CORRELATION BETWEEN PD-L1 PROTEIN AND KI-67 AND CLINICOPATHOLOGICAL FEATURES IN GLIOMA PATIENTS

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ABSTRACT

Objective: To analyse the correlation between programmed death receptor-ligand 1 (PD-L1) protein and Ki-67 and clinicopathological features in glioma patients.

Methods: Sixty cases of glioma resection specimens and 60 cases of adjacent tissue specimens were selected from the tumour center of our hospital from July 2013 to July 2014. The expression of PD-L1 and Ki-67 in glioma tissues was detected by immunohistochemistry to analyse the correlation between the expression of PD-L1 and Ki-67 and clinicopathological features. The relationship between the expression of PD-L1 and Ki-67 and the five-year survival rate of patients was analysed by the Kaplan-Meier survival curve.

Results: PD-L1 was mainly expressed in the cytoplasm or cell membrane, and yellow and brown particles were positive cells. The positive expression rate of PD-L1 in glioma tissues was 65.00% (39/60), which was significantly higher than that in normal brain tissues at 26.67% (16/60) (P<0.01). Ki-67 was mainly expressed in the nucleus and presented brown-yellow granules as positive cells. The positive expression rate of Ki-67 in glioma tissues was 68.33% (41/60), which was significantly higher than that in normal brain tissues at 30.00% (18/60) (P<0.01). PD-L1 expression in glioma tissues was correlated with necrosis, tumour size and WHO grade (P<0.05). The expression of Ki-67 was correlated with the WHO grade of tumour site (P<0.05). The expression of PD-L1 and Ki-67 was not correlated with age, gender, KPS score or whether there was any invasion of the cerebral lobe (P>0.05). The five-year survival rate of patients with a high expression of PD-L1 and Ki-67 was significantly lower than that of patients with low expression (P<0.05).

Conclusion: PD-L1 and Ki-67 are highly expressed in glioma tissues, which are related to the WHO grade of glioma and may play a potential role in the occurrence and development of glioma.

Keywords: Glioma, programmed death receptor ligand 1, Ki-67, clinicopathological features, correlation.

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Introduction

Glioma is the most common intracranial malignant tumour originating from the neuroepithelial tissue, and its incidence rate accounts for about 50%~55% of brain tumours, which is increasing year by year(1). In 2016, the degree of malignant glioma was divided into the I ~ IV levels by the world health organisation. Levels III and IV were defined as high-grade glioma and accounted for 40% ~ 50% of all glioma. This consists of invasive growth and its recurrence rate reaches almost 100%(2). At present, effective chemotherapy after surgical resection and radiotherapy is still the main treatment method, but the prognosis of glioma patients has not been significantly improved, so it is urgent to find a new treatment method to improve the clinical effect.

In recent years, studies have shown that glioma can promote the occurrence, progression, invasion and metastasis of glioma by down-regulating antigen-presenting cells, up-regulating the expression of anti-inflammatory proteins and increasing the mechanism of regulatory T cells and other immunosuppressive cells(3). By stimulating activated T lymphocytes, the body exerts a cellular immune response in the fight against cancer. In this process, the synergistic effect
of costimulatory molecules on the surface of T cells is required. Programmed death r-ligand (PD-L1) is a negative costimulatory regulatory molecule of the B7 family and plays an important role in the inhibition of T cell function. The PD-L1 can specifically bind with the receptor PD-1 to participate in the negative regulation of immunity and inhibit the function of T cells, thereby leading to the immune escape of tumour cells. Ki-67 is a nuclear antigen related to cell proliferation, which is closely related to the proliferation cycle of proliferating cells, and its expression changes can reflect tumour proliferation activity. In this study, the levels of PD-L1 protein and Ki-67 in glioma patients were detected, and the relationship between PD-L1 protein and Ki-67 was further analysed.

Data and methods

General information

Sixty cases of glioma resection specimens and 60 cases of corresponding para-tumour tissue specimens admitted to the tumour center of our hospital from July 2013 to July 2014 were selected.

Inclusion criteria:

All patients met the diagnosis of glioma in Guidelines for the diagnosis and treatment of central nervous system gliomas in China. Pathology confirmed that the patients with glioma were newly treated. They were at least 18 years old and expected to survive at least three months. All patients had not received chemoradiotherapy. There was no distant remover. We also required the informed consent of patients and their families and cooperation with treatment. This study was approved by the hospital ethics committee.

Exclusion criteria:

A patient with a history of immune system diseases or other malignant tumours could not participate. They were excluded if they had a severe postoperative intracranial infection combined with serious cardiac and liver renal insufficiency. Women who were pregnant and lactating were not included. Patients could not participate if they refused to follow the doctor’s instructions. Among them, 35 males and 25 females were aged from 45 to 70 years old, with an average age of 56.42±10.71 years old. Tumour tissues were in the frontal lobe in 20 cases and in other parts in 40 cases. Tumour ≤3 cm, 19 cases; >3 cm, 41 cases. I level, 16 cases; II level, 12 cases; III level, 15 cases; and IV level, 17 cases. The medical records were followed up by telephone and household registration, and the follow-up time was ≥60 months.

Laboratory reagents and instruments

PD-L1 monoclonal antibody was purchased from Abcam in the United States. Ki-67 monoclonal antibody was purchased from Wuhan Boster Biological Technology., LTD. The immunohistochemical kit was purchased from Beijing zhongshan jinqiao biotechnology co., LTD. EDTA antigen repair buffer was purchased from Maxim Biotech, USA.

The paraffin slicing machine and automatic embedding machine were purchased from Leica, Germany. The frozen centrifuge was purchased from Thermo scientific company, USA. The inverted fluorescence microscope was purchased from Lecai company, USA. The 4oC refrigerator was purchased from the Haier Group, China. The automatic image analyser was purchased from Shanghai peiqing science and technology co., LTD.

Inspection methods

The expression of PD-L1 and Ki-67 in glioma tissues of the two groups were detected by immunohistochemistry. The glioma tissues were fixed with 10% neutral formaldehyde wax, embedded in paraffin, cut into 3 μm slices and dewaxed.

The hydrated tissue sections were repaired with sodium citrate antigen repair solution at 100 oC for 15 minutes, then washed with a phosphate buffer in hydrogen peroxide for 15 minutes, added with primary antibody at 4 oC overnight, and washed with PBS to add secondary antibody to 37 oC for 40 minutes. Hematoxylin-eosin (HE) staining was performed and the histopathological features were observed under a microscope.

Results judgment

All the results were evaluated by two experienced doctors in the pathology department of our hospital. PD-L1 positive cells were mainly located in the cytoplasm or cell membrane of the glioma, which showed yellow and brown particles.

The positive rate of tumour cells was calculated by selecting selective tumour areas at 400 x field of view. The percentage of positive cells ≥ 75% was four points, 50% ~ 74% was three points, 10% ~ 49% was two points, 1% ~ 9% was one point.

According to the staining intensity score, three were sepia, two were brownish yellow, one was light yellow, and zero were colorless. When the two scores were added, 4~7 was classified as high expression and 0~3 as low expression. Ki-67 positive cells were mainly located in the nucleus and showed...
brown-yellow granules. The percentage of positive cells was >70% for three points, 30%~70% for two points and <30% for one point.

The staining intensity
Three points brown-yellow, two points yellow and one point light yellow. The two scores added 3 to 6 were divided into high expression, and 0 to 2 were divided into low expression.

Statistical methods
The enumeration data in this study were all expressed by [n (%)], and the percentage comparison was conducted by the χ² test and the Kaplan-Meier survival curve analysis of the relationship between the expressions of PD-L1 and Ki-67 and the five-year survival rate of patients. P<0.05 was considered statistically significant. All the data in this study were statistically analysed by a SPSS20.0 software package.

Results
Expression of PD-L1 and Ki-67 in glioma tissues
The results of immunohistochemistry showed that PD-L1 was mainly expressed in the cytoplasm or cell membrane, and yellow and brown particles were positive cells. The positive expression rate of PD-L1 in glioma tissues was 65.00% (39/60), which was significantly higher than that in normal brain tissues at 26.67% (16/60) (P<0.01).

The positive expression rate of Ki-67 in glioma tissues was 68.33% (41/60), which was significantly higher than that in normal brain tissues at 30.00% (18/60), and the difference was statistically significant (P<0.01). See Table 1, Figure 1.

Table 1: Expression of PD-L1 and Ki-67 in glioma tissues and normal brain tissues [n (%)].

<table>
<thead>
<tr>
<th>Group</th>
<th>Glioma tissues</th>
<th>Normal brain tissues</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High expression</td>
<td>39</td>
<td>16</td>
<td>1.257</td>
<td>0.001</td>
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<tr>
<td>Low expression</td>
<td>21</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High expression</td>
<td>41</td>
<td>18</td>
<td>2.369</td>
<td>0.001</td>
</tr>
<tr>
<td>Low expression</td>
<td>19</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Expression of PD-L1 and Ki-67 in glioma tissue and normal brain tissue.
A: PD-L1 expression in normal brain tissue; B: Low expression of PD-L1 in glioma tissue; C: High expression of PD-L1 in glioma tissue; D: Expression of Ki-67 in normal brain tissue; E: Low expression of Ki-67 in glioma tissues; F: High expression of Ki-67 in glioma tissues.

Correlation between the expression levels of PD-L1 and Ki-67 in glioma tissues and clinicopathological features
PD-L1 expression in glioma tissues was correlated with necrosis, tumour size and WHO grade (P<0.05). The expression of Ki-67 was correlated with the WHO grade of tumour site (P<0.05), and the expression of PD-L1 and Ki-67 was not correlated with age, gender, KPS score and whether there was any invasion of cerebral lobe (P>0.05). See Table 2.

Table 2: Correlation between the expression levels of PD-L1 and Ki-67 in glioma tissues and their clinicopathological features [n (%)].
The correlation between the expressions of PD-L1 and Ki-67 and the prognosis of glioma tissues

The five-year survival rates of patients in the PD-L1 and Ki-67 high expression group were significantly lower than those in the low expression group, with statistically significant differences (P<0.05), as shown in Table 3 and Figure 2~3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>5-year survival</th>
<th>χ²</th>
<th>P</th>
</tr>
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<tr>
<td>PD-L1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>39</td>
<td>10 (25.64)</td>
<td>5.833</td>
<td>0.016</td>
</tr>
<tr>
<td>Low</td>
<td>21</td>
<td>12 (57.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>41</td>
<td>11 (26.83)</td>
<td>5.396</td>
<td>0.020</td>
</tr>
<tr>
<td>Low</td>
<td>19</td>
<td>11 (57.89)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Correlation between the expression of PD-L1 and Ki-67 and prognosis.

Discussion

Gliomas are a type of highly heterogeneous tumour with invasive growth and unclear tumour boundaries, which are difficult to completely remove with surgery. Although patients often undergo surgical resection combined with postoperative radiotherapy and chemotherapy, glioma patients are still prone to recurrence after surgery, and the overall prognosis is poor(7). Immune escape can protect cancer cells from the immune system. The related mechanism is the regulation and change of the immune response at the tumour site. Anti-tumour immunotherapy can reduce the secretion of T cells by blocking the costimulatory-mediated co-stimulatory signaling pathway. Lymphocyte apoptosis and functional failure molecules promote tumour cell apoptosis and gradually eliminate tumour cell surface antigens and inhibit tumour growth(8, 9). In recent years, immunotherapy of glioma has also become a hot spot for clinical scholars.

PD-L1 is a 40k-Da transmembrane protein expressed in antigen-presenting cells, T cells, macrophages, cardiomyocytes and other cells and plays a role in inhibiting the immune system in tumour tissues, pregnancy and autoimmune diseases(10). When PD-L1 binds to PD-1, an inhibitory signal can be transmitted by PD-1, down-regulating the expression of some anti-apoptotic molecules and pro-inflammatory factors. The binding of PD-L1 to PD-1 during tumorigenesis can prevent tumour cells from being killed by the immune system by inhibiting the function of T lymphocytes, leading to tumour immune escape(11-12). In recent years, domestic and foreign studies have confirmed the positive expression of PD-L1 protein in a variety of tumour tissues, and it is believed that its expression level is closely related to the degree of tumour malignancy and the prognosis of patients(13). Some scholars have found that PD-L1 mRNA is highly expressed in all cell lines by detecting glioma cell lines, and the expression levels of PD-L1 mRNA are different in different pathological types of gliomas(14). Ki-67 is closely related to the cell cycle and is expressed in all increment cells but not in quiescent cells. It is a sensitive and specific cell proliferation marker, which is of great significance in determining the malignancy degree and prognosis of tumours(15). Previous studies have confirmed that Ki-67 is highly expressed in glioma cells but not in normal brain tissues, and its positive expression rate increases with the increase of glioma malignancy. In the prediction of tumour biological behavior,
the detection of the Ki-67 antigen can not only be used as a supplement to postoperative pathology but also can identify the grade of glioma\(^{(16)}\). The results of immunohistochemistry in this study showed that the expression of PD-L1 and Ki-67 in glioma cell tissues was significantly higher than that in normal brain tissues, and the expression of PD-L1 was correlated with necrosis, tumour size and WHO grade (\(P<0.05\)), and the expression of Ki-67 was correlated with the WHO grade of the tumour site (\(P<0.05\)). This suggested that there are high expressions of PD-L1 and Ki-67 in the body of glioma patients, and the expression levels gradually increase with the increase of tumour malignancies. Further analysis of Kaplan-Merier survival curve showed that the five-year survival rate of patients with high expressions of PD-L1 and Ki-67 was significantly lower than that of patients with low expression (\(P<0.05\)). These results suggested that the expression levels of PD-L1 and Ki-67 are related to the prognosis of patients and can be used as candidate tumour molecular markers for prognostic judgment.

In summary, PD-L1 and Ki-67 are highly expressed in glioma tissues, which are related to WHO grade of glioma and may play a potential role in the occurrence and development of glioma.

References


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