Determination of CD117 Expression in Glial Tumors and Its Comparison between High Grade and Low Grade Tumors

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Abstract:
Background and Objective:
Gliomas are the most common primary brain tumors. Despite therapeutic advances, the majority of gliomas do not respond either to chemo or radiotherapy. CD117, the gene product of c-kit has been shown to be expressed in cells of glial tumors. Because gastrointestinal stromal tumors (GISTs) that express CD117 respond dramatically to treatment with tyrosine kinase inhibitors, identification of glial tumors that express CD117 might open new therapeutic approaches for treatment of these tumors.

Material and Method:
In this study CD117 expression has been investigated in 69 gliial tumors of different types and grades. This protein was visualized by immunohistochemistry with commercially available antibody.

Results:
42% of the tumors were positive for CD117 expression. There was a statistically significant difference in CD117 immunoreactivity between high grade and low grade tumors (61.1% versus 21.2%, \( P=0.001 \)). 96.6% of the positive cases had cell membranous and/or cytoplasmic staining. All except two of the positive cases showed strong expression intensity. In 26.1% of cases, CD117 also expressed in endothelial cells of tumor vessels that 88.9% of them was in high grade tumors. Glioblastoma, anaplastic oligodendroglioma and anaplastic ependymoma showed the highest staining grade.

Conclusion:
We concluded that CD117 has an important role in growth of gliial tumors, especially high grade ones and that patients with CD117 expressing gliial tumors might benefit from tyrosine kinase inhibitors. This finding should be further studied.

Keywords: brain tumor/ CD117/ immunohistochemical staining

I. INTRODUCTION

CD117 is a transmembrane tyrosine kinase growth factor receptor (1). It is the product of c-kit gene expression (2). Its ligand is referred to as stem cell factor (SCF) (3). CD117 is expressed by a variety of normal human cell types, including germ cells, immature myeloid cells, and mast cells (1). Also it immunolocalized in a variety of neoplasms, the most notable of which is GISTs, where c-kit is felt to be a relatively specific and sensitive immunohistochemical marker of GISTs. If a GIST expresses CD117, it is generally treated with tyrosine kinase inhibitors (Gleevec) (4). Gliial tumors are the most common primary brain tumors. Despite therapeutic advances, the majority of gliomas do not respond either to chemo or radiotherapy (5) and investigation for new therapeutic approaches is indicated in these patients. The CD117/SCF signaling pathway is operative in astrocytes and neurons in normal developing and adult brain (3, 6-8) and CD117 expression might have potential therapeutic significance for brain tumors (3, 9). CD117 was detected in gliomas especially with higher levels in anaplastic forms and glioblastomas (3, 5, 10-13). The importance of this pathway is underscored by growth impairment by a tyrosine kinase inhibitor of human glioblastoma that was injected into mouse brain (14), and by several reports from the effect of thyrosine kinase inhibitor (imatinib) therapy on glioblastoma (15-17). As only a subset of patients seems to benefit from imatinib mesylate therapy and due to potential side effects and high costs of imatinib mesylate therapy, selection of the appropriate patients for this therapy is important. As a step toward this goal, the present study screened 69 human glioma samples of all major types and grades for CD117 expression.

II. MATERIAL AND METHOD

Formalin-fixed, paraffin-embedded tissue blocks were retrieved from 69 glial tumor cases of different types and grades from files of pathology department at Mobasher (Besat) hospital of Hamedan medical university. All cases were reviewed by pathologist for the accuracy of diagnosis according to the current World Health Organization criteria (18). Only there was one case of mixed glioma that excluded from study. CD117 expression was assessed immunohistochemically using the Polyclonal Rabbit Anti-Human CD117, c-kit (code A4502) and peroxidase labeled polymer (code K5007); Dako North America, Carpinteria, California, USA. For each sample the following steps were performed: 3 µm-thick tissue sections were deparaffinized.
Sections were microwave-treated for antigen retrieval in target retrieval solution (DAKO; code S1700) for 20 minutes at 95-99°C then incubated with 3% hydrogen peroxide in methanol for 15 minutes to block endogenous peroxidase activity. After 30 minutes incubation with the primary antibody (polyclonal rabbit anti-human CD117, c-kit, code A4502) at a 1/400 dilution in primary antibody dilution buffer, the sections were incubated with the peroxidase labeled polymer (DAKO; code K5007). After each step in the above staining procedure, the samples were carefully washed at least three times, each time with phosphate buffered saline (PBS) buffer (PH=7.4). Then, the sections were immersed for 10 minutes in diaminobenzidine (DAB) for chromogenic visualization, rinsed in distilled water briefly, counterstained with hematoxilin for 1 minute, dehydrated and mounted. As negative control, Rabbit Immunoglobulin Fraction (solid-phase absorbed, code X0936); Dako North America, Carpinteria, California, USA was used. As positive control, the reaction was tested on sections from GIST. The GIST contained cells that served as positive controls (tumor cells and interstitial cells of Cajal) as well as cells that were internal negative controls (smooth muscle cells of muscularis mucosa and muscularis propria). The stained tumor samples were numbered and assessed by pathologist who used light microscopy to determine the following goals:

- Presence (+) or absence (-) of CD117 immunostaining
- Expression location (cell membrane, cytoplasm, cell processes, nuclear
- Expression intensity (weak, strong)
- Percent of positively-stained cells (expression grade: 0=0%, 1+=1-10%, 2+=11-50%, 3+=51-75%, 4+>75%)

The comparison of CD117 expression between high and low grade tumors was evaluated with SPSS V16 soft ware and Chi square test.

III. RESULTS

We first were assessed GIST sample for the evaluation of the immunohistochemical staining quality used in the present study. The most convincing pattern of CD117 positivity is on featuring a membrane component in addition to a cytoplasmic one (19). CD117 immunoreactivity of the GIST sample was shown in figure 1. CD117 positivity was detected in 29 of the 69 tumor cases. There was a statistically significant difference in CD117 expression between high and low grade tumors. (61% versus 21.2%, P=0.001)(Tables 1).

Table 1

<table>
<thead>
<tr>
<th>Expression Grade</th>
<th>High Grade (%)</th>
<th>Low Grade (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1+</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>2+</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>3+</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4+</td>
<td>40</td>
<td>0</td>
</tr>
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</table>

Another important finding it was that in 18 cases, the CD117 was expressed in endothelial cells of tumor vessels (figure 4). The comparison of CD117 expression between high and low grade tumors was evaluated with SPSS V16 software and Chi square test.

IV. DISCUSSION

The gliomas are the most common primary tumors of the central nervous system (4) and their overall prognosis is poor (24), thus any effort for finding of novel therapies is indicated. In this study CD117 immunoreactivity was detected in 42% of gliomas that ranged from 16% to 75% in different previous studies (3, 5, 10-13, 25-27), including the 2005 study of Cetin et al. that found CD117 immunoreactivity in 75% of 52 assessed glial tumors (3) and the 2007 study of Gomes et al. on 179 gliomas of different types and grades with positivity rate of 15.6% (5). This wide range of positivity can arise from different properties of commercial antibodies, the dilution used in staining, antigen retrieval procedures or other issues. In the present study the DAKO rabbit anti-human CD117 antibody, which is reported to have a higher sensitivity compared to the Santa Cruz antibody for other types of tumors (22, 28), was used at a 1/400 dilution after antigen retrieval treatment. High grade tumors had a higher proportion of CD117 expression than low grade ones as in the previous studies (3, 10, 27). All three major types of gliomas expressed CD117 and the highest staining grades were found in glioblastomas, anaplastic oligodendrogliomas, and anaplastic ependymomas. Similar results achieved in Cetin et al. study for glioblastomas and anaplastic oligodendrogliomas (3). In our study, non of four pilocytic astrocytomas were CD117 immunoreactive as in Cetin et al. study (3). The high grade gliomas, especially glioblastoma, associated with a poor prognosis even after optimal treatment with function-saving surgical resection followed by both radiation and chemotherapy (17), thus now a day many investigations have focused on identifying target therapies. Our results also directed to this goal. In two studies the CD117 immunoreactivity was detected in endothelial cells of tumor vessels, especially in high grade ones. One of them was Gomes et al. study that they found CD117 staining in 22.3% of gliomas. In our study CD117 immunoreactivity was detected in endothelial cells of tumor vessels in 26.1% of cases that 88.9% of them was in high grade ones. The results of this and previous studies (8, 10, 12, 13, 26, and 27) suggest that CD117 may play a role in gliomagenesis and it may open new therapeutic approaches for treatment of gliomas. As only a fraction of patients seems to benefit from imatinib mesylate therapy and due to potential side effects...
and high costs of this therapy, selection of the appropriate patients is important. In several trial studies the effect of imatinib therapy for treatment of glioblastomas was investigated (15-17) and preclinical studies provide evidence that imatinib increases the chemo or radiosensitivity of glioblastoma cells in culture (29-33) suggesting that imatinib may enhance the activity of chemotherapeutic agents used to treat glioblastomas.

V. Conclusion

Regarding to the CD117 immunoreactivity in glial tumors and the significant difference in its expression between high and low grade tumors, we concluded that CD117 has an important role in growth of glial tumors, especially high grade ones. Therefore patients with CD117 expressing glial tumors might benefit from tyrosine kinase inhibitors similar to patients with GISTs. But more universal studies in the immunohistochemical and molecular levels, also evaluation of responsiveness to tyrosine kinase inhibitor therapy should be conducted.

VI. Acknowledgment

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VII. References


Table 1. CD117 expression in high grade and low grade glial tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Low grade</th>
<th>High grade</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td>CD117 +</td>
<td>CD117 -</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>11</td>
<td>24</td>
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<tr>
<td>Astrocytoma</td>
<td>7</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>(21.2%)</td>
<td>(78.8%)</td>
<td>(100%)</td>
</tr>
<tr>
<td>Oligodendroglioma</td>
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<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>(21.2%)</td>
<td>(78.8%)</td>
<td>(100%)</td>
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Pearson Chi- Square= 11.249
Table 2. CD117 staining grades in glial tumors

<table>
<thead>
<tr>
<th>Tumor type</th>
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<th>2+</th>
<th>3+</th>
<th>4+</th>
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<td>Anaplastic astrocytoma</td>
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<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Well differentiated astrocytoma</td>
<td>17</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
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<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ependymoma</td>
<td>1</td>
<td>2</td>
<td></td>
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</tr>
</tbody>
</table>

*Staining grade (percent of positive cells) was scored as: 0=0%, 1+=1-10%, 2+=11-50%, 3+=51-75%, 4+>75%*

Figure 1. CD117 immunostaining in a gastrointestinal stromal tumor (GIST) reveals strong and diffuse cytoplasmic and membranous staining pattern. Internal positive control, the interstitial cells of Cajal are also strongly stained (long arrows), whereas the internal negative control, muscularis mucosa, is not stained (short arrows). (Original magnification × 100)

Figure 2. CD117 immunoreactivity in glioblastoma. Neoplastic cells have strong cytoplasmic, membranous and cell processes staining. (Original magnification × 400)

Figure 3. CD117 staining in anaplastic oligodendroglioma. (Original magnification × 400)