Expression and Clinical Significance of Neuropilin-1 and Vascular Endothelial Growth Factor in Epithelial Ovarian Cancer

Fengjuan Liu², b
² Department of Gynecology, Affiliated Hangzhou First People’s Hospital, Zhejiang University School of Medicine, Zhejiang Hangzhou 310006, P.R. China
b lffjuan1019@163.com

Yubing Li¹, a*
¹ Department of Urology, The First Affiliated Hospital of Zhejiang Chinese Medicine University, Zhejiang Hangzhou 310006, P.R. China.
*Corresponding author: (E-mail: superbing1222@163.com)

Abstract
This paper explores the expression and clinical significance of Neuropilin-1 and vascular endothelial growth factor in epithelial ovarian cancer. Sixty female EOC patients who were treated in our hospital from July 2015 to December 2016 were selected as the research objects, and the expression of NRP-1 and VEGF in EOC tissues and normal tissues were observed by use the test method of Immunohistochemical technique (SP method), then the significance of NRP-1 and VEGF in the diagnosis of EOC was analyzed. In the ovarian cancer tissues of sixty cases EOC female patient, both NRP-1 and VEGF show a state of expression, the positive rates were 51 (85.00%) and 54 (90.00%) respectively, which is significantly higher than that of EOC women with normal ovarian tissue, and the differences have statistical significance (p<0.05). In the ovarian cancer tissues of EOC patients, the expression of NRP-1 was positively correlated with the expression of VEGF protein (r=0.646, p<0.05). NRP-1 and VEGF play an important role in the occurrence and transform of EOC, and it can be used as a new molecular marker to judge the prognosis of ovarian cancer patients.

Keyword: Neuropilin-1, Vascular endothelial growth factor, Epithelial, Ovarian cancer, Expression, Clinical

Expresión Y Significación Clínica De La Neuropilina-1 Y El Factor De Crecimiento Endotelial Vascular En El Cáncer De Ovario Epitelial

Resumen
Este artículo explora la expresión y la importancia clínica de la neuropilina-1 y el factor de crecimiento endotelial vascular en el cáncer de ovario epitelial. Se seleccionaron sesenta pacientes con EOC que fueron tratados en nuestro hospital entre julio de 2015 y diciembre de 2016 como objetos de investigación, y se observó la expresión de NRP-1 y VEGF en tejidos de EOC y tejidos normales mediante el uso del método de prueba de técnica inmunohistoquímica (SP Método), luego se analizó la importancia de NRP-1 y VEGF en el diagnóstico de EOC. En los tejidos de cáncer de ovario de sesenta casos de pacientes con EOC mujer, tanto NRP-1 como VEGF muestran un estado de expresión, las tasas positivas fueron 51 (85.00%) y 54 (90.00%) respectivamente, lo cual es significativamente más alto que el de mujeres EOC con tejido ovárico normal, y las diferencias tienen significación estadística (p <0,05). En los tejidos de cáncer de ovario de pacientes con EOC mujeres, la expresión de NRP-1 se correlacionó positivamente con la expresión de la proteína VEGF (r = 0.646, p <0,05). NRP-1 y VEGF desempeñan un papel importante en la aparición y transformación de EOC, y se puede usar como un nuevo marcador molecular para juzgar el pronóstico de los pacientes con cáncer de ovario.

Palabra clave: Neuropilina-1, Factor de Crecimiento Endotelial Vascular, Epitelial, Cáncer de Ovario, Expresión, Clínica

Ovarian cancer is the most lethal gynecologic malignant tumor in clinic, which have many histological types, including EOC, endometrioid tumor, squamous cell tumor, transitional cell tumor, clear cell tumor, serous tumor and so on, and EOC is the most common, accounting for more than 85% [1-2]. Because of the particularity and concealment of the anatomical position of ovary, making early pathological changes are not easy to be found in
clinical, and when most ovarian cancer patients find it has been the end stage, which has the characteristics of early metastasis, recurrence and drug resistance, and the five-year survival rate of female patients with ovarian cancer is only about 30% [3-4]. Therefore, the expression of NRP-1 and VEGF in cancerous tissues and normal tissues of EOC female patient were detected by SP method to analysis the relationship with clinico-pathological features of EOC, and the correlation of expression between NRP-1 and VEGF in EOC was analyzed to further explore the pathogenesis of EOC. The aim of this paper is to actively search for new EOC biological markers, which can not only improve the clinical diagnosis, but also provide scientific evidence for search therapeutic targets of EOC in clinical. The results are reported as follows.

1. Information and Methods

1.1. General Information

Sixty female EOC patients who were treated in our hospital from July 2015 to December 2016 were selected as the research objects. Inclusion criteria [5]: 1 All the selected patients were diagnosed as epithelial ovarian cancer by histopathological examination; 2 All patients received biological immunotherapy and chemotherapy before operation; 3. There is no history of other malignant tumors; 4. The patients in the group should be sober will and clear expression; 5. No other clinical trials were performed within a month; 6. Voluntarily accepted the treatment and signed the informed consent form. Exclusion criteria [6]: 1 Malignant tumor patients who received preoperative chemotherapy, radiotherapy or biological immunotherapy; 2 Patients with neurogenic diseases; 3 Patients who complicated with heart, liver, kidney and other organ diseases; 4 Patients who complicated with endocrine system diseases, hematological diseases and infectious diseases; 5 Clinical pathological data were missing; 6 Patients who could not receive follow-up. This study was approved by the medical ethics committee of our hospital. The maximum age of the sixty patients was 77 years, the youngest was 25 years old, the median age was 53 years, and the average age was (54.1 ± 11.3) years.

1.2. Reagent

SP immunohistochemical kit was purchased from Shanghai YanHui biological science and Technology Co., Ltd, and rabbit anti human NRP-1 polyclonal antibody and Rabbit anti human VEGF were purchased from Nanjing Biological SenBeiJia Technology Co., Ltd.

1.3. Research method

The experiment was carried out with microscope, case slicer, fluorescent quantitative PCR and other instruments. In the process of experiment, the specimens were fixed with conventional formalin and embedded in paraffin, and were made into 4μm continuous sections, then, Immunohistochemical staining and Quantitative fluorescence detection was performed. After immunohistochemical staining, looking for areas with more uniform microvascular distribution under the one hundred times the field of the microscope, and then, the pale brown endothelial cells or cells that are obviously distinguished from tumor and connective tissue components are selected as vascular counts and the number of microvessels in five field was recorded, and the average value was taken as the PCR value of the case. Specific technical roadmap as shown in the Figure 1

![Figure 1. Technology Roadmap](image)

1.4. Evaluation criterion
The NRP-1 and VEGF of the cytoplasm and membrane of tumor cells with pale brown granules were defined as positive, then the staining intensity and positive percentage of cells were expression evaluated. Among them, the number of positive cells that was less than 5% count 0 points, and 5% to 25% for 1, 5% to 25% for 2, and 50% for 3; No stain count 0 points, light yellow count 1 points, pale brown count 2 points and tan count 3 points; Add the two scores together after scoring, and 0 points showed strong positive (+++), 3~4 points showed moderate positive (++), 1~2 points showed weak positive (+).

1.5. Statistical methods

Statistical analysis using SPSS22.0, Enumeration data were described by N or rate, and chi-square test between groups was performed. Spearman rank correlation coefficient analysis was used to compare the correlation between NRP-1 and VEGF in ovarian cancer tissues. P < 0.05 indicated that the difference has statistically significant and P > 0.05 indicated that the difference not has statistically significant.

2. Result

2.1. The expression of NRP-1 in ovarian cancer tissues and normal tissues

NRP-1 positive staining was mainly located in the surface of ovarian cancer cells and vascular endothelial cells in ovarian cancer, in the ovarian cancer tissues of EOC female patient, 61.67% (37/60) were high expression, 23.33% (14/60) were low expression, 15% (9/60) were negative expression; in the normal tissues of EOC female patient, Immunohistochemical staining showed that only 3.33% (2/60) were low expression, and the remaining 96.67% (58/60) were negative expression. Details are shown in Figure 2.

![Figure 2. Expression of NRP-1 in normal ovarian tissues (A) and epithelial ovarian cancer tissues (B, C, D)](image)

2.2. The expression of VEGF in ovarian cancer tissues and normal tissues

The positive staining of VEGF protein was mainly located in the cytoplasm of ovarian cancer cells, in the ovarian cancer tissues of EOC female patient, 70.00% (42/60) were high expression, 20.00% (12/60) were low expression, 10.00% (6/60) were negative expression; in the normal tissues of EOC female patient, Immunohistochemical staining showed that 1.67% (1/60) were low expression, and the remaining 98.33% (59/60) were negative expression. Details are shown in Figure 3.

![Figure 3. Expression of VEGF in normal ovarian tissues (A) and epithelial ovarian cancer tissues (B, C, D)](image)

2.3. The positive expression of NRP-1 and VEGF in ovarian cancer tissues and normal tissues

The positive expression rate of NRP-1 and VEGF in ovarian cancer tissues was significantly higher than that in normal ovarian tissues. Among them, the positive expression rate of NRP-1 in ovarian cancer tissues of EOC female patient was 85% (51/60), and in ovarian normal tissues of EOC female patient was 3.33% (2/60), the difference between them has statistical significance (P<0.05); the positive expression rate of VEGF...
in ovarian cancer tissues of EOC female patient was 90.00% (54 / 60), and in ovarian normal tissues of EOC female patient was 1.67% (1 / 60), the difference between them has statistical significance (P<0.05). Details are shown in Table 1.

Table 1. The positive expression of NRP-1 and VEGF in ovarian cancer tissues and normal tissues

<table>
<thead>
<tr>
<th>Groups</th>
<th>The number of cases</th>
<th>NRP-1</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian normal tissue</td>
<td>60</td>
<td>51 (85.00%)</td>
<td>54 (90.00%)</td>
</tr>
<tr>
<td>Ovarian cancer tissue</td>
<td>60</td>
<td>2 (3.33%)</td>
<td>1 (1.67%)</td>
</tr>
<tr>
<td>(\chi^2)</td>
<td></td>
<td>5.172</td>
<td>5.498</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.007</td>
<td>0.002</td>
</tr>
</tbody>
</table>

2.4. Correlation analysis of expression of NRP-1 and VEGF in ovarian cancer tissues

From the correlation analysis of Spearman rank, it is show the expression of NRP-1 was positively correlated with the expression of VEGF in ovarian cancer tissues of EOC female patient (r=0.646, P<0.05). Details are shown in Table 2.

Table 2. The relativity of expression of NRP-1 and VEGF in ovarian cancer tissues

<table>
<thead>
<tr>
<th>NRP-1</th>
<th>VEGF</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.646</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0.646</td>
<td>0.001</td>
</tr>
</tbody>
</table>
3. Discussion

NRP-1 was first found in the nerve tissues of tadpoles of Xenopus laevis, which is a non-tyrosine kinase transmembrane multifunctional glycoprotein with a relative molecular mass of 130 000~140 000, and it consists of the transmembrane region, the intracellular region and a long extracellular region [7]. There are three different structural domains in the extracellular region, called a1 / a2, b1 / b2 and c, respectively. The a1 / a2 domain and the b1 / b2 domain are the binding sites of NRP-1 and ligand (Sema3A), the b1 / b2 domain is responsible for combining with VEGF 165 and it is related to the adhesion of NRP-1 mediated cells. The a1 / a2 region and the c region are responsible for the formation of NRP dimer, and the c structural domains of the near membrane is closely related to that NRP-1 conduct the signal of its ligands [8-9]. NRP-1 were high expression in various tumor tissues, and NRP-1 is not only distributed on tumor vascular endothelial cells, but also directly distributed on the surface of tumor cells, therefore, NRP-1 can play a role in the two parts of the tumor [10]. In the immune system, NRP-1 is mainly expressed in the plasma cells, thymocytes, regulatory T cells, dendritic cells [11]. In the human body, NRP-1 is mainly expressed on the surface of activated regulatory T cells, but in rats, NRP-1 is mainly expressed in static or activated regulatory T cells [12]. In malignant tumors, the high expression of NRP-1 usually predicts poor prognosis, which may be related to the expression of NRP-1 and tumor angiogenesis [13]. NRP-1 can participate in the regulation of tumor angiogenesis through the way of depending on VEGF or the way of independent effect on VEGF. NRP-1 can enhance the effect of VEGF receptors on VEGF to enhance the transmission of VEGF signals between cells, or in some tumor cells that do not express VEGF receptors and express NRP-1, NRP-1 can also combine with VEGF to induce proliferation of tumor cell [14]. Some scholars have found that the expression of NRP-1 or NRP-2 is closely related to the poor prognosis of breast cancer, and now, it has been evaluated as an independent prognostic factor in patients with breast cancer after surgery [15]. In addition, the expression level of NRPs in tumor cells is also related to invasiveness of clinical tumor, and in breast biopsies, breast cancer with high malignancy were found to express more NRP-1 than breast cancer with less malignancy [16]. NRP-1 interacts with multiple growth factors, such as VEGF, TGF-β, Hepatocyte growth factor and so on, which can promote the occurrence of cancer. These signal channel interact with each other, and NRP-1 plays a very important role in these responses, especially the interaction with TGF-β [17]. Some scholars have found that the TGF-β that has combined with αvβ3 integrin alpha is easily activated by NRP-1 or NRP-2. When the growth factor is low expression, activated TGF-β inhibits the growth of tumor cell, and when the growth factor is high expression, activated TGF-β promotes tumor metastasis to other places [18-19]. The results of this study showed that the positive expression of NRP-1 in ovarian cancer tissue was significantly higher than that in ovarian normal tissue, which indicated that NRP-1 is likely to be involved in the whole process of tumorigenesis, development and metastasis. VEGF is a highly specific angiogenic factor, which can regulate the activity of vascular endothelial cells, increase the permeability of tumor blood vessels and promote the development of solid tumors through combine with the VEGF receptor on the surface of vascular endothelial cells [20]. Tumor angiogenesis plays an important role in tumor growth and metastasis and is considered to be an important manifestation of tumorigenesis. Angiogenesis which as an important factor in the tumor ecosystem is involved in and affects the biological behavior of tumors [21]. The results of this study also showed that the positive expression of VEGF in ovarian cancer tissues was significantly higher than that in ovarian normal tissues, which point out that the difference of VEGF expression is the basis for identifying benign and malignant tumors [22-23]. VEGF is an important angiogenic factor in ovarian cancer and is also involved in the progression of ovarian cancer. Angiogenesis and the infiltration of tumor in ovarian cancer are closely related to high expression of VEGF [24].

In conclusion, NRP-1 and VEGF play an important role in the occurrence and metastasis of EOC [25], and can be used as a new molecular marker for the prognosis of patients with ovarian cancer [26].

Acknowledgements

The present study was sponsored by grants from the Zhejiang Provincial Traditional Chinese Medicine Science Research Foundation (no. 2016ZB036).

References


Chen C, Hu Y, Li L. (2016) “NRP1 is targeted by miR-130a and miR-130b, and is associated with multidrug resistance in epithelial ovarian cancer based on integrated gene network analysis”, Molecular Medicine Reports, 13(1), pp.188.


