Reproductive Sciences
2018, Vol. 25(9) 1446-1454

DOI: 10.1177/1933719118768704
journals.sagepub.com/home/rsx
SAGE

Decreased Expression of HOXA10 May Activate the Autophagic Process in Ovarian Endometriosis

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Abstract

Autophagy is a survival process that maintains homeostasis in all eukaryotic cells. Recent studies show an abnormal autophagic activity in endometriosis, but the role of autophagy is controversial. Homeobox A10 (HOXA10) is a transcription factor necessary for embryonic and adult uterine development, and studies indicate that its expression decreases in endometriosis. Homeobox A10 may negatively regulate autophagy in endometriosis. To test this hypothesis, we measured the expression levels of autophagic biomarkers (beclin-1 and LC3-II) and HOXA10 proteins by Western blotting and messenger RNA (mRNA) by quantitative real-time polymerase chain reaction. Furthermore, we evaluated the serum cancer antigen 125 (CA125) levels by immunoassay. Most tested autophagic biomarker proteins and mRNAs were upregulated, whereas HOXA10 protein and mRNA were decreased in ovarian endometriomas compared with eutopic endometria of women with endometriosis and normal endometria. Compared with normal endometrium, only protein expression levels of autophagic biomarkers were increased in the eutopic endometrium of women with endometriosis. Moreover, HOXA10 was found to have a significant negative correlation with autophagy ($P < .01$). Serum CA125 was at a high level in endometriosis and increased with elevated revised American Fertility Society staging (I-IV). There was a significant positive correlation between serum CA125 level and LC3-II protein level and/or LC3-II/LC3-I ratio ($P < .01$) and a significant negative correlation between serum CA125 level and HOXA10 gene level ($P < .01$). In conclusion, our studies support that the deficiency of HOXA10 may induce autophagy in endometriosis, and the relationship among CA125, autophagy, and HOXA10 in endometriosis requires additional research.

Keywords

ovarian endometriosis, autophagy, HOXA10, CA125

Introduction

Endometriosis is a chronic inflammatory disease, and it is defined by endometrial tissues outside the uterus, mainly on the ovary and pelvic peritoneum. Endometriosis affects 5% to 10% of all reproductive age women, and its principal clinical symptoms are progressive dysmenorrhea, infertility, and dyspareunia.[1] One main mechanism of endometriosis is an increased inflammatory response,[2] which has assigned tumor-like biological behaviors to ectopic endometrial tissues, including abnormal cell survival and proliferation,[3,4] increased invasiveness and vascularization,[5,6] and deficient apoptosis and differentiation.[7,8] These changes may determine the entire stepwise process of ectopic endometrial lesion formation and influence the pathogenesis of endometriosis through the autophagic response.

Autophagy acts as a basal catabolic process that induces the sequestration of cytoplasmic organelles or macromolecules in autophagosomes and subsequent degradation and recycling of their cargoes after fusion with lysosomes. Autophagy is widely involved in pathophysiological processes and presents complicated regulatory mechanisms especially in cancer.[9] In general, research suggests that deficient autophagy permits DNA damage and tumorigenesis, while autophagy maintains the survival of established tumors under metabolic stress.[10] Similarly,

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limited studies have revealed that the role of autophagy has 2 sides in ovarian endometriosis. Decreased autophagic level is detected in endometriosis and in vitro, which is associated with survival,[4] growth, invasion[11] and decreased apoptosis.[12] Conversely, autophagy can also serve as a survival mechanism for endometriosis in conditions of oxidative stress[13] and hypoxia.[14,15]

The homeobox (Hox/HOX) genes are identified as highly conserved transcription factors that mediate uterine development and function. Homeobox A10, a member of HOX genes, is involved in the regulation of inflammatory factors.[16] Studies show that decreased HOXA10 expression can activate the inflammatory response in endometriosis.[2,16] Homeobox A10 can also function in invasion property, tissue remodeling, angiogenesis, and apoptosis in both cancer and endometriosis.[17-19] Autophagic process is closely related to these pathophysiological changes,[20,21] which may participate in the formation and development of endometriosis.[7,22] Furthermore, HOX-mediated autophagy inhibition is reported to be evolutionarily conserved in both Drosophila and vertebrate cells.[23] These studies reveal the possibility that HOXA10 may influence the development of endometriosis in a boarder way than previously thought.

According to a literature review, the abovementioned pathophysiological relationship between HOXA10, autophagy, and endometriosis remains unreported, so we supposed that HOXA10 may function in endometriosis by negatively regulating autophagy. Thus, our study aims to investigate the mechanism of autophagy regulation in endometriosis.

Materials and Methods

Patients and Tissue Collection

Ovarian endometriotic tissues (OETs) and paired eutopic endometrial tissues (EETs) were obtained from 29 premenopausal women (aged 24-43 years) with ovarian endometriotic cysts who were undergoing laparoscopic ovarian endometriosis cystectomy and hysteroscopy. Normal eutopic endometrial tissues (NETs) were collected from 30 premenopausal women (aged 27-46 years) undergoing laparoscopic myomectomy and hysteroscopy or laparoscopic-assisted vaginal hysterectomy. All study participants had regular menstrual cycles and had been without the use of oral/intrauterine contraception or any hormonal therapy for at least 3 months prior to surgery. The phases of the menstrual cycle were confirmed according to histological examination. Most of samples were obtained in the late proliferative phase (days 13-14, 13/59) and early-to-mid secretory phase (days 15-23, 34/59), and samples in early-to-mid proliferative phase (days 1-12) and late secretory phase (days 24-28) each accounted for only 10.2%. Almost all patients (27/29) with endometriosis showed stage III or IV, as confirmed by the revised American Fertility Society (rAFS) score. The endometriotic tissues specimens were closely separated from inner cyst wall of ovarian endometriomas and had no contamination with ovarian tissues according to histologic examination. All tissues were divided into 2 parts. The first part was fixed with 4% paraformaldehyde for hematoxylin–eosin staining, and the second part was rapidly frozen in liquid nitrogen and stored at −80 °C for RNA and protein extraction. The study was approved by the Department of Obstetrics and Gynecology, The First Affiliated Hospital of Wenzhou Medical University, and written informed consent was obtained from all patients.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted with TRIzol reagent (Thermo Fisher Scientific, Waltham, Massachusetts), according to the manufacturer's instructions. RNA absorbance and concentration were checked with Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). To synthesize complementary DNA (cDNA), 1 μg of total RNA was reverse transcribed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. For quantitative real-time polymerase chain reaction, the mRNA levels of autophagic genes and HOXA10 were measured by StepOne Plus and SYBR Premix Ex Taq II (Takara Bio, Osaka, Japan), with the following primers: for microtubule-associated protein light chain 3 (LC3B): Forward 5′-CCGCACCTTCGAACAAAGAG-3′, Reverse, 5′-AAGCTGCTTCTCACCCTTGT-3′. For Beclin-1: Forward 5′-TAGACCGGACTTGGGTGACG-3′, Reverse, 5′-TAGACCCTTCCATCCCTCAGC-3′. For HOXA10: Forward 5′-TGCTTGGTGCTGGTTTAT-3′, Reverse, 5′-CCAGTCAGGACTTGACACTTA-3′. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH): Forward 5′-AAGAAGGTGGTGAAGCAGG-3′, Reverse, 5′-GTCAAAGGTGGAGGAGTGG-3′. The fold change in mRNA expression levels of the OET group and EET group over the NET group was obtained using the $2^{-\Delta\Delta CT}$ method. All calculated values of the above genes were normalized to the internal reference gene GAPDH values.

Western Blotting

Frozen tissue samples were ground up and homogenized in Radio Immunoprecipitation Assay (RIPA) tissue cell lysis buffer (Solarbio, Beijing, China) containing 1% phenylmethanesulfonylfluoride (Solarbio) proteinase inhibitors. Total protein concentrations were determined using enhanced bicinchoninic acid protein assay kit (Beyotime Biotech, Shanghai, China). Equal quantities of total proteins (30 μg) were separated by 12% sodium dodecyl sulfate polyacrylamide gels electrophoresis (SDS-PAGE) on a Mini-Protean III system (Bio-Rad, Hercules, California) and transferred to 0.45 mm/0.22 mm polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, Massachusetts). Then, the PVDF membranes were blocked for 2 hours at room temperature with 5% bovine serum albumin and subsequently incubated overnight with anti-LC3B (1:2000, ab192890; Abcam, Cambridge, United Kingdom), anti-beclin-1 (1:2000, ab207612; Abcam), or anti-HOXA10 (1:200, sc-17158; Santa Cruz Biotechnology, Delaware Ave

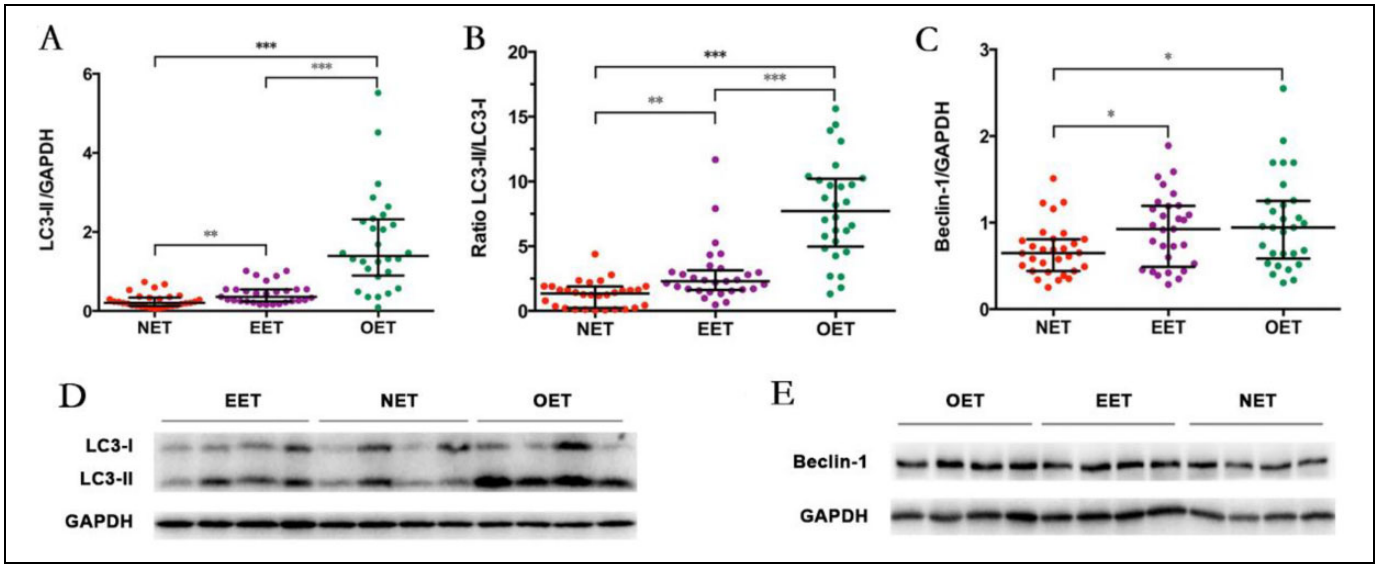

Figure 1. Autophagic proteins indicate an increased autophagic level in OET. Densitometric quantification of LC3-II protein (A), ratio LC3-II/LC3-I (B), and beclin-1 protein (C) in NET, EET, and OET groups evaluated by Western Blot. D and E, Representative immunoblots. All data are expressed as the mean (SD). *$P < .05$, **$P < .01$, ***$P < .001$, OET versus EET or NET, or EET versus NET. EET indicates eutopic endometrial tissue; NET, normal eutopic endometrial tissue; OET, ovarian endometriotic tissue; SD, standard deviation.

Santa Cruz , California) at 4°C overnight. The membranes were washed 3 times using Tris-buffered saline (TBS)–Tween 20 and then probed at room temperature for 1 hour with horseradish peroxidase (HRP)-conjugated goat anti-rabbit or goat anti-mouse secondary antibodies (1:10000; MultiSciences, Hangzhou, China). After 3 washes in TBS–Tween 20, the Western blot bands were detected using the WesternBright ECL HRP Substrate (Advansta, Inc, Menlo Park, California) and Super-Signal West Femto Substrate Trial Kit (Thermo Fisher Scientific). The Western blot bands were quantified using Image Lab Software (Bio-Rad) and normalized to GAPDH.

Immunoassay

Blood samples for cancer antigen 125 (CA125) measurement were collected before the surgery and were immediately centrifuged at 2000 rpm for 5 minutes. The supernatants were extracted to determine the serum CA125 concentrations using electrochemiluminescence immunoassays on an automatic Elecsys DXI 800 analyzer (Beckman Coulter, Inc, Fullerton, California). Normal concentrations of CA125 were less than 35 U/mL.

Hematoxylin–Eosin Staining

Formalin-fixed samples were dehydrated, embedded in paraffin, and sliced up into 4-μm paraffin sections. After dewaxing and hydrating, sections were dipped in 0.1% hematoxylin staining solution (Beyotime Biotech) for 5 minutes and 1% hydrochloric alcohol solution (Beyotime Biotech) for 5 seconds at room temperature. Then, sections were washed and treated with 1% eosin staining solution (Beyotime Biotech) for 1 minute. The sections were washed and mounted with Permount mounting medium (Beyotime Biotech). The optical microscope was used to identify the histological characteristics of OETs and endometrial tissues.

Statistical Analysis

The statistical analyses were performed with SPSS statistical software version 24.0 (SPSS, Inc, Chicago, Illinois). All results are presented as the mean (SD). The independent *t* test, Mann-Whitney *U* test, and Games-Howell test were used for quantitative variables. The χ^2 test and Fisher exact test were used for qualitative variables. Correlation coefficients were determined by spearman analysis, and a *P* value <.05 was considered statistically significant. *$P < .05$, **$P < .01$, ***$P < .001$.

Results

Autophagic Proteins Reveal Increased Levels in OET

To investigate whether autophagy activity is induced differently in OET, we detected the expression levels of LC3-II and the ratio of LC3-II to LC3-I, the most extensively detected autophagy-related protein. LC3-II is the only biochemical marker that is strictly related to completed autophagosomes, and the phenomenon that LC3-I is converted to LC3-II represents the formation of autophagosomes, for example, it predicts the induction of autophagy. As shown in Figure 1A, B, and D, both the LC3-II protein level and the ratio of LC3-II/LC3-I protein are significantly higher in OET than in EET and NET. Additionally, these markers are slightly but significantly increased in EET compared to NET.

We also measured levels of beclin-1, an additional autophagy-related marker, to further confirm the autophagic activity. Beclin-1 is an essential component that signals to

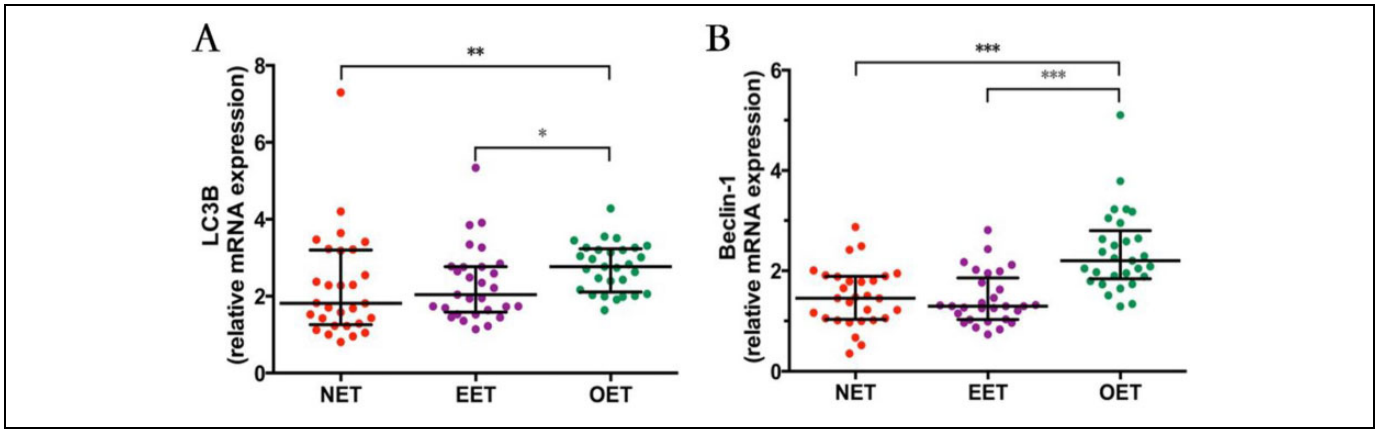

Figure 2. Autophagy-related genes are elevated in OET. Messenger RNA levels of beclin-1 (A) and LC3B (B) by quantitative real-time PCR analysis in NET, EET, and OET groups. All data are expressed as the mean (SD). *$P < .05$, **$P < .01$, ***$P < .001$, OET versus EET or NET, or EET versus NET. EET indicates eutopic endometrial tissue; NET, normal eutopic endometrial tissue; OET, ovarian endometriotic tissue; PCR, polymerase chain reaction; SD, standard deviation.

initiate autophagy and induces the autophagosome–lysosome fusion. As shown in Figure 1C and E, a significant decrease in beclin-1 protein level is observed in NET compared with EET and OET, but not in EET compared with OET, showing an upregulated autophagy activity in EET and OET.

Autophagy-Related Genes Are Elevated in OET

We have measured 2 autophagy core proteins, LC3-II and beclin-1, involved in the most crucial stages of the autophagic pathway, including induction, nucleation, expansion, and fusion. To further confirm the transcriptional level of these markers, we also evaluated the mRNA expression levels of the following autophagy-related genes: LC3B (LC3-II) and beclin-1. As shown in Figure 2A and B, the expression levels of LC3B and beclin-1 genes were significantly elevated in OET compared to EET and NET. However, we found no significant difference between the expression levels of LC3B and Beclin-1 genes in both EET and NET.

HOXA10 Expression Is Downregulated in OET

Because the ectopic endometrium tissues are exposed to microenvironments that include inflammatory responses and hormonal abnormalities, multiple genes are shown to have epigenetic aberrations and abnormal expression in endometriosis. Thus, we measured the expression of HOXA10, regulated by inflammation and sex hormones, acting on the regulation of endometrial cycle. In OET, the expression of the HOXA10 gene is significantly lower than in EET and NET (Figure 3A). There is a slight, nonsignificant increase in HOXA10 expression in NET compared with EET (Figure 3A). In accordance with its mRNA level, HOXA10 protein level is highly decreased or undetectable in OET compared with EET and NET and has no significant difference between EET and NET (Figure 3B and C).

CA125 Is Increased in Endometriosis and Is Related to rAFS Stages

Serum CA125 is broadly used to diagnose and stage ovarian endometriosis. In our study, it is obvious that serum CA125 levels of endometriosis group are higher than that of the control group (Table 1). In the endometriosis group, we also found that concentrations of serum CA125 significantly increase with the elevated staging (I-IV), which indicates that serum CA125 level is related to rAFS stages (Table 1).

The Correlation Among HOXA10, Autophagy, and Serum CA125 in All Tissues

First, we measured whether HOXA10 expression is related to autophagic level and found that the HOXA10 gene expression is significantly negatively correlated with the LC3B gene and beclin-1 gene ($R = -0.476$, $P < .001$; $R = -0.376$, $P < .001$; Table 2). We also found that at the protein level, HOXA10 expression was negatively correlated with the LC3-II protein and the LC3-II/LC3-I ratio ($R = -0.456$, $P < .001$; $R = -0.383$, $P < .001$), but not with the beclin-1 protein (Table 3). Thus, HOXA10 negatively regulates the autophagic process at the transcriptional and posttranslational level in both normal and/or abnormal EETs and OETs. We further investigated the correlation between serum CA125 level and HOXA10 expression or autophagic level. There is a significant positive correlation between serum CA125 and LC3-II protein and/or LC3-II/LC3-I ratio ($R = .307$, $P = .005$; $R = .405$, $P < .001$) and a significant negative correlation between serum CA125 and HOXA10 gene expression ($R = -0.304$, $P = .005$; Table 4).

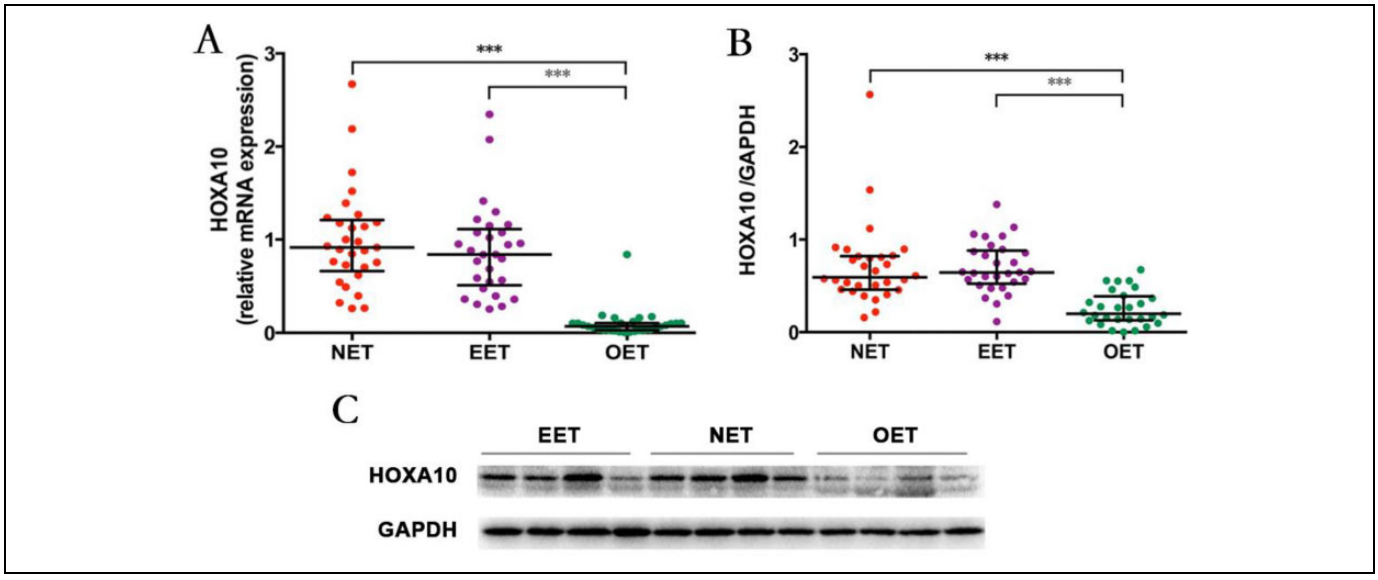

Figure 3. Homeobox A10 (HOXA10) is decreased in OET. A, Messenger RNA levels of HOXA10 measured by real-time PCR analysis in NET, EET, and OET. B, Densitometric quantification of HOXA10 protein by Western Blot analysis in NET, EET, and OET groups. C, Representative immunoblot of HOXA10. All data are expressed as the mean (SD) ***$P < .001$, OET versus EET or NET, or EET versus NET. EET indicates eutopic endometrial tissue; NET, normal eutopic endometrial tissue; OET, ovarian endometriotic tissue; PCR, polymerase chain reaction; SD, standard deviation.

Table 1. Demographic Data of Endometriosis and Control Groups.

	Control	Endometriosis	Stage I-II	Stage III	Stage IV
N	30	29	3	15	11
Age	38.53 ± 4.69	32.86 ± 5.44	33.67 ± 9.07	32.20 ± 5.14	33.55 ± 5.30
P	<.001		.961[a]	1.000[b]	.796[c]
BMI	22.82 (3.04)	21.59 (3.08)	19.48 (1.68)	21.50 (1.80)	22.30 (4.42)
P	.129		.290[a]	.250[b]	.839[c]
Parity	1.43 (0.62)	0.93 (0.59)	1.00 (1.00)	0.80 (0.41)	1.09 (0.70)
P	.003		.940[a]	.988[b]	.455[c]
% of infertile women	6.67	24.14	66.67	20.00	18.18
P	.133		.172	.176	1.000
Serum CA125	24.96 (21.56)	88.07 (69.56)	39.17 (12.22)	55.91 (39.78)	142.34 (74.64)
P	<.001		.416[a]	.003[b]	.009[c]

Abbreviation: BMI, body mass index.
[a]Comparison between stage I-II and stage III.
[b]Comparison between stage I-II and stage IV.
[c]Comparison between stage III and stage IV.

Table 2. Correlation Coefficient (R) Between HOXA10 Gene and Autophagy-Related Genes in All Groups.

	LC3B mRNA	Beclin-1 mRNA
HOXA10 mRNA	−0.476[a]	−0.376[a]

Abbreviations: HOXA10, homeobox A10; mRNA, messenger RNA.
[a]$P < .001$.

Table 3. Correlation Coefficient (r) Between HOXA10 Protein and Autophagic Proteins in All Groups.

	LC3-II Protein	LC3-II/LC3-I Ratio	Beclin-1 Protein
HOXA10 protein	−0.456[a]	−0.383[a]	0.002

Abbreviations: HOXA10, homeobox A10; mRNA, messenger RNA.
[a]$P < .001$.

Table 4. Correlation Coefficient (r) **B**etween Serum CA125 and HOXA10 and Autophagic Biomarker in All Groups.

	LC3B mRNA	LC3-II Protein	LC3-II/LC3-I Ratio	Beclin-1 mRNA	Beclin-1 Protein	HOXA10 mRNA	HOXA10 Protein
Serum CA125	−0.009	0.307[b]	0.405[a]	−0.027	−0.002	−0.304[b]	−0.151

Abbreviations: CA125, cancer antigen 125; HOXA10, homeobox A10; mRNA, messenger RNA.
[a]$P < .001$.
[b]$P < .01$.

No significant correlations between serum CA125 and other biomarkers are found (Table 4).

Discussion

The pathogenesis of ovarian endometriosis is still not well understood, but the most widely accepted theory remains Sampson implantation hypothesis, which does not explain why some, but not most, women have endometriosis. Recently, abundant evidence indicates that interaction of the frequency and quantity of menstrual blood reflux with molecular and cellular alterations appears to decide the occurrence and development of endometriosis.

Autophagy is a homeostatic process that maintains the survival of various cells in normal or stress-induced conditions and is involved in pathophysiological processes such as inflammatory diseases.[21,24] In this context, we have revealed that NET and EET are characterized by a decrease in LC3-II protein level, conversion of LC3-I to LC3-II, and beclin-1 protein level compared to OET. In accordance with their protein levels, a significant transcriptional induction of LC3B and beclin-1 is observed in OET compared with NET and EET. Additionally, LC3-II protein, ratio of LC3-II/LC3-I, and Beclin-1 protein are increased in EET compared with NET, while their mRNA levels are not significantly different, showing a possible post-translational level regulation of autophagy genes in human endometrial tissues. Taken together, these findings indicate that a remarkable upregulation of autophagy activity in OET is involved in the pathogenesis of endometriosis and related to reduced apoptosis. Moreover, EETs with altered autophagic level, regarded as the source of lesions, may be more prone to act on the initiation and progression of endometriosis.

The HOX genes share the typical spatial expression in both the embryonic and adult female genital tracts, among which the HOXA10 gene plays a predominant role in differentiating embryonic tissues to uterus during development and persistently functions in the adult endometrial tissues.[25] Interestingly, previous studies show that although weakly expressed, the expression of HOXA10 is observed in ovarian endometriosis and other endometriotic lesions such as peritoneal, lung, and rectosigmoid endometriosis.[18,26] Our study confirms that the HOXA10 gene and protein are weakly expressed in OETs in comparison with EETs and normal endometrial tissues. The detection of HOXA10 protein outside its normal spatial domain (ie, myometrium and endometrium) indicates that HOXA10 might play a role in the formation of endometrial tissues at ectopic locations and is related to the pathogenesis of endometriosis.[25] However, the HOXA10 gene and protein levels are slightly decreased in EETs compared with normal endometrial tissues. Downregulation of HOXA10 contributes to the decidualization deficiency and endometriosis-related infertility.[16,27] Otherwise, impaired decidualization lead to the higher content of endometrial stromal cells in endometriosis-derived endometrium than that is found in normal endometrium.[28] Considering that endometrial stromal cells have a primary role in the implantation of endometrial tissues outside the uterus,[22] studies have assigned adhesive and invasive function to HOXA10-deficient refluxed endometrium, which will develop into endometriotic lesions.

Although there is a negative correlation between HOXA10 and endometriosis as demonstrated in our study and previous reports,[18,29] the mechanism of how HOXA10 influences endometrial cells is still not clear. In our study, a negative correlation between HOXA10 expression and the level of autophagy was found for the first time. It suggested that downregulated HOXA10 activates autophagic process in endometriosis, and this correlation coincides with the evolutionary conservation of HOX-mediated autophagy inhibition.[23] Meanwhile, HOXA10 may negatively regulate the autophagy through control of various downstream genes such as β3-integrin,[30] FK506 binding protein 4,[31] and the Wnt/β-catenin axis.[32] Besides, there are 2 main mechanisms that could explain why decreased HOXA10 induces autophagy and contributes to the endometriosis: inflammation and apoptosis. Because of a pelvic inflammatory condition in endometriosis, the peritoneal microenvironment is remarkably rich in cytokines, chemokines, prostaglandins, and excessive macrophages.[33,34] These abnormal molecules and cells induce the invasion and implantation of endometrial cells and the formation of ovarian endometriotic lesions through controlling tissue remodeling. Additionally, they create an abundant vascular supply for the development and maintenance of ovarian endometriotic lesions.[35,36] Consequently, the presence of inflammatory responses, matrix remodeling, and angiogenesis in OETs stimulates autophagy that may fuel mitochondrial metabolism and metastasis in endometriotic stromal cells or serve as a survival process under metabolic stress, resulting from a high proliferation rate and hypoxia or nutrient deprivation from insufficient vascularization.[9,37] Moreover, inflammation may self-regulate in a positive feedback loop in endometriosis through downregulating the HOXA10 gene. Research shows that the cytokine IL-β can induce the downregulation of the HOXA10 gene and in turn enhance the effect of prostaglandin E_2, resulting in development of endometriosis.[27,38] The deficiency of HOXA10

would stimulate autophagy by inducing adhesion, invasion, angiogenesis, growth, and survival of endometriotic lesions through multiple inflammation-associated factors and signaling pathways.[39-45] In fact, HOXA10 deficiency-induced inflammation can also contribute to mitochondria damage and excessive release of substances such as mitochondrial ROS and oxidative mitochondrial DNA.[46] Consequently, endometriotic cells will have a strong and persistent mitochondrial ROS-induced oxidative stress. In this condition, cytoprotective autophagy is induced to eliminate damaged mitochondria and inhibit inflammation in endometriosis.[13,20,46] Therefore, the autophagic response in endometriosis, acting as both a pathogenic and survival mechanism, has been attributed to the inflammatory response promoted by the HOXA10 deficiency-induced overexpression of inflammation.

Autophagy and apoptosis are 2 interconnected processes that often occur in the same cell in response to stress and prove to be mutually inhibitory in most conditions.[47] Recent research confirms that increased autophagic processes are attributed to deficiency of the apoptotic death program in endometriotic cells, that is, activated autophagy can repress apoptosis in endometriosis.[13,47] Evidence shows that decreased expression of the HOXA10 gene inhibits apoptosis in breast cancer and endometriosis, and the following major mechanisms explain this: the first is the inhibition of caspase-3-mediated intrinsic apoptotic pathways through HOXA10 deficiency-mediated decrease of calpain 5 and increase of prostaglandin E_2[17,43]; the second is HOXA10 deficiency-mediated posttranscriptional degradation of p53.[19] Furthermore, inefficiency to exert apoptosis can contribute to increased autophagy, and low p53 levels can trigger autophagy by activating the autophagy-initiating UNC-51-like kinase 1 complex.[13,48] Therefore, HOXA10 deficiency promotes the survival and maintenance of endometriotic lesions through inducing autophagy.

The abnormalities of HOXA10 expression and autophagic levels are also observed in the eutopic endometrium of patients with endometriosis, which may propose that HOXA10 deficiency-mediated induction of autophagy can also lead to increased inflammatory response and decreased apoptotic process in eutopic endometrium; on the other hand, altered endometrial tissues are prone to develop into endometriotic lesions when they exist outside the uterus. This is a new perspective for the role of HOXA10 in the eutopic endometrium.

Our study also shows that concentrations of serum CA125 are higher in the endometriosis group than in the control group. Furthermore, we found a moderate negative correlation between serum CA125 and HOXA10 gene expression and a moderate positive correlation between serum CA125 and LC3B protein expression. Previous research shows that serum CA125 has a positive association with cyclooxygenase-2 (a key enzyme in prostaglandin E_2 synthesis) and has mesothelin-binding ability.[49,50] Increased serum CA125 is related to active inflammatory responses and the development of endometriosis. We suppose that CA125 may promote the progression of endometriosis through HOXA10 deficiency and autophagy activation. Serum CA125 is well recognized as the nonsurgical tool to diagnose and stage ovarian endometriosis, especially moderate to severe stages of endometriosis.[51] Our results show that with elevated staging (I-IV), serum levels of CA125 also gradually increased.

This study has some limitations. Surgeries were usually operated during the nonmenstrual period for the patients in this study, as a result, most of samples were obtained in the late proliferative phase and early secretory phase. The paucity of cases, especially cases of early proliferative stage and late secretory stage, led to the inability to calculate possible change trends in HOXA10 and autophagy-related proteins with the menstrual cycle. In the future, we will increase the sample sizes and analyze the cyclic change of HOXA10 levels and autophagic markers in various endometriotic lesions and the corresponding eutopic endometrium. What's more, it has been reported that the histologic ectopic endometrial structure was different (refer to glandular differentiation and whether or not the gland exist in the tissue) in superficial endometriosis, deeply infiltrating endometriosis, and ovarian endometriomas.[52] Therefore, it's possible that ovarian lesions have different ratios of endometriotic cell types than lesions found at other sites. What we focused on in this study is ovarian endometriosis; we did not collect specimens from other lesions. As far as we know, no study in English has been found to detect the level of autophagic markers in the different endometriotic cell types. In the near future, our teams are planning to make experiment in vitro to explore the relationship among HOXA10, autophagic marker, and CA125 in different types of endometriotic cells in endometriosis.

In conclusion, our results show that the autophagic process is upregulated in ovarian endometriomas, as evaluated by LC3-II and beclin-1 mRNA and protein expression levels. The HOXA10 expression is suppressed in ovarian endometriomas and is related to the levels of autophagic indicators, which may contribute to the upregulated autophagy in endometriosis. Serum CA125 is related to the stage of endometriosis and may function as an indicator of HOXA10 and autophagic levels. Future investigations about HOXA10 and autophagy through inflammation and apoptosis will provide a reliable and comprehensive experimental system to dissect the above pathophysiologic network.

Authors' Note

X.L. carried out most of the study and drafted the article. J.B. and X.H. screened patients and collected their biological samples. Y.J. and L.C. carried out some histological studies. J.Z. conceived and designed the study. F.Z. carried out the analysis and interpretation of data. All participated in writing the article.

Acknowledgments

The authors acknowledge laboratory medicine staff and pathology department staff. The authors also acknowledge help from collaborating physicians in tissue accrual (FengDi Chen, Yan Hu, XueQing Wu, Ya Lin, Kai Zhou, Qian Zhang).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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