Expression of CyclinD1 and Ki-67 proteins in gliomas and its clinical significance

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Abstract. – OBJECTIVES: To investigate the expression of cyclinD1 and Ki-67 proteins in gliomas and its significance.

METHODS: The immunohistochemistry was used to detect the expression of cyclinD1 and Ki-67 proteins in 18 cases of normal brain tissues, 32 cases of low-grade gliomas, and 24 cases of high-grade gliomas.

RESULTS: The cyclinD1 positive ratio in normal brain tissues, low-grade gliomas, and high-grade gliomas were 4/18, 15/32, and 18/24, respectively, with statistically significant difference (p < 0.05). Differences were significant by pairwise comparison between normal brain tissue with high-grade gliomas and low-grade gliomas with high-grade glioma groups (p < 0.01). However, there was no significant difference between normal brain tissue with low-grade gliomas. The Ki-67 positive ratio in normal brain tissues, low-grade gliomas, and high-grade gliomas were 5/18, 21/32, and 20/24, respectively. The difference among three tissues was statistically significant (p < 0.05). Differences were significant by pairwise comparison between normal brain tissue with low-grade gliomas and normal brain tissue with high-grade glioma group (p < 0.01). There is no difference between low-grade gliomas and high-grade gliomas (p > 0.05). Spearman’s rank correlation confirmed that cyclinD1 and Ki-67 was positively correlated in low-grade gliomas and high-level brain tumor (p < 0.05), but no correlation in the normal brain tissue (p > 0.05).

CONCLUSIONS: The expression of CyclinD1 and Ki-67 increased in gliomas, suggesting that both may play an important role in the occurrence of gliomas.

Key Words: Glioma, CyclinD1, Ki-67, Immunohistochemistry, Expression.

Introduction

Glioma is the most common intracranial malignant tumor with clinical characteristics such as easy recurrence, poor prognosis, and high mortality rate, which has greatly threatened the human health. Currently, surgical resection approach of glioma-combined radiotherapy and chemotherapy cannot obtain satisfactory results. Therefore, exploring the new treatments becomes the research focus of majority of medical workers. CyclinD1 is an important cell cycle regulatory protein, which plays a positive regulatory role during the key restriction point G1/S transition of the cell cycle. Ki-67 is a specific marker of cell proliferation, which is significantly associated with tumor histologic grade. This study aimed to investigate the expression of cyclinD1 and Ki-67 in glioma and correlation between them, revealing its clinical significance.

Patients and Methods

Clinical Data

From 2005 to 2011, 56 cases of patients with pathologically confirmed glioma have been chosen and underwent surgery in our hospital, including 35 males and 21 females, aged from 12 to 75 years at an average of 53 years old. All the cases were index cases without preoperative radiotherapy, chemotherapy, and biological therapy. According to WHO (2000), classification criteria of nervous system tumors: 32 cases with low level (including 27 cases of astrocytoma, 3 cases of oligodendrocytoma, and 2 cases of ependymoma); 24 cases with high level (including 14 cases of glioblastoma and 10 cases of anaplastic astrocytoma). The postoperative pathological section was taken as the experimental group. Another 15 cases of normal brain tissue under intracranial decompression were collected as a control group.

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Experimental Methods

CyclinD1 (ab6152) and Ki-67 (ab15580) monoclonal antibodies were purchased from Abcam Company (San Francisco, CA, USA); fluorescent secondary antibody goat anti-rabbit IgG (A0526), Hoechst33342 staining solution (C1022), and Antifade mounting medium (P0126) were purchased from Beyotime Institute Biotechnology Company (Shanghai, China). Experimental procedure: paraffin was sliced and baked at 60°C for 60 min. After deparaffinization of xylene and gradient ethanol, it was washed twice by distilled water for 5 min each time. The paraffin section was dipped into 0.01 mol/l sodium citrate solution at pH 6.0, under high microwave output in 2 min and low microwave output in 15 min for antigen retrieval. The retrieved slice was added with 3% H2O2 to remove endogenous enzymes. It was added with 10% goat serum and enclosed, drying surrounding water with filter paper, then added with primary antibody (1:50) at 4°C overnight. The next day it was added with fluorescently labeled secondary antibody (1:200) and incubated in a side-sway shaker at room temperature for 1 h. It was three times by phosphate buffered saline (PBS) for about 5 min each time. Cell nucleus was stained by Hoechst at room temperature for 10 min, and then washed three times by PBS in each time about 5 min, finally, observed under fluorescence microscope.

Standard of Criterion

Under high-power fluorescence microscope, the observed cells with fluorescence emission were positive cells, and the fluorescence intensity was expressed as follows: no fluorescence (−); weak fluorescence (+); bright fluorescence (++); shiny fluorescence (+++ ∼ ++++). Positive cells account for more than 25% of the total tumor cells showing the positive expression, while < 25% shown as negative expression.

Statistical Analysis

SPSS13.0 for Windows statistical package (SPSS Inc., Chicago, IL, USA) has been used for statistical analysis. χ²-test was used to analyze the differences between groups. Spearman’s rank correlation method was used for correlation analysis. p < 0.05 was considered statistically significant.

Results

Immunohistochemical Results

Immunohistofluorescence staining results showed that cyclinD1 and Ki-67 proteins were expressed in glioma cell nucleus, while there was no protein expression in the cytoplasm and cell membrane (as shown in Figure 1).

CyclinD1 Protein Expression

Distribution and positive ratio of cyclinD1 protein expression in normal brain tissue, low-grade glioma, and high-grade glioma tissues were shown in Table I. The differences among three groups were statistically significant (χ² = 11.681, p < 0.01). There was no statistically significant difference for positive ratios between the normal brain tissue and low-grade glioma tissue (χ² = 2.972, p > 0.05). The difference of positive ratios between normal brain tissue and high-grade glioma tissue was statistically significant (χ² = 11.486, p < 0.01). Comparing the positive

Figure 1. Expression of cyclinD1 and Ki-67 proteins in high-grade glioma cell nucleus.
Table I. Positive ratios of cyclinD1 protein in normal brain tissue, low-grade glioma, and high-grade glioma tissues.

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain tissue</td>
<td>18</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>22.2</td>
</tr>
<tr>
<td>Low-grade glioma</td>
<td>32</td>
<td>17</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>46.9</td>
</tr>
<tr>
<td>High-grade glioma</td>
<td>24</td>
<td>6</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>75.0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>74</td>
<td>37</td>
<td>24</td>
<td>9</td>
<td>4</td>
<td>50.0</td>
</tr>
</tbody>
</table>

There was no correlation for cyclinD1 and Ki-67 protein expression in normal brain tissue (see Table III).

### Ki-67 Protein Expression

Distribution and positive ratio of Ki-67 protein expression in normal brain tissue, low-grade glioma, and high-grade glioma tissues were shown in Table II. The differences among three groups were statistically significant ($\chi^2 = 13.784$, $p < 0.01$). There was statistically significant difference for positive ratios between the normal brain tissue and low-grade glioma tissue ($\chi^2 = 6.611$, $p < 0.01$). The difference of positive ratios between normal brain tissue and high-grade glioma tissue was statistically significant ($\chi^2 = 13.176$, $p < 0.01$). Comparing the positive ratios between low-grade glioma and high-grade glioma tissues, there was no statistically significant difference ($\chi^2 = 2.193$, $p > 0.05$).

### Correlation Between CyclinD1 and Ki-67

Using Spearman’s rank correlation method for analysis, cyclinD1 and Ki-67 protein expression were positively correlated in the low-grade glioma and high-grade glioma tissues, whereas there was no correlation for cyclinD1 and Ki-67 protein expression in normal brain tissue (see Table III).

### Discussion

More and more evidences suggest that changes in the cell cycle play a crucial role in pathogenesis of glioma. CyclinD1, as an important cell cycle regulatory protein, plays a positive regulatory role during transition of the G1/S restriction point in the cell cycle. Numerous studies have demonstrated that CyclinD1 is closely related to the occurrence of cancer. CyclinD1 overexpression plays an important role in promoting the occurrence of basal cell carcinoma. In addition, cyclinD1 overexpression is related to advanced squamous cell carcinoma of larynx cancer, but irrelevant to sex, age, grading cancer, histo-differentiation, anatomical position, etc. This work has demonstrated that expression of cyclinD1 in normal brain tissue, low-grade glioma, and high-grade glioma tissues are significantly different ($\chi^2 = 11.681$, $p < 0.01$). But, there is no difference for the cyclinD1 protein expression in normal brain tissue and low-grade gliomas ($\chi^2 = 2.972$, $p > 0.05$). However, compar-

Table II. Positive ratios of Ki-67 protein in normal brain tissue, low-grade glioma, and high-grade glioma tissues.

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Positive ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain tissue</td>
<td>18</td>
<td>13</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>27.8</td>
</tr>
<tr>
<td>Low-grade glioma</td>
<td>32</td>
<td>11</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>65.6</td>
</tr>
<tr>
<td>High-grade glioma</td>
<td>24</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>6</td>
<td>83.3</td>
</tr>
<tr>
<td>Subtotal</td>
<td>74</td>
<td>28</td>
<td>12</td>
<td>22</td>
<td>12</td>
<td>62.2</td>
</tr>
</tbody>
</table>

Table III. Correlation of cyclinD1 and Ki-67 in normal brain tissue, low-grade glioma, and high-grade glioma tissues.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal brain tissue</th>
<th>Low-grade gliomas</th>
<th>High-grade gliomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>CyclinD1/Ki-67</td>
<td>r-value</td>
<td>0.089</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.605</td>
<td>0.026</td>
</tr>
</tbody>
</table>
ing the positive rates in low-grade glioma and high-grade glioma tissues, the difference was statistically significant ($\chi^2 = 4.482, p < 0.05$), showing that the cyclinD1 is closely related to occurrence of gliomas, while there is higher protein expression in malignant glioma tissues in accordance with previous studies. The immunofluorescence staining results show that cyclinD1 is expressed in nucleus of tumor cells, but not in the cytoplasm and cell membrane.

Proliferating cell nuclear antigen Ki-67 is a specific marker of cell proliferation, and its function is closely related to mitosis, indispensable in cell proliferation, but its exact mechanism is unclear. This report demonstrates that the expressions of Ki-67 in normal brain tissue, low-grade glioma and high-grade glioma tissues were significantly different ($\chi^2 = 13.784, p < 0.01$), indicating that Ki-67 is related to the occurrence of glioma. By pairwise comparison among groups, it is found that there is no difference for the expressions of Ki-67 protein in low-grade glioma and high-grade glioma tissues ($\chi^2 = 2.193, p > 0.05$), but the difference is significant compared to the normal group ($\chi^2 = 6.611, p < 0.01$), suggesting that Ki-67 plays an important role during the process from normal tissues to tumorigenesis. Comparing positive rates of normal brain tissue and high-grade glioma tissue, the difference is significant ($\chi^2 = 13.176, p < 0.01$). Immunofluorescence staining findings show that Ki-67 protein is expressed in nucleus of tumor cells, but not in cytoplasm and cell membrane.

The simultaneous expression of CyclinD1 and Ki-67 has been found in a variety of tumors, which can be used as an important indicator for preliminary identification of tumor grade and prognosis judgment. This study shows that CyclinD1 and Ki-67 have a positive correlation in low-grade glioma and high-grade glioma tissues, suggesting that both of them may synergistically promote the development of glioma and, therefore, can be used as indicator for judging the prognosis of glioma. Ling et al. found that reducing cyclinD1 protein levels can effectively treat the tumors induced by Notch-1, indicating that cyclinD1 plays an important role in tumor formation. Therefore, cyclinD1 may become a new target for the clinical treatment of cancer.

Conclusions

In summary, the studies on the expression of CyclinD1 and Ki-67 in glioma tissues and the correlation between them can provide target gene objectives for further clinical treatment of glioma disease.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

11) Ling H, Jolicoeur P. Notch-1 signaling promotes the cyclinD1-dependent generation of mammary tumor-initiating cells that can revert to bi-potential progenitors from which they arise. Oncogene 2013; 32: 3410-3419.