Correlation Analysis between Clusterin and Pathological Factors Related to Endometrial Hyperplasia

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Abstract
This article aims to investigate the relationship between clusterin (CLU) and pathological factors related to endometrial hyperplasia. Methods a total of 110 patients treated in our hospital from July 2016 to December 2017 were selected as subjects. The CLU in the specimens of the subjects was determined by immunohistochemistry and correlated with pathological factors. Record and statistics related data. Results In terms of age, the positive rate of CLU expression was 75.61% in patients ≥50 years of age, and the positive rate of CLU expression was 43.48% in patients <50 years of age. The expression rates of CLU (+) and (++) were 28.57% and 3.57%, 31.03% and 3.45%, 28.57% and 21.43% in proliferative, proliferative, atypical hyperplasia and cancerous stages, respectively, 24.00% and 68.00%, respectively, with statistical significance (P < 0.05). Of the 25 patients with endometrial cancer, 5 had tumor emboli, and the rates of CLU expression (+), (++), and (-) were 0.00%, 60.00 and 40.00%, respectively. There were 20 cases of patients with CLU (+), (++), and (-) were 30.00, 70.00 and 0.00% respectively. The difference between the two groups was not statistically significant (P < 0.01). In the FIGO staging of endometrial cancer, there were 14 patients with stage Ia, 21.43%, 64.29% and 14.28% of patients with CLU (+), (++), and (-) Cases, the odds were 14.29%, 85.71% and 0.00% respectively; the patients in stage II were 4 cases, the odds were 50.00%, 50.00% and 0.00% respectively; there was no significant difference between the two groups (P> 0.05). There was no correlation between the degree of tumor differentiation and the infiltration of muscular layer in endometrial cancer patients. The difference was not statistically significant (P> 0.05). Conclusions the development of endometrial hyperplasia is associated with the expression of Clusterin, and there is also a correlation between CLU overexpression and endometrial cancer.

Key words: Clusterin, Endometrial Hyperplasia, Pathological Factors

1. Introduction
In medicine, the periodic changes of human normal endometrium are controlled by the hypothalamus-pituitary-ovary axis. If the regulation pathway is out of balance, it will lead to abnormal changes of endometrium. This change may lead to gynecological diseases in patients, and a few patients may develop endometrial cancer. Endometrial cancer is one of the three major malignant tumors of female genital tract, accounting for 20% to 30% of all malignant tumors of female genital tract. Large-scale studies have shown that effective early diagnosis and treatment of endometrial cancer can lead to a 5-year survival rate of 93% and a total survival rate of 98%. Therefore, the screening and early diagnosis of endometrial cancer should be paid more attention. Finding new tumor-related markers is very important for the early diagnosis, efficacy evaluation and prognosis estimation of endometrial cancer. It has also become the focus of basic researchers and clinicians.

Clusterin (CLU) is a sulfated glycoprotein of heterodimer, which has universality and high conservativeness. It exists widely in many tissues and cells of the body. It can play a variety of functions such as promoting cell aggregation, regulating reproduction, transporting lipids, promoting tissue repair, immune regulation, etc. It is also related to many pathological conditions of the body, such as aging, neurodegenerative diseases and cancer. We studied the expression of plexus protein in various tissues of endometrium, and explored the relationship between plexus protein and endometrial proliferative diseases with clinic pathological parameters.
2. Clinical Materials and Methods

2.1. General Clinical Data

From July 2016 to December 2017, 110 cases of diagnostic curettage or hysterectomy were selected, including 28 cases of endometrial hyperplasia, 29 cases of endometrial hyperplasia, 28 cases of atypical endometrial hyperplasia and 25 cases of endometrial cancer. All cases were clinically diagnosed according to the diagnostic criteria of diseases related to the eighth edition of gynecology and obstetrics textbook. Female reproductive tract pathology (6th edition) and WHO Classification of Tumors’ of Female Reproductive Organs published by Surgery Publishing House are the diagnostic basis. The inclusion criteria of the cases were: (1) over 18 years old; (2) no hormone therapy, radiotherapy, chemotherapy and other biological treatment before operation; (3) no psychiatric disorders; (4) this study was approved by the hospital ethics committee of our hospital, and the patients in the group were required to sign an informed consent.

2.2. Method

1) Experimental Method

Immunohistochemical staining was performed by MaxVision one-step method with Abcam Hong Kong Company as the reagent manufacturer. Immunohistochemical operation was carried out strictly according to the instructions, as follows: tissue wax block was cut continuously at 4um, dewaxed; newly prepared 3% hydrogen peroxide inactivated endogenous enzymes for 15min, microwave oven heated for 15min, antigen repaired and natural cooling. Then non-immune sheep serum was dripped to block the non-specific binding in tissues, and then one anti (ER, PR/CLU monoclonal antibody, CLU monoclonal antibody 1:200), two anti (sheep anti-mouse IgG), and DAB were dripped in turn for 3-5 minutes. Hematoxylin was used for re-dyeing, dehydration, transparency and neutral gum sealing, and then observed under a microscope. PBS was used to replace primary antibody as a negative control.

2) Judgement of Positive Results in Staining Results

The positive expression of CLU was yellowish brown granules in the cell membrane and cytoplasm of glandular epithelial cells. Immunohistochemical sections were examined by two senior doctors by double blind method. According to the depth of staining and the number of positive cells, the positive cells were divided into: non-positive cells (-), positive cells 10% or very light staining (+), with a score of 0; positive cells 10% - 50% or light brown (+), with a score of 1; positive cells 50% - 75% or between dark and light brown (++), with a score of 2; positive cells 7.5% or dark brown (+++), with a score of 3.

2.3. Statistical Method

All the data in this study were analyzed by statistical software SPSS 19.0. Measurement data were measured by mean (±standard deviation). The proportion of counted data (%) was expressed by t-test. The difference between groups was analyzed by variance analysis. The counted data were all tested by _2 test. P < 0.05 was regarded as the difference with statistical significance.

3. Results

3.1. The Relationship between CLU Expression and Age

A total of 110 subjects were enrolled in this study, with an average age of 47.65 ± 7.30 years. According to pathological types, 28 cases of endometrial proliferative stage (age 45.25 ± 6.50 years), 29 cases of endometrial hyperplasia (age 46.55 ± 6.30 years), 28 cases of atypical endometrial hyperplasia (age 46.80 ± 7.50 years), and 25 cases of endometrial cancer (age 46.40 ± 8.20 years).

The expression of CLU was positive in 41 patients older than or equal to 50 years old, of which 75.61% were CLU positive, while 69 patients younger than 50 years old, 43.48% were CLU positive. The difference between the two groups was statistically significant (P < 0.01). The results were shown in Table 1. From the data in the table, it can be seen that the expression of CLU in people older than 50 is significantly higher than that in people younger than 50, which indicates that the expression of CLU may be related to the age of patients. In addition, there was no significant difference in CLU expression among different pathological types (such as endometrial proliferative stage, endometrial hyperplasia, atypical endometrial hyperplasia, and endometrial cancer) (P > 0.05). The results were shown in Table 1.
Table 1. Relationship between CLU expression rate and age of patients at different pathological stages [n (%)]

<table>
<thead>
<tr>
<th>Pathological typing</th>
<th>Age</th>
<th>Number of cases</th>
<th>CLU expression</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>Endometrial proliferative phase</td>
<td>&lt;50</td>
<td>23</td>
<td>15 (65.22)</td>
<td>8 (34.78)</td>
<td>0.004</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td>≥50</td>
<td>6</td>
<td>4 (66.67)</td>
<td>2 (33.33)</td>
<td></td>
</tr>
<tr>
<td>Atypical hyperplasia of endometrium</td>
<td>&lt;50</td>
<td>22</td>
<td>14 (63.64)</td>
<td>8 (36.36)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>≥50</td>
<td>8</td>
<td>5 (62.50)</td>
<td>3 (37.50)</td>
<td></td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>&lt;50</td>
<td>10</td>
<td>1 (10.00)</td>
<td>9 (90.00)</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>≥50</td>
<td>16</td>
<td>1 (6.25)</td>
<td>15 (93.75)</td>
<td></td>
</tr>
<tr>
<td>Summary</td>
<td>&lt;50</td>
<td>69</td>
<td>39 (56.52)</td>
<td>30 (43.48)</td>
<td>10.75</td>
</tr>
<tr>
<td></td>
<td>≥50</td>
<td>41</td>
<td>10 (24.39)</td>
<td>31 (75.61)</td>
<td></td>
</tr>
</tbody>
</table>

3.2. Pathological Examination of Endometrial Proliferative Diseases in Different Stages

The pathological features of endometrial proliferative diseases at different stages are shown in Fig. 1. Images were a proliferative stage, B endometrial hyperplasia, C endometrial atypical hyperplasia, D endometrial cancer, and immunohistochemical CLU detection were positive. As seen in the figure, the cytoplasm of endometrial glandular epithelial cells was yellowish brown, and the number and intensity of positive cells stained from A to D gradually increased and increased.

![Figure 1](image_url)

**Figure 1.** Pathological pictures of endometrial proliferative diseases at different stages

3.3. The Relationship between the Expression of CLU and the Stages of Endometrial Proliferative Diseases

From the pathological type, 28 cases were in endometrial proliferative stage, and the expression rates of CLU (-), (+) and (++) were 67.86%, 28.57% and 3.57% respectively; 29 cases of endometrial hyperplasia, the expression rates of CLU (-), (+) and (++) were 65.52%, 31.03% and 3.45% respectively; 28 cases of atypical endometrial hyperplasia, the expression rates of CLU (-), (+) and (++) were 85.71%, 21.43%. There were 25 cases of endometrial cancer, and the expression rates of CLU (-), (+) and (++) were 8.00%, 24.00% and 68.00% respectively. There was a significant difference between the data ($2 = 23.976, P < 0.001$). From the above data, we can see that the expression of CLU in endometrial proliferation, endometrial hyperplasia, endometrial atypical hyperplasia, and endometrial cancer showed a gradual upward trend (Table 2).

3.4. The Correlation between CLU Expression and Parameters Related to Endometrial Cancer

The 110 patients enrolled in this study, 25 were endometrial cancer patients. The expression rate of CLU was 100.00%, but the number of positive cells was different. The number of CLU positive cells was compared with the pathological parameters of endometrial cancer as shown in Table 3. Of the 25 patients, 5 had tumor thrombus. The probability of CLU expression (-), (+) and (++) were 40.00%, 0.00% and 60.00%, respectively. The probability of CLU expression (-), (+) and (++) in 20 patients without tumor thrombus was 0.00%, 30.00% and 70.00%, respectively. The difference between the two groups was statistically significant (P < 0.01).

In FIGO staging of endometrial cancer, there were 14 patients in stage Ia, 14.28%, 21.43% and 64.29% in CLU expression (-), (+) and (++), 7 patients in stage Ib, 0.00%, 14.29% and 85.71% in FIGO staging, 4 patients...
in stage II, 0.00%, 50.00% and 50.00% in FIGO staging, respectively. There was no significant difference between the two groups (P > 0.05).

Table 2. Relationship between CLU expression and stages of endometrial hyperplasia [n (%)]

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Number of cases</th>
<th>CLU Expression</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>By Stages</td>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Endometrial proliferative phase</td>
<td>28</td>
<td>19 (67.86)</td>
<td>8 (28.57)</td>
<td>1 (3.57)</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td>29</td>
<td>19 (65.52)</td>
<td>9 (31.03)</td>
<td>1 (3.45)</td>
</tr>
<tr>
<td>Atypical hyperplasia of Endometrium</td>
<td>28</td>
<td>14 (50.00)</td>
<td>8 (28.57)</td>
<td>6 (21.43)</td>
</tr>
<tr>
<td>Atypical hyperplasia of endometrium</td>
<td>25</td>
<td>2 (8.00)</td>
<td>6 (24.00)</td>
<td>17 (68.00)</td>
</tr>
</tbody>
</table>

The clinical results showed that there were 3 cases of endometrial cancer with poor differentiation, and the probability of CLU expression (-), (+) and (+++) were 0.00%, 33.33% and 66.67%, respectively. The probability of CLU expression (-), (+) and (+++) in 10 cases of endometrial cancer was 10.00%, 20.00% and 70.00%, respectively. The probability of CLU expression (-), (+) and (+++) in 12 cases of high differentiation was 10.00%, 20.00% and 70.00% respectively, 8.33%, 25.00% and 66.67% respectively. There was no significant difference among different differentiation groups (P > 0.05).

In addition, there were 5 cases without infiltration, the probability of CLU expression (-), (+) and (+++) were 0.00%, 40.00% and 60.00% respectively; 7 cases with infiltration < 1/2, the probability of CLU expression (-), (+) and (+++) were 0.00%, 14.28% and 85.71% respectively; 13 cases with infiltration > 1/2, the probability of CLU expression (-), (+) and (+++) were 15.38%, 23.08% and 61.53%, respectively. There was no significant difference between groups with different infiltration (P > 0.05).

Table 3. Comparison of the correlation between CLU expression and endometrial cancer-related parameters [n (%)]

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Number of cases</th>
<th>CLU Expression</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor thrombus</td>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>2 (40.00)</td>
<td>0 (0.00)</td>
<td>3 (60.00)</td>
</tr>
<tr>
<td>no</td>
<td>20</td>
<td>0 (0.00)</td>
<td>6 (30.00)</td>
<td>14 (70.00)</td>
</tr>
<tr>
<td>FIGO staging</td>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Ia</td>
<td>14</td>
<td>2 (14.28)</td>
<td>3 (21.43)</td>
<td>9 (64.29)</td>
</tr>
<tr>
<td>Ib</td>
<td>7</td>
<td>0 (0.00)</td>
<td>1 (14.29)</td>
<td>6 (85.71)</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>0 (0.00)</td>
<td>2 (50.00)</td>
<td>2 (50.00)</td>
</tr>
<tr>
<td>Differentiation of tumors</td>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>3</td>
<td>0 (0.00)</td>
<td>1 (33.33)</td>
<td>2 (66.67)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>10</td>
<td>1 (10.00)</td>
<td>2 (20.00)</td>
<td>7 (70.00)</td>
</tr>
<tr>
<td>Highly differentiated</td>
<td>12</td>
<td>1 (8.33)</td>
<td>3 (25.00)</td>
<td>8 (66.67)</td>
</tr>
<tr>
<td>Infiltration of muscular layer</td>
<td></td>
<td>(-)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>No infiltration</td>
<td>5</td>
<td>0 (0.00)</td>
<td>2 (40.00)</td>
<td>3 (60.00)</td>
</tr>
</tbody>
</table>
4. Discussion

Clusterin, a single-copy gene was isolated from goat testicular reticular duct fluid by Blaschuk et al. in 1983 and located at 8p21-p12 [1, 2]. It has two main protein subtypes: nuclear clump protein (nCLU) and secretory clump protein (sCLU), nuclear clump protein (nCLU) can promote apoptosis; secretory clump protein (sCLU) can inhibit apoptosis, which determines that CLU has two opposite biological effects. CLU exists in various tissues and body fluids. Konrad Lutz et al. found that the main source of plexus proteins in uterus is epithelial cells and endothelial cells [3]. After studying the uterine mucus of women with endometriosis and non-endometriosis, it is believed that there are different dominant Clusterin receptors in uterine glands, blood vessels and smooth muscle cells. Another study, Brown et al. [4], found the expression of cluster proteins in the late secretory phase, and suggested that progesterone levels decreased, tissue regeneration, and epithelial cells may promote the increase of cluster proteins. The results of Ahn et al. on mouse endometrial adenocarcinoma suggest that plexus protein is an estrogen-related gene [5], and plexus protein can be detected in endometrial epithelial cells, mainly in secretory cells. The typical clinical manifestation of endometrial hyperplasia is abnormal uterine bleeding, as well as other symptoms such as abnormal vaginal discharge, abdominal pain and uterine effusion. It is a kind of non-physiological and non-invasive endometrial hyperplasia. It is mainly due to the change of gland structure and the ratio of gland to stroma, which leads to the increase of endometrial volume [6, 7]. For endometrial proliferative diseases, the main reason is that there is no antagonistic estrogen stimulation for a long time. In this study, the expression rate of CLU in normal endometrial proliferative phase was 3.57%, endometrial hyperplasia was 3.45%, and atypical endometrial hyperplasia was 21.43%. It showed that the expression of CLU was related to endometrial proliferative diseases.

The expression of CLU was abnormal in various tumors [8-16], high in kidney, breast, lung, ovary, liver and colon, but low in neuroblastoma, esophagus and pancreas. CLU is intricately related to tumors and is involved in the formation and metastasis of tumors [17]. In the study of constructing breast cancer MCF-7 cell line (MCF-7CLU) with over-expression of CLU, MCF-7 and MCF-7CLU were transplanted into ovariectomized female mice at the age of 6 weeks. The mice were euthanized after 10 weeks. The results showed that the growth of cancer in MCF-7 cell line was slow, while the growth of cancer in MCF-7CLU cell line was rapid, and lung metastasis and lymphatic metastasis occurred in the early stage of cancer, which confirmed that CLU played a significant role in the growth and metastasis of cancer [18]. Gregory et al. considered that CLU was closely related to the occurrence [19], development and prognosis of ovarian cancer, but there was little information about the correlation between CLU expression and endometrial cancer. In this project, the expression of CLU in various endometrium was investigated by immunohistochemical method. It was found that the expression rate of CLU in endometrial cancer tissue was 68.00%, which was high. Compared with other groups of endometrial proliferative diseases, there was a significant difference (P < 0.001), indicating that the overexpression of CLU was related to endometrial cancer.

The process of endometrial hyperplasia is proliferative stage, proliferative stage, atypical proliferative stage and cancer. In the process of normal endometrial hyperplasia and the occurrence and development of proliferative diseases, CLU shows a gradual increasing trend in each stage of endometrial hyperplasia and development, which indicates that the development of endometrial proliferative diseases is related to the expression of clusterin, and the overexpression of Clusterin is also related to endometrial cancer. Huang Rong et al. inhibited the expression of sCLU gene in ovarian cancer SKOV3 by RNA interference technology, and found that it could significantly reduce the proliferation and invasion ability of ovarian cancer SKOV3 cells, and increase the apoptotic rate, which provided a new target for the treatment of ovarian cancer. So there may also be some unknown mechanisms in endometrial cancer. Some drugs or techniques can interfere with the expression of CLU gene in endometrial cancer, so as to inhibit the proliferation, invasion and even induce apoptosis of cancer cells. CLU gene may become an attractive target in targeted gene therapy of endometrial cancer. At present, there are few clinical studies in this area. Such targets need to be explored in the future. Therefore, we hope that clusterin can be used as a molecular biomarker, and may be a potential marker of endometrial cancer. In the near future, CLU will be used as a screening index for endometrial cancer. It can be used for early detection of endometrial cancer cases through blood and intrauterine mucus detection, and even for the development of targeted gene therapy targeting CLU gene in patients with endometrial cancer. Therapy has a new way to make patients get early, effective treatment and improve prognosis, which need a large number of samples to study and verify.

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Proteotoxicity

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