Original Article

Overexpression of CD44 and EpCAM may be associated with the initiation and progression of epithelial ovarian cancer

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Received December 28, 2016; Accepted March 9, 2017; Epub April 1, 2017; Published April 15, 2017

Abstract: Objective: Cluster of differentiation 44 (CD44) and epithelial cell adhesion molecule (EpCAM) play an essential role in cancer initiation and progression via inducing tumor cells proliferation, differentiation, invasion and migration. This study was to investigate the protein expression levels of CD44 and EpCAM in epithelial ovarian cancer and to evaluate the correlation between expression of these markers and the clinical pathological features, as well as the correlation between CD44 and EpCAM expression. Methods: The expression of CD44 and EpCAM in 50 formalin-fixed paraffin-embedded (FFPE) human epithelial ovarian cancer specimens and 15 FFPE normal ovary specimens was examined. Clinical and pathological parameters were collected, including age, clinical stage, status of lymph node metastasis, histological type and histological grade. The Statistical Package for Social Sciences version 17.0 was used for all statistical analyses. Results: The levels of CD44 and EpCAM expression in epithelial ovarian cancer were increased compared with normal ovary tissues. Moreover, the increased CD44 and EpCAM protein expression were closely related with International Federation of Gynecology and Obstetrics (FIGO) stage and histological grade, lymph node metastasis, whereas had no statistically significant association with age, histological type. A significantly positive correlation between CD44 and EpCAM expression was detected in epithelial ovarian carcinoma tissues. Conclusions: Overexpression of CD44 and EpCAM may be involved in the pathogenesis of epithelial ovarian cancer and associated with the initiation and progression of epithelial ovarian cancer. These results further indicated that CD44-EpCAM-targeted therapy might be a potential strategy in epithelial ovarian cancer.

Keywords: Epithelial ovarian cancer, CD44, EpCAM, immunohistochemistry

Introduction

Ovarian carcinoma is the most frequent gynecologic malignancy resulting in cancer-related death. Epithelial ovarian cancer constitutes 85%-90% cases of ovarian carcinoma. According to GLOBOCAN estimates, an estimated 238,700 new ovarian cancer cases and 151,900 deaths occurred in 2012 worldwide [1]. The lack of early stages specific symptoms contribute to the high mortality of ovarian carcinoma. As a consequence, more than 70% patients are diagnosed at advanced stages when it presents extensive local invasion and intraperitoneal metastasis. In spite of the cytoreduction surgery and platinum-paclitaxel chemotherapy as the standard treatment have been performed for advanced stage disease, the majority patients finally relapse and the 5-year overall survival rate only 45% [2]. Therefore, it is necessary to investigate the biological behavior of ovarian carcinoma to explore the molecular target-directed therapies which inhibit the local invasion and distant metastasis and represent a new treatment modality.

Cluster of differentiation 44 (CD44), initially identified as a leukocyte antigen, is a single-span transmembrane glycoprotein with three functional domains, including an intracellular domain, an extracellular domain and a transmembrane domain [3]. CD44 is encoded by the highly conserver gene about 60 kb of length located in chromosome 11 in human. The CD44
gene is composed of 20 exons and 19 introns [4]. CD44 is the principal receptor for hyaluronan (HA). The binding of HA with CD44 induces tumor cell proliferation, differentiation, invasion, and migration, leading to the progression and metastasis of tumors [5]. In addition, it has been reported that CD44 plays a critical role in promoting chemotherapy resistance in cancer cells and animal models [6, 7].

Epithelial cell adhesion molecule (EpCAM, also known as CD326) was initially identified in 1979 as a predominant antigen in human colon carcinoma tissue. EpCAM is a type I transmembrane glycoprotein, calcium-independent, homophilic, epithelial-specific intercellular adhesion molecule [8], with a molecular weight of 39-42 kDa, and contains a large extracellular domain, a single transmembrane region, and a short intracellular domain of 26 amino acids [9]. EpCAM is not only expressed in human normal epithelium, with the exception of squamous epithelium and of specific epithelial cells of adult hepatocytes and keratinocytes, but also abundantly expressed in various human epithelial neoplasms [10]. It is more frequently positive expression in tumor cells than in the normal epithelia. In addition to mediating cell adhesion, enough evidence revealed that EpCAM also participate in cellular signaling, cell proliferation, migration, invasion and differentiation [11].

As overexpression of CD44 and EpCAM plays a dominant role in development and progression of human cancers, we hypothesized that CD44 and EpCAM might have a correlation in epithelial ovarian cancer progression. In this study we evaluated the status of CD44 and EpCAM expression in epithelial ovarian cancer tissues by immunohistochemistry. Furthermore, we analyzed the association of protein expression with clinicopathological features and the possible relevance between CD44 and EpCAM, which would provide a promising joint molecular therapeutic target for epithelial ovarian carcinoma.

Materials and methods

Tissue specimens

50 formalin-fixed paraffin-embedded (FFPE) epithelial ovarian cancer samples, 15 FFPE normal ovarian epithelial tissue samples which obtained from the normal ovaries during surgery for other gynecological diseases were collected in this study. The screening specimens were obtained from April 2013 to December 2015 in the Department of Pathology of the Second Hospital of Jilin University. None of the epithelial ovarian cancer patients had taken chemotherapy or radiotherapy prior to their surgery. All hematoxylin-eosin slides were reviewed by two pathologists to evaluate histological grade and histological type according to World Health Organization (WHO) criteria to achieve a consensus diagnosis. The patient signed informed consent and the present study was carried out with the approved of the Medical Ethics Committee of the Second Hospital of Jilin University.

Immunohistochemistry

FFPE tissue specimens were cut into 4 µm-thick sections, mounted and baked at 60°C for 1 hour. The tissue sections were dewaxed in xylene, rehydrated in graded ethanol, and incubated in 3% H2O2 for 5 minutes to block endogenous peroxidase activity. Antigen retrieval was performed by heating the slides in a pressure cooker in 10 mM citric acid buffer (pH 6.0) at 100°C for 15 minutes. The slides were rinsed in 0.1 M Tris-HCl (PBS, pH 7.6) for 5 minutes 3 times and then immersed in PBS containing 10% goat serum to block non-specific binding for 30 minutes at room temperature. The slides were incubated overnight at 4°C with the primary antibody against either CD44 (rabbit anti-human monoclonal antibody, 1:100, Abcam, MA, USA) or EpCAM (mouse anti-human monoclonal antibody, 1:200, Abcam, MA, USA). After washing in PBS for 5-minute 3 times, slides were incubated with horseradish-peroxidase-labeled goat anti-mouse/rabbit IgG/HRP conjugated polymer (PV-6000, ZSGB-BIO) for 20 minutes at 37°C. After three 5-minute washes in PBS, staining was visualized by immersed slides in the solution of 3,3-diaminobenzidine tetrahydrochloride with hydrogen peroxidase. Subsequently the slides were rinsed with distilled water and counterstained with hematoxylin solution, dehydrated, cleared and sealed. Negative control slides were processed in the same way except that the primary antibody was substituted by phosphate buffered saline solution.
CD44 and EpCAM in human epithelial ovarian cancer

Evaluation procedures

EpCAM and CD44 immunostaining were seen in the cell membrane of epithelial ovarian cancer. All slides were assessed independently by 2 investigators who were blinded to clinicopathologic information. Immunostaining was evaluated in a series of randomly selected five high-power fields (200 × magnification) and 100 tumor cells were counted in each field. Staining intensity was graded as follows: 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining. The staining extent was graded according to the proportion of positive tumor cells as follows: score 0, 0-5% positive tumor cells; 1, 6-25% positive tumor cells; 2, 26-50% positive tumor cells; 3, 51-75% positive tumor cells; 4, 76-100% positive tumor cells. The staining intensity score was multiplied with the staining extent score, resulting in the semi-quantitative immunoreactivity score that indicated the expression level: 0-2, negative; 3-4, weak positive; 6-8, moderate positive; 9-12, strong positive.

Statistical analyses

Statistical analysis was carried out using the Statistical Package for Social Sciences version 17.0 (SPSS 17.0, IBM, Chicago, IL, USA). Chi-square test or Fisher’s exact test was used to evaluate the correlation of EpCAM or CD44 expression with clinicopathologic parameters. The correlation between EpCAM expression and CD44 expression was investigated using Spearman correlation analysis. For all comparisons, $P<0.05$ was considered to be statistically significant.

Results

Clinical pathology information

The clinicopathological characteristics of patients were gathered from the patients’ medical records and pathology reports and are shown in Table 1. The diagnosis of histological type and grade were performed according to the Classification of Ovarian Cancer (WHO 2004). Among 50 cases of epithelial ovarian carcinoma, 36 cases were serous cystadenocarcinoma, 9 cases were mucinous cystadenocarcinoma and 5 cases were endometrioid carcinoma. Regarding tumor histological grade (tumor differentiated), 15 cases were well differentiated (Grade 1), 15 cases were moderately differentiated (Grade 2) and 20 cases were poorly differentiated (Grade 3). The stage of epithelial ovarian carcinoma was categorized using the International Federation of Gynecology and Obstetrics (FIGO) standards, 17 patients were stage I, 8 patients were stage II, 17 patients were stage III, 8 patients were stage IV. 23 of the 50 patients had lymph node metastasis. The mean age of the patients was 55.3 (range 26-78).

CD44 protein expression and its associations with clinicopathological features in epithelial ovarian carcinoma

CD44 immunostaining was observed in the membrane of the epithelial ovarian cancer cells, the representative of immunohistochemical staining are shown in Figure 1. CD44 immunoreactivity was detected in 32 tissue sections (64%), but not detected in normal ovarian epithelial tissue. As shown in Table 2, there was a significant difference of CD44 expression between normal ovary and ovarian cancer ($P<0.05$). This result verified that the level of

Table 1. Association analyses between expression of EpCAM and CD44 and the clinical pathological characteristics in epithelial ovarian cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>EpCAM Positive (%)</th>
<th>P</th>
<th>CD44 Positive (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥55</td>
<td>27</td>
<td>23 (85.2)</td>
<td>0.523</td>
<td>17 (63)</td>
<td>0.869</td>
</tr>
<tr>
<td>&lt;55</td>
<td>23</td>
<td>17 (73.9)</td>
<td></td>
<td>15 (65.2)</td>
<td></td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>36</td>
<td>30 (83.3)</td>
<td>0.466</td>
<td>22 (61.1)</td>
<td>0.636</td>
</tr>
<tr>
<td>Mucinous</td>
<td>9</td>
<td>7 (77.8)</td>
<td></td>
<td>7 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>5</td>
<td>3 (60)</td>
<td></td>
<td>3 (60)</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well-moderately</td>
<td>30</td>
<td>20 (66.7)</td>
<td>0.03</td>
<td>15 (50)</td>
<td>0.012</td>
</tr>
<tr>
<td>Poorly</td>
<td>20</td>
<td>20 (100)</td>
<td></td>
<td>17 (85)</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>25</td>
<td>17 (68)</td>
<td>0.034</td>
<td>12 (48)</td>
<td>0.018</td>
</tr>
<tr>
<td>III-IV</td>
<td>25</td>
<td>23 (92)</td>
<td></td>
<td>20 (80)</td>
<td></td>
</tr>
<tr>
<td>Lymphatic metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27</td>
<td>18 (66.7)</td>
<td>0.028</td>
<td>13 (48.1)</td>
<td>0.011</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>22 (95.7)</td>
<td></td>
<td>19 (86.3)</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square test or Fisher’s exact test was used to evaluate the correlation of EpCAM or CD44 expression with clinicopathologic parameters. The correlation between EpCAM expression and CD44 expression was investigated using Spearman correlation analysis. For all comparisons, $P<0.05$ was considered to be statistically significant.
CD44 and EpCAM in human epithelial ovarian cancer

CD44 protein expression was closely associated with epithelial ovarian cancer.

As shown in Table 1, the immunohistochemical expression of CD44 in epithelial ovarian cancer significantly correlated with FIGO stage and tumor differentiation (histological grade), lymph node metastasis (P<0.05). No significant association was found between CD44 expression and age, histological type in 50 epithelial ovarian cancer patients (P>0.05). CD44 expression was higher in patients with FIGO advanced stages (III-IV) and poorly differentiated (G3) than in those with FIGO early stages (I-II) and moderately differentiated and well-differentiated (G1-G2). The percent positive rate of CD44 expression in patients with lymph node metastasis was higher than in those without lymph node metastasis.

Table 2. Fisher's exact test analyses of CD44 expression in epithelial ovarian cancers and normal ovaries

<table>
<thead>
<tr>
<th></th>
<th>CD44 expression</th>
<th>Positive rate (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>EOC</td>
<td>50</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: EOC: epithelial ovarian cancer.

EpCAM protein expression and its associations with clinicopathological features in epithelial ovarian carcinoma

Forty cases (80%) of epithelial ovarian carcinoma showed immunoreactivity for EpCAM protein, but only 4 cases (26.7%) of normal ovarian epithelial tissue showed immunoreactivity for EpCAM protein. As shown in Figure 2, the typical EpCAM staining was localized in cell membrane. In fact, expression of EpCAM was significantly increased in the epithelial ovarian ca-
CD44 and EpCAM in human epithelial ovarian cancer

Furthermore, we assessed the correlation between EpCAM expression and clinico-pathological characteristics. The data are summarized in Table 1. There was a positive correlation between EpCAM expression and FIGO stage and tumor differentiation (histological grade), lymph node metastasis (P<0.05). However, EpCAM expression exhibited no relationship to age, histological type in 50 epithelial ovarian cancer patients (P>0.05). The percentage of cases with EpCAM expression is higher in lymph node metastasis patients than in those without lymph node metastasis. Increased EpCAM protein expression was more frequently observed in patients with FIGO advanced stages (III-IV) and poorly differentiated (G3) compared to those with FIGO early stages (I-II) and moderately differentiated and well-differentiated (G1-G2).

Table 3. Chi-square test analyses of EpCAM expression in epithelial ovarian cancers and normal ovaries

<table>
<thead>
<tr>
<th></th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive rate (%)</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EOC</td>
<td>50</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>80</td>
<td>12.668</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>26.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Association between EpCAM and CD44 expression in epithelial ovarian carcinoma

<table>
<thead>
<tr>
<th>CD44 expression</th>
<th>EpCAM expression</th>
<th>Total</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>29</td>
<td>3</td>
<td>0.354</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>11</td>
<td>7</td>
<td>0.012</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>40</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Representative immunohistochemical staining of EpCAM in human epithelial ovarian carcinoma and normal ovary tissue specimens. A. Mild staining of EpCAM in normal ovary tissue. B. Weak expression (+) of EpCAM in epithelial ovarian carcinoma tissue. C. Moderate expression (++) of EpCAM in epithelial ovarian carcinoma tissue. D. Strong expression (+++) of EpCAM in epithelial ovarian carcinoma tissue.
Correlated protein expression of EpCAM and CD44 in epithelial ovarian carcinoma

The aforementioned analysis indicated a similar pattern of EpCAM and CD44 immunostaining intensity, we sought to detect if EpCAM and CD44 immunostaining might be correlate to each other. In 40 epithelial ovarian cancer samples with EpCAM immunostaining, 29 (29/40=72.5%) samples exhibited a positive CD44 expression. In 10 epithelial ovarian cancer samples with negative EpCAM expression, 7 (7/10=70%) samples exhibited a negative CD44 expression. EpCAM protein level positively correlated with CD44 protein level, demonstrating a significantly positive association between the two molecules (Table 4, \( r=0.354, P=0.012 \)).

Discussion

CD44, as a type of cell-surface adhesion molecules, mediates multiple pathological and physiological processes, including malignancy development, cell adhesion, angiogenesis, wound healing and inflammation. Studies have suggested that CD44 is overexpression and promotes cells migration and metastasis for human solid tumors, including breast carcinoma and ovarian carcinoma [12, 13]. Several studies have shown that the overexpression of CD44 enhance the capacity of proliferation and carcinogenesis in renal cancer and gastric cancer [14, 15]. Up to date, the relationship between CD44 expression and clinical significance in epithelial ovarian cancer remains controversial. The present study suggested that the levels of CD44 expression were increased in epithelial ovarian cancer tissues compared with normal ovarian epithelial tissues, and increased of CD44 expression was significantly associated with FIGO stage and tumor differentiation, lymph node metastasis. The results show that CD44 expression may be involved in disease pathogenesis.

It has been reported that EpCAM was activated via proteolysis, and the activated EpCAM as a mitogenic signal transducer mediated the cell proliferative [16]. EpCAM, as a carcinoma-associated antigen, exerts carcinogenesis via upregulating the proto-oncogene c-myc and the cell cycle regulating genes cyclin A and E, which affect the cell cycle progression and enhance cell proliferation and metabolism. In addition, it has been shown that EpCAM also promotes cell proliferation and tumorigenesis by participating in the nuclear Wnt signaling pathway [17]. EpCAM expression was reported to be correlated with tumor differentiation, stage of disease and metastasis in several human carcinomas, including gastric cancer, breast cancer and so on [18, 19]. Furthermore, it was reported that EpCAM was highly overexpressed in primary, recurrent, and metastatic epithelial ovarian cancer specimens [20]. The current study showed the level of EpCAM expression in epithelial ovarian cancer was higher than in normal ovary, and identified a significantly positive association between EpCAM overexpression and FIGO advanced stages, poor differentiation and lymph node metastasis.

In summary, the present study demonstrated that CD44 and EpCAM expression were closely associated with the occurrence, development, invasion and metastasis of human epithelial ovarian cancer, and there was a positive correlation between CD44 and EpCAM expression. Joint detection of CD44 and EpCAM is conductive to a comprehensive judgment of the malignance degree and metastatic potential of epithelial ovarian cancer. Our results further indicated that CD44-EpCAM-targeted therapy might be a potential strategy in epithelial ovarian cancer.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (No.81272875) and Jilin Provincial Science and Technology Funds (No.20150204041YY).

Disclosure of conflict of interest

None.

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References

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