Plasma MicroRNAs Can be a Potential Diagnostic Biomarker for Endometriosis

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Abstract

Plasma microRNAs are considered to be potential diagnostic biomarkers for endometriosis. Increasing evidence has shown that a huge amount of miRNAs are abnormally expressed in endometriosis plasma and play irreplaceable roles in diagnosis. The aim of the our study was to identify the differential expression of circular miRNA by reviewing the PubMed, ScienceDirect, and Cochrane databases between normal women and women with endometriosis and analyzing the miRNA data downloaded from the GEO database. Because of the differential miRNA expression in this review, we evaluated the diagnostic values of the differentially expressed miRNAs, particularly during the menstrual phases. According to the cut-off criteria with |log 2 FC|>1.0 and $P < 0.05$, 36 differentially expressed miRNAs were identified, including 13 upregulated miRNAs and 23 downregulated miRNAs. We developed miR-155, miR-574, miR-23a, and miR-520d via a Venn diagram. Functional enrichment analysis considered that the target miRNAs might be involved in various pathways related to endometriosis, including neurotrophin, Hippo, oocyte meiosis, ubiquitin mediated proteolysis, HTLV-Infection, FoxO, and Rap1 signaling pathways. CTNNB1, MYC, and ES R1 of transcription factors were related to the differentially expressed miRNAs. In summary, our study suggested that a four-miRNA could be included as a prognostic marker in endometriosis.

Introduction

MicroRNAs (miRNAs) are composed of 21-23 nucleotides. miRNAs have the characteristics of high conservation, timing, and tissue specificity. miRNAs are stable in serum and may be used as non-invasive diagnostic indicators of diseases [1Chen L,2015]. Two small non-coded RNAs, namely, Lenin4 and elet-7, which have been recognized as being related to various human diseases, were found in *Caenorhabditis elegans* for the first time by Lee in 1993 [2Lee RC1993, 3Qasquinelli AE2000]. Global expression profiling studies have identified hundreds of misaligned miRNAs in several diseases. miRNAs are involved in a number of steps, such as inclusion, addition, transfer out of the nucleus, processing in the fine cytoplasm, and translation or stimulation [4LIM LP2005].

miRNAs mature by not fully binding to the 3' end of the non-coding region of the target gene,

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inhibition of their translation, or binding to RNA silent complexes composed of multiple proteins. Target gene expression can be suppressed by completely binding to the non-coding region of the target gene 3'[5Teague EM2010, 6Caporali A2011]. It is important that miRNA can target multiple mRNA expressions, and one mRNA can be regulated by multiple miRNAs at the same time through this complex post-transcription regulatory network [7Bartel DP2004, 8Lagos-Quintana M2001]. miRNA is involved in almost all pathophysiological processes in the body. To date, more than 1,881 miRNA precursors, encoding more than 2500 miRNAs, have described as mature miRNA in humans. With the increased understanding of the mechanism of action of miRNAs and the study of the biogenesis, function, role, and characterization of miRNA, candidate biomarkers for many diseases have emerged, such as cancer, coronary artery disease, and gynecological diseases, including endometriosis [9-13Mitchell2008,Small2011,Lawrie2008,Alessandra2010, Vodolazkaia2012]. Therefore, miRNAs have substantial potential as promising markers for diagnosis, prognosis and personalized targeting.

Endometriosis (EMS) refers to a common estrogen-dependent chronic disease in the endometrium (glands and interstitial substances) that occurs in other parts of the uterus and affects nearly 10 % of women of childbearing age [14-16Giudice2004,Tamaresis2014,Giudice2010]. This disease primarily causes pelvic pain and infertility. The prevalence of this disorder is an estimated global average of 176 million individuals, in whom the diagnosis is delayed by 7 years, and the mean diagnostic age is 32.5-36.4 years, depending on the research population[17Tokushige2011]. Although significant progress has been made in the study of the etiology and pathogenesis of EMS, unfortunately, compared to other chronic diseases, it is difficult to diagnose, and the diagnosis of EMS is often delayed because there are currently no accurate, accessible and non-invasive diagnostic tools. Early diagnosis and treatment of EMS remain difficult. Therefore, it is urgent to further explore the etiology and pathogenesis of EMS to identify specific and sensitive detection indicators and treatment targets and provide new ideas and strategies for early clinical diagnosis and treatment [18Burney2012]. In several studies, a specific miRNA has been identified as a potential biomarker of the disease. These and other miRNAs have been associated with target genes and functional pathways in the disease-specific pathophysiology. The occurrence of endometriosis involves various factors, such as hormones, inflammatory factors, and hypoxic microenvironments. In recent years, studies have shown that tiny RNA also plays an important role in the development of endometriosis. There are differences in the expression of miRNA between ectopic endometrial tissue cells and normal tissue cells. These differences in the expression of miRNA may be related to the occurrence and development of EMS. The expression pattern of miRNA in the endometrium in endometriosis is based on patients and control women as well as different individuals who have endometriosis. miRNA may be an attractive candidate for new diagnostic markers and treatment interventions for endometriosis. These small non-coding molecules have become attractive candidates as new biomarkers for early non-invasive diagnosis [19-22Jia2013,Fessbender2010,Cho2015,Cosar2016]. Study of this disease may lead to valuable benefits for patients by reducing the recurrence rates in terms of prognosis and improvements.

In this study, a systematic review was conducted of the key serum miRNAs predicted for endometriosis diagnosis. GEO (Gene expression omnibus) is a gene expression database created and maintained by the NCBI (National Biotechnology Information Center of the United States). The purpose of this study is to identify miRNA data downloaded from the GEO database to determine serum differences between normal women and patients with endometriosis. In miRNA

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high-throughput analysis, miRNA target genes are shown to be differentially expressed and their function is annotated, and a miRNA feature that can effectively diagnose endometriosis is constructed. In addition, the TFactS database was analyzed using analytical transcription factors. This study shows the importance of miRNA in the diagnosis of endometriosis.

Methods and materials

Systematic review

A systematic review was conducted of all the pertinent studies that were identified in the electronic PubMed, ScienceDirect, and the Cochrane Central Register of Controlled Trials (CENTRAL) databases that examined plasma microRNAs as potential diagnostic biomarkers for endometriosis from 1966 to January 2019. The search strategy included the terms miRNA, microRNA, circular, blood, serum, and plasma. The search was concluded by (1) perusal of the reference sections of all relevant studies in English and (2) a manual search of the key journals and abstracts from the major annual meetings in the fields of endocrinology and obstetrics and gynecology. Articles were excluded from the analysis that lacked adequate disease-matched control groups. The control groups consisted of women without endometriosis.

Screening of the endometriosis miRNA expression dataset

The Series Matrix file of GSE46735 was downloaded from the GEO (Http://www.ncbi.nlm.nih.gov/geo/) database. The inclusion criteria were as follows: (1) Diagnosis of endometriosis and a normal female control group; (2) sample sequencing data and clinical information of miRNA; and (3) processed bold parts in the properties of natural cells. The platforms included GPL 15634 (Applied Biosystems Human TaqMan Low Density Array (TLDA, v2 .0, Card A)) and GPL 15647 (Applied Life, dispensers TaunqMan Dense). The datasets of GSE46735 were used to recalibrate the relationship between control women and women with quiet division endometriosis (N = 8 in each group). Each programmed soft space in the early portfolio included the public and personal spaces of the natural class (N = 47 total spaces). The better case is better than the better. RNA was made available to study the identified microRNAs. miRNA sequencing data were processed using R language packets. The difference between endometriosis and normal female blood samples expressed by miRNAs was analyzed by Lima packets in R. Multiples in individual expression (FCs) were used to calculate miRNA and express miRNA considerations and GT with $|\log2\ FC|>1.0$ and $P < 0.05$. Importantly, differential expression of miRNA at different stages of the menstrual cycle was associated with the diagnosis of endometriosis. Differentiated expression of the miRNA spectrum was normalized by log2 conversion. We used FunRich (Http://www.Funrich.org) to obtain the overlapping differential expression of miRNA among GSE46735. A Vern diagram and volcano map were also constructed by FunRich. We used Heml 1.0 (Http://hemi.biocuckoo.org/down.php) to obtain the differential expression of miRNA among GSE 46735. A heatmap was also constructed by Heml.

Prediction of the functional enrichment of microRNA target genes in endometriosis serum

Identification of miR target genes was performed with Targetscan (Http://www.targetscan.org/), miRanda (http://miranda.org.uk), miRDB (Http://www.mirdb.org/mirdb/), Pictar (https://pictar.mdc-berlin.de), miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/), and RNA22 (http://cbcsrv.watson.ibm.com/rna22.html/) online analysis tools. To improve the reliability of the bioinformatic analysis, a Venn diagram was used to identify overlapping target genes. Then, The Date for Annotation, Visualization and Intrusion analyze the overlapping gene bioinformatic tool (DAVID) (Https://david.ncifcrf.gov/) was used. DAVID is the web-based,

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onlined and bioinformatic tool designed to provide investigators with a complete series of functional annotation tools to identify biological mechanisms associated with a number of genes or proteins. GO (Gene Ontogirl) and KEGG (Kyoto Engineering of Genes and Genomes) pathway events were analyzed for particular genes. A P-value < 0.05 was set as the cut-off for significance.

Analysis of the regulation of miRNA targeting in endometriosis serum was used to determine up- and downregulation of miRNA (Essaghir et al.) using the TFactS database (2010) (Http://www.tfacts.org/). Only four indicators (P value, Q value, E value, and FDR) were used in advance to indicate that a value which was less than 0.05 was considered to be a reliable transcription factor scope. State on translation factors (TF) of target DEMs of up and down-protected miRNA regulated objects where we have to go to the special study.

Results

1 Systematic review

The electronic search strategy identified 160 potentially relevant articles (PubMed, 136; ScienceDirect, 24; and Cochran Library, 0), which filtered articles by the title, summary, full text, or a combination of these factors. Of these 160 articles, 84 articles were excluded because of not meeting the inclusion criteria after reading the abstracts. The full studies of the remaining 76 studies, which focused on miRNAs used in the diagnosis of endometriosis, were then carefully read. An additional 65 articles were excluded because the sample originated from peritoneal fluid or urine. In particular, we excluded trials as follows: data on the diagnosis of endometriosis via a blood test identifying microRNAs were not available from the papers and could not be obtained from the investigators by e-mail contact and the microRNA detection method used reverse transcriptase quantitative real-time PCR. Eleven studies that investigated the role of miRNA expression changes as blood biomarkers in endometriosis samples were included. Eleven studies analyzed the expression of miRNAs by comparing endometriosis cases vs healthy controls [19, 21-31 Jia2013,Cho2015,Cosar2016,Suryawanshi2013,Wang2013,Wang2016,Nothnick2016,Maged2018, Wang2018,Rekker2015,Pateisky2018,Shakiba2008]. A total of 472 endometriosis serum samples and 357 normal corresponding serum samples were collected (some articles studied serum and some studies studied plasma; for simplicity, we used serum instead of serum/plasma). The subjects' age in the studies ranged from 26 to 53 years old. Table 1 summarizes the quality of the trials included in the review.

By summarizing 11 studies that studied the difference in the expression of miRNA in peripheral blood between endometriosis patients and normal individuals, the expression of miRNA was obtained, and the increased expressions included： miRNA-365， -125b, -150, -342, -143, -145, -500a, -451a, -18a, -154, -196b, -378a, -33a, -199a, -122, -4645, -636, -24-2, -3127, -185, -542, -502, -296, -550a, -424, -451a, -16, -191, -195, -1978, -1979, -4284, -1973, and -1974； the decreased expressions included：miRNA-let7，-135a, -200a, -141, -363, -6755, -145, -141, -542, -9, -889,-432, -1381, -410, -584, -99b, -127, -30c, -215, and -17. Quantitative real-time polymerase chain reaction detected the expression in blood and peritoneal fluid (PF) samples for miR-122 and miR-199a, and serum miR-122 and miR-199a detected endometriosis with a sensitivity of 95.6 and 100.0 and specificity of 91.4 and 100, respectively. MiR-199a ($P<0.05$) and miR-122 could be used to distinguish between severe and mild endometriosis. MiR-199a was closely related to pelvic adhesion and lesions ($P <0.05$) and was also related to hormone mediated signaling pathways. Moreover, it was confirmed that the best combinations of miR-199a, miR-122,

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miR-145 and miR-542-3p were reliable in terms of sensitivity and specificity, and the tested feature lines (Receiver Opera Charitable Curve, ROC) Under the Curve Area (Area Under Curve, AUC) was 0.994 (95 % CI: 0.984 to 1.000). In addition, the AUC associated with miR-17-5p, miR-20a, and miR-22 was 0.9 (95 % CI: 0.8-1.0). At the same time, the combination of serum le-7b, 7D and 7f during the proliferation period could be used as a diagnostic marker for endometriosis according to the differential expression of circulating miRNA between the endometriosis and control groups. The level of miRNA varied with the time of blood collection, and miR-200a and miR-141 have potential as new non-invasive biomarkers of endometriosis. In addition, the plasma levels of miR-200a, miR-200b and miR-141 varied with the sampling time; thus, the sampling time is critical. The specificity and sensitivity of plasma miR-17-5p, miR-20a and miR-22 in the diagnosis of phase III/IV endometriosis were 90.0 and 70.0, respectively [19Jia2013].

2 GEO analyses

In the present study, 242 differentially expressed miRNAs in GSE46735 were identified in the plasma of endometriosis samples compared to control samples. Among the differentially expressed miRNAs, 124 miRNAs were upregulated, while 118 miRNAs were downregulated. The hierarchical clustering heat map is shown in Fig1, and the volcano map is shown in Fig2. The identified miRNAs were well distinguished from differentially expressed miRNAs.

Has-miR-155-5p, hsa-miR-128-3p, hsa-miR-1-3p, and hsa-miR-532-5p of the upregulated differentially expressed miRNAs (LogFC>1, P<0.05) and hsa-miR-574-3p, hsa-miR-23a-3p, hsa-miR-520d-5p, hsa-miR-433-3p, hsa-miR-485-5p, and hsa-miR-122-5p of the downregulated differentially expressed miRNAs (LogFC<-1, P<0.05) were significantly different. With respect to patients who provided blood samples in the early proliferative, late proliferative and mid luteal phases of the menstrual cycle (n = 47 total plasma samples), the cycle phase was verified according to the hormonal profile. RNA was extracted from each sample, and the expression of microRNAs was assessed using TaqMan Low Density Human miRNA arrays. Has-miR-155, hsa-miR-218, hsa-miR-301b, hsa-miR-128, hsa-miR-532-5p, hsa-miR-22, hsa-miR-1, hsa-miR-339-5p, and hsa-miR-143 in early proliferation; has-miR-155, hsa-miR-218, hsa-miR-532-5p, hsa-miR-22, hsa-miR-1, hsa-miR-339-5p, hsa-miR-331-5p and hsa-miR-362-3p in late proliferation; and has-miR-155, hsa-miR-218, hsa-miR-301b, hsa-miR-128, hsa-miR-133a, and hsa-miR-143 in the mid luteal phase were upregulated and differentially expressed (LogFC>1, P<0.05). Has-miR-574-3p, hsa-miR-23a, hsa-miR-500, hsa-miR-98, hsa-miR-7f, hsa-miR-451, hsa-miR-122, hsa-miR-520d-5p, hsa-miR-15a and hsa-miR-409-5p in early proliferation, hsa-miR-574-3p, hsa-miR-23a-3p, hsa-miR-98, hsa-miR-122, hsa-miR-874, hsa-miR-381, hsa-miR-520d-5p, hsa-miR-452, hsa-miR-369-3p, hsa-miR-224, hsa-miR-502-5p, hsa-miR-320, hsa-miR-433 and hsa-miR-23b in late proliferation and has-miR-574-3p, hsa-miR-382, hsa-miR-23a, hsa-miR-10b, hsa-miR-485-5p, hsa-miR-520d-5p, hsa-miR-433, hsa-miR-452, hsa-miR-130b and hsa-miR-874 in the mid luteal phase were downregulated and differentially expressed (LogFC<-1,P<0.05) and were significantly different. The consistently upregulated and downregulated genes in independent cohorts in all three phases were identified using Venn analysis, and a Venn diagram was generated by FunRich (Fig3). As a result, we identified has-miR-155-5p as having unregulated expression and hsa-miR-574-3p, hsa-miR-23a-3p, and hsa-miR-520d-5p as having downregulated expression.

3 Target prediction and function analysis.

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The target genes of four miRNAs were predicted using the TargetScan, miRDB, RNA22, miRWalk and miRanda online analysis tools. Thirty-nine overlapping genes of miR-155-5p, 4 overlapping genes of miR-574-3p, 70 overlapping genes of miR-23a-3p, and 107 overlapping genes of miR-520d-5p were identified. Enrichment analysis of the target genes was subsequently performed to elucidate the biological function of the consensus target genes.

The biological processes (BP) were mainly enriched in axon guidance, peptidyl-tyrosine phosphorylation, negative regulation of an extrinsic apoptotic signaling pathway, positive regulation of cell migration, positive regulation of transcription, liver development, transmembrane receptor protein tyrosine kinase signaling pathway, positive regulation of transcription from RNA polymerase II promoter, retinal ganglion cell axon guidance, and negative regulation of transcription from RNA polymerase II promoter (Fig4-1). The cellular components (CC) were significantly enriched in the cell-cell adherens junction, transcriptional repressor complex, cell surface, protein-DNA complex, Golgi membrane, cytoplasm, cytosol, nucleus, nucleoplasm, and perinuclear region of cytoplasm (Fig4-2). The KEGG pathways that were primarily significantly enriched were the neurotrophin signaling pathway, hippo signaling pathway, oocyte meiosis, ubiquitin mediated proteolysis, HTLV-Infection, FoxO signaling pathway, Rap1 signaling pathway, pathways in cancer, signaling pathways regulating pluripotency of stem cells, and osteoclast differentiation (Fig4-3). In addition, the molecular functions (MF) were mainly enriched in ubiquitin protein ligase binding, insulin-like growth factor receptor binding, chromatin binding, ubiquitin protein ligase activity, SMAD binding, transcription corepressor activity, transcriptional repressor activity, protein binding, and RNA polymerase II core promoter proximal region sequence-specific DNA binding (Fig4-4).

4 Analysis of the transcription factors (TFs) of target genes

The corresponding TFs were analyzed and compared. The results showed that there were 61 TFs corresponding to the target. Among them, 56 genes correspond to upregulated relating TFs and 17 genes correspond to downregulated relating TFs. Comparative analysis suggested that of the 61 TFs, 12 TFs were shared between the two target genes, accounting for 19.67 % of the total TFs. In addition, the TFs corresponding to the regulation of miRNA target genes showed high specificity, consistent with the results of the GO analysis, and enriched the regulation of miRNA target genes during the regulation of transcription. According to the results of the analysis, the main TFs with credibility E values and cross ratios were not more than 0.05, and only the TFs CTNNB1, MYC, and ES R1 had a hard cross rate.

Discussion

Endometriosis is the main cause of pelvic pain and low fertility. However, it is difficult for the diagnosis of endometriosis, and there is no clear diagnostic biomarker. Laparoscopy is currently the gold standard for the endometriosis diagnosis; however, it is traumatic. Many clinicians evaluate a series of clinical symptoms of endometriosis prior to seeking a definitive diagnosis by laparoscopy. Moreover, experimental treatment drugs have significant side effects and are typically not completely eradicated [31, 32Shakiba2008,Johnson2013]. At the same time, 70-75% of visually diagnosed lesions are confirmed histologically in laparoscopy, thus hindering their widespread use [33Nnoaham2011]. In addition, CA125 is only 21-50% sensitive for the diagnosis of endometriosis. Therefore, there is a need to develop a non-invasive diagnostic test for endometriosis. In recent years, studies on miRNAs have shown that their expression levels are closely related to the occurrence, development and metastasis of EMS; thus, it miRNAs are

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expected to be non-invasive diagnostic markers for EMS.

miRNA achieves the regulation of EMS through its regulation[34Pan2008]. Using TaqMan microRNA chips to detect changes in serum miRNA expression levels in EMS patients and healthy control groups, studies showed that miR-199 and miR-122 were increased in the serum of EMS patients compared to healthy control groups[24Wang2013]. miR-141, miR-9, MiR-145, and miR-542-3p were downregulated, and miR-199 and miR-122 could be used to distinguish between severe and mild EMS patients. In addition, the area under the ROC curve measured jointly by miR-199, miR-122, miR-145 and miR-542-3p was 0.994, and the sensitivity and specificity were 93.22% and 96.00%, respectively. It was proven that the combined detection of miR-199, miR-122, miR-145 * and miR-542-3p as non-invasive biomarkers of EMS had significant diagnostic significance. At the same time, it was found that 27 miRNAs were differentially expressed in the serum of EMS patients compared to the healthy control group using TaqMan microRNA chips [19Jia2013]. After testing with Real-time PCR, it was found that miR-17-5p, miR-20a and miR-22 showed significant downward expression, indicating that these miRNAs can be used as serum markers to diagnose endometriosis. EMS is characterized by the growth of the endometrium outside the endometrium. This process is closely related to factors such as vascular endothelial growth factor-A, which regulates angiogenesis, and thrombin-sensitive protein, miR-222, and miR-17-5p, which regulate the expression of angiogenic factors and play important roles in the pathogenesis of EMS. MiR-199a can inhibit the invasion of endometrial stromal cells by inhibiting the IKKβ/NF-κB signaling pathway and decreasing IL-8 expression, and it can be used as a serum marker for metastasis in EMS patients [36, 37Dai2012,Ramon2011]. Therefore, circulating miRNA can be used as a biomarker for the early diagnosis of small and mild endometriosis.

Because of the differential expression of miRNAs in the review, we downloaded a dataset from the GEO database to validate the exact plasma miRNA levels. Unexpectedly, we found that miR-155, miR-128, miR-1 and miR-532 of the upregulated miRNAs and miR-574, miR-23a, miR-520d, miR-433, miR-485 and miR-122 of the downregulated miRNAs were differentially significantly expressed and associated with the diagnosis of endometriosis patients in the present study. With respect to the phases of the menstrual cycle, we found that miR-155, which upregulated miRNA expression, and miR-574, miR-23a, and miR-520d, which downregulated miRNAs expression, could be used as a multi-marker based model to provide more powerful information for the prediction of EMS in patients. When it is performed enrichment analysis of the four-miRNAs for the prediction and function analyses of biological processes, cellular components, KEGG pathways, and molecular function. Furthermore, we also assessed the miRNA target genes during the regulation of transcription. The results of the functional enrichment analysis implied that the three target genes of miRNAs related to endometriosis might be involved in various pathways, including neurotrophin, Hippo, oocyte meiosis, ubiquitin mediated proteolysis, HTLV-Infection, FoxO, and Rap1 signaling pathways. Unsurprisingly, only the transcription factors CTNNB1, MYC, and ES R1 agreed with this conclusion.

In short, in recent years, with the in-depth study of miRNAs, the different stages of disease occurrence and development have been shown to be accompanied by changes in miRNA expression, and a deep understanding of miRNAs helps to scientifically grasp the internal mechanism of disease occurrence. In addition, miRNA expression levels are expected to be important markers for disease diagnosis, treatment selection, efficacy evaluation, and prognosis

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evaluation. In summary, a number of miRNAs have been found to be differentially expressed in the plasma of women with endometriosis, and the mechanism of serum miRNA dysregulation remains unknown. To date, as indicated by the different results from the review and microarray datasets, circulating miR-155, miR-574, miR-23a, and miR-520d may be powerful biomarkers for diagnosis of endometriosis, accurate chemotherapy and targeted therapy; however, additional research is required to determine the repeatability and consistency of the results. These findings provide new insights into the early diagnosis and detection of endometriosis.

Conclusions

Comprehensive analysis of the pooled data provides strong evidence that circulating unregulated miR-155 expression and downregulated miR-574, miR-23a, and miR-520d expression are significantly associated with the diagnosis of endometriosis. Abnormal expression of aberrant miR-155 and low expressions of miR-574, miR-23a, and miR-520d may be promising diagnostic biomarkers for non-invasive endometriosis testing.

Author Contributions

Zhuo zhihong contributed to the conception of the study. Zhuo zhihong and Wang chuhan contributed significantly to analysis and manuscript preparation. Zhuo zhihong, Gao li, and Yu huimi performed the data analyses and wrote the manuscript.

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Conflict of Interest

No conflict of interest

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