Pathogenetic gene changes of eutopic endometrium in patients with ovarian endometriosis

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To the Editor: Endometriosis refers to the endometrial tissue (interstitial substance and gland) with growth function occurring in the uterine lining of uterine cavity and the places outside myometrium. It often leads to pain, infertility, nodules or masses and other symptoms and signs, and thus, brings a heavy burden on individuals and society. Traditional blood reflux theory, immune theory, inflammatory theory, hormone theory, etc., cannot fully elucidate the pathogenesis of endometriosis. Recent studies have shown that there is some specificity in eutopic endometria of patients with endometriosis themselves, making it possible that endometrial fragments adhere, invade, and grow outside of the uterine cavity.[1,2] Eutopic endometria may be one of the key factors in the occurrence of endometriosis. This study intends to use the whole exome sequencing (WES) and gene chip sequencing technology to explore the role of eutopic endometria of patients with ovarian endometriosis (OEM) in the pathogenesis of endometriosis.

Case group inclusion criteria: patients with OEM who received surgical treatment in our hospital. Control group inclusion criteria: patients who underwent hysterectomy due to cervical lesion. Exclusion criteria: patients who received drug therapy before surgery; patients with comorbidity of other benign and malignant uterine or ovarian diseases; patients with family history of Lynch syndrome; patients with other systemic malignancies and immune system diseases.

Three patients with sporadic OEM and three control patients were selected for WES from the Peking Union Medical College Hospital. Peripheral venous blood from both case group and control group, the eutopic endometria and OEM lesions of OEM patients, as well as the endometrial tissues of non-endometriosis patients, were collected.

High-throughput sequencing adopted Hiseq2500 sequencing platform of Illumina, resulting in 101 bp paired-end (PE) reads, with an average of about 15-G data produced by one sample. We orderly carried out whole exome sequence capture, biological information analysis and the functional prediction.

In the result of WES, the genes where somatic cell gene mutation was located shared by endometrium tissues and OEM cyst tissues in OEM patients were made into gene chips. A group of patients with OEM was selected from the pathological database for the validation. Enrichment analysis and high-throughput sequencing in object region were performed followed by biological information analysis to explore the relationship between related somatic gene mutations and OEM. The clinical features of the three patients with OEM and the three patients in the control group are shown in Supplementary Table 1, http://links.lww.com/CM9/A25.

An analysis of single nucleotide variant (SNV) mutations was performed in the tissues of the six included patients, and only the mutations that could potentially alter protein coding in the gene coding regions and splicing regions were selected. There was no overlap among the mutation sites of ectopic lesions and eutopic endometria of the above patients as well as those of endometria of patients in the control group.

Somatic mutations present in eutopic endometria and OEM lesions of the three endometriosis patients were compared and analyzed. The results showed that each patient had an average of a total of 25 mutations, most of which were intergenic region (IGR) site mutations. And five, five, and three common mutations were found in OEM patients one, two, and three, respectively. The 13 mutation sites were located in ten genes. The genes were respectively located at positions of 76131597, 11036963,

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Table 1: Information of common mutations which were repeatedly found in at least two patients.

Number of patients with mutations	Chromosome	Location	Gene	Reference sequence	Nucleotide change
2	chr6	160961136	*LPA*	NM_005577.2	c.5673A>G
5	chr6	161007495	*LPA*	NM_005577.2	c.4114C>G
7	chr6	161007537	*LPA*	NM_005577.2	c.4072C>G
4	chr9	78910240	*PCSK5*	NM_001190482.1	c.3236A>C
5	chr9	78910272	*PCSK5*	NM_001190482.1	c.3268G>A
4	chr9	78936357	*PCSK5*	NM_001190482.1	c.3824G>A
2	chr9	78936491	*PCSK5*	NM_001190482.1	c.3958A>G
3	chr9	78969058	*PCSK5*	NM_001190482.1	c.5097C>A

Number of patients with mutations	Amino acid change	Type of mutation	Mutation Region	Polyphen2* prediction	SIFT* prediction	CONDEL* prediction
2	p.Ile1891Met	Missense	EX37	–	TOLERATED	Neutral
5	p.Leu1372Val	Missense	EX26	–	DAMAGING	Neutral
7	p.Leu1358Val	Missense	EX26	–	DAMAGING	Neutral
4	p.Glu1079Ala	Missense	EX26	BENIGN	DAMAGING	Neutral
5	p.Gly1090Ser	Missense	EX26	BENIGN	DAMAGING	–
4	p.Gly1275Asp	Missense	EX30	BENIGN	TOLERATED	Neutral
2	p.Lys1320Glu	Missense	EX30	BENIGN	DAMAGING	–
3	p.Asp1699Glu	Missense	EX36	BENIGN	TOLERATED	–

* Software names for mutation prediction. –: Not applicable.

161032852, 33395267, 78790207, 75212411, 23805712, 190878808, 112632495, and 104195858, and the genes were *DTX2, BAGE4, BAGE3, BAGE2, BAGE5, LPA, AQP7, PCSK5, SEC14L1, CST2, FRG1, HECTD4,* and *ZFYVE21*, respectively. No common mutation sites or common genes were seen among the three patients.

Gene chips were customized according to these ten SNV mutated genes and 18 patients finally met the criteria for sample verification. In the analysis of non-silent mutations, there were a total of 105 non-silent mutations in the 36 samples from these 18 patients, of which 86 non-silent mutations (82%) were common mutation of eutopic endometrial and ectopic lesion of OEM patients.

Two or more common mutations were found in 11 patients (61%), all of which were missense mutations. These mutations were respectively located at three sites of *LPA* and five sites of *PCSK5* [Table 1]. Among them, there were eight patients with at least one *LPA* mutation site. Similarly, there were nine patients with at least one site mutation in the five sites of *PCSK5* gene.

Although there are many theories about the pathogenesis of endometriosis, none of them explain all aspects of it. Endometriosis is a histological benign disease with metastases, infiltration, recurrence, and other malignant behaviors. Previous studies have found that eutopic endometria of patients with endometriosis have many abnormalities, which may be closely related to the pathogenesis of endometriosis.[3] The purpose of this study is to explore the possible abnormal changes associated with the pathogenesis of endometriosis at the gene level in eutopic endometria of the patients with OEM using WES and gene chip methods.

In this study, we performed WES on eutopic endometria and ectopic lesions in the three patients with OEM and further identified ten related genes (*DTX2, BAGE4, BAGE3, BAGE2, BAGE5, LPA, AQP7, PCSK5, SEC14L1, CST2, FRG1, HECTD4*, and *ZFYVE21*). These sites and genes did not overlap with the mutation sites found in normal endometrium tissues of the control group, but they provided clues for us to seek mutations.

A gene chip containing the above ten genes was customized in this study, and eutopic endometrial and endometriotic lesions of 18 patients with OEM were verified and detected. These mutations were respectively located at three sites of *LPA* and five sites of *PCSK5*, suggesting that eutopic endometrial *LPA* and *PCSK5* mutations may have a significant correlation with the pathogenesis of OEM.

Apolipoprotein an (Apo [a]) encoded by *LPA* is an important part of lipoprotein an (LP [a]). Some studies have found that LP (a) is related to inflammatory factors, such as tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-6 (IL-6), and monocyte chemoattractant protein (MCP-1).[4,5] There is no literature report on the relationship between LP (a) or Apo (a) and endometriosis. LP (a) may promote oxidative stress and be related to patients' eutopic endometrial changing to cause the formation of OEM lesions. Further investigation for the expression of *LPA* in endometrial, peritoneal, and ovarian tissues may help to elucidate the mechanism of action of LP (a) in the pathogenesis of endometriosis.

PCSK5, as a protein precursor converting enzyme, plays a processing and activating role for the precursors of multiple cytokines and adhesion factors, such as matrix metalloproteinase, N-cadherin, insulin-like growth factor, and these factors are closely related to the occurrence of

endometriosis.[6-8] However, further studies are needed to explore the expression of *PCSK5* and the effect of *PCSK5* on relevant cytokines, as well as the possible role of *PCSK5* in the pathogenesis of endometriosis.

We found that *LPA* and *PCSK5* site mutations were common to eutopic endometria and endometriosis lesions in 66.7% (12/18) of the patients in this study. Whether they can serve as molecular markers of auxiliary diagnosis or molecular diagnosis or not, or what specific clinical manifestations do the ovarian chocolate cysts with the two kinds of mutations have, are pending further in-depth study.

In this study, WES was used to sequence and analyze the ectopic endometrium and OEM tissues of OEM patients. Ten genes of somatic mutations were screened out in three pairing samples for OEM. And we identified *LPA* and *PCSK5* genes with the gene chips, suggesting that they may be related to the occurrence of OEM, which provides not only new ideas for the molecular mechanism of endometriosis but also new clues for the diagnosis of endometriosis.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

None.

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