p53 Protein expression in central nervous system neoplasms

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Abstract

Aims: To demonstrate, immunohistochemically, p53 protein expression in a selection of central nervous system tumours; to investigate the relation between p53 expression and that of the proliferation related antigen, PCNA.

Methods: Surgical specimens from 86 central nervous system tumours were routinely fixed, paraffin wax embedded, and immunostained with a monoclonal (PAb 1801) and a polyclonal antibody (CM1) against p53 protein and a monoclonal antibody against PCNA (PC10). Normal brain samples obtained at necropsy and 10 surgically obtained samples of gliotic brain parenchyma were also immunostained.

Results: p53 protein expression was observed in 35 of 86 brain tumours, suggesting frequent p53 gene mutation. p53 protein alterations were associated with all grades of malignancy in tumours displaying solely astrocytic differentiation, with the exception of pilocytic astrocytomas. In those showing oligodendroglial or ependymal differentiation they appeared to be restricted almost to only high grade lesions. No p53 immunoreactivity was observed in normal or gliotic brain tissue; p53 altered expression was not related to the percentage of PCNA labelled cells.

Conclusions: The use of sophisticated gene amplification techniques or highly sensitive immunohistochemical methods might be useful in distinguishing between reactive and neoplastic astrocytic lesions, and in the identification of malignant progression in other non-astrocytic glial tumours. Tumours with very similar histogenetic differentiation features might actually be a genetically heterogeneous group with possible different clinical courses.

The p53 gene is one of the most extensively investigated tumour suppressor genes (TSG), and there is good evidence that it is a frequent target for genetic abnormalities in most tumours. In general, mutations of the p53 gene result in an abnormal protein which, being much more metabolically stable than the wild protein, accumulates in the nucleus, reaching the threshold of immunohistochemical detection. Therefore, the immunohistochemical demonstration of the abnormal presence of the p53 gene product in histological sections of neoplasms may be regarded as an indication of a possible mutation of the p53 gene itself. Several neoplasms have been thoroughly investigated for altered p53 gene expression, including lung, breast, and colorectal cancers, and Hodgkin’s and non-Hodgkin’s lymphomas.

p53 gene alterations have been reported in a few cell lines and biopsy specimens of glioblastoma multiforme and medulloblastomas, and loss of heterozygosity at the 17p region—the chromosomal region where the p53 gene is located—have been reported both in low and high grade astrocytomas.

Methods

Surgically obtained specimens of 86 central nervous system tumours were investigated, including six pilocytic astrocytomas, 15 low grade astrocytomas, six pleomorphic xanthoastrocytomas (PXA), 10 anaplastic astrocytomas, eight oligodendrogliomas, two anaplastic oligodendrogliomas, seven ependymomas, six anaplastic ependymomas, one anaplastic mixed glioma, 21 glioblastoma multiforme (GBM), and four medulloblastomas. One case of PXA showed three recurrences, the last one with features of a giant cell GBM. All samples were routinely fixed and paraffin wax embedded. Neoplasms were classified according to Burger et al. No stereotactic biopsy specimen was included in the present series. One to three different paraffin wax blocks were investigated for each case. Normal brain samples obtained at necropsy, performed within four hours of death, and surgically obtained samples of normal brain tissue (two cases of Sturge-Weber-Krabbe disease) or reactive gliosis surrounding non-neoplastic (one arterovenous malformation, one granulomatous disease, one old haemorrhage); and neoplastic non-gliial tumours (three metastases, two cerebellar haemangioblastomas) were also immunostained at the same time.

p53 protein was immunolocalised by using the monoclonal antibody PAb 1801 (Oncogene Science) and the polyclonal antiserum CM1 (Novocastra Laboratories) as previously described. PCNA was immunostained with PC10-murine IgG2 (Dako) as previously described. A non-specific mouse IgG monoclonal antibody was used in all experiments as a negative control. To minimise the degree of variability of PCNA immunostaining all sections were immunostained by the same person, under strictly determined conditions, in batches of 20 sections each.
Appropriate positive controls (sections of a p53 positive small cell lung cancer as well as normal tissues with well characterised proliferative compartments, like tonsils and colonic mucosa) were run concurrently to verify the extent and the degree of immunostaining. PCNA immunostaining was scored by counting at least 500 cells in more than 10 high power representative fields. In cases where heterogeneous intratumoral staining was found, examined fields included those with the highest percentage and those with the lowest percentage of stained cells. Tumours were independently and blindly scored by at least two observers. Inter- and intra-observer variation was low (about 5%). The percentage of positive stained cells was recorded as PCNA labelling index (LI).

Statistical tests were performed using the Microstat statistical software (Ecosoft, Inc.) run on an Olivetti 286 PC. Frequency tables were tested for association using the $\chi^2$ test.

**Results**

p53 immunoreactivity was clearly evident as granular or homogeneous nuclear staining. Similar results, as far as the number of positive cases was concerned, were obtained with the monoclonal antibody PAb 1801 and the polyclonal antiserum CM1. With the latter the number of immunoreactive cells in a given tumour was slightly higher. Staining intensity was sometimes variable, and various degrees of intratumoral heterogeneity of p53 positive cell distribution were seen. Astrocytomas frequently showed a minor percentage of p53 immunoreactive nuclei, while anaplastic astrocytomas and GBM showed a rather high percentage of immunostained nuclei (figs 1 and 2). In GBM both small and giant cells were p53 positive, and faint cytoplasmic staining was seen in mitotic cells. Medulloblastomas showed either a diffuse immunostaining pattern of most cells, or a more focal staining (fig 3). Every stained nucleus was considered positive, independently of intensity. No immunoreactive cells were seen in normal or reactive nervous tissue, nor in the vascular lesions and in haemangioblastomas. p53 immunostaining was present in two of the malignant metastases, as well as in the sections of small cell lung cancer used as positive controls. Results are summarised in the table.

PCNA immunolabelled nuclei were clearly and easily identified. Staining intensity was sometimes variable and only clearly stained cells were considered positive, independent of staining intensity, as suggested by Hall et al.25 Median PCNA-LI for the various groups of neoplasms are reported in the table. Median PCNA-LI values were used to separate each group of tumours in subgroups with high and low PCNA-LI. No significant differences could be found concerning the prevalence of p53 immunoreactivity among the subgroups with high and low PCNA-LI.

**Discussion**

Central nervous system tumours show a spectrum of lesions with different degrees of aggressiveness and there is good evidence that low grade tumours may evolve to high grade neoplasms.10 Neoplastic progression is considered to be the result of several events, including loss of differentiation, increase in
p53 expression in brain neoplasms

Figure 3
Medulloblastoma. Several p53 immunoreactive cells are seen throughout the neoplasm. Immunohistochemistry for p53 protein with light nuclear counterstain. (PAI 1801 monoclonal antibody).

p53 overexpression, and loss of tumour suppressor gene (TSG) products. Lack of differentiation and increased proliferative activity are considered to be a consequence of loss of TSG products leading to a more pronounced genetic instability.

In central nervous system tumours, loss of differentiation is readily appreciated and forms the basis of the usual diagnostic grading system. Proliferative activity of brain neoplasms can be evaluated on histological material using monoclonal antibodies against cell-cycle related antigens, like Ki67 antigen and PCNA. On the other hand, the role of the p53 gene in neoplastic progression of brain tumours has been investigated in a few cases, mostly using molecular biology methods, but no extensive study exists on the immunohistochemical demonstration of its product.

Our data show that abnormal p53 expression is found both in low and in high grade astrocytomas, although in the latter groups of neoplasms the prevalence of p53 overexpression is slightly higher than in the former. Pilocytic astrocytomas were always unreactive. Low grade oligodendrogliomas and ependymomas were usually p53 negative, while their malignant (anaplastic) counterparts were frequently p53 positive. Venter and Tomas using restriction fragment length polymorphism analyses, found loss of heterozygosity for loci on chromosome 17 in low grade astrocytomas (one out of five cases) and in glioblastomas (three out of 18 cases), but not in oligodendrogliomas, mixed oligo-astrocytomas, and ependymomas regardless of histological grade. None of the tumours of the series of Venter and Tomas showed evidence of structural abnormalities of the p53 gene: however, very subtle genetic abnormalities (such as point mutations) may have escaped detection. Similar results have been obtained by James et al., who found loss of heterozygosity in eight out of 24 astrocytic tumours of various grades of malignancy, but not in oligodendrogliomas, mixed oligo-astrocytomas, and ependymomas.

Data regarding loss of heterozygosity for loci on chromosome 17 and our immunohistochemical data suggest that p53 gene alterations are a common genetic lesion in brain tumours. p53 gene alterations seem to be associated with all grades of malignancy in tumours displaying solely astrocytic differentiation, while they are almost restricted to high grade lesions in tumours showing oligodendroglial or ependymal differentiation.

Normal and reactive glial tissue was consistently p53 negative, as similarly reported by Chang et al.

The above data suggest that the use of sophisticated gene amplification techniques or highly sensitive immunohistochemical methods—both of which may be used for small biopsy specimens—may provide future diagnostic tools in the differential diagnosis between reactive and neoplastic astrocytic lesions, and in the identification of malignant progression in other non-astrocytic glial tumours. p53 gene or protein alterations are common genetic alterations in most human neoplasms, and indeed it has been suggested that they could be regarded as possible diagnostic markers of the malignant phenotype of various human neoplasms.

The inability to detect p53 altered expression or loss of heterozygosity for loci on chromosome 17 in all astrocytic tumours may partially be due to the limited sensitivity of our technical methods, or to a real lack of p53 gene lesions in a percentage of tumours. This latter hypothesis implies that even astrocytic tumours with very similar differentiation features might indeed be a genetically heterogeneous group. It would be very interesting to compare the clinical course of astrocytic neoplasms with and without p53 abnormal protein expression. In fact, neoplasms with p53 protein alterations should have a more uncontrolled cell proliferation, which is a well accepted prognostic variable in several neoplasms, including central nervous system tumours.

Our data, showing that p53 protein altered
expression may also be demonstrated on routinely fixed and wax embedded material, could lead to a new approach to the evaluation of possible genetic heterogeneity of human brain tumours with similar histology.

We have found p53 altered expression in two out of four medulloblastomas. Loda et al reported p53 mRNA and protein overexpression in one medulloblastoma cell line and xenograft, and focal p53 protein immunoreactivity in one out of five tumours.13 These data point to genetic heterogeneity of the tumours of the medulloblastoma group. Alterations in p53 expression may have a role in facilitating progression of certain clones of medulloblastoma cells, and this could have prognostic value.15

Our data show that altered p53 expression is not related to the percentage of PCNA labelled cells. In central nervous system tumours, PCNA expression has been associated with tumour grade22 and Ki67 labelling index,31 and it has been shown that in cell lines of GBM p53 wild protein down-regulates PCNA expression.14 In this study we have shown that there is no association between high PCNA-LI and p53 altered expression. The lack of association between the two variables, which we have also shown in breast cancers, may be due to an alteration of the p53 dependent PCNA gene control or may underscore other still unknown control mechanisms of the PCNA gene.

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