of endometriosis [11]. lncRNAs are kinds of noncoding/limited-coding RNAs with the length longer than 200 nucleotides [12], and aberrant expression of lncRNAs is indicated to be associated with endometrial hyperplasia [13]. MALAT1, a well-known lncRNA, could repress the expression of hypoxia-inducible factor-1α (HIF-1α), which exacerbated the autophagy of endometrial stromal cells and further promoted the pathogenesis of endometriosis [14].

In light of the crosstalk between different subtypes of RNAs, the competing endogenous RNA (ceRNA) network is proposed as different subtypes of RNAs competing for a limited pool of miRNAs [15]. In detail, miRNAs can repress the expression of their target genes or lncRNAs by targeting the miRNA response elements (MREs) on the 3′-untranslated region (UTR), coding sequence (CDS), or the 5′-UTR at the post-transcriptional level, and each miRNA can target numerous mRNAs or lncRNAs, while the vast majority of mRNAs or lncRNAs also harbor a certain number of MREs and are thus repressed by their corresponding miRNAs, which leads to the competition for the finite pool of miRNAs between lncRNAs and mRNAs [16, 17]. Fortunately, all ceRNA networks can be predicted using bioinformatics methods according to the principle of complementary base pairing. For example, the ceRNA network has been typically estimated in various physical disorders, including cancer, atherosclerosis, and central nervous system diseases, which provides insights for diseases in both clinical treatment and mechanism study [18–20]. In regards to the gynecologic disorders, lncRNA HOTAIR has been demonstrated to upregulate CCND1 and CCND2 by sponging miR-206 in ovarian cancer [21], and lncRNA MALAT1 could suppress tumor growth and progression through inhibiting PHF19 via sponging miR-211 [22].

Although many studies have suggested the indispensable role of ceRNA in various diseases, to the best of our knowledge, it has been seldom discussed in endometriosis.

To investigate the regulatory mechanism of different subtypes of RNAs in endometriosis, we collected the high throughput sequencing profiles of lncRNAs, miRNAs, and mRNAs to generate a new dataset, which was used to identify the differentially expressed RNAs (DERNAs) and construct the ceRNA network. The fitness of the ceRNA network was evaluated from 4 aspects of node degree, topological coefficient, closeness centrality, and betweenness centrality [23], which were all indicators of the social network analysis showing the relationship between RNAs. The biological function of the specific RNAs involved in the ceRNA network was enriched by using bioinformatics analysis through platforms of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). The ceRNA network identified in this study would contribute to a better understanding of the pathogenesis of endometriosis-associated infertility and shed insights for etiological studies.

RESULTS

Characteristics of study subjects

The ectopic and eutopic endometrium from 14 infertile women with endometriosis were collected in this study, among whom the miRNAs and lncRNAs were profiled in 7 patients from the Chinese PLA general hospital, and the lncRNAs were detected in the rest 7 patients from the Fondazione Italiana Endometriosi. The median age of the patients used for miRNA/lncRNA profiling is lower than that of the mRNA group, but the difference was of no statistical significance (26 (25, 37) *vs.* 36 (34, 38), $P > 0.05$). The distribution of the r-ASF stage between the ectopic and eutopic endometrium is similar (Table 1). These data indicate that samples involved in the combined dataset are of high homogeneity, which is comparable for the investigation of RNA interactions.

Identification of differentially expressed RNAs in endometriosis

To investigate the crosstalk between different subtypes of RNAs in endometriosis, we identified the dysregulated mRNAs, lncRNAs, and miRNAs, respectively. Three datasets regarding the genome-wide expression of mRNAs, lncRNAs, and miRNAs in the eutopic and ectopic endometrium were retrieved from the Gene Expression Omnibus (GEO): GSE105764, GSE105765, and GSE25628. In total, we found 22278 mRNAs, 20402 lncRNAs and 1014 miRNAs, among which 687 DEmRNAs, 263 DEmiRNAs, and 313 DElncRNAs were

Table 1. Characteristics of 14 enrolled endometriosis patients from two datasets.

Variables	mRNA/lncRNA	miRNA	*Z/F* Value	*P* Value
N	7	7		
Age (yrs)*	26 (25, 37)	36 (34, 38)	-1.54	0.12
r-AFS stage			0.24	0.59
Phase III (%)	4 (57.14)	2 (28.57)		
Phase IV (%)	3 (42.86)	5 (71.43)		

*, Median (P25, P75).

determined using limma analysis with rigorous data quality control (Figure 1), including 427 mRNAs, 161 lncRNAs, and 119 miRNAs that were upregulated, and 260 mRNAs, 152 lncRNAs, and 144 miRNAs that were downregulated. The top 10 dysregulated mRNAs, lncRNAs as well as miRNAs were visualized in Figure 2, and the full list of these RNAs could be referred to the Supplementary Tables 1–3.

Construction of the lncRNA-miRNA-mRNA triple network in endometriosis

To explore the regulatory mechanism of RNA crosstalk in endometriosis, we constructed a triple network using miRNAs as a bridge between mRNAs and lncRNAs. In total, we identified 44290 mRNAs and 168170 lncRNAs that were targeted by the dysregulated miRNAs (Supplementary Figures 1 and 2). Furthermore, we extracted the DEmiRNAs that were associated with both mRNAs and lncRNAs to construct the lncRNA-miRNA-mRNA triple network, and, as a result, 9496 mRNAs, 11211 lncRNAs and 259 miRNAs were identified (Supplementary Figure 3).

To investigate the functional implication of the triple network, we adopted the functional annotation and enrichment analysis for RNAs involved in the triple network, and four aspects of biological process, molecular function, cellular component, and signaling pathway were investigated based on GO and KEGG.
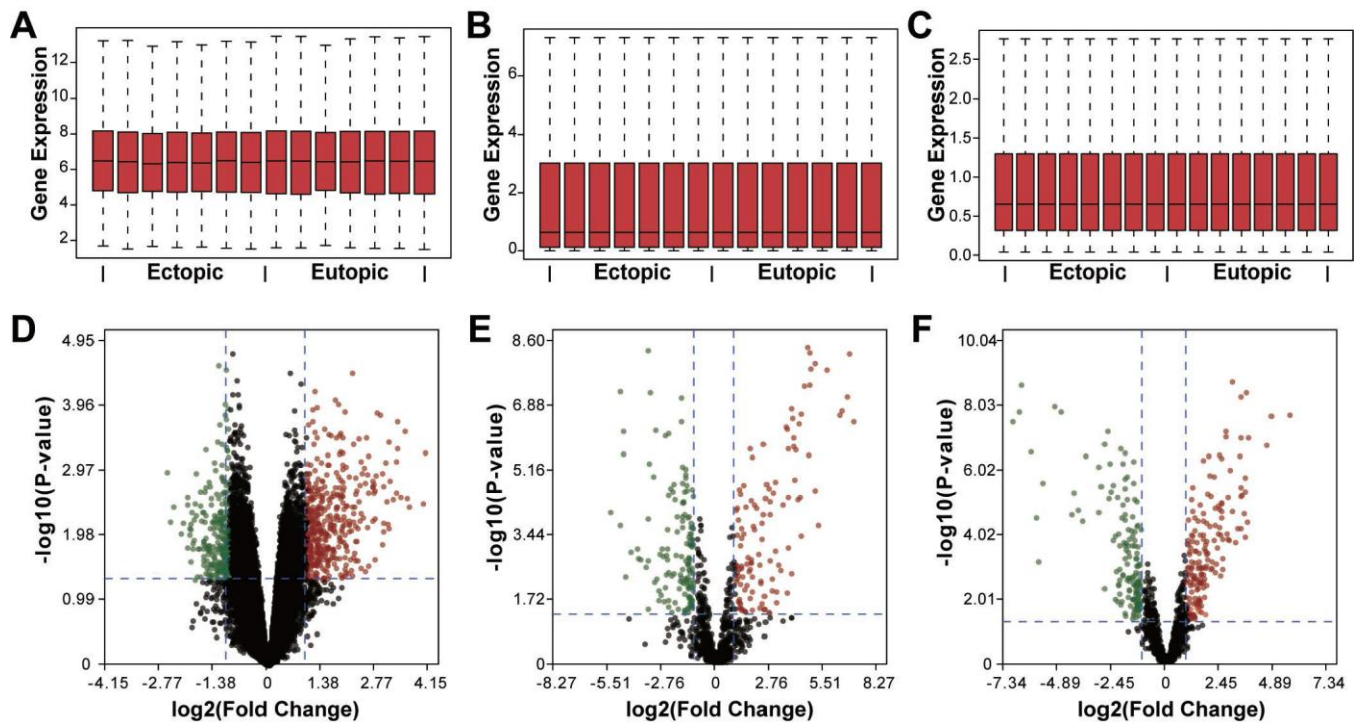

Figure 1. Identification of differentially expressed RNAs in endometriosis. (**A–C**) Box plots showing the expression of mRNAs, miRNAs, and lncRNAs. (**D–F**) Volcano plots of differentially expressed mRNAs, miRNAs, and lncRNAs. Red dots, upregulation, green dots, downregulation, black dots, rational expression.

We found that the RNAs involved in the triple network were supposed to distribute in different organelles and play crucial roles in the developmental process, and these RNAs also regulate the catalytic activity and molecular function. The related signaling pathways are cell cycle and biosynthesis of unsaturated fatty acids (Figure 3). Thus, the RNAs in the triple network may contribute to the development of endometriosis through inducing disorders of cell cycle and metabolic processes.

Identification of the ceRNA network in endometriosis

To identify the ceRNA network in endometriosis, we extracted the aberrantly expressed mRNAs and lncRNAs in the triple network. Utilizing the hypergeometric test, 172 DEmRNAs, 86 DElncRNAs, and 68 DEmiRNAs were determined, and 6871 DEmRNA-DElncRNA-DEmiRNA interactive items were identified in the ceRNA network (Figure 4).
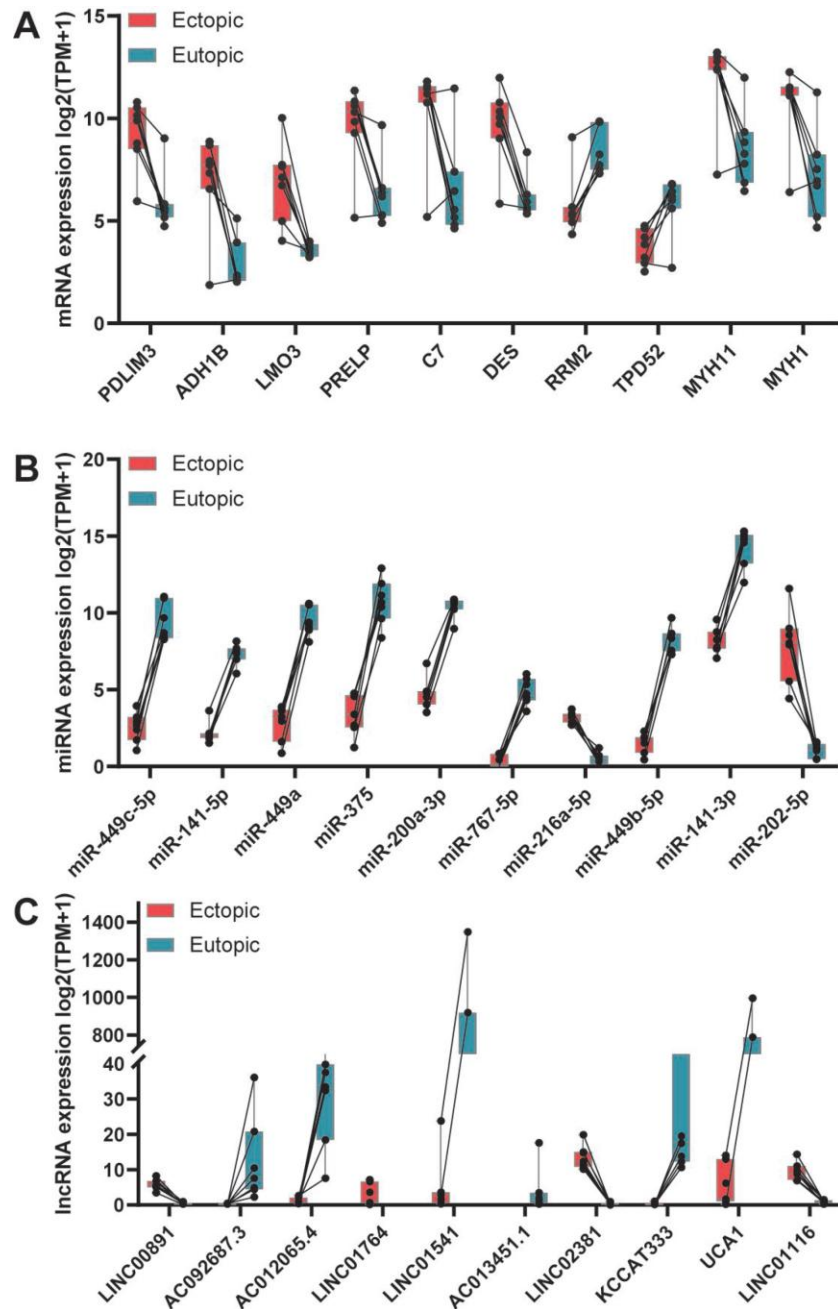

Figure 2. Top 10 dysregulated RNAs in ectopic endometrium comparing with that in eutopic endometrium. (**A**) Top 10 dysregulated mRNAs. (**B**) Top 10 dysregulated miRNAs. (**C**) Top 10 dysregulated lncRNAs.
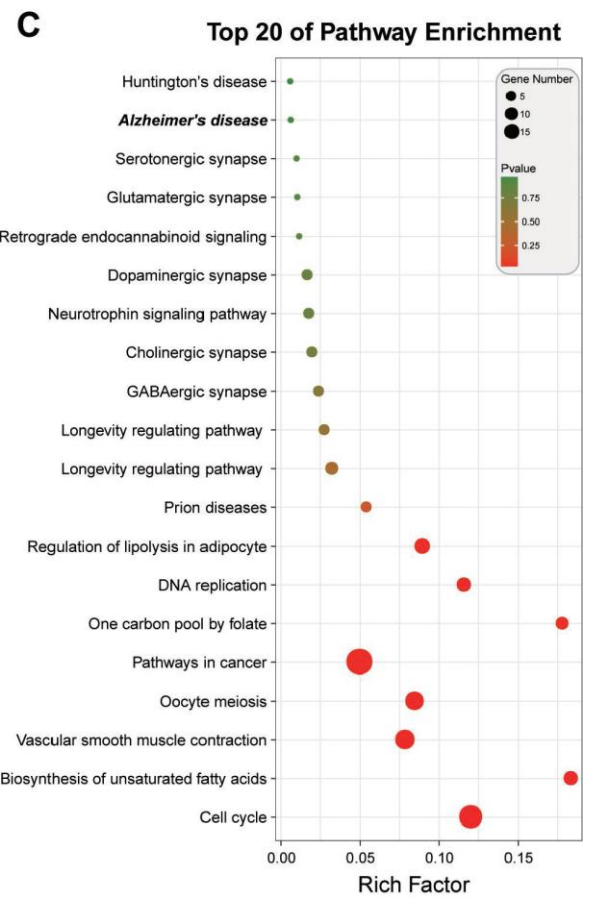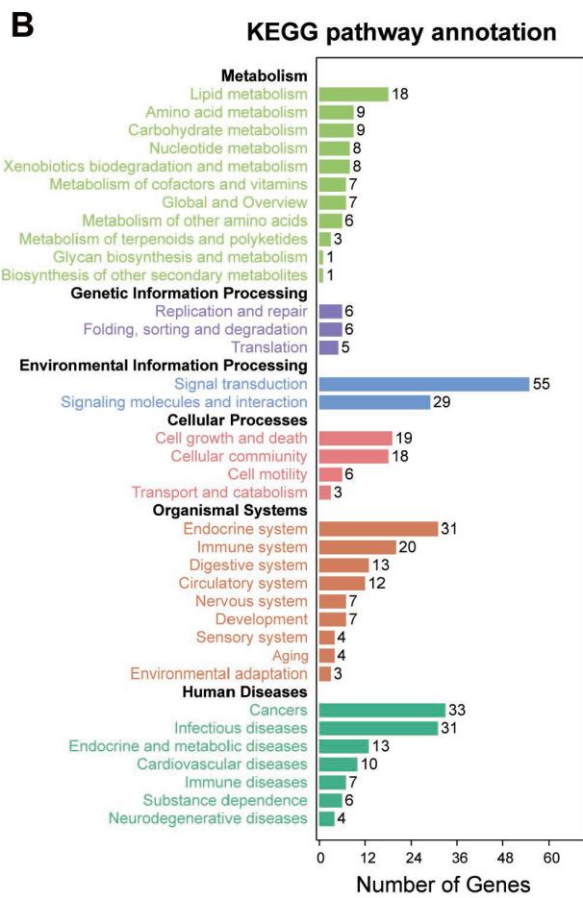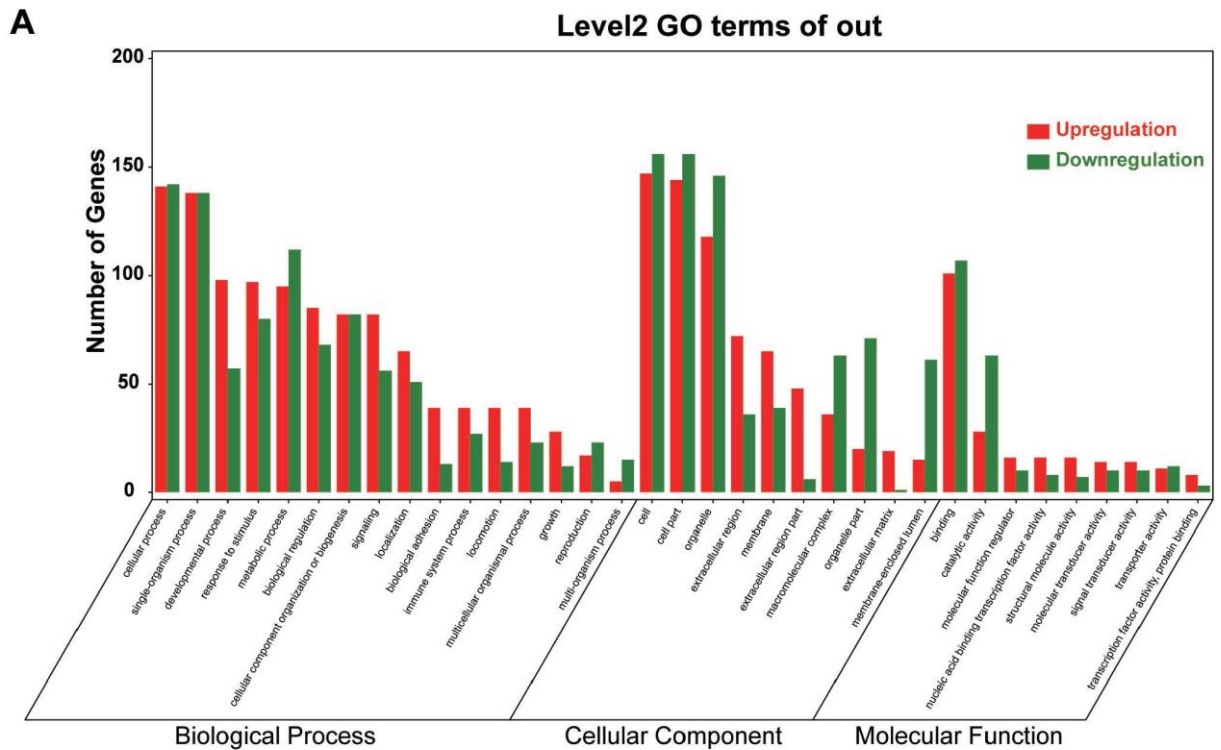
Figure 3. Functional annotation for RNAs in the triple network. (**A**) Gene Ontology analysis from 3 aspects of biological process, molecular function, and cellular components. (**B**) KEGG pathway annotation and enrichment. (**C**) Top 20 signaling pathways derived from KEGG signaling pathway enrichment.

Fitness assessment and functional annotation of the ceRNA network

To explore the fitness of the ceRNA network, we conducted the topological assessment from 4 aspects of node degree, topological coefficient, closeness centrality, and betweenness centrality (Figure 5). As previously indicated, the node degree reflects the possibility for a node to be targeted by other nodes, the topological coefficient explains the number of neighbors shared by a pair of nodes, the closeness centrality implies the potential for a node to be a center, and the betweenness centrality suggests the role for a node in connecting different modules [23]. Herein, the R squares of node degree, topological coefficient, closeness centrality, and betweenness centrality are 0.749, 0.749, 0.905, and 0.883, indicating the high efficiency of the ceRNA network.

Additionally, we adopted functional annotation for RNAs in the ceRNA network (Figure 6), and five functional groups were identified, including the p53 signaling pathway, cell cycle, DNA replication, PPAR signaling pathway, and biosynthesis of unsaturated fatty acids. Importantly, a set of hub genes such as PTTG1, BUB1, AURKA, CCNB1, CDK1, PPP2R5C, and CDC25A were determined as crucial regulators of cell proliferation and oocyte maturation, which contributed to the maintenance of menstrual cycle. Therefore, these data demonstrate that the ceRNA network constructed in this study is of high accuracy in exploring the mechanism of endometriosis, and the critical regulators
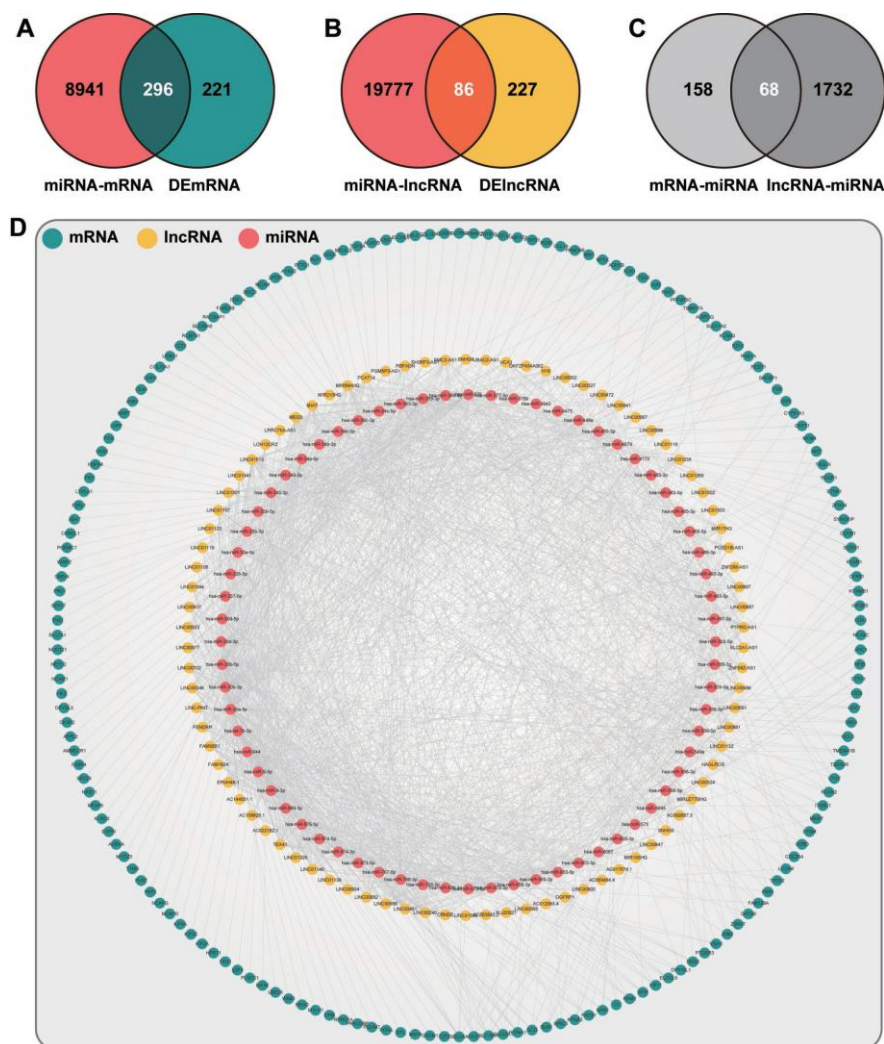

Figure 4. The layout of the ceRNA network. (**A**) Venn diagram of mRNAs. Red, mRNAs targeted by DEmiRNAs. Blue, mRNAs differentially expressed in endometriosis. (**B**) Venn diagram of lncRNAs. Red, lncRNAs targeted by DEmiRNAs. Yellow, lncRNAs differentially expressed in endometriosis. (**C**) Venn diagram of miRNAs. Light gray, miRNAs related to intersection mRNAs in (**A**). Dark gray, miRNAs related to intersection lncRNAs in (**B**). (**D**) The topological layout of ceRNA network based on DEmRNAs, DElncRNAs, and DEmiRNAs.

identified in the ceRNA network are potential biomarkers for the diagnosis of endometriosis.

DISCUSSION

Endometriosis is one of the most common gynecologic diseases with high risk for infertility and gynecologic cancer, particularly in patients who underwent surgical operation of the uterus [24]. However, the pathogenesis of endometriosis is not fully clarified. In general, it is widely accepted that endometriosis could be induced by various factors, and pelvic inflammation has been identified as a prerequisite for the occurrence of endometriosis [25]. With the development of transcriptomics, recent studies confirmed the essential role of different subtypes of RNAs in endometriosis, and a set of well-studied RNAs, including mRNAs, lncRNAs, and miRNAs, had been identified as crucial regulators of endometriosis. For example, NLRC5 and P62 were significantly upregulated in the ectopic endometrium, while the elevation of NLRC5 corresponded to autophagy inhibition in endometriosis, which could be used as indicators for the diagnosis of endometriosis [26]. As a transcriptional regulator, circulating miR-370-3p was demonstrated to repress cell proliferation by downregulating steroidogenic factor 1 (SF1) in endometriosis [27]. CDK6 was found to upregulate the expression of lncRNA AC002454.1 and further accelerate cell cycle, which promoted the progression of endometriosis [28]. Besides, our previous work also showed an indispensable role of let-7 families in promoting nanoparticle phagocytosis in lung cancer [29–31]. Thus, it is necessary to grasp the RNA characteristics at the genome-wide level, which will provide evidence for the mechanism study as well as the clinical treatment of endometriosis.
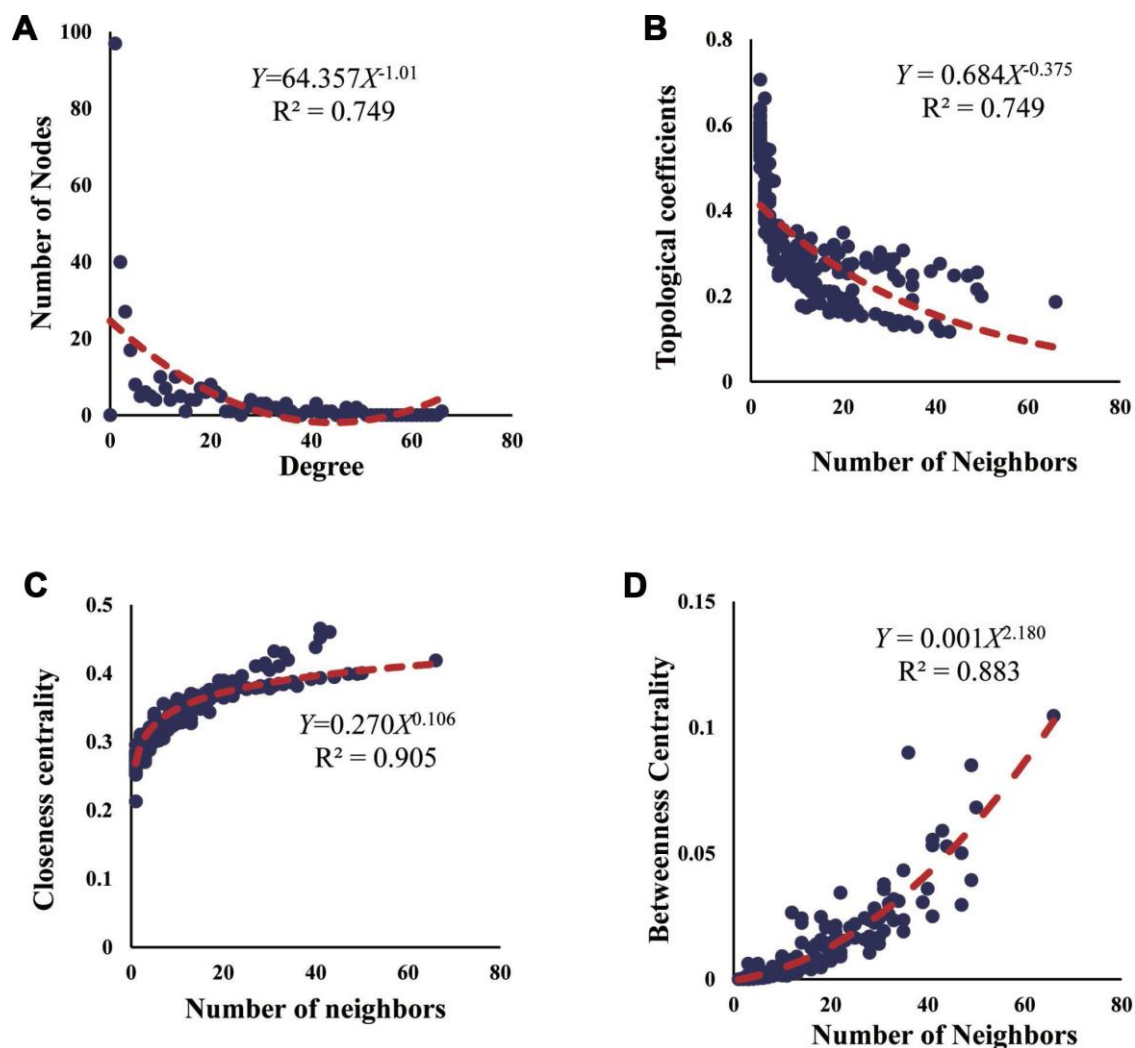

Figure 5. Fitness assessment of the ceRNA network. (**A–D**) Assessment of ceRNA network from 4 aspects of node degree, topological coefficient, closeness centrality, and betweenness centrality.

Using publicly available datasets, we recombined a new dataset containing the expression profiles of mRNAs, lncRNAs, and miRNAs. Then, we identified the dysregulated RNAs in the ectopic endometrium comparing with that in the eutopic endometrium. In total, 687 DEmRNAs, 263 DEmiRNAs, and 313 DElncRNAs were determined, among which 427 mRNAs, 161 lncRNAs, and 119 miRNAs were upregulated, and 260 mRNAs, 152 lncRNAs, and 144 miRNAs were downregulated. Of the 687 DEmRNAs, the top 10 mRNAs, including LMO3, DES, ADH1B, PDLIM3, ADH7, RRM2, PRELP, TPD52, MYH11, and MYH1,

were shown in Figure 2. Interestingly, PRELP has been demonstrated to be upregulated in patients with endometriosis [32]. Meanwhile, most of the top 10 DEmiRNAs have also been validated in endometriosis [33]. miR-202, for example, was found to promote endometriosis by inhibiting SOX6 [34], and downregulation of miR-375 alleviated the suppression of endothelin 1 (EDN1) in ectopic stromal cells, which in turn contributed to the development of endometriosis [35]. Moreover, direct transfection of miR-449b-3p into endometrial stromal cells inhibited the proliferation and formation of tubular structures in human umbilical vein
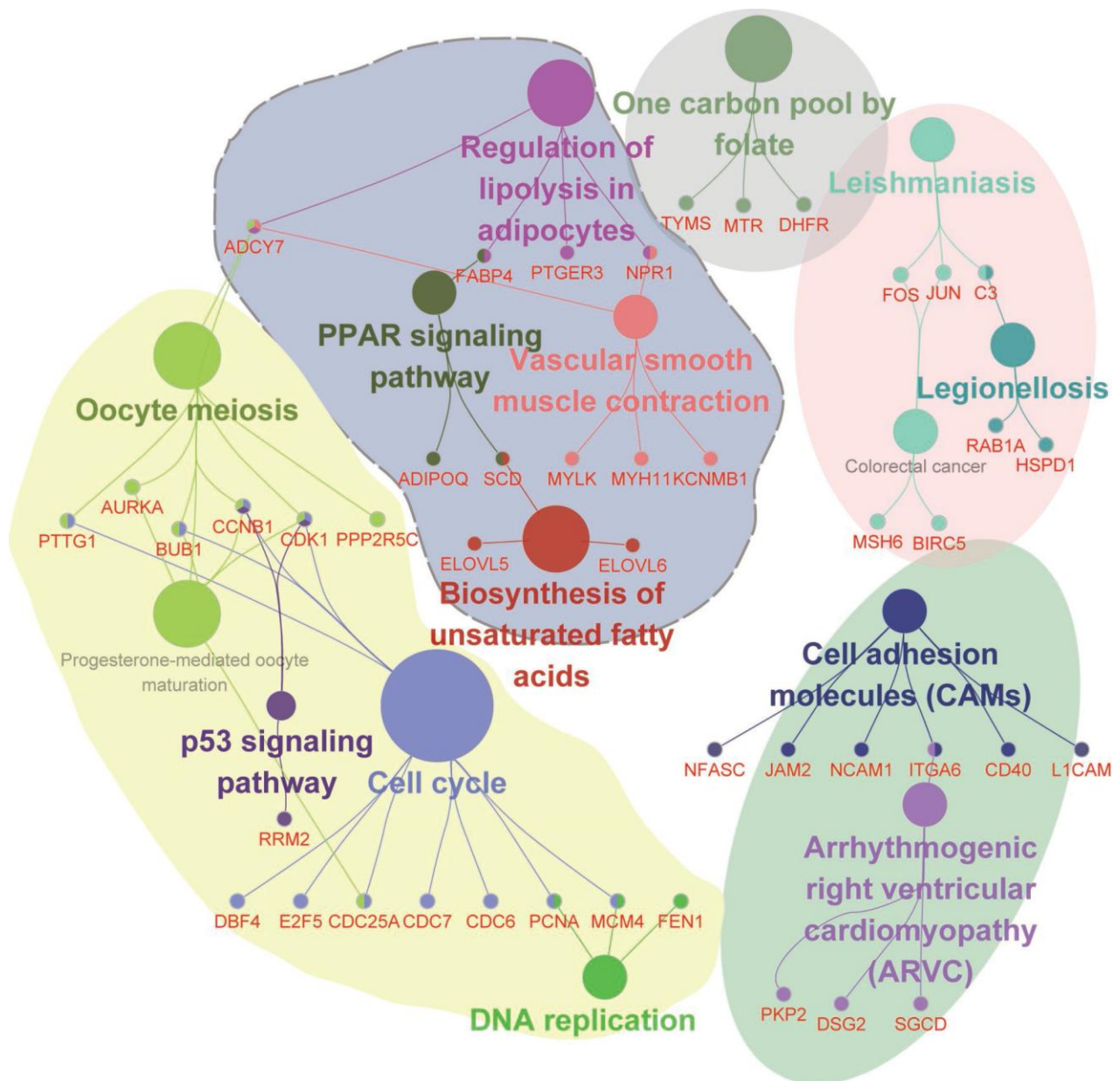

Figure 6. Functional enrichment analysis for RNAs involved in the ceRNA network. Functional enrichment modules are represented in different colors.

endothelial cells [36]. For miR-216a-5p and miR-767-5p, available studies concerning their roles in endometriosis were not found. Similarly, there are no studies concerning the top 10 dysregulated lncRNAs in endometriosis, but the DElncRNA H19 has been reported as an efficient regulator in altering stromal cell growth via IGF signaling pathway in the endometriosis [37].

Furthermore, we constructed a triple network and performed functional annotation for RNAs in the triple network. As shown in Figure 4, signaling pathways of cell cycle and DNA replication (KEGG id: ko04110 and ko03030) were determined with high-risk factors, gene numbers, and shallow *P* values. Consistent with previous studies, dysregulation of cell cycle and DNA replication adversely affected the proliferation of endometrial stromal cells, and several dysregulated genes such as CDC2, PCNA, and CCN1 have also been previously investigated [38]. Specially, PCNA was crucial in the maintenance of cell proliferation, and downregulation of PCNA promoted the proliferation and invasion of endometrial stromal cells [39]. CCN1 was a secretory cysteine-rich matricellular protein that highly expressed in human ectopic endometriotic lesions, as a regulator of estrogen at downstream, CCN1 controled cell proliferation and neovascularization [40]. However, Tang L and colleagues found that there was no difference in CDC2 expression between ectopic and eutopic endometrium [41], so further studies are still needed to confirm the regulatory mechanism of CDC2 in endometriosis.

For the downstream investigation, we constructed the ceRNA network based on the RNA triple network. A total of 6871 DEmRNA-DElncRNA-DEmiRNA interactive items were identified, but only two studies concerning the ceRNA regulatory mechanism in endometriosis were found, which all focused on the circRNA-related ceRNA network [42, 43], so it was hard to provide convincing evidence from previous studies for the putative ceRNA network. Indeed, the RNAs in the ceRNA network have mostly been demonstrated to be associated with endometriosis, and the biological processes concluded from the functional annotation were also highly consistent with the pathogenesis of endometriosis. Cell adhesion molecules (CAM), for example, were a kind of cell surface transmembrane glycoproteins that promoted cell-to-cell adhesion and directly affect cell proliferation, differentiation, extension, and migration, which were essential for the occurrence and development of endometriosis [44]. As a bridge connecting the PPAR signaling pathway and the lipolysis in adipocytes signaling pathway, FABP4 had been demonstrated to manipulate the metabolism and differentiation of

endometrial stromal cells [45], so the circulating fatty acid level may influence the pathological procedure of endometriosis. Consistently, the folate and the biosynthesis of unsaturated fatty acids have been identified in the functional enrichment analysis, which could be linked to endometriosis development. Also, the P53 signaling pathway acted as a guardian in tumor suppression [46], where three relevant genes were identified in endometriosis and maybe ligaments for studying the relationship between endometriosis and endometrial cancer. At last, we conducted the fitness assessment for the ceRNA network. As indicated by R squares of the number of nodes, betweenness centrality, closeness centrality, and topological coefficient, the ceRNA network was of high efficiency and could be used for guiding future studies.

Since this is cross-sectional research, which does limit the causal relationship proposed in this study. Moreover, a total of 14 patients were recruited to construct the ceRNA network, and this may lead to Type II errors due to the limited sample size. Although several interactions of the ceRNA network have been determined in previous studies, the ceRNA network is still needed to be verified using further experiments either *in vitro* or *in vivo*. Importantly, several factors, including the calculating parameters and software versions, may influence the reproducibility of the ceRNA network and should be strictly set according to our descriptions in future studies

To sum up, we construct a ceRNA network with high efficiency and precision based on the genome-wide expression profiles of mRNAs, lncRNAs, and miRNAs in endometriosis. The crosstalk between RNAs of different subtypes, including CDK1, CCNB1, E2F5, and PCNA along with their corresponding miRNAs and lncRNAs, is speculated to promote the development of endometriosis, which provides insights and perspectives for the investigation of endometriosis-associated infertility.

MATERIALS AND METHODS

Patients

A total of 14 infertile women with endometriosis were enrolled in this study. All patients were confirmed by histological examination and diagnosed with endometriosis at moderate to severe stage using the revised American Fertility Society (r-AFS) classification standards [47, 48], women in the secretory phase of the menstrual cycle and without any hormonal treatment history were included in the study. The clinical samples of the eutopic or ectopic ovarian endometrial tissues were collected from

women who underwent surgery for infertility, pelvic pain symptoms, or adnexal masses during laparoscopy surgery or curettage before the laparoscopic procedure, the baseline characteristics including age and r-AFS stage were also listed in Table 1. A total of 7 out of the 14 patients from Rome offered samples for mRNA detection, and the remaining patients from China at the Chinese PLA general hospital denoted samples for miRNA/lncRNA sequencing. All patients were well informed and admitted about the purpose of this study.

Data quality control and dataset recombination

The high throughput sequencing profiles of miRNAs (GSE105764) and lncRNAs (GSE105764), as well as the mRNA microarray dataset (GSE25628), were retrieved from GEO publicly available at https://www.ncbi.nlm.nih.gov/geo/. All data were normalized using the quantile normalization method, in which RNAs with missing values were excluded. The homogeneity of all samples was checked using cluster analysis and principal component analysis, where heterogeneously distributed samples were removed.

Identification of differentially expressed RNAs

The expression patterns of mRNAs, lncRNAs and miRNAs were compared using bioinformatics analysis through R software version 3.4.1 for windows, and the DERNAs were identified using the following criteria: limma comparison method, P-value < 0.05, and the absolute value of ($\log_2$(fold change)) ≥ 1. The differentially expressed RNAs were summarized and used for the construction of the ceRNA network.

Construction of the mRNA-miRNA-lncRNA triple network

Firstly, the putative regulatory patterns of miRNA-lncRNA and miRNA-mRNA were determined according to the principle of complementary base pairing. Using miRNA as a bridge, we constructed a triple network among miRNAs, lncRNAs, and mRNAs. In detail, the mRNAs targeted by the differentially expressed miRNAs were predicted using microT scoring method (paring score ≥ 0.8) via the DIANA-microT-CDS plugin in DIANA platform (available online at http://www.microrna.gr/microT-CDS) [49], and the lncRNAs repressed by the dysregulated miRNAs were identified through the lncBase database version 2.0, a database elaborated information for predicted and experimental verified miRNA-lncRNA interactions [50]. Finally, the mRNA-miRNA-lncRNA interactions were visualized using a topological network in Cytoscape version 3.7.1.

Visualization and fitness assessment of the ceRNA network

To identify the ceRNA network, we extracted the DEmRNAs and DElncRNAs in the triple network, and we used the hypergeometric test to evaluate the significance of the shared miRNAs between each mRNA and lncRNA, a P-value < 0.05 was considered as statistically significant, and it was calculated as follows:

$$P = 1 - \sum_{t=0}^{x} \frac{\binom{t}{K}\binom{N-t}{M-K}}{\binom{N}{M}}$$

Where K is the number of miRNAs interacted with mRNAs, N represents the number of miRNAs interacted with lncRNAs, M is the number of miRNAs in the genome, x is the number of miRNAs shared by mRNAs and lncRNAs [51].

Fitness assessment of the ceRNA network was performed using the NetworkAnalyzer plugin in Cytoscape version 3.7.1 [23], several properties including the degree of node, topological coefficient, betweenness centrality, and closeness centrality were used to investigate the efficiency of the model. The specific calculation methods could be referred to the manual of SocNetV V2.0, a software for visualizing social networks.

Functional annotation and enrichment analysis for differentially expressed RNAs

Functional enrichment analysis was performed from 4 aspects of biological process, molecular function, cellular component, and signaling pathway using GO and KEGG database through ClueGO plugin in Cytoscape version 3.7.1 [52], the analytical parameters were set as default: two-side hypergeometric test, P-value of pathway enrichment ≤ 0.05, Kappa score of GO term/pathway network connectivity ≥ 0.4, GO tree interval between 3 and 8, and the number of genes involved in each cluster $\geq$ 3. For cluster analysis of different functional modules, we used EnrichmentMap plugin in Cytoscape Version 3.7.1 [53], which was used to show the pathways as an enrichment map and further highlight the overlaps among different pathways. GO and KEGG were available online at http://geneontology.org/ and https://www.kegg.jp/, respectively.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

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REFERENCES

1. Baranov V, Malysheva O, Yarmolinskaya M. Pathogenomics of endometriosis development. Int J Mol Sci. 2018; 19:1852.
https://doi.org/10.3390/ijms19071852
PMID:29937493

2. Wheeler JM. Epidemiology of endometriosis-associated infertility. J Reprod Med. 1989; 34:41–46.
PMID:2704007

3. Ozkan S, Murk W, Arici A. Endometriosis and infertility: epidemiology and evidence-based treatments. Ann N Y Acad Sci. 2008; 1127:92–100.
https://doi.org/10.1196/annals.1434.007
PMID:18443335

4. D'Hooghe TM, Debrock S, Hill JA, Meuleman C. Endometriosis and subfertility: is the relationship resolved? Semin Reprod Med. 2003; 21:243–54.
https://doi.org/10.1055/s-2003-41330 PMID:12917793

5. Saavalainen L, Lassus H, But A, Tiitinen A, Härkki P, Gissler M, Pukkala E, Heikinheimo O. Risk of gynecologic cancer according to the type of endometriosis. Obstet Gynecol. 2018; 131:1095–102.
https://doi.org/10.1097/AOG.0000000000002624
PMID:29742675

6. Bougie O, Yap MI, Sikora L, Flaxman T, Singh S. Influence of race/ethnicity on prevalence and presentation of endometriosis: a systematic review and meta-analysis. BJOG. 2019; 126:1104–15.
https://doi.org/10.1111/1471-0528.15692
PMID:30908874

7. Wu CQ, Albert A, Alfaraj S, Taskin O, Alkusayer GM, Havelock J, Yong P, Allaire C, Bedaiwy MA. Live Birth Rate after Surgical and Expectant Management of Endometriomas after In Vitro Fertilization: A Systematic Review, Meta-Analysis, and Critical Appraisal of Current Guidelines and Previous Meta-Analyses. J Minim Invasive Gynecol. 2019; 26:299–311.e3.
https://doi.org/10.1016/j.jmig.2018.08.029
PMID:30717864

8. Lin SC, Lee HC, Hsu CT, Huang YH, Li WN, Hsu PL, Wu MH, Tsai SJ. Targeting anthrax toxin receptor 2 ameliorates endometriosis progression. Theranostics. 2019; 9:620–32.
https://doi.org/10.7150/thno.30655
PMID:30809297

9. Chen Q, Hang Y, Zhang T, Tan L, Li S, Jin Y. USP10 promotes proliferation and migration and inhibits apoptosis of endometrial stromal cells in endometriosis through activating the Raf-1/MEK/ERK pathway. Am J Physiol Cell Physiol. 2018; 315:C863–72.
https://doi.org/10.1152/ajpcell.00272.2018
PMID:30281322

10. Bjorkman S, Taylor HS. Erratum: MicroRNAs in endometriosis biological function and emerging biomarker candidates. Biol Reprod. 2019; 101:1179.
https://doi.org/10.1093/biolre/ioz219
PMID:31875878

11. Sahin C, Mamillapalli R, Yi KW, Taylor HS. microRNA Let-7b: A Novel treatment for endometriosis. J Cell Mol Med. 2018; 22:5346–53.
https://doi.org/10.1111/jcmm.13807
PMID:30063121

12. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009; 10:155–59.
https://doi.org/10.1038/nrg2521
PMID:19188922

13. Tanos V, Ariel I, Prus D, De-Groot N, Hochberg A. H19 and IGF2 gene expression in human normal, hyperplastic, and malignant endometrium. Int J Gynecol Cancer. 2004; 14:521–25.
https://doi.org/10.1111/j.1048-891x.2004.014314.x
PMID:15228427

14. Liu H, Zhang Z, Xiong W, Zhang L, Du Y, Liu Y, Xiong X. Long non-coding RNA MALAT1 mediates hypoxia-induced pro-survival autophagy of endometrial stromal cells in endometriosis. J Cell Mol Med. 2019; 23:439–52.
https://doi.org/10.1111/jcmm.13947 PMID:30324652

15. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011; 146:353–58.
https://doi.org/10.1016/j.cell.2011.07.014
PMID:21802130

16. Karreth FA, Pandolfi PP. ceRNA cross-talk in cancer: when ce-bling rivalries go awry. Cancer Discov. 2013; 3:1113–21.
https://doi.org/10.1158/2159-8290.CD-13-0202
PMID:24072616

17. Qi X, Zhang DH, Wu N, Xiao JH, Wang X, Ma W. ceRNA in cancer: possible functions and clinical implications. J Med Genet. 2015; 52:710–18.
https://doi.org/10.1136/jmedgenet-2015-103334
PMID:26358722

18. Xu J, Li Y, Lu J, Pan T, Ding N, Wang Z, Shao T, Zhang J, Wang L, Li X. The mRNA related ceRNA-ceRNA landscape and significance across 20 major cancer types. Nucleic Acids Res. 2015; 43:8169–82.
https://doi.org/10.1093/nar/gkv853 PMID:26304537

19. He L, Chen Y, Hao S, Qian J. Uncovering novel landscape of cardiovascular diseases and therapeutic targets for cardioprotection via long noncoding RNA-miRNA-mRNA axes. Epigenomics. 2018; 10:661–71.
https://doi.org/10.2217/epi-2017-0176
PMID:29692219

20. Yang C, Wang L, Sun J, Zhou JH, Tan YL, Wang YF, You H, Wang QX, Kang CS. Identification of long non-coding RNA HERC2P2 as a tumor suppressor in glioma. Carcinogenesis. 2019; 40:956–64.
https://doi.org/10.1093/carcin/bgz043
PMID:30809632

21. Chang L, Guo R, Yuan Z, Shi H, Zhang D. Lncrna hotair regulates ccnd1 and ccnd2 expression by sponging mir-206 in ovarian cancer. Cell Physiol Biochem. 2018; 49:1289–303.
https://doi.org/10.1159/000493408
PMID:30205383

22. Tao F, Tian X, Ruan S, Shen M, Zhang Z. miR-211 sponges lncRNA MALAT1 to suppress tumor growth and progression through inhibiting PHF19 in ovarian carcinoma. FASEB J. 2018; 32:6330–43.
https://doi.org/10.1096/fj.201800495RR
PMID:29874124

23. Assenov Y, Ramírez F, Schelhorn SE, Lengauer T, Albrecht M. Computing topological parameters of biological networks. Bioinformatics. 2008; 24:282–84.
https://doi.org/10.1093/bioinformatics/btm554
PMID:18006545

24. Lundberg FE, Iliadou AN, Rodriguez-Wallberg K, Gemzell-Danielsson K, Johansson AL. The risk of breast and gynecological cancer in women with a diagnosis of infertility: a nationwide population-based study. Eur J Epidemiol. 2019; 34:499–507.
https://doi.org/10.1007/s10654-018-0474-9
PMID:30623293

25. Chiang AJ, Chang C, Huang CH, Huang WC, Kan YY, Chen J. Risk factors in progression from endometriosis to ovarian cancer: a cohort study based on medical insurance data. J Gynecol Oncol. 2018; 29:e28.
https://doi.org/10.3802/jgo.2018.29.e28
PMID:29400021

26. Zhan L, Yao S, Sun S, Su Q, Li J, Wei B. NLRC5 and autophagy combined as possible predictors in patients with endometriosis. Fertil Steril. 2018; 110:949–56.
https://doi.org/10.1016/j.fertnstert.2018.06.028
PMID:30316442

27. Hu Z, Mamillapalli R, Taylor HS. Increased circulating miR-370-3p regulates steroidogenic factor 1 in endometriosis. Am J Physiol Endocrinol Metab. 2019; 316:E373–82.
https://doi.org/10.1152/ajpendo.00244.2018
PMID:30576245

28. Liu J, Wang Y, Chen P, Ma Y, Wang S, Tian Y, Wang A, Wang D. AC002454.1 and CDK6 synergistically promote endometrial cell migration and invasion in endometriosis. Reproduction. 2019; 157:535–43.
https://doi.org/10.1530/REP-19-0005 PMID:30884467

29. Zhang L, Li J, Hao C, Guo W, Wang D, Zhang J, Zhao Y, Duan S, Yao W. Up-regulation of exosomal mir-125a in pneumoconiosis inhibits lung cancer development by suppressing expressions of ezh2 and hnrnpk. RSC Advances. 2018; 8:26538–48.
https://doi.org/10.1039/C8RA03081B

30. Zhang L, Hao C, Zhai R, Wang D, Zhang J, Bao L, Li Y, Yao W. Downregulation of exosomal let-7a-5p in dust exposed- workers contributes to lung cancer development. Respir Res. 2018; 19:235.
https://doi.org/10.1186/s12931-018-0949-y
PMID:30497474

31. Zhang L, Hao C, Yao S, Tang R, Guo W, Cong H, Li J, Bao L, Wang D, Li Y, Yu X, Duan S, Yao W. Exosomal miRNA Profiling to Identify Nanoparticle Phagocytic Mechanisms. Small. 2018; 14:e1704008.
https://doi.org/10.1002/smll.201704008
PMID:29516679

32. Araujo FM, Meola J, Rosa-E-Silva JC, Paz CC, Ferriani RA, Nogueira AA. Increased expression of ID2, PRELP and SMOC2 genes in patients with endometriosis. Braz J Med Biol Res. 2017; 50:e5782.
https://doi.org/10.1590/1414-431x20175782
PMID:28678915

33. Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, Print CG, Hull LM. MicroRNA-regulated pathways associated with endometriosis. Mol Endocrinol. 2009; 23:265–75.
https://doi.org/10.1210/me.2008-0387
PMID:19074548

34. Zhang D, Li Y, Tian J, Zhang H, Wang S. MiR-202 promotes endometriosis by regulating SOX6 expression. Int J Clin Exp Med. 2015; 8:17757–64.
PMID:26770366

35. Rekker K, Tasa T, Saare M, Samuel K, Kadastik Ü, Karro H, Götte M, Salumets A, Peters M. Differentially-

expressed mirnas in ectopic stromal cells contribute to endometriosis development: the plausible role of mir-139-5p and mir-375. Int J Mol Sci. 2018; 19:3789.
https://doi.org/10.3390/ijms19123789
PMID:30487429

36. Liu Y, Chen J, Zhu X, Tang L, Luo X, Shi Y. Role of miR-449b-3p in endometriosis via effects on endometrial stromal cell proliferation and angiogenesis. Mol Med Rep. 2018; 18:3359–65.
https://doi.org/10.3892/mmr.2018.9341
PMID:30066926

37. Ghazal S, McKinnon B, Zhou J, Mueller M, Men Y, Yang L, Mueller M, Flannery C, Huang Y, Taylor HS. H19 lncRNA alters stromal cell growth via IGF signaling in the endometrium of women with endometriosis. EMBO Mol Med. 2015; 7:996–1003.
https://doi.org/10.15252/emmm.201505245
PMID:26089099

38. Shao J, Zhang B, Yu JJ, Wei CY, Zhou WJ, Chang KK, Yang HL, Jin LP, Zhu XY, Li MQ. Macrophages promote the growth and invasion of endometrial stromal cells by downregulating IL-24 in endometriosis. Reproduction. 2016; 152:673–82.
https://doi.org/10.1530/REP-16-0278 PMID:27624484

39. Olivares C, Ricci A, Bilotas M, Barañao RI, Meresman G. The inhibitory effect of celecoxib and rosiglitazone on experimental endometriosis. Fertil Steril. 2011; 96:428–33.
https://doi.org/10.1016/j.fertnstert.2011.05.063
PMID:21683949

40. Zhao Y, Li Q, Katzenellenbogen BS, Lau LF, Taylor RN, Bagchi IC, Bagchi MK. Estrogen-induced CCN1 is critical for establishment of endometriosis-like lesions in mice. Mol Endocrinol. 2014; 28:1934–47.
https://doi.org/10.1210/me.2014-1080
PMID:25321413

41. Tang L, Wang TT, Wu YT, Zhou CY, Huang HF. High expression levels of cyclin B1 and Polo-like kinase 1 in ectopic endometrial cells associated with abnormal cell cycle regulation of endometriosis. Fertil Steril. 2009; 91:979–87.
https://doi.org/10.1016/j.fertnstert.2008.01.041
PMID:18353325

42. Shen L, Zhang Y, Zhou W, Peng Z, Hong X, Zhang Y. Circular RNA expression in ovarian endometriosis. Epigenomics. 2018; 10:559–72.
https://doi.org/10.2217/epi-2017-0079
PMID:29334789

43. Zhang M, Ren C, Xiao Y, Xia X, Fang X. Expression profile analysis of circular rnas in ovarian endometriosis by microarray and bioinformatics. Med Sci Monit. 2018; 24:9240–50.

https://doi.org/10.12659/MSM.913885
PMID:30566420

44. Choi HJ, Park MJ, Kim BS, Choi HJ, Joo B, Lee KS, Choi JH, Chung TW, Ha KT. Transforming growth factor β1 enhances adhesion of endometrial cells to mesothelium by regulating integrin expression. BMB Rep. 2017; 50:429–34.
https://doi.org/10.5483/BMBRep.2017.50.8.097
PMID:28760197

45. Goetz LG, Mamillapalli R, Taylor HS. Low body mass index in endometriosis is promoted by hepatic metabolic gene dysregulation in mice. Biol Reprod. 2016; 95:115.
https://doi.org/10.1095/biolreprod.116.142877
PMID:27628219

46. Mantovani F, Collavin L, Del Sal G. Mutant p53 as a guardian of the cancer cell. Cell Death Differ. 2019; 26:199–212.
https://doi.org/10.1038/s41418-018-0246-9
PMID:30538286

47. Lin SY, Lee RK, Hwu YM, Lin MH. Reproducibility of the revised American Fertility Society classification of endometriosis using laparoscopy or laparotomy. Int J Gynaecol Obstet. 1998; 60:265–69.
https://doi.org/10.1016/S0020-7292(97)00247-6
PMID:9544711

48. Rock JA, and ZOLADEX Endometriosis Study Group. The revised American Fertility Society classification of endometriosis: reproducibility of scoring. Fertil Steril. 1995; 63:1108–10.
https://doi.org/10.1016/S0015-0282(16)57556-6
PMID:7720925

49. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Vlachos IS, Vergoulis T, Reczko M, Filippidis C, Dalamagas T, Hatzigeorgiou AG. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. Nucleic Acids Res. 2013; 41:W169–73.
https://doi.org/10.1093/nar/gkt393 PMID:23680784

50. Paraskevopoulou MD, Georgakilas G, Kostoulas N, Reczko M, Maragkakis M, Dalamagas TM, Hatzigeorgiou AG. DIANA-LncBase: experimentally verified and computationally predicted microRNA targets on long non-coding RNAs. Nucleic Acids Res. 2013; 41:D239–45.
https://doi.org/10.1093/nar/gks1246 PMID:23193281

51. Zhang Y, Xu Y, Feng L, Li F, Sun Z, Wu T, Shi X, Li J, Li X. Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers. Oncotarget. 2016; 7:64148–67.
https://doi.org/10.18632/oncotarget.11637
PMID:27580177

52. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009; 25:1091–93.
https://doi.org/10.1093/bioinformatics/btp101
PMID:19237447

53. Merico D, Isserlin R, Stueker O, Emili A, Bader GD. Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. PLoS One. 2010; 5:e13984.
https://doi.org/10.1371/journal.pone.0013984
PMID:21085593
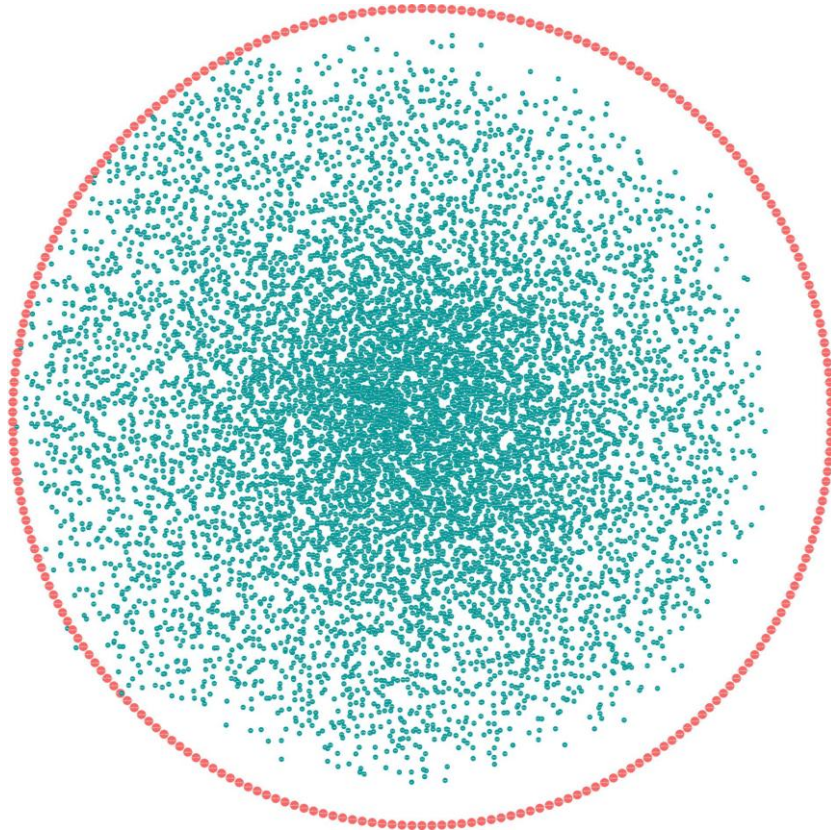
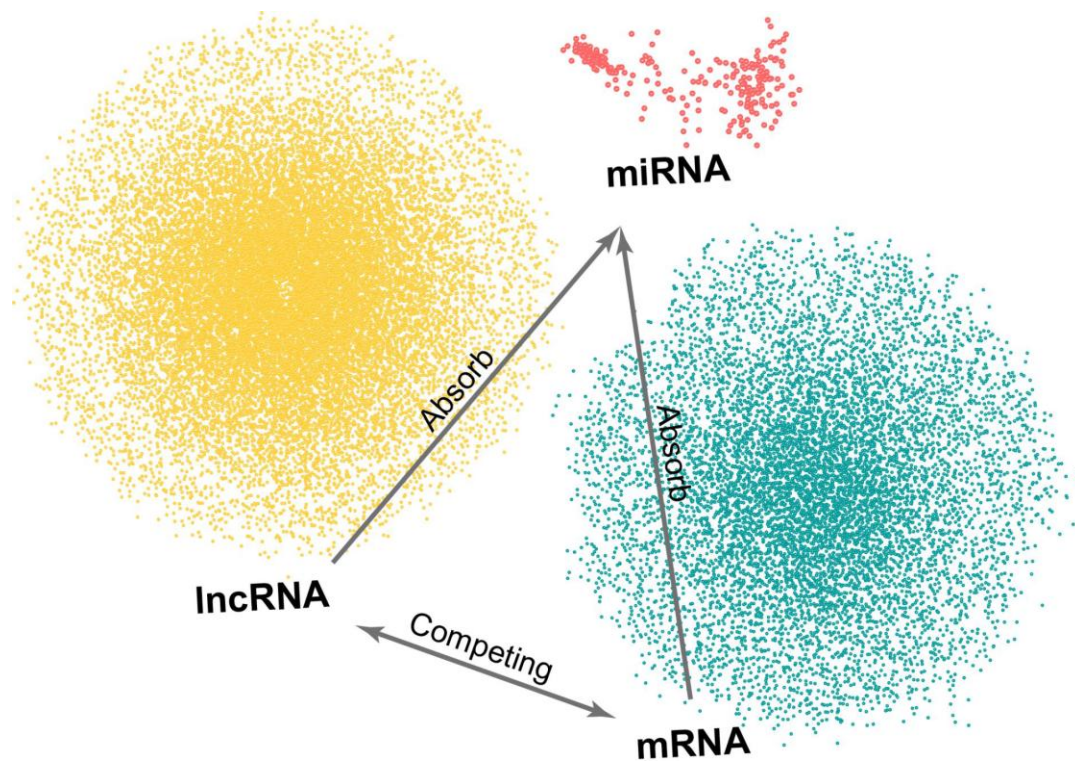**Supplementary Figure 1. Target genes of the differentially expressed miRNAs in endometriosis.** Pink dots, miRNA, blue dots, mRNA.
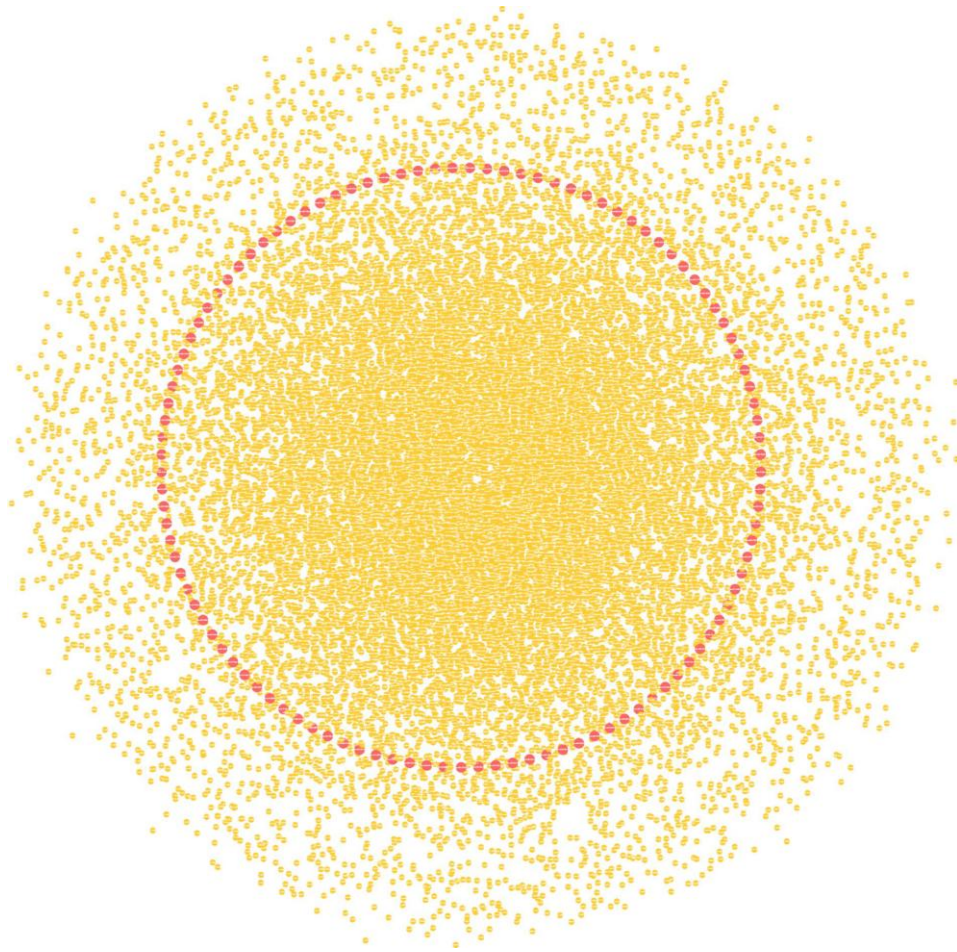
Supplementary Figure 2. Target lncRNAs of the differentially expressed miRNAs in endometriosis. Pink dots, miRNA, yellow dots, lncRNAs.

Supplementary Figure 3. Triple network of mRNA-miRNA-lncRNA in endometriosis. Pink dots, miRNA, yellow dots, lncRNAs, blue dots, mRNA.

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 1–3

Supplementary Table 1. Full list of differentially expressed genes in ectopic endometrium.

Supplementary Table 2. Full list of differentially expressed miRNAs in ectopic endometrium.

Supplementary Table 3. Full list of differentially expressed lncRNAs in ectopic endometrium.