p53 protein and bcl-2 expression in glioblastomas. Pathological correlations in a comprehensive series

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Abstract

p53 and bcl-2 are two well-known antiapoptotic factors associated with gliomas, and mostly astrocytic tumors. In the present study we assessed, by immunohistochemistry, the expression of these two factors in a series of 50 glioblastomas. The correlations between their expression and several tumor-related factors (age, location, recurrence, proliferating potential) were investigated. Our results suggest that a valuable correlation between these factors cannot be found. This is in discrepancy with some literature data, which emphasize strong relations of p53 and bcl-2 in oncogenesis spreading or final prognosis. Further studies are needed, with unique, standard procedures for these evaluations.

Keywords: p53, bcl-2, glioblastoma, immunohistochemistry.

Introduction

Glioblastoma is the most threatening tumor in the brain pathology. Despite extensive research, few are the possibilities to assess accurately the exact length or to imagine new therapeutic ways.

We intended to study a large series of glioblastomas of the adult regarding the expression of p53, bcl-2 and Ki-67, in order to associate them with clinical data and to make possible some outcome correlations.

p53 immunoreactivity is well known as a characteristic of low-grade, as well as anaplastic astrocytomas and also secondary glioblastomas. Its detection by immunohistochemical methods is due to the fact that the non-mutated protein has a very short half-life, being not detectable in routine determinations and only the abnormal or mutated variant of p53 becomes visible by this method [1, 2].

The TP53 gene seems to be frequently the site of mutations or deletions in astrocytic tumors [3, 4].

In some studies, long-term survival in low-grade astrocytomas seems to be influenced by the positivity to p53 [5].

Regarding the relationship between p53 expression in glioblastomas and their therapy response, survival or recurrence delay are conflicting. Recent studies show that only a better response to therapy and a consequently longer survival are expected in tumors having TP53 mutations and high p53 expression [6, 7].

On the other way, the bcl-2 protein, a well-known antiapoptotic factor, is overexpressed in gliomas of any histological grade [8] and with certitude in glioblastomas [9].

Some doubts exist regarding the relationship between these proteins expression and clinical factors.

Recent studies found even some correlations with the location of the tumor (supra or infratentorial) and the p53 expression [10].

Material and methods

Archived material from 59 cases with well-documented primary glioblastomas, 29 females and 30 males, were taken into study. Ages ranged from 30 to 73 years (mean 53.30, standard deviation 11.32) were comparable with literature data.

Follow-up data were available for 43 patients. The paraffin blocks were sectioned at 4 µm and stained with routine hematoxylin-eosin and Masson trichrome.

After a careful examination by two pathologists, significant sections were stained for immunohistochemistry with antibodies to p53, bcl-2, and Ki-67 (Table 1).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Specificity</th>
<th>Source</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>bcl-2</td>
<td>124</td>
<td>Cytoplasmic protein of bcl-2 gene</td>
<td>DAKO, Glostrup, Denmark</td>
<td>1:40</td>
</tr>
<tr>
<td>p53</td>
<td>DO7</td>
<td>Nuclear product of TP53 gene</td>
<td>Neomarkers</td>
<td>1:50</td>
</tr>
<tr>
<td>Ki-67</td>
<td>MIB1</td>
<td>Nuclear cell proliferation factor</td>
<td>DAKO, Glostrup, Denmark</td>
<td>1:50</td>
</tr>
</tbody>
</table>

Counting of reactivity for p53 was done on 100 cells at 400×. The result was expressed as a percentage of black nuclei.

The bcl-2 reactivity was also done by counting 100 cells and expressed as positive cytoplasms from the total. p53 protein was counted also as a percentage of dark nuclei, and was considered as being positive
only when more than 50% of the cells were positive, as described elsewhere [11], cited by [7].
Ki-67 was counted as the percentage of nuclei in most positive areas of the tumor. The data were analyzed using Stat View 3 software.

Results and discussions
p53 was found positive in 48 of 59 tumors. The degree of positivity was variable, with extreme values between 2% and 90%, with a low mean value, of 13.55%. Only two cases had maximal values (Figure 1).
It is generally known that p53 expression, as a consequence of TP53 mutation or overexpression, is a hallmark of secondary glioblastomas, and primary glioblastomas are devoid of it [12]. This makes curious our findings, of various degrees of positivity in a series of only primary tumors.
The p53 overexpression seems to be related, at least in children, to a negative prognosis, regardless histological or clinical factors [13].
Other studies affirm it as a favorable prognosis factor [14] or completely unrelated to the overall evolution [15].
We did not find any relationship to the degree of proliferation or clinical evolution. Only a significant decrease with age was conspicuous, the lowest values being found in elder (p = 0.0248, r = 0.297). This result is in contradiction with recent studies, which found positivity for p53 mostly in elder group of age [16].
bcl-2 was positive in variable degrees, with a mean value of 9.09% (Figure 2).
Sometimes reactive cells adjacent to the tumors were found to be more positive than the tumor, were only scattered lymphocytes proved to be positive (Figure 3).
A significant negative correlation was found between age and bcl-2 expression (p = 0.0087, r = -0.4). bcl-2 is well known as an antiapoptotic factor, not found, normally, in any of brain or cerebellum astrocytes [17].
It contrast, it appears positive in gliomas, but with a decrease in its expression parallel to the anaplastic changes [18].
In our case, in contrast with these findings, the positivity decreased with age, parallel to a decrease in Ki-67 values. Therefore, a coincident increase in both values was not obvious.
Even though some previous studies found a relationship between values of p53 and bcl-2 [19], but several other studies [20–22] did not find a significance in the expression of p53 protein and some clinical (length of survival, recurrence interval) or laboratory parameters (mitotic index, Ki-67 expression, etc). Our study confirmed the latter variant.
Ki-67 is considered as increasing with age. In our case we found a very variable expression, from 1–2% to over 50% (median, 22.68%) (Figure 4).
The lowest values were found in recurrent cases, which had previous fractioned radiation therapy in standard doses (60 Gy).
Regarding the relationship with age, we found completely inverse values as compared with literature data. If in the decade 31–40 years the mean values were 30.6%, we found values of 22.5% for 41–50 years, 24.7% for 51–60 years and only 17.9% for 61–70 years decade, respectively.
Lower Ki-67 values were not statistically significant in relation to survival, the values being between 2 and 14 months. This finding is in contradiction with some recent data, which found a signification correlation between these parameters [22].
Recent studies found a positive correlation between p53 and MIB-1 label index, at least in children [23].
Even though both p53 and MIB-1 values were parallel in decreasing with age, we could not find a statistically significant relationship between the two values in our adult series.
None of the three variable showed particular values related to tumor localization, supra or infratentorial, or of the lobe involved.

Conclusions
bcl-2 and p53 seem not to be related to histological tumor aggressivity. In contrast, a clear decrease in their values was noticed with increasing age. This could be related to a decrease in Ki-67 values with the same pattern.
Therefore, glioblastomas in the elder seem to be less aggressive than in young people, even though our clinical data and survival follow-up do not support a global influence on the survival.
Radiation therapy considerable reduces the proliferation activity, also without an overall improvement in the length of evolution.

References
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Figure 1 – Intense positivity for p53 in a glioblastoma. About 90% of cells nuclei express the protein (IHC, ob. ×40)

Figure 2 – bcl-2 expression in a glioblastoma. Numerous cytoplasms show positive reaction (IHC, ob. ×40)

Figure 3 – The picture shows two cerebral giri, the above one being almost normal, the other one having intense cortical infiltration by a glioblastoma. bcl-2 shows strong positivity in the scattered reactive astrocytes of the normal cortex and is negative in the tumor. The few small intratumoral cells which are positive represent lymphocytes (IHC, ob. ×20)

Figure 4 – A focus of intense Ki-67 immunoreaction in a glioblastoma (IHC, ob. ×40)


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