Roles of EPX in endometrial cancer and its clinical significance

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Abstract

Objective To investigate the diagnostic and prognostic value of EPX in endometrial cancer and its clinical significance. Methods A total number of 90 endometrial cancer tissues and the adjacent tissue were included in this study, and the expression of EPX in tumor tissues and the adjacent tissues were examined by RT-qPCR and IHC methods; the correlation between the expression of EPX and the clinical pathological characteristics of the patients were analyzed, and the diagnostic value of EPX in patients with endometrial cancer were evaluated. Results EPX was down-regulated in endometrial cancer. ROC curve analysis suggested that the AUC of EPX was 0.8852 (95% CI, 0.8379–0.9324). Increased expression of EPX may predict better prognosis of the patients. Conclusion EPX were aberrantly expressed in endometrial cancer, and EPX has the potential to become a diagnostic and prognostic marker as well as therapeutic target for the treatment of endometrial cancer.

Key word: eosinophil protein-x, endometrial cancer, prognosis, diagnostic
Introduction

Nowadays, endometrial cancer has become the most common type of female cancer, and it is also one of leading cause of cancer-related deaths among women worldwide (1-3). At current stage, the pathogenesis of endometrial cancer remains unclear, and most of the patients were diagnosed at later stage when they first came to the hospital, leading to poor prognosis (5-year survival rate <20%) (4,5). Thus, to identify novel biomarkers and therapeutic targets is in a great need for the diagnosis, prognosis and treatment of endometrial cancer.

Eosinophils are a group of blood cells characterized by the acidophilic stains. Like many other type of leukocytes, eosinophils can migrate to various tissues and organs to maintain the local homeostasis. Increasing evidences indicated that the degranulation of eosinophil or tumor-associated tissue eosinophilia (TATE) have been associated with the prognosis of several types of solid tumors (6-9). eosinophil protein-X (EPX, also known as eosinophil derived neurotoxin (EDN)) is one of the four basic granule proteins in eosinophil proteins (10), however, the roles of either eosinophils or EPX in endometrial cancer has not yet been discussed.

In the present study, we will focus on the diagnostic and prognostic value of EPX in endometrial cancer and its clinical significance. Our results may provide novel biomarkers for the early diagnosis and prognosis of endometrial cancer.

Material and methods

Patients

A total number 90 endometrial cancer tissues and the adjacent tissue were collected between 42 and 78 from patients who have been clinically diagnosed as endometrial cancer in Gynaecology & Obstetrics department of our hospital. Patients who have received chemo or radiotherapy were excluded from this study. This study has been proved by the ethics committee of our hospital, and informed consent was obtained from each patient. The clinical pathology information of the patients was
shown in Table 1.

Table 1. Clinical information of the patients

<table>
<thead>
<tr>
<th></th>
<th>Cases (n)</th>
<th>EPX</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;60</td>
<td>55</td>
<td>18</td>
<td>0.0050</td>
</tr>
<tr>
<td>Age &lt;60</td>
<td>35</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Tumor size &gt;3 cm</td>
<td>55</td>
<td>15</td>
<td>0.0427</td>
</tr>
<tr>
<td>Tumor size &lt;3 cm</td>
<td>35</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>77</td>
<td>25</td>
<td>0.3368</td>
</tr>
<tr>
<td>Low</td>
<td>13</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Lymph node methasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47</td>
<td>15</td>
<td>0.5975</td>
</tr>
<tr>
<td>No</td>
<td>43</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>TNM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>57</td>
<td>19</td>
<td>0.7706</td>
</tr>
<tr>
<td>Low</td>
<td>33</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

**Real-time quantitative PCR**

The total RNAs were isolated from the tissues samples by TRIzol (Invitrogen, Thermo Fisher Scientific, MA, USA). Real-time quantitative PCR(qRT-PCR) was performed on an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the One Step SYBR®PrimeScript™ RT-PCR Kit II (Takara, Dalian, China) with the thermocycling profiles as follow: 95 °C 30 sec, 40 cycles of 95 °C5 sec and 60 °C 30 sec. GAPDH was used as the internal control.

**Immunohistochemical analysis**
Cancer tissue and the adjacent tissue were embedded with paraffin and sectioned into 4mm slides for the immunohistochemistry analysis. IHC was performed with Ready-to-Use Immunohistochemistry Hypersensitivity UltraSensitive™ S-P kit (Maxim, Fuzhou, China) following the manufacturer’s protocols. Briefly, the tissue sections were deparaffinized and rehydrated, and then heat-fixed with the protein-blocking solution. Sections were subsequently incubated with primary antibodies (anti-EPX, Boster, Wuhan, China), followed by HRP-labeled secondary antibody. Diaminobenzidine (DAB) was used for colorization.

**Histology scoring**

The histological scoring was performed by two researchers independently. The intensity of the staining was categorized as 0-3 (negative 0, weak 1, moderate 2, and strong 3). The positive area of the immunostaining was characterized as 0 (<10%), 1 (10-25%), 2 (25-50%), 3 (>50%). The final score was calculated by multiplying the intensity of the staining with the positive area. The final scores of 0-3 represents negative and 4-9 represents positive.

**Statistical analysis**

Statistical analysis was performed using SPSS 17.0 software. Data were presented as the means ± standard deviation, and the two independent sample T-test was performed to draw a comparison between groups. Correlation between the expression of the proteins (immunohistochemical scores) and clinical pathology parameters of the patients were analyzed by Chi-square test. Kaplan-Meier analysis was performed to compare the OS curves in the different groups, and P<0.05 indicated statistically significant difference.

**Results**

**Decreased mRNA expression of EPX in endometrial cancer tumor samples**

First of all, to investigate the roles of EPX as diagnostic marker for endometrial cancer, the expression of EPX in tumor samples of patients with endometrial cancer and the adjacent using RT-qPCR methods. It was observed that the expression of EPX
was significantly decreased in tumor samples compared with the adjacent tissues (Fig. 1, \( P < 0.01 \)); moreover, receiver operating characteristic (ROC) curve was drawn to evaluate whether the expression of EPX can distinguish the tumor sample from the adjacent tissue. As shown in figure 2, the AUC of EPX was 0.8852 (95% CI, 0.8379–0.9324).

![Figure 1](image)

Figure 1. Expression of EPX in endometrial cancer tissue and the adjacent tissue

**\( P < 0.01 \) vs. the control group.

**Decreased protein expression of EPX in endometrial cancer tumor samples**

Next, to further investigate the roles of EPX in endometrial cancer, the expression of EPX in tumor tissues and the adjacent tissues were examined by IHC methods. As shown in Table-1, we observed that the positive rate of EPX was significantly decreased in tumor tissue compared with the adjacent tissue; moreover, the levels of EPX was correlated with the age and the tumor size of the patients.
Increased expression of EPX may indicate better prognosis of patients with endometrial cancer

Finally, the roles of EPX in the prognosis of patients with endometrial cancer were also evaluated. The overall survival (OS) of the patients was calculated. As shown in Figure 3, the EPX+ has shown better OS compared with the EPX- group.

Discussion

In this study, we have explored the roles of EPX in the pathogenesis of
endometrial cancer. We discovered that EPX was down-regulated in endometrial cancer tissue and EPX has the potential to become a diagnostic and prognostic marker as well as therapeutic target for the treatment of endometrial cancer.

The roles of eosinophilia in different types of tumors, including colon tumors, oral squamous cell cancer, esophageal SCC, nasopharyngeal cancer, penile cancer, laryngeal cancer, bladder cancer, and prostate cancer have been discussed (11-13). EPX is one of the four basic granule proteins in eosinophil proteins, and previous studies mainly focused on the roles on of EPX in inflammatory disease i.e asthma, chronic rhinosinusitis (14-16), and investigations on the roles of EPX in cancers were limited. In the present study, we explored the roles of EPX in endometrial cancer. In this study, we examined the expression of EPX in tumor tissue and the adjacent tissue. It was observed that the expression of EPX was significantly decreased in tumor tissue compared with the adjacent tissue (P<0.01); furthermore, ROC curve analysis suggested that the AUC of EPX was 0.8852 (95% CI, 0.8379–0.9324), indicating that EPX is a good biomarker to distinguish tumor tissue of endometrial cancer from the adjacent tissue. These data suggested that EPX may serve as a diagnostic marker for the early diagnosis of endometrial cancer.

The roles of EPX in the prognosis of endometrial cancer were also discussed in the present study. By IHC staining, the positive rate of EPX was significantly lower in tumor tissue compared with the adjacent normal tissue; moreover, we analyzed the relationship between EPX expression and clinical pathology parameters of the patients, and observed that the expression of EPX was closely associated with the age and tumor size of the patients; finally, patients with increased expression of EPX has shown better OS compared with the EPX negative patients. Taken together, our results proved that EPX was down-regulated in endometrial cancer, and the level of EPX in the cancer tissue may predict the prognosis of patients.

In summary, EPX was aberrantly expressed in endometrial cancer, furthermore, The expression of EPX exhibit a negative correlation with the development of the disease and is positively correlated with the prognosis of the patients. Our results have
provided novel evidence for the application of EPX as a novel therapeutic target and prognostic marker for the treatment and evaluation of the clinical outcomes of endometrial cancer.

References


