Accumulation of Wild Type p53 Protein in Human Astrocytomas

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Materials and Methods

Abstract

We have previously described 10 astrocytomas with accumulation of p53 protein but no mutations in p53 exons 5–8, and we have suggested that they might represent overexpression of wild type protein or mutations in less conserved regions of the gene. To investigate these possibilities further, we studied the tumors with immunohistochemistry for wild type and mutant p53 protein and showed that all cases stained with the wild type PAb 1801 antibody but only one case stained with the mutant-specific PAb 240 antibody. To support the hypothesis that the accumulated p53 protein is wild type in most cases, we used single-strand conformation polymorphism analysis and DNA sequencing to evaluate p53 exons 4, 9, and 10 and did not detect mutations at these loci. Although the product of the MDM2 oncogene binds wild type p53 and may account for p53 accumulation, slot-blot analysis of these astrocytomas did not detect MDM2 gene amplification. Thus, evidence suggests that some astrocytomas may accumulate wild type p53 protein but not as a result of MDM2 gene amplification. Furthermore, PAb 1801 immunohistochemistry may not be an adequate method of screening astrocytomas for p53 mutations.

Introduction

Mutations of the p53 tumor suppressor gene are common in astrocytomas and may be associated with elevated levels of p53 protein (1, 2). Recent studies of other human cancers, however, have suggested that p53 gene alterations are not necessarily associated with immunohistochemically detectable p53 protein and that high levels of p53 protein may exist without mutations in exons 5–8 (3–5). We recently studied 34 astrocytomas using immunohistochemistry on fixed, embedded tissues with the monoclonal antibody PAb 1801 to demonstrate p53 protein accumulation, and SSCP1 and DNA sequence analysis of exons 5–8 to reveal mutations in the p53 gene (2). Ten tumors had p53 protein accumulation but no mutations by SSCP. We postulated that these cases might have elevated levels of wild type p53 protein or p53 mutations outside of the conserved exons. To determine whether the accumulated protein was wild type or mutant, we used immunohistochemistry on frozen tumor tissues with antibodies PAb 240, which is mutant specific, and PAb 1801, which reacts with both wild type and mutant proteins. To confirm that these tumors did not harbor mutations in the less-conserved regions of the p53 gene, we performed SSCP on exons 4, 9, and 10, since rare mutations have been reported in these exons. Finally, because the product of the MDM2 oncogene binds to p53 protein, MDM2 gene amplification could lead to increased levels of MDM2 protein and, in turn, wild type p53 protein (6, 7). We therefore used slot-blot analysis of tumor and normal DNA to assay these tumors for MDM2 gene amplification.

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2 To whom requests for reprints should be addressed, at Molecular Neuro-Oncology Laboratory, CNY6, Massachusetts General Hospital, Charlestown, MA 02129.
3 The abbreviations used are: SSCP, single strand conformation polymorphism; WHO, World Health Organization; PCR, polymerase chain reaction.
Results and Discussion

To determine whether the 10 astrocytomas with p53 protein accumulation and no conserved region mutations had wild type or mutant protein, we studied these tumors with immunohistochemistry using two antibodies, PAb 1801 and PAb 240. PAb 1801 is a human-specific monoclonal antibody that recognizes an epitope near the NH₂ terminus of both wild type and mutant p53 protein (10). PAb 1801, however, does not recognize most nonsense mutations (2). In this study, immunohistochemistry on frozen sections showed that all seven available cases were positive with the PAb 1801 antibody (Fig. 1, left), confirming our previous results with this antibody on formalin-fixed, paraffin-embedded tissues (2). In most cases, approximately one-third of cells stained with PAb 1801. Positive reaction product was observed only in nuclei, not in cytoplasm, and varied in intensity, with some nuclei staining more strongly than others. PAb 240 is a monoclonal antibody that reacts with an epitope in the middle of only mutant forms of mouse and human p53 protein (11). Immunohistochemistry with PAb 240 showed positive staining in only one case (case 18), with moderately strong nuclear staining in scattered cells (Fig. 1, right). No cytoplasmic staining was noted with PAb 240. The positive control cell line HTB26 showed moderate to strong nuclear staining with both PAb 1801 and PAb 240 antibodies. The normal cerebellum, the negative control cell line HTB5, and omission of the primary antibody all resulted in no immunohistochemical reaction. The results suggest that the accumulated p53 protein may be wild type in six of the seven studied astrocytomas.

To confirm that these cases did not have p53 mutations outside of exons 5–8, we performed SSCP analysis on exons 4, 9, and 10. Previous studies of astrocytomas that have included exons 2–11 have shown that p53 mutations are exceedingly rare outside of exons 5–8, with only one exon 4 mutation, one exon 9 mutation, and no mutations in other nonconserved exons in 128 astrocytomas (1, 12–14). In other human cancers, mutations outside of exons 5–8 are uncommon but have also been noted in exons 4 and 9 (9, 15). Five of the nine available astrocytomas (cases 12, 18, 150, and 228) had SSCP migration shifts in exon 4, but sequencing showed that these four shifts were caused by the common codon 72 polymorphism (CCC/CGC). No other migration alterations were noted (Fig. 2), making it unlikely that these astrocytomas have mutations in p53 coding regions. Intronic mutations cannot be excluded in these cases but have been noted only rarely in astrocytomas (1, 16) and other human tumors (17). It is possible that our one case (case 18) with positive PAb 240 staining has an intronic mutation. For the remaining cases, however, these data provide further support that the accumulated p53 protein may be wild type.

In summary, increasing evidence argues that wild type p53 protein levels may increase after various types of DNA damage, and such accumulation may represent a common response to DNA injury (26). In the same time, it remains possible that elevated wild type p53 protein may be associated with mutations in intronic sequences that are not examined by current molecular genetic techniques.

Wild type p53 protein has a short half-life and is generally not detectable by immunohistochemistry (27), whereas mutant p53 proteins often have extended half-lives (28) and can be detected immunohistochemically. In our cases, therefore, the wild type protein could have an extended half-life. One possible explanation is that wild type
p53 is bound by another protein which elongates its half-life. A candidate for such a protein is the p90 product of the MDM2 oncogene. p90 binds to both wild type and mutant p53 protein and has transforming properties (6). MDM2 amplification has been noted in approximately one-third of human sarcomas (7). For these reasons, we evaluated our cases for MDM2 gene amplification. Slot-blot analysis demonstrated equal amounts of MDM2 gene in tumor and blood DNA in the nine astrocytomas studied (Fig. 3). Another study has recently reported a similar lack of MDM2 amplification in human astrocytomas (29). Other mechanisms must therefore exist to produce accumulation of wild type p53 protein in a subset of human astrocytomas. Recent data have suggested that increased protein stability is a more likely mechanism for wild type p53 protein accumulation than p53 overexpression (26). Further elucidation, however, awaits the identification of other human proteins that bind p53 and clarification of factors that control p53 expression (30).

The present findings provide further evidence that immunohistochemistry using the PAb 1801 antibody does not correlate well with p53 gene analysis in human astrocytic tumors. We have previously shown that PAb 1801 does not stain astrocytoma cells with nonsense mutations (2). In turn, the present findings indicate that PAb 1801 may not reliably distinguish wild type from mutant protein in human astrocytomas. Jaros et al. (31) recently demonstrated that PAb 1801 immunopositivity correlates with poor clinical survival in astrocytoma patients and attributed the immunopositivity to presumed mutations in the p53 gene. An alternative explanation may be that PAb 1801 immunopositivity reflects accumulated wild type p53 protein and that tumors with high levels of normal p53 protein are less susceptible to the DNA-damaging effects of radiation and chemotherapy than those tumors with p53 gene mutations (30). The biological basis of clinical correlations with PAb 1801 immunohistochemistry may therefore need to be reevaluated in light of the present data.

References


Fig. 3. Slot-blot analysis of MDM2 gene in blood (N) and tumor (T) DNA from cases 10 and 238. MDM2 gene dosage is the same for equal amounts (300 ng of blood) and tumor DNA in the first column. Dilutions of the tumor DNA appear in the second and third columns.
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