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***Observational Study***

**Clinical significance and role of up-regulation of SERPINA3 expression in endometrial cancer**

Zhou ML *et al*. Role of SERPINA3 expression in endometrial cancer

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**Abstract**

***BACKGROUND***

Serpin peptidase inhibitor, clade A member 3 (SERPINA3) belongs to the serpin family with an inhibitory activity against proteases. Its aberrant expression has been observed in a wide range of tumor cells. However, its clinical significance and biological function in endometrial cancer have been rarely studied. We designed a study to determine the levels of SERPINA3 and its significance in patients with endometrial cancer.

***AIM***

To investigate the clinical significance and role of SERPINA3 expression in endometrial cancer cells.

***METHODS***

Eightyendometrial tissue samples collected from patients with endometrial cancer were included in an observation group and 80 paraffin-embedded tissues samples collected from patients with normal endometrial tissues undergoing myomectomy were employed as a control group between January 2014 and December 2018. The expression of SERPINA3 mRNA was detected by quantitative polymerase chain reaction (PCR) for all endometrial tissues included in the study.

***RESULTS***

The positive expression rate of SERPINA3 protein in endometrial cancer cells was 71.25% in the observation group, which was significantly higher than that in the control group (31.25%; *P* < 0.05). There was no correlation between SERPINA3 protein in endometrial cancer cells and the age range at which women experienced menopause (*P* > 0.05). However, it was associated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis (*P* < 0.05). Pathological grade, clinical stage, vascular invasion, and lymph node metastasis were independent prognostic factors for endometrial cancer.

***CONCLUSION***

The follow-up study of SERPINA3 can be used as a prognostic biomarker for endometrial cancer and as one of the targets for bio-targeted therapy for endometrial cancer.

**Key words:** Serpin peptidase inhibitor, clade A member 3; Endometrial cancer; Quantitative polymerase chain reaction; Expression

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**Core tip:** Serpin peptidase inhibitor, clade A member 3 (SERPINA3) is considered to be associated with several cancers. In this study, the clinical significance and role of SERPINA3 expression in endometrial cancer were investigated. It was found that the expression of SERPINA3 was up-regulated in endometrial cancer and expression of SERPINA3 in endometrial cancer was correlated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis. SERPINA3 has the potential to be used as a biomarker for prognosis and a specific target for targeted therapy in patients with endometrial carcinoma.

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**INTRODUCTION**

Endometrial cancer is a common malignancy which affects women in China. The incidence of endometrial cancer is just behind that of cervical cancer. It is also the representative malignant cancer of the female reproductive system with an elevated incidence rate in the United States[1]. Many patients are unaware of its onset and development due to the hidden cause and non-specific signs and symptoms in the early stages of the disease. As a result, a great proportion of women are diagnosed with endometrial cancer at an advanced stage, which causes them to miss the important opportunities for appropriate and effective interventions. In most cases, endometrial cancer presents as vaginal bleeding in postmenopausal women; and in non-menopausal women, it is characterized by increased menstrual loss and longer or irregular periods.

Therefore, cancer detection should be carefully used to effectively control the development of cancer, promote the early management intervention, and improve the prognosis accordingly. Currently, the most basic methods for detection of endometrial cancer include diagnostic curettage, adjuvant detection by B-mode ultrasonography, hysteroscopy, and nuclear resonance imaging[2,3]. Surgical therapy is the main intervention for most women with endometrial cancer. The follow-up intervention should focus on the non-invasive, accurate strategies for detecting the above mentioned therapies. Recently, more studies increased their interest in targeted therapy targeting cancer specific genes. Serpin peptidase inhibitor clade A member 3 (SERPINA3) is one of them. The related studies[4,5] revealed that SERPINA3 and its ectopic expression were identified in several cancers, *e.g.*, liver, prostate, lung, and breast cancers. However, few studies investigated SERPINA3 in patients with endometrial cancer. The present study examined the gene expression of SERPINA3 and its clinical significance in endometrial cancer.

**MATERIALS AND METHODS**

***Sample collection***

Eightyendometrial tissue samples collected from patients (aged 54-68 years, mean age 61.5 ± 3.1 years) with endometrial cancer were included in an observation group and 80 paraffin-embedded tissues samples collected from patients (aged 52-69 years, mean age 60.2 ± 2.9 years) with normal endometrial tissues undergoing myomectomy were employed as a control group between January 2014 and December 2018. The tissue samples (6 cm × 4 cm × 2 cm for both groups) were collected with the informed consent of the donors and relatives and with complete follow-up information and clinical data. All the patients were chemoradiotherapy, hormone therapy, and antitumor therapy naïve.

***Tissue microarray construction***

Tissue microarrays were constructed and exposed to the dye. The most representative tumor regions were marked on haematoxylin and eosin stained section slides. This step was also applicable for normal endometrial tissues. Two focus points were selected per tissue block. Targeted tissue samples were extracted from the donor blocks by a fine hollow needle of 1.0 mm in diameter and then injected into the recipient paraffin blocks. The placement of each tissue sample on the coordinate grid of the recipient block was done according to the research aim and the requirements for the study. Once the recipient block was filled, it was cut to generate 4 um thick slices for analysis.

***Immunohistochemical staining***

The slices were baked at 70 °C for a total of 1 h. Xylene was used to remove paraffin wax. Then, hydration was performed in graded ethanol. For antigen retrieval, 0.01 mmpl/L of sodium citrate buffer (pH = 6) was added and incubated at 100°C for 10 min. The sections were then incubated with rabbit anti-human polyclonal antibody (dilution, 1:50) at 4 °C overnight, followed by incubation with goat anti-rabbit (IgG) secondary antibody (1:200) at room temperature for 1 h. Diaminobenzidine was used for staining. Hematoxylin stain was applied in the secondary staining step. At last, proper neutral balsam was added to seal the tissue. The scoring criteria were: a score of 0 was considered to reflect the positive expression rate of 0 to 10%, score of 1 reflect 10% to 30%, score of 2 reflect 30% to 60%, and score of 3 reflect >60%. If the staining results were not consistent between the two tissue cores, the higher score was considered only. The expression was scored by two pathologists double-blindly. The consistent sores between the two pathologists were involved in the final analysis. Scores of 0 to 1 were defined as low expression and scores of 2 to 3 were defined as high expression.

***Real-time quantitative polymerase chain reaction***

Total RNA was extracted using TRIZOL reagent. This step was done by strictly following standard instructions (PrimeScript RT, USA). A Rim eScriptRT kit was used for reverse transcription to obtain cDNA. The reaction condition was 37 °C for 30 min and 85 °C for 5 s. The ABI 7300 real-time PCR system was used for amplification. The primers used are as follows: SERPINA3 upstream primer, 5’-TGCCAGCGCACTCTTCATC-3’ and SERPINA3 downstream primer, 5’-TGTCGTTCAGGTTATAGTCCC; GAPDH upstream primer, 5’-TGCGAGTACTCAACACCAACA-3’ and GAPDH downstream primer, 5’-GCATATCTTCGGCCCACA-3’. The overall reaction system consisted of 0.8 μL of GAPDH or AERPINA3 upstream and downstream primers, 7.6 μL of cDNA, 10 μL of SYBR Prem ixEx TaqTM, 0.4 μL of Rox Reference Dye (50×), and 0.4 μL of Rox Reference Dye II (50×). PCR reaction conditions were predenaturation at 95°C for 30 s and 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 31 s. The obtained data were input into Excel for calculation and analysis.

***Statistical analysis***

Statistical analyses were performed with SPSS19.0 software using “*X2*” and “*t”* reflecting results of the test. The count data are presented using percentages and the measured data are presented as the mean ± standard deviation. Significance level was considered at *P* < 0.05.

**RESULTS**

***Expression of SERPINA3 protein***

A comparison of SERPINA3 protein expression between the observation group and the control group showed that the positive expression rate of SERPINA3 protein in endometrial cancer cells was 71.25% (57/80) in the observation group, which was significantly higher than that in the control group (31.25%, 25/80; *P* < 0.05).

***Relationship between SERPINA3 expression and pathologic features of endometrial cancer***

SERPINA3 protein expression in endometrial cancer cells was associated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis (*P* < 0.05), rather than with the age range at which women experienced menopause (*P* > 0.05, Table 1).

***Factors influencing the prognosis of patients with endometrial cancer***

Pathological grade, clinical stage, vascular invasion, and lymph node metastasis were independent prognostic factors for endometrial cancer (Table 2).

**DISCUSSION**

Rising prevalence of endometrial cancer has been reported in China over the last decades, and accordingly the research work on endometrial cancer went further and more detailed. However, the exact cause of endometrial cancer is still unknown[6-8]. Currently, the research of endometrial cancer shows increasing interest in how prognosis can be improved by early diagnosis and effective treatment. The assessment of biomarkers may predict the prognosis of endometrial cancer, and should be further studied and interpreted, and studies of biomarkers and bio-targeted therapy for endometrial cancer should be warranted. SERPINA3, an important member of the serine peptidase inhibitors family, has been identified and validated in several cancer cells. However, very few studies have examined SERPINA3 in endometrial cancer cells[9]. As a typical acute-phase protein, SERPINA3 is regulated by inflammatory cytokines so that its expression is increased in the inflammatory response. The promoter of SERPINA3 is transcriptionally activated by three transcription factors and the transcriptional level of SERPINA3 is influenced by single nucleotide polymorphisms within the promoter[10-12]. Single nucleotide polymorphisms are expressed by regulating the components of genetic sequence when the inflammatory response occurs. Once the inflammatory response occurs, it is benefit for the synthesis of SERPINA3. Nowadays, studies have produced inconsistent and even contradictory results on the expression of SERPINA3. The relevant studies[13-15] showed that overexpression of SERPINA3 predicts that damage tends to occur in the body contributing to decreased cell adhesion ability and inhibition of apoptosis. It is revealed that the up-regulation of SERPINA3 is positively associated with malignant tumors.

Few studies have reported the relationship between SERPINA3 protein expression and the incidence of endometrial cancer in China. The current study revealed that pathological grade, clinical stage, vascular invasion, and lymph node metastasis of endometrial cancer were strongly associated with the expression of SERPINA3 (*P <* 0.05). Recently, many studies demonstrated that SERPINA3 could be used as one of the biomarkers in predicting prognosis of several cancers. For instance, SERPINA3 is one of the proteins in signal transduction pathways of the inflammatory response. A studyof clinical relapsed ovarian tumors showed that the up-regulation of SERPINA3 was associated with disease progression and chemical resistance[16]. Moreover, another study showed that methylation of the 5' region may account for up-regulation of SERPINA3 in placental disease. Researchers also found that overexpression of SERPINA3 in JEG-3 human choriocarcinoma cells could prevent apoptosis[17]. Furthermore, one study established that SERPINA3 levels were increased in patients with liver, pancreatic, and prostate cancers. Research studies on endometrial cancer have increased in recent years[18]. What is certain is that p53 mutations and subsequent overexpression of human epidermal growth factor receptor 2 (HER2) were common molecular events in endometrial cancer. According to Villalba *et al*[19], an analysis of SERPINA3 expression in normal tissues and endometrial cancer tissues and in different surgical/pathological stages through immunohistochemistry and fluorescence quantitative real-time polymerase chain reaction (FQ-PCR) suggested that the up-regulation of SERPINA3 was strongly associated with factors such as poor differentiation and high surgical/pathological stage. In addition, it predicted a poor prognosis of endometrial cancer.

Endometrial cancer is a malignant tumor that lacks clear diagnosis and optimal treatment. There is currently no conclusive evidence available for the postoperative healing, and the overall survival rate has not been improved fundamentally, although hysterectomy is performed as the main treatment for most women with this cancer. Alternatively, effective postoperative bio-targeted therapy should be given to improve survival and delay recurrence subsequent to surgery in patients who experienced hysterectomy for endometrial cancer[1,20]. Based on the present study, lymph node metastasis and vascular invasion were independent prognostic factors influencing prognosis of endometrial cancer, and SERPINA3 played an important role in the prognosis of endometrial cancer. This further strengthens that SERPINA3 is one of biomarkers for predicting poor prognosis of endometrial cancer. Estrogen-independent type II carcinoma is the more common type of endometrial cancer. Activity of HER2 is increased and cell growth and apoptosis are regulated through the PI3K/AKT pathway in this population. Whether a synergistic interplay exists between SERPINA3 and HER2 in the development of type II endometrial carcinomas should be further investigated[21,22].

In conclusion, SERPINA3 has the potential to be used as a biomarker for prognosis and a specific target for targeted therapy in patients with endometrial carcinomas based on a deepening understanding of endometrial carcinoma through research.

**ARTICLE HIGHLIGHTS**

***Research background***

The expression profile of serpin peptidase inhibitor clade A member 3 (SERPINA3) in patients with endometrial cancer has rarely been studied. In the present study, we detected the protein levels of SERPINA3 in patients with endometrial cancer and the correlation between the expression of SERPINA3 and the prognosis for endometrial cancer. The result revealed that SERPINA3 expression was significantly up-regulated in endometrial cancer and was closely correlated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis. These findings indicated that SERPINA3 can be used as a prognostic biomarker for endometrial cancer and as one of the targets for bio-targeted therapy for endometrial cancer.

***Research motivation***

The results of the present study may provide insight into the application of SERPINA3 as a predictor of clinical outcomes and a potential therapeutic target for endometrial cancer.

***Research objectives***

The present study aimed to assess the significance of SERPINA3 levels in patients with endometrial cancer.

***Research methods***

The present study examined tissue samples which were available from patients with endometrial cancer and patients with normal endometrial tissues. Tissue microarrays were constructed and immunohistochemical staining was performed. The expression of SERPINA3 mRNA was detected by quantitative PCR. The data were analyzed with SPSS19.0 software.

***Research results***

We found that SERPINA3 expression was higher in endometrial cancer compared to normal tissues and up-regulated SERPINA3 was closely correlated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis in endometrial cancer.

***Research conclusions***

Assessment of tumor *vs* normal tissues showed that the expression of SERPINA3 was up-regulated in endometrial cancer. The SERPINA3 protein level in endometrial cancer cells was associated with pathological grade, clinical stage, vascular invasion, and lymph node metastasis, rather than with the age range at which women experienced menopause. SERPINA3 has the potential to be used as a biomarker for prognosis and a specific target for targeted therapy in patients with endometrial carcinomas.

***Research perspectives***

Further studies are warranted to improve our understanding of the role of SERPINA3 in endometrial cancer.

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**Table 1 Relationship between SERPINA3 expression and pathologic features of endometrial cancer**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Variable** | ***N*** | **SERPINA3 protein** | ***X*2** | ***P*-value** |
| - | + |
| Pathological grade | I | 45 | 19 | 26 |  9.774 | <0.05 |
| II | 27 | 4 | 23 |
| III/IV | 8 | 0 | 8 |
| Clinical stage | G1 | 21 | 10 | 11 | 6.351 | <0.05 |
| G3 | 32 | 5 | 27 |
| G2/G3 | 27 | 8 | 19 |
| Menopause | Yes | 41 | 20 | 21 | 0.864 | >0.05 |
| No | 39 | 15 | 24 |
| Vascular invasion | ≤50% | 52 | 7 | 45 | 16.953 | <0.05 |
| >50% | 28 | 16 | 12 |
| Lymph node metastasis | Positive | 24 | 1 | 23 | 10.115 | <0.05 |
| Negative | 56 | 22 | 34 |

“-” indicates the negative expression of SERPINA3; “+” indicates the positive expression of SERPINA3.

**Table 2 Factors influencing the prognosis of patients with endometrial cancer**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Factor** | **B** | **SE** | **df** | ***P*** | **95% CI for Exp (B)** |
| Pathological grade | 0.6567 | 0.0149 | 1 | 0.000 | 0.5385-0.8865 |
| Clinical stage | 0.7337 | 0.0140 | 1 | 0.000 | 0.5689-0.8561 |
| Vascular invasion | 0.7685 | 0.0208 | 1 | 0.000 | 0.4414-0.9836 |
| Lymph node metastasis | 0.6605 | 0.0145 | 1 | 0.000 | 0.5176-0.9074 |

B: Regression coefficient; CI: Confidence index; Exp (B): Odds ratio.