Expression and clinical significance of YKL-40 protein in epithelial ovarian cancer tissues

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Key words: ovarian neoplasms, YKL-40, clusterin, prognosis

Background and Objective: Overexpression of YKL-40 has been detected in the sera from patients with various kinds of malignant tumors, including epithelial ovarian cancer. Moreover, YKL-40 expression is closely related to clinical phenotypes of some malignant tumors. This study was to investigate the expression and clinical significance of YKL-40 protein in epithelial ovarian cancer tissues. Methods: Protein expression of YKL-40 was detected by immunohistochemistry (IHC) using tissue microarray (TMA) consisting of 86 specimens of epithelial ovarian cancer and 20 specimens of normal ovarian tissues. The correlations of YKL-40 expression to clinical features and prognosis, as well as to the expression of clusterin protein in epithelial ovarian cancer were evaluated. Results: The expression of YKL-40 in all normal ovarian tissues was negative or at low levels. In 74 evaluable specimens of epithelial ovarian cancer, overexpression of YKL-40 was detected in 42 cases (56.8%). YKL-40 expression was closely associated with the clinical stage of epithelial ovarian cancer (p < 0.0001). The overall survival time in patients with overexpression of YKL-40 was significantly shorter than that in patients with normal expression of YKL-40 (p = 0.0389). Moreover, expression of YKL-40 protein was positively correlated with that of clusterin protein in epithelial ovarian cancer (p < 0.0001). Conclusion: YKL-40 may be used as a new molecular marker to predict the prognosis of epithelial ovarian cancer.

Ovarian cancer is one of the three major malignant tumors of female genital organs, and its peak incidence is at age 45 or above. Its five-year survival rate is no more than 30%, because most patients with ovarian cancer are preliminarily diagnosed at advanced stages (FIGO III–IV).

Currently, only a few molecular markers are clinically used to predict the prognosis of ovarian cancer. YKL-40, which belongs to the mammalian 18 glucoprotein family, is recently found highly expressed in sera from patients with epithelial ovarian cancer or other malignant tumors, and is closely related to malignant phenotypes of some tumors. However, the protein expression of YKL-40 and its prognostic value in epithelial ovarian cancer are still unclear. In addition, it is suggested that the YKL-40 gene may be a downstream gene of clusterin. In the present study, we used immunohistochemistry combined with tissue microarrays to examine the protein expression of YKL-40 in 86 patients with epithelial ovarian cancer. Furthermore, we analyzed the correlation of YKL-40 expression to clinicopathological characteristics and to the expression of clusterin in these patients.

Patients and Methods

Patients. Eighty-six patients with epithelial ovarian cancer treated by surgery in the Department of Gynecology and Obstetrics of the First Affiliated Hospital, Sun Yat-sen University from 1994 to 2003 were selected. None of the patients received radiotherapy, chemotherapy or biotherapy prior to surgery. Data of survival time and clinicopathological parameters were collected. The median age of the patients was 50.5 years. There were 56 cases of serous adenocarcinoma, 25 cases of mucoid adenocarcinoma and five cases of other types of tumors. Using the Silverberg grading system, 19 cases were grade G1, 49 cases were grade G2 and 18 cases were grade G3. According to international FIGO staging criteria, 21 cases were stage I, 10 cases were stage II, 46 cases were stage III and nine cases were stage IV. In addition, 20 specimens of normal ovaries from exairesis for non-ovary diseases in the Department of Gynecology and Obstetrics of the First Affiliated Hospital, Sun Yat-sen University from 1994 to 2003 were used as control. Diagnoses for all specimens were confirmed by HE staining.

Main instruments and reagents. The tissue chip maker was purchased from Laboratory of Tumor Genetics of National Human Genome Research Institute, National Institute of Health (USA). The paraffin section cutter was bought from Leica Company (Germany). The microscope and photography equipments were products of Olympus (Japan). Mouse anti-human YKL-40 mono-

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clonal antibody was purchased from San Diego Company (USA). Mouse anti-human clusterin monoclonal antibody was bought from Lake Placid Company (USA). Immunohistochemistry SABC kit and DAB coloring kit were products of Dako Company (Japan), including goat serum blocking solution, antigen repair solution, biotin labeled goat anti-mouse IgG and streptavidin-biotin-peroxidase complex SABC.

Preparation of tissue microarray and judgments of results. Firstly, the typical sites of lesions were selected from the donor paraffin section under microscopy. Secondly, the desired tissues from the donor paraffin block were chosen, inserted into the hole on the receptor paraffin block to prepare the tissue chips. Serial sections of 4 μm thick were obtained from the paraffin block of tissue chips using the section cutter. HE staining was performed to examine whether each point of tissue in the tissue microarray was corresponding to the donor tissue. The microarray was excluded if any of the following conditions occurred: an excess of undesired tissues, insufficiency of desired cell population and lack of representativeness due to the deviation in tissue localization and material selection or tissue shedding, for example, dislocation or detachment of tissue chips during preparation. The exclusion was recorded, and was not included into result judgments and statistical analysis.

Immunohistochemical staining. SABC immunohistochemical staining was adopted and performed according to the instruction provided by the manufacture. YKL-40 antigen was repaired with protease K at room temperature for 15 min. The section was incubated with monoclonal anti-YKL-40 antibody at a working dilution of 1:100 at 4°C overnight. A known positive section of breast cancer tissues was used as positive control. Brown to yellow particles in cytoplasm were considered as positive staining of YKL-40 protein. The results were semi-quantitatively judged based on the 9-score method. According to the intensity of staining, negative staining was score 0, weakly positive staining was score 1, moderately positive staining was score 2 and strong positive staining was score 3. At least five visual fields per section were analyzed in 400-fold magnification, and the number of positive cells in 100 cells of each visual field was counted. Positive cells less than 1% were score 0, 1–10% was score 1, 11–50% was score 2 and more than 50% was score 3. Multiplication of the above two scores was recorded as the final staining result (0–9 score). The staining density of all 20 control samples were negative (score 0) or weakly positive (score 1) for YKL-40, therefore the final scores of normal expression of YKL-40 protein were defined as overexpression of YKL-40 (Fig. 1B–D).

Statistical methods. Statistical analyses were performed using SPSS10.0 software. Numeration data were analyzed with χ² test. The Kaplan-Meier survival curve was plotted and the difference in the survival rate was analyzed using the log-rank test. p < 0.05 was considered statistical significance.

Results

Expression of YKL-40 protein in specimens of epithelial ovarian cancer tissues. YKL-40 protein was detected in 74 out of 86 tissue chips of epithelial ovarian cancer tissues using immunohistochemistry. Twelve tissue chips were excluded due to the lack of representativeness or tissue shedding. YKL-40 protein was overexpressed in 42 (58.3%) out of 74 epithelial ovarian cancer specimens.

YKL-40 protein expression in epithelial ovarian cancer was significantly correlated to the clinical stage (χ² = 25.553, p < 0.001). The majority of cases (65.6%) with normal expression of YKL-40 were at early stages of ovarian cancer, while most cases (90.5%) with overexpression of YKL-40 were at advanced stages of ovarian cancer. No significant association was observed between YKL-40 expression and tumor histological type, pathological grade and age (p > 0.05) (Table 1).

Correlation of YKL-40 expression to culsterin protein expression in epithelial ovarian cancer. Normal expression of clusterin was found in the majority (75.0%) of cases with normal expression of YKL-40 protein, while overexpression of clusterin was found in most cases (82.3%) with overexpression of YKL-40 protein. The expression of these two proteins had a highly significant positive correlation (χ² = 37.3, p < 0.0001).

Discussion

YKL-40 is a heparin and chitin-binding lectin. Under physiological conditions, YKL-40 is produced by macrophages, neutrophils, chondrocytes and vascular smooth muscle cells. It is highly expressed in inflammation associated diseases, especially in various malignant tumors.8

Recently, YKL-40 has been found to be a potential serum tumor marker for the evaluation of genesis, progression and prognosis of many kinds of human malignant tumors, such as ovarian cancer.3-5, 9,11 Johansen et al.9 revealed that in sera from patients with 13 different types of tumors (2,500 cases), YKL-40 provides better independent information of survival than many of other tumor markers, such as Her2, arcino-embryonic antigen, CA125, prostate specific antigen and lactate dehydrogenase. Dupont et al.2 show that serum YKL-40 is more sensitive than CA125 and CA15-3 in the diagnosis of ovarian cancer. Patients with early stage ovarian cancer whose serum YKL-40 level is higher than 80μg/mL have significant poorer prognosis than those whose YKL-40 is lower than 80 μg/mL. High-level expression of serum YKL-40 is found negatively correlated with short-term survival in patients with recurrent ovarian cancer.10 Furthermore, a high level of serum YKL-40 is suggested to associate with resistance towards secondary chemotherapy of patients with ovarian cancer.11 However, there has been no report on protein expression of YKL-40 in primary ovarian cancer tissues and its clinical significance.

In this study, YKL-40 protein was found to be overexpressed in 60% of primary epithelial ovarian cancer tissues, and positively
Expression and clinical significance of YKL-40 protein in epithelial ovarian cancer tissues

Figure 1. Normal protein expression of YKL-40 and overexpression of clusterin in epithelial ovarian cancer (IHC ×200). (A) Normal expression of YKL-40 is shown in the cytoplasm of all tumor cells in a case of grade G2 ovarian cancer patient. (B) Overexpression of clusterin is shown in a case of grade G1 ovarian cancer patient. The cytoplasm of 60% tumor cells is moderately positive. (C) Overexpression of clusterin is shown in another grade G1 ovarian cancer patient. The cytoplasm of all tumor cells is strongly positive. (D) Overexpression of clusterin is shown in a case of grade G2 ovarian cancer patient. The cytoplasm of all tumor cells is moderately positive.

Table 1  Correlation of YKL-40 protein expression to clinicopathological features of 74 patients with epithelial ovarian cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases</th>
<th>YKL-40 protein [cases (%)]</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50.5</td>
<td>37</td>
<td>15 (41)</td>
<td>22 (59)</td>
</tr>
<tr>
<td>&gt;50.5</td>
<td>37</td>
<td>17 (46)</td>
<td>20 (54)</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>53</td>
<td>22 (42)</td>
<td>31 (58)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>21</td>
<td>10 (48)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>Histological grade (Silveberg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>7</td>
<td>4 (57)</td>
<td>3 (43)</td>
</tr>
<tr>
<td>G2</td>
<td>44</td>
<td>15 (34)</td>
<td>29 (66)</td>
</tr>
<tr>
<td>G3</td>
<td>23</td>
<td>13 (57)</td>
<td>10 (43)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early stage (I + II)</td>
<td>25</td>
<td>21 (84)</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Advanced stage (III + IV)</td>
<td>49</td>
<td>11 (22)</td>
<td>38 (78)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chi-square test
corrected with the clinical stage of the disease, suggesting that upregulation of YKL-40 protein expression is very important in malignant progression of ovarian cancer. In addition, the median survival time of ovarian cancer patients with overexpression of YKL-40 was 33 months, which was significantly shorter than that in patients with normal expression of YKL-40 (69 months), implying a close correlation of overexpression of YKL-40 to poor prognosis of ovarian cancer patients. Thus, YKL-40 expression appears to have a predictable value for ovarian cancer. Currently, there are only a few studies on the gene function of YKL-40 and the molecular mechanism of YKL-40 in tumor genesis and progression remains unclear. Malinda et al.\(^\text{12}\) propose that YKL-40 is a potential migration factor for endothelial cells, playing an important role in genesis of new vessels by regulating cell morphology via promoting formation of branched tubules. Additionally, YKL-40 also participates in malignant progression of tumors via reconstitution and degradation of the extracellular matrix. Therefore, we hypothesize that upregulation of YKL-40 protein expression in ovarian cancer may promote tumorigenesis and progression through increasing tumor angiogenesis or extracellular matrix degradation.

Recently, Lau et al.\(^\text{6}\) have exhibited that introduction of the clusterin gene into H2-P, a primary hepatocellular carcinoma (HCC) cell line, can significantly upregulate the protein expression of YKL-40, promote migration of tumor cells in vitro and tumor generation and metastasis in vivo. In addition, in primary and metastatic HCC tissues, YKL-40 protein expression is closely related with clusterin, which is in line with our previous study.\(^\text{6}\) Our finding also reveals a positive correlation between the expressions of YKL-40 and clusterin, suggesting that upregulation of YKL-40 expression in epithelial ovarian cancer may be due to an increase of clusterin. Further investigation on the exact mechanism of elevation of YKL-40 in human malignant tumors needs to be performed.

**Acknowledgements**

Grants: National Natural Science Foundation Project (No. 30772334); Guangdong Technology Projects (No. 2004B35001004, No. 2005A30801001).

**References**