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DNA methylation profiles of ovarian cysts resemble ovarian tissues but not endometrial tissues

Xiaohui Zhang¹, Xiaojing Zhao², Jiapo Wang¹, Yifang Zhang³ and Jinhong Chen^{1*}

Abstract

Introduction Endometriosis is a heritable, complex chronic inflammatory disease, for which much of the causal pathogenic mechanism remain unknown. Despite the high prevalence of ovarian chocolate cyst, its origin is still under debate.

Methods Prevailing retrograde menstruation model predicts that ectopic endometrial cells migrate and develop into ovarian chocolate cyst. However, other models were also proposed. Genome-wide association studies (GWASs) have proved successful in identifying common genetic variants of moderate effects for various complex diseases.

Results A growing body of evidence shows that the remodeling of retrograde endometrial tissues to the ectopic endometriotic lesions involves multiple epigenetic alterations, such as DNA methylation, histone modification, and microRNA expression. Because DNA methylation states exhibit a tissue specific pattern, we profiled the DNA methylation for ovarian cysts and paired eutopic endometrial and ovarian tissues from four patients. Surprisingly, DNA methylation profiles showed the ovarian cysts were closely grouped with normal ovarian but not endometrial tissues.

Conclusions These results suggested alternative origin of ovarian cysts or strong epigenetic reprogramming of infiltrating endometrial cells after seeding the ovarian tissue. The data provide contributing to the pathogenesis and pathophysiology of endometriosis.

Keywords Ovarian endometriosis, DNA methylation, Tissue of origin

Background

Ovarian endometriosis has a high prevalence and affects millions of women worldwide. The origin of endometriosis is still puzzling. It is generally considered to be derived from endometrium [1], however, other hypotheses were also provided. For instance, a latest report suggested that ~60% of endometriosis may originate from fallopian tube [2]. Ovarian origin recently, researchers found and confirmed the existence of cells expressing two germ cell line specific proteins (deadbox polypeptide4/interferon induced trans, membraneprotein3, ddx4/iftm3) in ectopic ovarian EMS lesions, suggesting that ovary-originated stem cell may contribute to the occurrence

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and progression of ovary EMS lesions [3]. To answer this question, we resorted to paired DNA methylation assay for ovarian endometriosis (ovarian endometrioma), normal ovarian and endometrial tissues in a small pilot cohort.

Epigenetic modifications regulate the transcriptional process, and abnormalities lead to various diseases. Among the most investigated and vital modifications, DNA methylation was linked to alterations in endometrial function/dysfunction-related genes, modulating cell proliferation, inflammation/immune response, angiogenesis, and steroid hormone response. These findings offer a deep understanding of epigenetic reprogramming and steroid hormone effects in endometrium aiding in the pathogenesis and pathophysiology of endometriosis [4–6].

Previous reports have shown that the endometriosis pathophysiology involves aberrant promoter methylation of several genes, for instance, HOXA10 [7], steroidogenic factor 1 [8] and aromatase [9]. As a new research area, genome-wide DNA methylation analysis can clarify the relationship between DNA methylation state and endometriosis. Our study utilized a microarray-based approach to determine the accurate DNA methylation level. The abnormal methylation expression between ovarian endometriosis cells, eutopic endometrium and ovarian tissues was analyzed.

Methods

Sample collection

Endometrium was biopsied from 4 fertile women receiving surgery for histologically confirmed unilocular/multilocular ovarian endometriosis (2:2). Normal ovarian tissue and eutopic endometrium of the same patient were used as controls. Specimens were obtained from subjects without hormone-based treatments at least 1 quarter before specimen acquisition and menstruation just clean, that is, endometrial hyperplasia. Upon surgical dissection, partial tissue was cryopreserved for microarray analyses. Endometrium and cysts were rinsed by Dulbecco's modified eagle's medium (DMEM) comprising of glutamine, 50 mg/ml streptomycin, and 50iu/ml penicillin (all from Invitrogen, Paisley, UK), and subsequently cut to a size of 1mm³. Then, ESC was isolated via screening with 70 mm nylon mesh after 2 h of collagenase (Sigma, Mo, USA) procession in a shaking incubator at 37°C. The filtrate was washed three times. The human investigation committee of Tongji University endorsed the research.

DNA methylation analysis

Illumina 850k methylation EPIC Bead Array data was processed through a standard pipeline using the

ChAMP package in R. The ChAMP can load raw intensity data in the form of IDAT or matrix containing methylation value (beta) of each probe [10]. After filtering and normalization, the 12 samples were combined into an array including only overlapping CpG sites and finally 743,800 probes were included. Principal component analysis (PCA) was firstly run on normalized methylation data to evaluate the similarity of methylation profile in 12 samples.

Bumphunter method was implemented to identify differentially methylated regions (DMR) within ChAMP (ChAMP.DMR function). The methylation beta value of each DMR was calculated using the mean value of all the probes that included in each DMR.

The heatmap was drawn using all the DMRs generated from ChAMP.DMR function. The $|\log_2(\text{FoldChange})| > 0.5$ and Family-wise error rate (FWER) adjusted $p\text{-value} < 0.05$ was considered a statistical significance threshold. Therefore, the DMR regions with a $|\log_2(\text{FoldChange})| > 0.5$ and $\text{FWER} < 0.05$ was considered as significantly differentially methylated regions. The DMRs region were annotated using ChIPseeker package in R bioconductor. The genomic regions for significantly differentially methylated regions were annotated with the definition of TSS3000 (3000 upstream and downstream of transcription initiation locus respectively) as the promoter region. Gene ontology (GO) functional enrichment analysis was done for the genes associated with differential methylation regions. And adjusted $p\text{-value} < 0.05$ was considered a statistical significance threshold.

Quantitative reverse transcription PCR

Total RNA was extracted from ectopic and eutopic samples. cDNA was obtained after reverse transcription reversal by Evo M-MLV RT Kit (Accurate Biology Co. Ltd, AG11601). The qRT-PCR was performed by Evo M-MLV One Step RT-PCR Kit (Accurate Biology Co. Ltd, AG11607). Each target gene was compared to β -actin. The expression of target mRNA was calculated based on 2- $\Delta\Delta\text{Ct}$ method.

Statistical analysis

All the statistical analyses were performed using R software (version 4.0.1). P-values less than 0.05 were considered statistically significant. The Kolmogorov-Smirnov test was applied to evaluate the normality of the distribution of the variables. For qRT-PCR, statistical analyses were conducted using a student t-test (data with normal distribution) or Mann-Whitney test (data with skewed distribution) as appropriate by GraphPad Prism software 9.0 (GraphPad Software Inc).

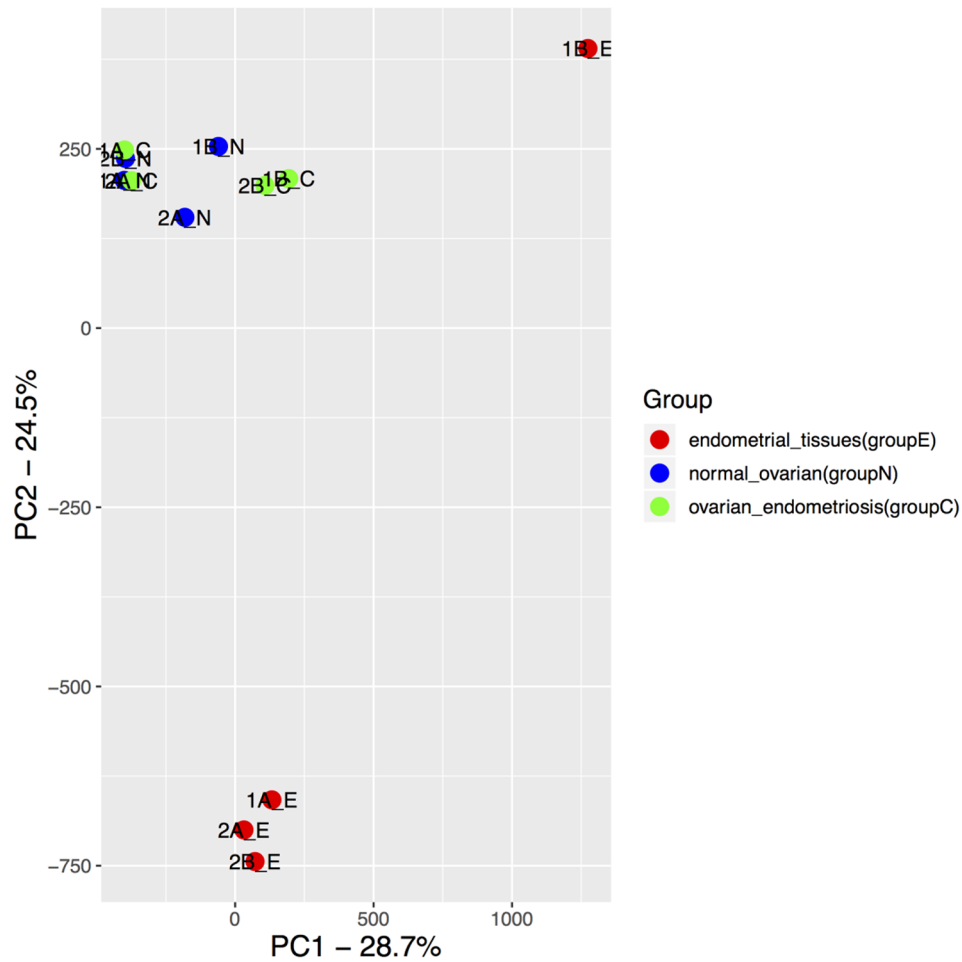


Fig. 1 PCA plot of DNA methylation data from endometriosis, normal ovarian and endometrial tissues

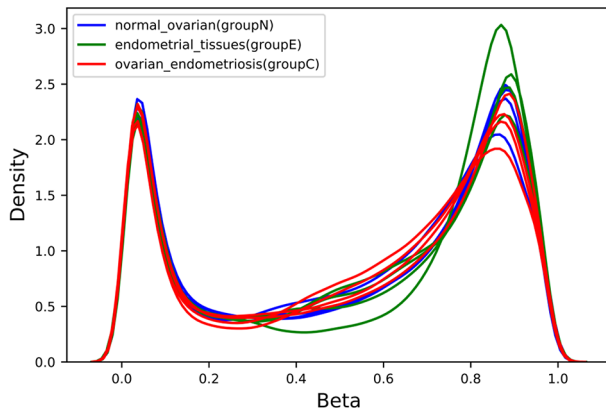


Fig. 2 One of endometrial sample was located further away from other samples as an outlier, and this sample also showed a slightly different normalized beta distribution compared with other samples

Results

DNA methylation profiles showed the ovarian cysts were closely grouped with normal ovarian but not endometrial tissues

Total of 12 samples from 4 patients, each with endometriosis, ovarian and endometrial tissues were profiled with illumina 850k methylation array. Principal component analysis clearly showed that endometriosis samples were tightly clustered with samples of ovarian tissue (Fig. 1). One of endometrial sample was located further away from other samples as an outlier, and this sample also showed a slightly different normalized beta distribution compared with other samples (Fig. 2). Then we plotted the heatmap of DNA methylation beta value matrix after normalization, and clustered the samples by hierarchical clustering. Again, 1B_E exhibited a hybrid DNA methylation profile.(Fig. 3) Therefore, we removed

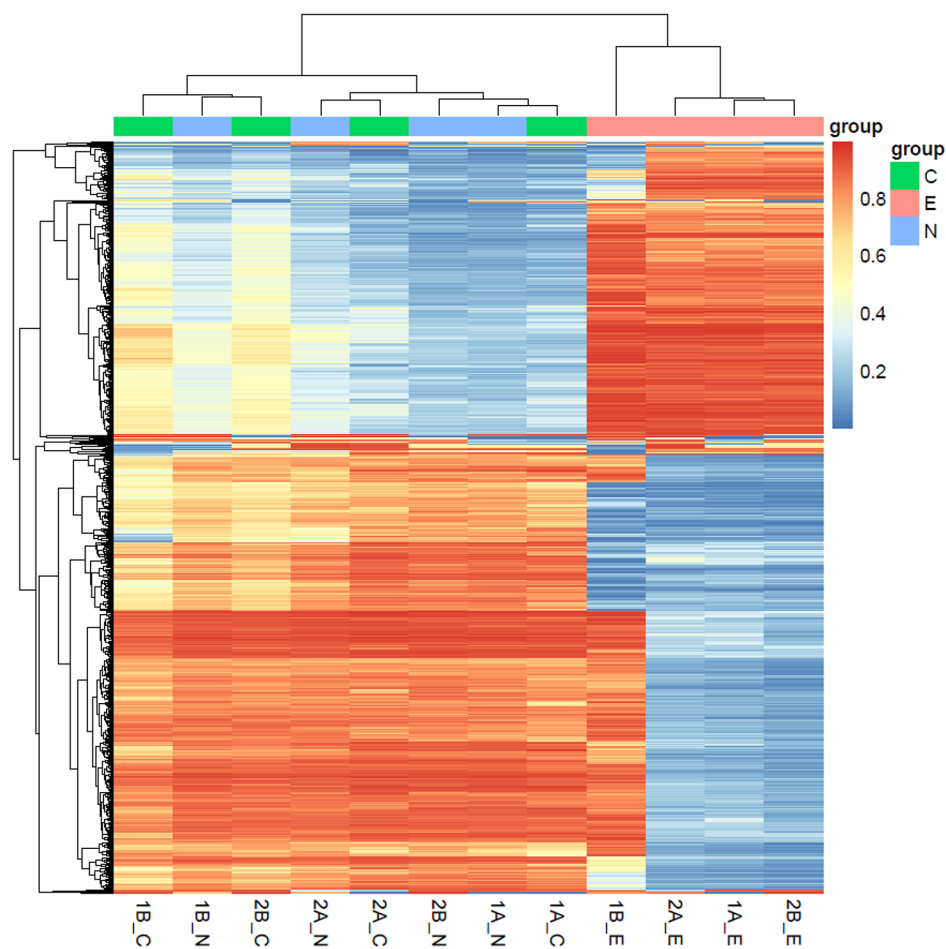


Fig. 3 Hierarchical clustering analysis of 12 samples based on methylation levels. Top 1000 CpGs with the highest variance among 12 samples were included. Color mapping from blue to red indicates methylation level from low to high. Group C: ovarian endometrial cysts; N: normal_ovarian; E: eutopic endometriosis

this sample for the downstream analyses. To confirm this result, we also performed PCA on our samples as well as publically available DNA methylation data on healthy endometrial biopsies [11], and as expected, healthy endometrial samples from public data were closer to our endometrial samples (Fig. 4).

As for DMRs analysis, we used the software ChAMP to explore the DMRs and the methylation beta value of each DMR was calculated using the mean value of all the probes that included in each DMR. The heatmap was generated using the beta value of DMRs. And from the heatmap we can see the ovarian endometrial cysts (group C) and normal_ovarian (group N) are clustered together, suggesting that the methylation profile of the cyst is more similar to ovarian tissues compared with endometrial tissue (Fig. 5).

We finally got 431 DMRs (with p -value <0.05) using the champ.DMR function. But the counts of DMRs with $|\log_2(\text{FoldChange})| > 0.5$ and adjusted p -value <0.05 was 55 (Fig. 6). Figure 7 shows the GO analysis of Genes with

lower methylation levels in eutopic endometriosis (group E) compared with in ovarian endometrial cysts (group C). And Fig. 8 shows the GO analysis of Genes with higher methylation levels in eutopic endometriosis (group E) compared with ovarian endometrial cysts (group C).

Abnormal epigenetic profiles of genes involving receptors, signaling pathways and immune responses

Most of previous studies compared the endometriosis with endometrial tissues; therefore we analyzed differential DNA methylation between endometriosis and endometrial tissues and identified the differentially methylated regions (Table S1). In comparison with eutopic endometriosis, many genes in ovarian endometrial cysts (choESC) had different degrees of methylation, high and low (Tables 1 and 2), With acquired methylation profiles data, we identified the detailed features of aberrantly methylated genes in choESC using gene cards (the humor gene database) analyses. Depending on pathway analysis of screened genes, there are abnormalities of relevant

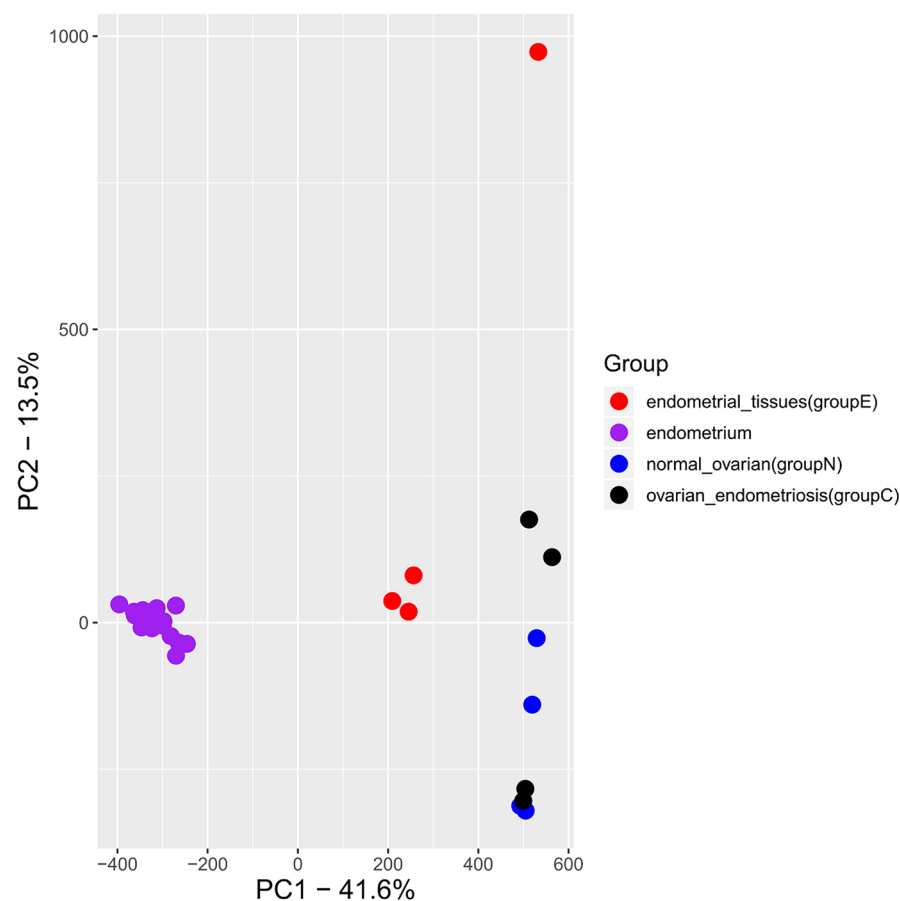


Fig. 4 Endometriosis samples closely resembled normal ovarian tissues, but not endometrial tissues

signal transduction pathways involving endometriosis onset and progression, developmental processes, human early embryonic development, regulation of caspase, tyrosine, and mRNA metabolic processes et al. It indicated an abnormal expression pattern of choESC in peritoneal environment.

GO term enrichment analysis showed that the hypomethylation genes in ovarian endometrial cysts were primarily engaged in embryonic organ development and embryonic organ development, stem cell population maintenance, et al. And the hypermethylation genes in ovarian endometrial cysts were primarily engaged in mRNA destabilization, female uterus and genitalia development, et al. The function of genes related to steroid binding, transcription factor activity and receptor activity. (Figures 7 and 8).

We compared our differentially methylated sites and only found consistent results on hyper-methylation on ESR1 [1, 12]. Consistently, downregulation of ESR1 mRNA level in endometriosis was also reported in few studies [12, 13]. Then we searched for the literature for the differentially methylated genes we identified and examined whether the associated expression patterns

were observed. Indeed, we observed consistent results on 5 genes: TNFSF13B, FOXP1, TCF21, BST2 and STRA6 [14–20]. Taken together, despite little overlap with previously characterized genes, the characterized methylated genes in endometriosis were consistent with the reported expression changes in endometriosis (Table 3). RT-PCR results showed that ER1, STAR6 and PEMT were significantly downregulated in ectopic tissues, and BST2, TCF21 and FOXP1 were significantly upregulated in ectopic tissues (Fig. 9). In summary, we validated the top candidate genes in endometriosis which might be regulated by DNA methylation.

Discussion

In this work, we presented a very small cohort of paired DNA methylation analysis of normal ovarian, endometrial and endometriosis samples. Despite the very small sample size, results were very clear that endometriosis samples closely resembled normal ovarian tissues, but not endometrial tissues. Such results suggested that, instead of migrating from endometrial tissues, endometriosis might be originated from ovarian tissues. Such results did not rule out the possibility that endometriosis

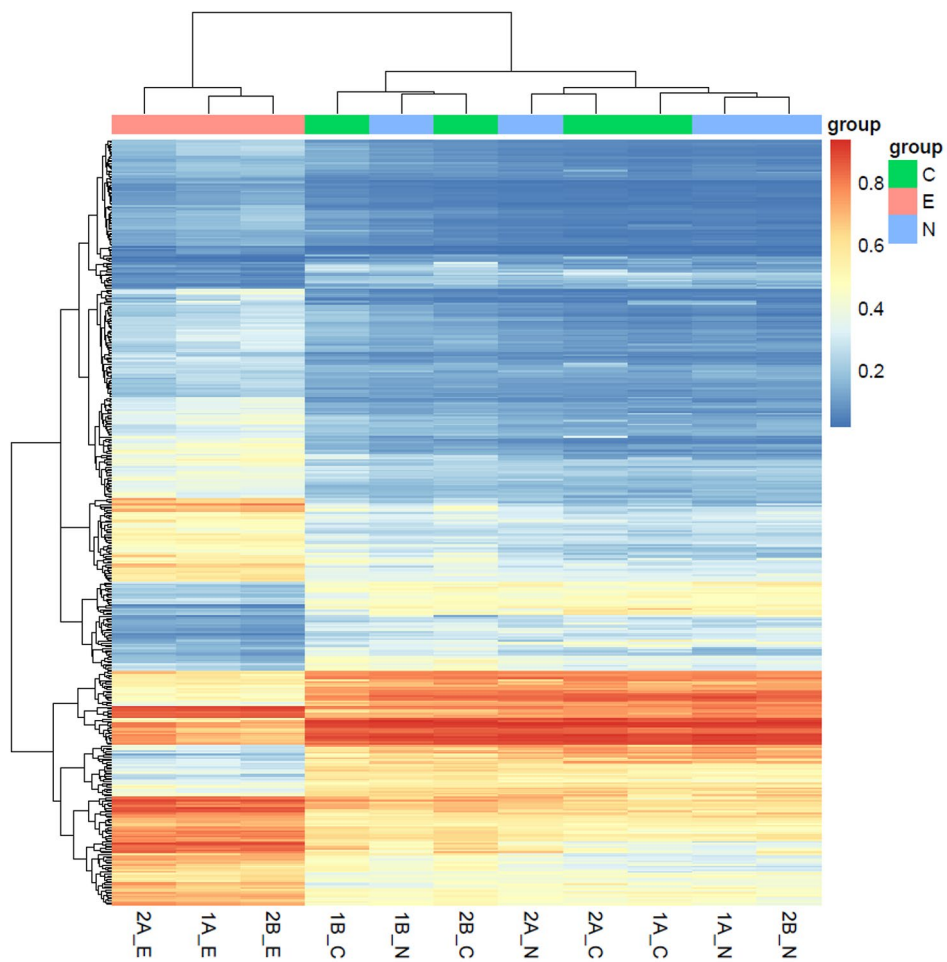


Fig. 5 Hierarchical clustering analysis of the three groups based on DMRs. Color mapping from blue to red indicates methylation level from low to high. Group C: ovarian endometrial cysts; N: normal_ovarian; E: eutopic endometriosis

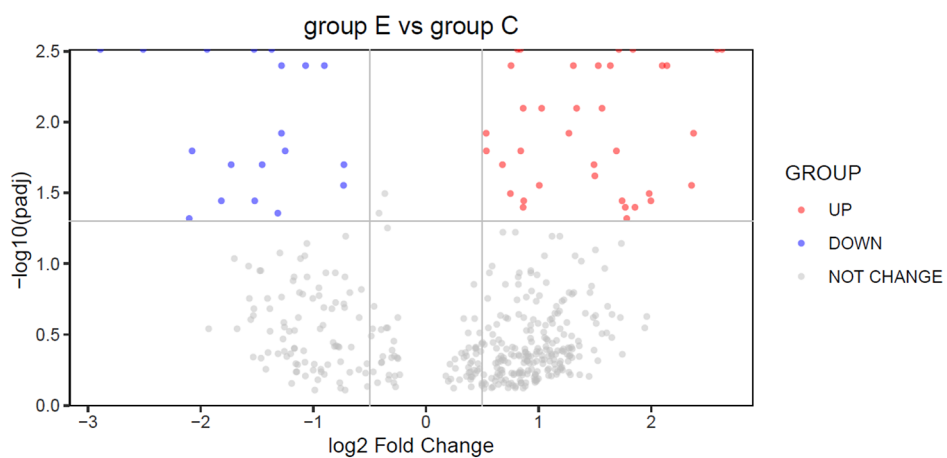


Fig. 6 Volcano plot of $\log_2(\text{fold change})$ against $-\log_{10}(\text{p.adj})$ of DMRs. The red blue spots stand for DMRs with adjusted p-value < 0.05 and $\log_2\text{FC} > 0.5$, and blue spots stand for DMRs with adjusted p-value < 0.05 and $\log_2\text{FC} < -0.5$, grey spots stand for non-significant DMRs. The horizontal dash line denotes adjusted p-value < 0.05 ; the vertical dash lines denote $|\log_2\text{FC}| > 0.5$. Group C: ovarian endometrial cysts; E: eutopic endometriosis

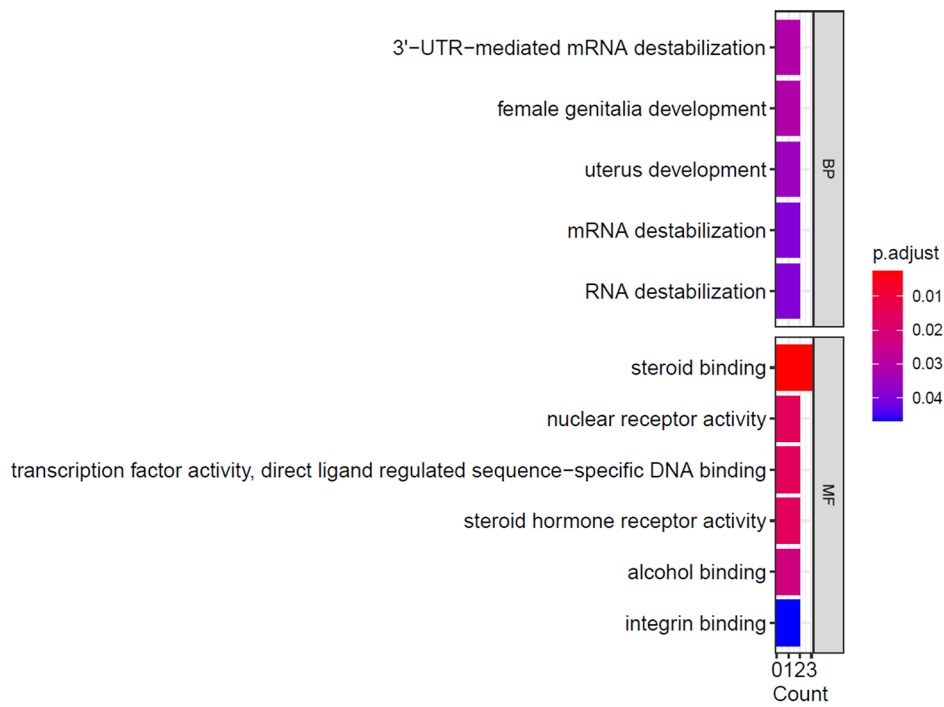


Fig. 7 Significantly enriched term by the genes with higher methylation levels in ovarian endometrial cysts (group C). Genes with higher methylated levels in eutopic endometriosis (group E) compared with ovarian endometrial cysts (group C) GO analysis

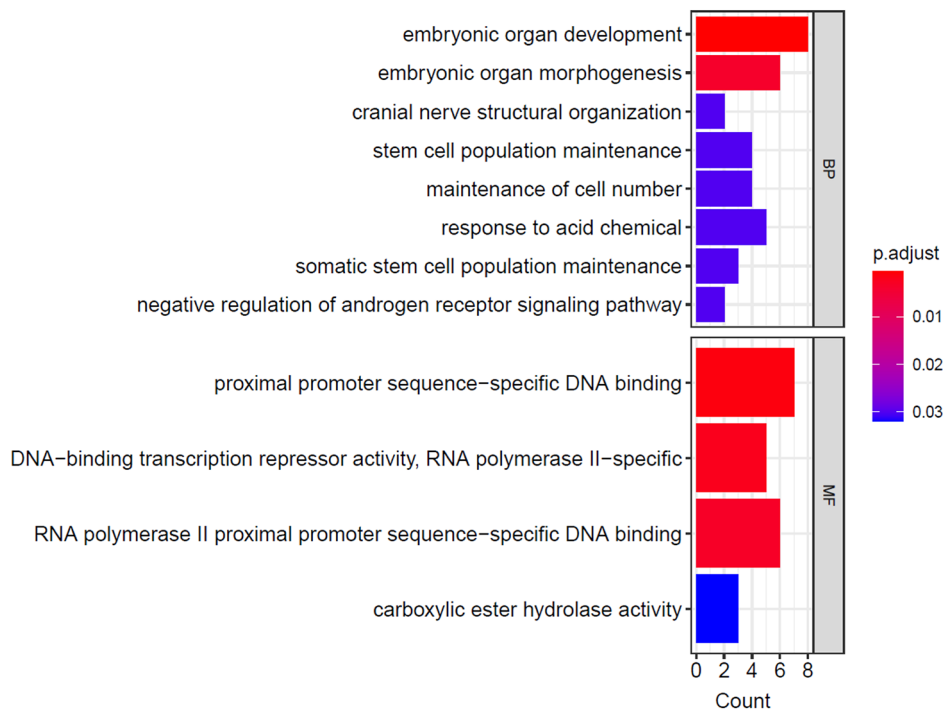


Fig. 8 Significantly enriched term by the genes with lower methylation levels in ovarian endometrial cysts (group C)

Table 1 Hypomethylation in endometrial tissues compared the endometriosis

| Gene Symbol | Gene name | DMR value (Endometrial vs. Cyst) | Delta_beta | Log2 Fold Change Ratio | P value | Function |
|-------------|--|----------------------------------|------------|------------------------|---------|--|
| HOXB1 | homeotic genes B1 | 3.749 | 0.386 | 0.813 | 0 | Important growth and development regulatory genes regulator of retinoic acid response elements (RAREs) |
| MCC | membrane compartment of Can1 | 3.709 | 0.288 | 2.587 | 0 | one cytoplasm membrane microdomain regulating metabolism, cellular morphogenesis, signaling cascades, and mRNA degradation. |
| TMEM101 | transmembrane protein 101 | 3.695 | 0.297 | 2.629 | 0 | upregulation and increase the expression of NF-κB in ovary cancer. |
| CASS4 | Homo sapiens Cas scaffolding protein family member 4 | 3.624 | 0.515 | 1.530 | 0.004 | Possible docking protein which may play a role for tyrosine-kinase-based signaling related to cell adhesion. Regulates FAK activity, focal adhesion integrity, and cell spreading. |
| BST2 | bone marrow stromal cell antigen 2 | 3.612 | 0.450 | 1.713 | 0 | Regulating Type-I interferon and interleukin-6 generation |
| FABP3 | Fatty Acid Binding Protein 3 | 3.150 | 0.298 | 2.098 | 0.004 | During the early stages of pregnancy, upregulating cell growth and proliferation in uterine endometrium |
| FAIM2 | Fas Apoptotic Inhibitory Molecule | 3.052 | 0.317 | 2.376 | 0.012 | specifically protecting cells from Fas-induced apoptosis |
| SHANK2 | SH3 and multiple ankyrin repeat domains protein | 3.051 | 0.353 | 2.140 | 0.004 | A protein-coding gene relating to autism |
| TCF21 | Transcription Factor 21 | 3.030 | 0.433 | 1.563 | 0.008 | augmenting steroidogenic factor (SF) 1 and estrogen receptor β (ERβ) |
| PRDM1 | positive regulatory domain 1 | 2.983 | 0.431 | 1.269 | 0.012 | increased IL-10+Th17 cells are significantly |
| MIR365A | MicroRNA 365a | 2.974 | 0.378 | 0.842 | 0.016 | involving posttranscriptional modulation of genes via regulating mRNA stabilization and translation |
| FOXP1 | Forkhead Box P1 | 2.849 | 0.443 | 1.492 | 0.02 | activating Wnt/β-cateninsignaling pathway in endometriosis |
| LOC339803 | LOC339803 | 2.841 | 0.199 | 2.358 | 0.028 | Enhancing HCC invasion and migration via miR-30a-5p/ SNAIL1. In human atherosclerotic lesions, serve as potential biomarkers for lesion hypoxia. |
| ZNF311 | Zinc Finger Protein311 | 2.833 | 0.330 | 1.838 | 0 | upregulated in grade-dependend glioma patients of adverse outcome |
| RNASE1 | Ribonuclease A Family Member 1 | 2.773 | 0.364 | 1.027 | 0.008 | a key player in regulation of vascular homeostasis |
| LYPLAL1 | Long non coding RNA lysophospholipase-like 1 | 2.771 | 0.206 | 1.338 | 0.008 | Long non coding RNA lysophospholipase-like2 |
| PAX2 | Paired Box Homeotic Gene 2 | 2.670 | 0.291 | 1.741 | 0.036 | Its mutations contribute to optical nerve glioma and kidney dysplasia |
| NUP210L | Nucleoporin 210 | 2.663 | 0.328 | 0.868 | 0.036 | Encoding a membrane-spanning glycoprotein which participates in formation of nuclear pore complex |
| SLC1A2 | Solute Carrier Family 1 Member 2 | 2.657 | 0.329 | 1.856 | 0.04 | Encoding a solute transporter protein |
| MX2 | MX Dynamin Like GTPase 2 | 2.646 | 0.252 | 1.770 | 0.04 | Encoding a protein of dynamin and GTPases families, with nuclear and cytoplasmic forms. |
| SVIL | Supervillin | 2.632 | 0.369 | 0.863 | 0.04 | Encoded product contributes to myosin II assembly and disintegration of focal adhesions. |
| TERC | Telomerase RNA Component | 2.614 | 0.227 | 1.500 | 0.024 | Encoding telomerase. |
| PROCA1 | Protein Interacting With Cyclin A1 | 2.588 | 0.259 | 1.982 | 0.032 | Enables cyclin binding activity. Predicted to be involved in arachidonic acid secretion and phospholipid metabolic process |

Table 1 (continued)

| Gene Symbol | Gene name | DMR value (Endometrial vs. Cyst) | Delta_beta | Log2 Fold Change Ratio | P value | Function |
|-------------|--|----------------------------------|------------|------------------------|---------|---|
| ARHGAP25 | Rho GTPase Activating Protein 25 | 2.577 | 0.300 | 1.637 | 0.004 | Encoding negative regulators of Rho GTPases. |
| DAW1 | Dynein Assembly Factor With WD Repeats 1 | 2.569 | 0.226 | 0.679 | 0.02 | Maybe the upstream of some processes, including cerebrospinal fluid circulation; determination of left/right symmetry; and outer dynein arm assembly. |
| MAGI2-AS3 | MAGI2 Antisense RNA 3 | 2.563 | 0.264 | 1.783 | 0.048 | An RNA Gene, affiliated with the lncRNA class. Diseases associated with MAGI2-AS3 include Nephrotic Syndrome, Type 15 and Breast Cancer. |
| TNFSF13B | TNF Superfamily Member 13b | 2.488 | 0.179 | 2.000 | 0.036 | modulating B cells proliferation and differentiation. |
| HOXA2 | Homeobox A2 | 2.3473 | 0.330 | 0.864 | 0.008 | Encoding a DNA-binding transcription factor governing morphogenesis, and differentiation. |
| SGIP1 | SH3GL Interacting Endocytic Adaptor 1 | 2.103 | 0.196 | 1.691 | 0.016 | May involve in clathrin-mediated endocytosis and energy homeostasis. |
| ASCL2 | Achaete-Scute Family BHLH Transcription Factor 2 | 2.028 | 0.257 | 0.838 | 0 | related pathways are Embryonic and Induced Pluripotent Stem Cells and Lineage-specific Markers and Human Early Embryo Development. |
| EPM2AIP1 | EPM2A Interacting Protein 1 | 2.013 | 0.133 | 1.007 | 0.028 | MLH1 and EPM2AIP1 genes share a common promoter whose methylation has been shown to affect both genes. |
| UBD | Ubiquitin D | 1.818 | 0.140 | 1.309 | 0.004 | Encoded protein participates in aggresomes formation, mitotic regulation, and dendritic cell maturation. |
| RNF39 | RING finger protein 39 | 1.6484 | 0.201 | 0.535 | 0.012 | Its variants were linked to viral diseases and autoimmune diseases. |
| TBX3 | T-Box Transcription Factor 3 | 1.567 | 0.272 | 0.756 | 0.004 | Regulating developmental processes. |
| PON1 | Paraoxonase 1 | 1.390 | 0.180 | 0.537 | 0.016 | Displaying lactonase and ester hydrolase activity. |
| ZIC1 | Zic Family Member 1 | 1.157 | 0.138 | 0.750 | 0.032 | Encoding a C2H2-type zinc finger proteins. |

was indeed seeded by endometrial cells, but drastic DNA methylation reprogramming resulted in highly similar DNA methylation profiles to the ovarian tissues.

A recent report confirmed genome-wide DNA methylation profiles of endometriosis tissue subtypes, focusing on promoters [21]. They found a large number of methylation differences compared with the eutopic endometrium and verified with clinical data. At the same time, Yoshiaki et al. revealed a more accurate genome-wide DNA methylation map, which was derived from the homogenous ESCs of eutopic endometrium and ovarian endometrium cysts with endometriosis. Some differentially methylated or expressed genes (Nr5a1, star, STRA6 and HSD17B2) were involved in steroidogenesis, also verified in numerous clinical specimens by independent methods [20]. Our strategy was to investigate whether there were methylation differences between multilocular ovarian cysts and unilocular ovarian cysts and normal ovarian tissues.

Many genes in choESC acquired from endometrium cysts had different degrees of methylation. Basing on acquired methylation profiles, specific features of aberrantly methylated genes in choESC were evaluated using gene cards (the humor gene database) analysis. There are abnormalities of relevant signal transduction pathways in endometriosis pathogenesis and progression. Abnormal methylation status exists in genes related to proliferation and apoptosis, and immune responses, indicating an abnormal expression of choESC in peritoneal environment. Meanwhile, choesc showed abnormal differentiation, such as neurogenesis and embryogenesis. This means that choesc may have differentiated into other types of cells, suggesting the existence of abnormal developmental processes. The above findings add the possible involvement of DNA epigenetic modifications in endometriosis pathogenesis.

We compared our differentially methylated sites and only found consistent results on hyper-methylation on

Table 2 Hypermethylation in endometrial tissues compared the endometriosis

| Gene Symbol | Gene name | DMR value (Endometrial vs. Cyst) | Delta_beta | Log2 Fold Change Ratio | P value | Function |
|------------------|--|----------------------------------|------------|------------------------|---------|---|
| SHF | Src Homology 2 Domain Containing F | -2.107 | -0,172 | -1.249 | 0.016 | May enable phosphotyrosine residue binding activation, and participate in apoptosis. |
| ESR1 | Estrogen Receptor 1 | -2.177 | -0.296 | -1.520 | 0.016 | Encoding estrogen receptor, vital for hormone binding, and transcriptional activation. |
| ESR2 | Estrogen Receptor 2 | -2.280 | | | 0.036 | Encoding the estrogen receptor 2. |
| RBM24 | RNA Binding Motif Protein 24 | -2.414 | -0.249 | -1.816 | 0.036 | Involved in several processes, including negative regulation of cytoplasmic translation, and regulation of mRNA metabolic process. |
| LMO7DN | LIM Domain 7 downstream neighbour | -2.487 | -0.334 | -0.728 | 0.02 | Involved in lung cancer prognosis. |
| ZC3H12D | Zinc Finger CCCH-Type Containing 12D | -2.541 | -0.327 | -1.454 | 0.02 | Predicted to enable endoribonuclease activity and mRNA binding activity, and negatively modulate G1/S transition and cell growth |
| CACNB2 | Calcium Voltage-Gated Channel Auxiliary Subunit Beta 2 | -2.550 | -0.234 | -1.729 | 0.02 | Encoding a subunit of voltage-dependent calcium channel proteins. |
| C17orf107(CHRNE) | Cholinergic Receptor Nicotinic Epsilon Subunit | -2.563 | -0.197 | -2.101 | 0.048 | After binding acetylcholine, AChR undergoes an alteration in conformation opening an ionotransduction channels across the plasma membrane |
| HAND2-AS1 | HAND2 Antisense RNA 1 | -2.605 | -0.383 | -1.315 | 0.044 | Predicted to be involved in positive regulation of gene expression, and possess a positive effect on cardiac right ventricle morphogenesis. |
| PEMT | Phosphatidylethanolamine N-Methyltransferase | -2.800 | -0.346 | -0.731 | 0.028 | Converting phosphatidylethanolamine to phosphatidylcholine by sequential methylation. |
| ZNF22 | Zinc Finger Protein22 | -2.837 | -0.259 | -1.282 | 0.012 | Modulating cell migration, adhesion, and cycle. |
| STRA6 | Signaling Receptor And Transporter Of Retinol STRA6 | -3.033 | -0.430 | -1.369 | 0 | Encoding a membrane protein responsible for retinol metabolism. |
| LINC00460 | Long Intergenic Non-Protein Coding RNA 460 | -3.090 | -0.351 | -1.068 | 0.004 | play vital roles in the pathogenesis, tumorigenesis, and angiogenesis of cancers. |
| LRMDA | Leucine Rich Melanocyte Differentiation Associated | -3.270 | -0.442 | -1.282 | 0.004 | This gene encodes a leucine-rich repeat protein. |
| EMX2OS | EMX2OS | -3.272 | -0.445 | -1.943 | 0 | Modulating ovarian cancer cells through miR-654-3p/AKT3/PD-L1. |
| RNF19A | RING finger protein 19 A | -3.461 | -0.448 | -1.526 | 0 | Interacting with alpha synuclein in neurons. |
| HOXC4 | Homeobox C4 | -3.463 | -0.435 | -2.510 | 0 | Related to immunodeficiency with Hyper-Igm, and lymphoma. |
| TFAMP1 | Transcription Factor A, Mitochondrial Pseudogene 1 | -3.641 | -0.391 | -0.901 | 0.004 | Encoding a mitochondrial transcription factor. |
| FMN1 | Formin 1 | -4.035 | -0.407 | -2.890 | 0 | Modulating development of adhesion junction and linear actin polymerization. |

ESR1. Consistently, downregulation of ESR1 mRNA level in endometriosis was also reported in few studies. Then we searched for the literature for the differentially methylated genes we identified and examined whether the associated expression patterns were observed. Indeed, we observed consistent results on 5 genes:

TNFSF13B, FOXP1, TCF21, BST2 PEMT and STRA6. Increased TNFSF13B B lymphocyte stimulator protein was identified in serum of endometriosis subjects [14]. Suppressing FOXP1 reverted the endometrium cell phenotype, involving decreased collagen gel contraction, cell growth and migratory movement [15]. TCF21 could

Table 3 The differentially methylated genes were consistent with the reported expression changes in endometriosis

| Gene Symbol | Gene name | DMR value (Endometrial vs. Cyst) | Delta_beta | Log2 Fold Change Ratio | P value | Function | |
|------------------|---|----------------------------------|------------|------------------------|---------|--|---|
| Hypomethylation | | | | | | | |
| BST2 | bone marrow stromal cell antigen 2 | 3.612 | 0.450 | 1.713 | 0 | Regulating type-I interferon and interleukin-6 generation Modulating embryo-maternal immune. | |
| TCF21 | Transcription Factor 21 | 3.030 | 0.433 | 1.563 | 0.008 | Interacting with upstream stimulatory factor 2, transactivating SF-1 and Erβ promoters, and modulating estrogen pathway and fibrosis in endometriosis. | |
| FOXP1 | Forkhead Box P1 | 2.849 | 0.443 | 1.492 | 0.02 | FOXP1 activating Wnt/β-catenin signaling pathway in endometriosis. Its knockdown reverted endometrium cell phenotypes. | |
| TNFSF13B | TNF Superfamily Member 13b | 2.488 | 0.179 | 2.000 | 0.036 | Modulating B cells proliferation and differentiation. Increased in serum of endometriosis subjects. | |
| Hypermethylation | | | | | | | |
| ESR1 | Estrogen Receptor 1 | -2.177 | -0.296 | -1.520 | 0.016 | Encoding estrogen receptor, vital for hormone binding, and transcriptional activation. | |
| STRA6 | Signaling Receptor And Transporter Of Retinol STRA6 | -3.033 | -0.430 | -1.369 | 0 | Vital for retinol binding protein, the retinol uptake into cells. Downregulating STRA6 enhanced endogenous estradiol synthesis. | |
| PEMT | Phosphatidylethanolamine N-Methyltransferase | -2.800 | | -0.259 | -1.282 | 0.028 | Converting phosphatidylethanolamine to phosphatidylcholine by sequential methylation. Its polymorphism may induce infertility of endometriosis women. |

transactivate SF-1 and Erβ promoters in ESCs [16], modulating estrogen pathway and fibrosis of endometriosis [17]. rs4244593 of PEMT-related polymorphism modulated the choline or phospholipids generation, inducing infertility of endometriosis women [19]. STRA6 is vital for retinol binding protein, and retinol uptake into cells.

The correlation analysis between DNA methylation and transcriptomes identified an anticipated positive relation, as well as a negative relation. It is impossible to characterize the close relationship between DNA methylation

and mRNA expression due to the lesser gene coverage of beadchip specifications and the analysis of only two CPGs per gene. In genome-wide observation, a complex network may exist between DNA methylation and transcription.

Altogether, this study provides basic DNA methylation data on ovarian endometriosis compared with ovarian tissue and eutopic endometrium using untreated cultured ESCs. We believe that this kind of new information will contribute to the future research on treatment strategies and preventive drugs for endometriosis.

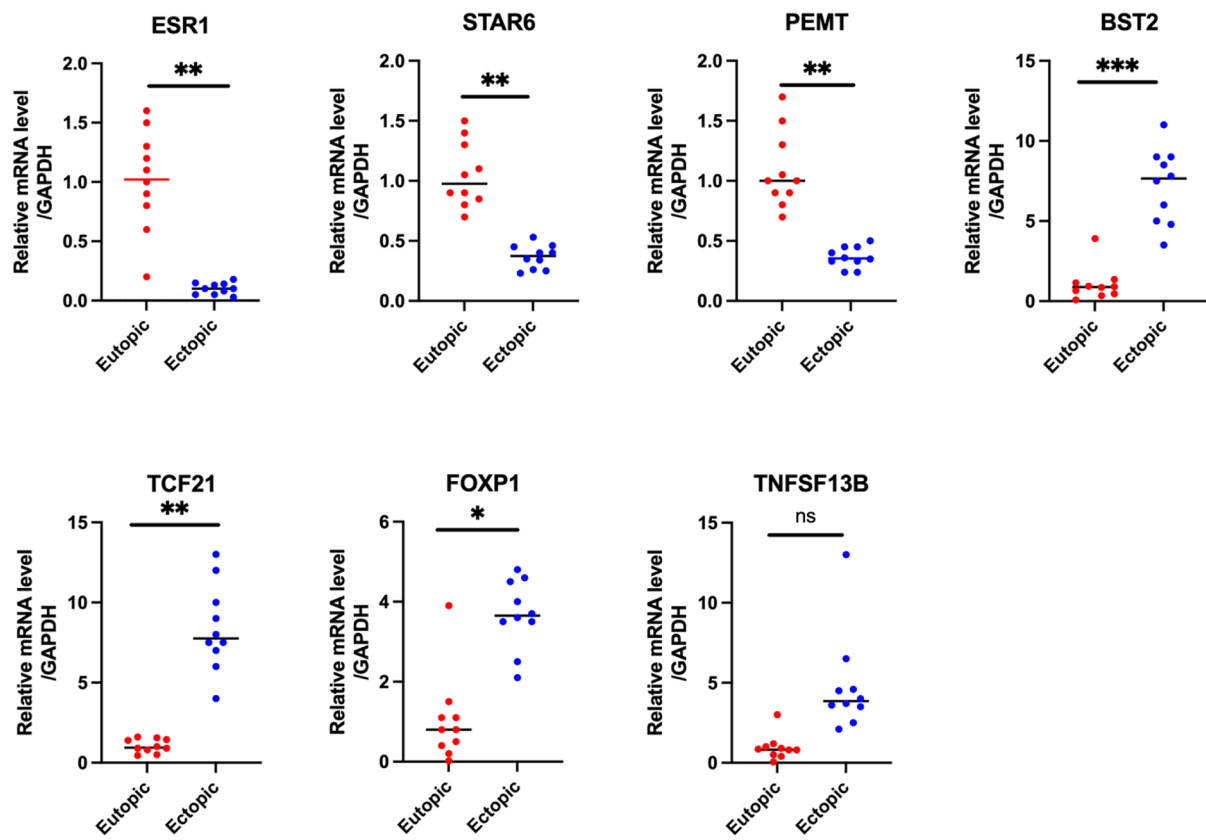


Fig. 9 qRT-PCR validation of the gene expression of the candidate genes. ESR1, STAR6 and PENT were significantly downregulated in ectopic tissues, and BST2, TCF21 and FOXP1 were significantly upregulated in ectopic tissues. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, ns, not significant

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13048-024-01440-1>.

Supplementary Material 1

Acknowledgements

This work is funded by National Natural Science Foundation of China (31871332) and ShanghaiTech University. We thank ShanghaiTech University and Y. Zhang for the HPC platform and technical support.

Author contributions

XHZ and JC conceived the study. XHZ and XZ performed the data analysis. XHZ, JW and JC performed the sample collection. XHZ, XZ and YZ wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work is funded by National Natural Science Foundation of China (31871332).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 6 March 2024 / Accepted: 19 May 2024

Published online: 06 June 2024

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