Expression levels of PTEN, HIF-1α, and VEGF as prognostic factors in ovarian cancer

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Abstract. – OBJECTIVE: Epithelial ovarian cancer is associated with high mortality, mostly because of delayed diagnosis, necessitating the search for novel diagnostic and prognostic markers. Here we evaluated the association between expression levels of phosphatase and tensin homolog (PTEN), hypoxia-inducible factor (HIF)-1α, and vascular endothelial growth factor (VEGF), and ovarian cancer.

PATIENTS AND METHODS: Expressions of these proteins were assessed by immunohistochemistry in 21 specimens of normal ovary tissues and 76 specimens of ovarian cancer tissues. Associations with pathological characteristics and prognosis were determined using chi-square test, Kaplan-Meier method, log-rank test, and Cox regression model.

RESULTS: Expression of PTEN in ovarian cancer tissue was negatively associated with clinical stage and differentiation degree. A reverse trend was observed in association between expression of HIF-1α and VEGF, and the clinical stage of the disease. PTEN expression negatively correlated with HIF-1α and VEGF expression levels, whereas both latter positively correlated with each other. The overall survival of patients with positive PTEN expression was significantly longer than that of those with negative expression; the opposite trend was observed with HIF-1α and VEGF. The differentiation degree and expressions of HIF-1α and PTEN were dependent predictors, whereas VEGF expression, clinical stage and lymph node metastasization were independent prognostic factors in these patients.

CONCLUSIONS: PTEN, HIF-1α, and VEGF were found to be prognostic markers in ovarian cancer, with VEGF also being as an independent prognostic factor. Combined detection of their expression levels may be useful for determination of the degree of malignancy, metastasis, and prognosis of ovarian cancer.

Key Words: Ovarian cancer, PTEN, HIF-1α, VEGF, Prognosis.

Introduction

Epithelial ovarian cancer is a common gynecological malignancy. Its incidence rate is the third highest among other gynecological malignancies, following cervical cancer and endometrial cancer. Furthermore, ovarian cancer is associated with the highest mortality, mostly because clinical symptoms are not manifested clearly during the early stages of cancer. When manifested, the cancer is usually spread into the abdominal cavity and may even cause pleural effusions. Because of delayed diagnosis, the 5-year survival rate of these patients is very low.

At present, ovarian cancer markers, such as CA125 and CEA, are used for cancer screening. Their diagnostic value is not sufficient, though, and more precise markers are needed to diagnose ovarian cancer, and determine its metastasization or recurrence.

Cancers develop because of inactivation of tumor suppressor genes, activation of proto-oncogenes, or because of modulation of apoptosis-related genes. These processes lead to an imbalance between cell proliferation, differentiation, and apoptosis. Some of these genes hold potential as future markers for ovarian cancer.

Located on the chromosome 10, the phosphatase and tensin homolog (PTEN) is the first identified tumor suppressor gene with phosphorylase activity that is closely related to tumor development. PTEN expression is absent or reduced in many cancers or precancerous lesions.

The hypoxia-inducible factor (HIF)-1α is the transcription factor activated by hypoxic stress. This transcription factor regulates a panel of downstream genes, including the vascular endothelial growth factor (VEGF). HIF-1α has been documented as a contributor to the cancer deve-
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Development and occurrence. The aforementioned downstream target gene VEGF is a mitogen for vascular endothelial cells. Cancer cells are known to secrete high levels of VEGF, thereby promoting tumor angiogenesis and production of tumor stroma.

These three factors have been associated by previous studies. Thus it was found that, in gastric cancer, PTEN negatively regulates HIF-1α through the PI3K/AKT/FARP signalling pathway, leading to decreased expression of VEGF. There are no similar findings about ovarian cancer. It is possible that PTEN, HIF-1α, or VEGF can be useful as markers of ovarian cancer.

The aim of this study was to evaluate a potential association between PTEN, HIF-1α, and VEGF, and clinicopathological characteristics and prognosis of patients with ovarian cancer.

Patients and Methods

Patients' Specimens

Seventy-six tissue specimens were obtained from patients with primary ovarian cancer who had been admitted from October 2007 to September 2010 to our hospital. The study was approved by the Ethical Committee of the Fourth Hospital of Hebei Medical University. In addition, we procured specimens from 21 patients with uterine fibroids; these specimens were used as normal ovarian tissue controls. Control patients had undergone hysterosalpingo-oophorectomy in the same time period as patients with ovarian cancer.

Patients with ovarian cancer patients had a median age of 52.9 years (range 32 to 72 years; 34 patients ≤ 50 years old and 42 patients >50 years old). None of these patients had received radiotherapy or chemotherapy before the operation, and their clinical and pathological files were complete. All patients were followed up until September 2015. Among them, there were 34 cases of serous cystadenocarcinoma, 24 cases of mucinous cystadenocarcinoma, and 18 cases of endometrial carcinoma. Furthermore, 26 specimens represented well differentiated cancer, whereas 50 specimens were from moderately or poorly differentiated ovarian cancer.

Patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2000 staging system. The histopathological grade of the cancer was determined by the World Health Organization (WHO) classification standard. Thirty-eight cases were in stages I and II, and the remaining 38 cases in stages III and IV of the disease. There were 40 cases presenting with lymph node metastasis, and 36 cases with no lymph node metastasis.

Immunohistochemistry

Rabbit anti-human monoclonal VEGF and HIF-1α antibodies, rabbit anti-human PTEN polyclonal antibody, PV-6001 detection reagents, and DAB kit were purchased from Beijing Zhongshan Jinqiao Reagent Co., Ltd (Beijing, China).

The specimens were fixed in 10% formalin, embedded in 5 μm sections of paraffin, processed by conventional dewaxing to water, and further soaked for 5 min in a 3% hydrogen peroxide solution at room temperature to eliminate endogenous peroxidase activity. Anti-PTEN, HIF-1α, and VEGF antibodies were added, and slides were incubated overnight at 4°C. This was followed by three washes with phosphate-buffered saline, each wash going for 2 min. Then, the PV-6001 reagent was added, and slides were incubated at 37°C for 15-20 min, with three more washes afterwards. The slides were finally stained with DAB for approximately 5 min to achieve the desired staining intensity, which was verified under a microscope. The stain was removed with distilled water, and the slides were counterstained with hematoxylin, dehydrated, cleared in xylene, sealed with neutral gum, and observed under a light microscope.

As per literature recommendations, five fields were randomly selected from each section at high magnification. A semi-quantitative analysis was carried out to rank the slides based on staining intensity and number of positive cells. The staining intensity was ranked as cells with no color (0 points), cells stained pale yellow (1 point), cells stained yellow-brown (2 points), or cells stained brown (3 points). The number of positive cells was ranked as 0 points (positive cells constituting <10% of total cells), 1 point (10-49% positive cells), 2 points (50-74% positive cells), and 3 points (≥75% positive cells). These two scores were combined. The threshold was 2 points (i.e. scores 0-2 were considered negative, and >2 were considered positive).

Statistical Analysis

All statistical analyses were performed using the SPSS13.0 (SPSS Inc., Chicago, USA) statistical software. Qualitative data were analyzed using the chi-square test. The Kaplan-Meier method was used to explore the correlations among expressions of PTEN, HIF-1α, and VEGF. The difference in survival curves was evaluated by a log-rank test. The Cox regression model was...
Results

Expressions of PTEN, HIF-1α, and VEGF in Ovarian Cancer and Normal Ovarian Tissue

PTEN was primarily expressed in the cytoplasm, HIF-1α in the cytoplasm or nucleus, and VEGF in the cytoplasm or cytomembrane (Figure 1). The percentages of specimens positive for PTEN, HIF-1α, and VEGF were, respectively, 100, 0, and 14.29% in normal ovarian tissue specimens, and 36.84, 60.53, and 68.42% in specimens of ovarian cancer tissue (p < 0.05, ovarian tissue vs. normal tissue).

Relationships Between PTEN, HIF-1α, and VEGF Expressions, and Clinicopathological Parameters in Ovarian Cancer Tissue

Expression of PTEN in ovarian cancer tissue was associated with clinical stage or differentiation degree, but not with histological type, age, or lymph node metastasization (Table I). Furthermore, PTEN expression levels were higher in stages I/II than in stages III/IV, as well as in G1 stage vs. G2/G3 stage (Table I). These differences were statistically significant (Table I).

About HIF-1α and VEGF, their expressions were associated with clinical stage, differentiation degree, and lymph node metastasization, but not with histological type or age (Table I). In contrast to PTEN, HIF-1α and VEGF expression levels were higher in stages III/IV vs. stages I/II (Table I), and in G2/G3 stage vs. G1 stage (Table I). Also, expressions of both these proteins were higher in specimens with lymph node metastases, compared with specimens without lymph node metastasization (Table I). As with PTEN, these differences reached statistical significance (Table I).

Correlations Among PTEN, HIF-1α and VEGF Expressions in Ovarian Cancer Tissue

The occurrence of ovarian cancer is a result of a concerted effect of multiple genes⁹. Therefore, we assumed that expressions of the three genes of interest might be related to each other. To test for this association, a Spearman rank correlation analysis was performed (Table II). We observed that PTEN expression was negatively correlated to HIF-1α or VEGF expressions (Table II), while HIF-1α expression was positively correlated with VEGF expression (Table II).

![Figure 1](image.png)

**Figure 1.** Positive expression of PTEN, HIF-1α, and VEGF in ovarian cancer tissue. A. PTEN; B. HIF-1α; C. VEGF. Magnification ×400.

used for multivariate analysis. The p < 0.05 was considered statistically significant.
Association Between PTEN, HIF-1α and VEGF Expressions, and Clinical Prognosis in Patients with Ovarian Cancer

Here we analyzed the association between PTEN, HIF-1α, and VEGF expression levels, and clinical prognosis in patients with ovarian cancer. This association was probed by the Kaplan-Meier method and log-rank test. Our analyses indicate that the overall survival of patients with positive PTEN expression was significantly longer than that of patients with negative PTEN expression (Table III; Figure 2A). In contrast, positive expression levels of HIF-1α or VEGF were associated with markedly shorter overall patient survival (Table III; Figure 2B and 2C). The aforementioned associations were statistically significant.

In addition, the survival benefit was observed in patients with lower clinical stage (p = 0.001), better differentiation degree (p = 0.022), and without lymph node metastasization (p = 0.009).

Multivariate Analysis of Prognostic Factors

To further explore the prognostic factors in ovarian cancer, the factors with statistical significance in the Kaplan-Meier survival analysis were subjected to a multivariate Cox survival analysis. The latter analysis revealed that differentiation degree and expression levels of HIF-1α and PTEN were dependent predictors of prognosis of ovarian cancer, whereas VEGF expression, clinical stage, and lymph node metastasization were independent prognostic factors (Table IV).

Discussion

Cancer is a polygenic disease, whose occurrence and development involve multiple pathways and numerous genes\(^1\). Combined detection of several markers of ovarian cancer can provide more
information about underlying mechanisms of cancer proliferation, invasion, and metastasization.\(^1\)

One of candidate markers is PTEN. It is a recently discovered tumor suppressor gene that regulates cell cycle, induces apoptosis in tumor cells, and inhibits their growth, invasion, and metastasization.\(^12\). PTEN inhibits proliferation of cancer cells by activating the FAK pathway, suppresses cancer cell growth by negatively regulating the MAPK pathway, and induces cancer cell apoptosis by suppressing the PI3K pathway.\(^13\). Genetic abnormalities of PTEN gene (mutation, heterozygous loss, hypermethylation, microsatellite instability, transcription modification) lead to a “silencing” of gene expression.\(^14\). Furthermore, low PTEN expression on protein level plays a role in the progression of prostate cancer, endometrial cancer, and ovarian cancer.\(^15\)-\(^17\). Loss of heterozygosity in PTEN was reported in as high as 45% of primary ovarian malignancies. Abnormal PTEN gene leads to diminished levels of this protein in the cell.\(^19\)-\(^20\). Our findings showed that PTEN protein expression in ovarian cancer was significantly lower than that in normal ovarian tissues, indicating that down-regulation of PTEN protein is closely associated with progression of ovarian cancer, which is consistent with previous reports.\(^19\)-\(^20\). Furthermore, we observed a negative relationship between the differentiation degree of ovarian cancer and PTEN protein expression levels, which is also consistent with previous reports.\(^21\). Thereby, absent or low expression of PTEN may be a marker of ovarian cancer. In addition, PTEN protein expression was associated with the clinical stage, but not with lymph node metastases. It can be speculated that gene deletion may be a late event in the pathogenesis of ovarian cancer and, therefore, may occur on a specific stage of cancer.

HIF-1α plays an important role in cancer cell metabolism, angiogenesis, and metastasization; its expression is regulated by multiple factors.\(^22\). HIF-1α is expressed in most cancers, and its expression is associated with proliferation, invasion, and metastasization. The high HIF-1α expression is most commonly associated with poor prognosis, as has been shown in colon, breast, and pancreatic cancers.\(^25\)-\(^27\). In our work, expression of HIF-1α was markedly higher in ovarian cancer than in normal ovarian tissue. Other studies demonstrated similar findings.\(^29\). This suggests that HIF-1α could be used as an early diagnostic marker for ovarian cancer screening. HIF-1α was overexpressed at advanced clinical stages, in poorly differentiated ovarian cancer and patients with lymph node metastases. Therefore, high expression levels of HIF-1α may serve as a prognostic marker in ovarian cancer.

### Table III. Analysis of prognostic value of proteins of interest in ovarian cancer patients.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Cases</th>
<th>Survival</th>
<th>Survival rate(^1) (%)</th>
<th>Median overall survival (months)</th>
<th>(\rho^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>48</td>
<td>9</td>
<td>18.75</td>
<td>26.33</td>
<td>0.008</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
<td>12</td>
<td>42.86</td>
<td>43.27</td>
<td></td>
</tr>
<tr>
<td>HIF-1α</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>13</td>
<td>43.33</td>
<td>48.13</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive</td>
<td>46</td>
<td>8</td>
<td>17.39</td>
<td>25.90</td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>24</td>
<td>10</td>
<td>41.67</td>
<td>51.80</td>
<td>0.005</td>
</tr>
<tr>
<td>Positive</td>
<td>52</td>
<td>11</td>
<td>21.15</td>
<td>24.30</td>
<td></td>
</tr>
</tbody>
</table>

Footnote: \(^1\)5-year survival rate; \(^2\)log-rank test.

### Table IV. Independent prognostic factors for overall survival in patients with ovarian cancer.

<table>
<thead>
<tr>
<th>Determinants</th>
<th>Wald</th>
<th>(p)</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF expression</td>
<td>4.960</td>
<td>0.026</td>
<td>2.016</td>
<td>1.088-3.737</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>10.117</td>
<td>0.001</td>
<td>2.456</td>
<td>1.412-4.271</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>7.696</td>
<td>0.006</td>
<td>2.180</td>
<td>1.257-3.780</td>
</tr>
</tbody>
</table>

Footnote: RR, relative risk; CI, confidence interval.
VEGF is a vascular growth factor that promotes the growth of vascular endothelial cells. It can also promote proliferation of vascular endothelial cells, improve vascular permeability, modulate the extracellular matrix, induce angiogenesis, and promote tumor growth and metastases. Moreover, VEGF has an impact on proliferation and apoptosis of tumor cells. Increased VEGF expression promotes proliferation of tumor cells, and accelerates invasion and metastasis of tumor cells. Generally, VEGF is overexpressed in malignant tumors and vascular endothelial cells. In our study, VEGF expression levels positively correlated with clinical stage, differentiation degree, and lymph node metastasis. These observations indicate that VEGF expression is increased in the process of tumor growth, which leads to the formation of blood vessels, the appearance of a large number of structural and functional abnormalities in tumor blood vessels, and the promotion of tumor progression and metastases. Notably, VEGF expression was also observed in normal ovarian tissues in our research, although this expression was not strongly positive. Indeed, expression levels of VEGF were significantly lower in normal ovarian tissue than in ovarian cancer specimens, which is consistent with literature reports. These results suggest that VEGF plays an important role in the progression of ovarian cancer and is closely related to malignancy, invasion, and metastasis. Therefore, detection of VEGF expression could be used as a clinical indicator of the clinical stage of ovarian cancer.

Despite many reports being published on expression levels of PTEN, HIF-1α, and VEGF in various cancers, the observations have not been consistent. By inducing the expression of VEGF and glycolysis-related enzymes, HIF-1α can increase the supply of blood, oxygen, and energy to the tumor, and attenuate hypoxia. In our study, HIF-1α expression was positively correlated with VEGF expression, similar to previous publications. PTEN inhibits expression of HIF-1α. Therefore, loss of PTEN gene upregulates HIF-1α expression and activates the PI3K/Akt pathway to induce VEGF expression and tumor angiogenesis. Not surprisingly, PTEN was negatively correlated with both HIF-1α and VEGF in our study. Other investigators reported that deletion of PTEN gene increases HIF-1α expression, supporting the role of PTEN as a negative regulator of carcinogenesis.

Our univariate and multivariate survival analyses demonstrated that positive expressions
of PTEN, HIF-1α, and VEGF were prognostic factors in ovarian cancer patients, with VEGF expression also being an independent prognostic factor. Low expression of PTEN and high expressions of HIF-1α and VEGF were associated with poor prognosis and significantly lower survival rate.

Conclusions

PTEN, HIF-1α and VEGF were found to be prognostic markers in ovarian cancer, with VEGF also being as an independent prognostic factor. Combined detection of expression levels of PTEN, HIF-1α, and VEGF may be useful for determination of the degree of malignancy, metastasis, and prognosis of ovarian cancer.

Conflict of interest

The authors declare no conflicts of interest.

References

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