p53 Mutations Are Associated with 17p Allelic Loss in Grade II and Grade III Astrocytoma

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Abstract

Loss of genetic material on the short arm of chromosome 17 is observed in approximately 40% of human astrocytomas (WHO grades II and III) and in approximately 30% of cases of glioblastoma multiforme (WHO grade IV). Previous studies of glioblastoma multiforme have shown that the p53 gene, located on the short arm of chromosome 17, is frequently mutated in these glioblastomas. To explore whether lower-grade astrocytomas are also associated with corresponding mutations of the p53 gene, we have investigated a series of 22 human astrocytomas of WHO grades II and III both for loss of heterozygosity on chromosome 17p and for p53 mutations. Mutations in the conserved regions of the p53 gene were identified by single strand conformation polymorphism analysis of exons 5, 6, 7, and 8 and were verified by direct DNA sequencing of the polymerase chain reaction products. p53 mutations were observed in 3 of 8 grade II astrocytomas and 4 of 14 grade II astrocytomas. In all 22 tumors, allelic loss of the short arm of chromosome 17 was investigated by restriction fragment length polymorphism analysis. One-half of the grade II astrocytomas (4 of 8) and grade III astrocytomas (7 of 14) exhibited allelic loss on chromosome 17p. Mutations in the p53 gene were exclusively observed in tumors with allelic loss on 17p. Our results show that p53 mutations are not restricted to glioblastoma multiforme and may be important in the tumorigenesis of lower-grade astrocytomas and that p53 mutations in lower-grade astrocytomas are associated with loss of chromosome 17p. These findings are consistent with a recessive mechanism of action of p53 in WHO grade II and III astrocytoma tumorigenesis.

Introduction

Astrocytic tumors are the most common adult, primary human central nervous system tumors. According to histopathological criteria, these tumors can be assigned different grades of malignancy: astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), and GBM (WHO grade IV) (1). Molecular genetic analyses have revealed genomic alterations characteristic of the different grades. Loss of portions of the short arm of chromosome 17 are seen in lower-grade (grades II and III) and high-grade (grade IV) tumors (2-4), while loss of chromosome 10 and amplification of the epidermal growth factor receptor gene are essentially restricted to glioblastoma multiforme (5-11).

The p53 gene is a tumor suppressor gene located on the short arm of chromosome 17. Mutations in the conserved regions of the p53 gene, which span exons 5 to 8, have been identified in numerous common human malignancies, such as carcinomas of the breast, lung, and colon (12-14). These mutations are often associated with corresponding loss of the other copy of chromosome 17 (13, 15), thus fulfilling the classic paradigm of recessive tumor suppressor gene inactivation (16). In addition, p53 mutations can act in a “dominant negative” manner, without loss or mutation of the other allele (13, 15). In several human tumors, it has been suggested that p53 mutations are relatively late steps in tumorigenesis (15, 17). However, the recent identification of germline p53 mutations in the hereditary cancer syndrome of Li-Fraumeni suggests that p53 mutations may be important in tumor initiation as well (18, 19).

In brain tumors, previous molecular genetic studies have shown p53 mutations in GBM, anaplastic astrocytoma, oligodendroglioma, and medulloblastoma but not in grade II astrocytic tumors (20-23). Mashiyama et al. (21) found no p53 mutations in six “low grade astrocytomas” but did not specify whether these were adult, supratentorial grade II astrocytomas or juvenile grade I pilocytic astrocytomas. Chung et al. (22) described p53 mutations in 2 of 5 astrocytomas, WHO grades II and III. Fults et al. (23) detected p53 mutations in 5/14 anaplastic astrocytomas, but in 0/6 grade II astrocytomas.

Because loss of heterozygosity on chromosome 17p is frequently observed in lower-grade astrocytomas, we explored the possibility that p53 mutations may occur in grade II and III astrocytomas. To investigate this hypothesis, we analyzed 22 astrocytic tumors (8 grade II astrocytoma and 14 grade III anaplastic astrocytomas) for mutations in the conserved regions of the p53 gene by SSCP analysis and direct DNA sequencing. In addition, we evaluated all tumors for loss of heterozygosity of chromosome 17p by Southern blot restriction fragment length polymorphism analysis to assess the relationship of p53 mutations to allelic loss on chromosome 17p.

Materials and Methods

Tissue Specimens and Histopathology. Tumor and blood samples were obtained from patients biopsied at the Massachusetts General Hospital, Boston, and at the University Hospital of Zurich, Switzerland. All tumors were classified by the same neuropathologists and graded according to WHO guidelines. The presence of tumor necrosis was used as the major criterion to distinguish between anaplastic astrocytoma (grade III) and glioblastoma multiforme (grade IV). Because grade I pilocytic astrocytomas are a different clinicopathological entity, they were not examined in the present study. Five of the cases (cases 50, 58, 62, 78, and 114) had been included in a previous study (22).

SSCP Analysis and Direct Sequencing. DNA from human tissue was
isolated as described (24). Exons 5, 6, 7, and 8 of the p53 gene were amplified from 200 ng of genomic DNA by the polymerase chain reaction in a total volume of 10 µl containing 2.5 pmol of each primer, 50 µM deoxyribonucleotide triphosphates, 1 µl of [32P]dCTP (Amersham; specific activity, 3000 Ci/mmol), 10 mM KCl, 1 mM MgCl2, and 0.5 unit Taq polymerase (Perkin Elmer-Cetus). Thirty-five cycles with denaturation (95°C) for 50 s, annealing (63°C for exons 5, 6, 7, 58°C for exon 8) for 50 s, and extension (72°C) for 70 s were performed with an automated Thermal Cycler (Perkin Elmer-Cetus). Primer sequences for the exons of interest (exons 5 to 8) were designed as described elsewhere (24). Selected cases were then examined using exon 8 primers with the following sequences: 5'-CTGCTCTTGCTTCTCTTTT and 5'-TCTCCTCCACCGCTTCT. Amplification products were denatured (95°C, 10 min) and separated on a 6% acrylamide nondenaturing gel containing 10% glycerol. Gels were run at 7 W for 13–15 h at room temperature. A second polymerase chain reaction amplification was performed for sequencing of those exons which showed an abnormal migration pattern on SSCP gels. For sequencing, 1 µg of genomic DNA was amplified in a total volume of 100 µl. The reaction contained 12.5 pmol of each primer, 200 µM deoxyribonucleotide triphosphates, 10 mM Tris (pH 8.8), 50 mM KCl, 1 mM MgCl2, and 2.5 units Taq polymerase. Amplification conditions were as described above. Amplification products were separated on a 4% agarose gel and the amplified bands were electroeluted, purified with Elutip-d columns (Schleicher & Schuell), and ethanol precipitated. DNA sequencing was performed according to a standard protocol as described previously (22). Both strands of all exons with SSCP mobility shifts were analyzed.

DNA Probes and RFLP Analysis. We used a panel of four DNA probes to the short arm of chromosome 17 [p144D6 (D17S34), pYNZ22.1 (D17S57), pYNH37.3 (D17S28), and LB17.3 (no locus designation)] and three probes for the long arm [NF13.0 (no locus designation), LEW206 (D17S57), and pTHH59 (D17S4)]. Probes were purchased from the American Tissue Type Collection, except for LB17.3, which was kindly provided by Y. Nakamura. The following combinations of DNA markers and restriction enzymes were used: p144D6 (D17S34) and TaqI; pYNZ22.1 (D17S57) and RsaI; pYNH37.3 (D17S28) and TaqI; and LEW206 (D17S57) and MspI; pTHH59 (D17S4) and TaqI. RFLP analysis was performed a described elsewhere (24).

Results

SSCP analysis revealed eight tumors with abnormal migration patterns in one of the examined p53 exons. Four of these cases were WHO grade II astrocytomas (4 of 8) and four were WHO grade III anaplastic astrocytomas (4 of 14). Representative SSCP data are shown in Fig. 1A and Fig. 2. Exon 5 was affected in cases 62 and 256, exon 6 in cases 24 and 156, exon 7 in cases 88 and 358, and exon 8 in case 516 (Table 1). Case 256 had two point mutations in exon 5 (Fig. 1, C and D). Corresponding blood DNAs for each case were also examined. In case 156, SSCP analysis of exon 6 in blood DNA also revealed an abnormal migration pattern (see Fig. 2), suggesting the presence of either germline mutation or polymorphism.

Direct DNA sequencing identified point mutations resulting in amino acid sequence alterations in all cases with atypical SSCP migration (see Table 1) with the exception of case 156. In this case, codon 213 of exon 6 showed a silent mutation which was also present in the germline. Thus, p53 mutations in codons 5 to 8 were found in 38% of grade II astrocytomas (3 of 8 cases) and 29% of grade III astrocytomas (4 of 14 cases). Exons 5 to 8 were also sequenced in three cases which had normal SSCP migration patterns (cases 58, 78, and 114); all exons proved to be wild type.

All tumor samples included in this study were informative for at least one marker on the short arm of chromosome 17. Four of 8 (50%) WHO grade II astrocytomas and 7 of 14 WHO grade III (50%) astrocytomas showed loss of heterozygosity on chromosome 17p. All cases with genetic loss on chromosome 17p had lost heterozygosity for all informative 17p markers. A representative case is shown in Fig. 1B.

p53 mutations in exon 5 to 8 were observed in 7 of 11 tumors with loss of heterozygosity on 17p. Representative data are shown in Fig. 1. C and D. p53 mutations were not detected in any tumor which maintained both 17p alleles. The association of p53 mutations with loss of heterozygosity on 17p was statistically significant (Fisher exact test, P < 0.01).

Discussion

By using SSCP analysis and DNA sequencing, we have demonstrated p53 mutations in 3 of 8 grade II astrocytomas and 4 of 14 grade III anaplastic astrocytomas. Southern blot RFLP analysis showed that 50% of astrocytomas and anaplastic astrocytomas had LOH of 17p (4 of 8 grade II and 7 of 14 grade III). These results show that loss of chromosome 17p is more common in lower-grade astrocytomas than had been suggested previously (2, 4, 5, 10). In addition, the percentage of LOH 17p does not change from grade II to grade III astrocytoma, implying that loss of chromosome 17p may be involved in the early steps of astrocytoma tumorigenesis. All cases with p53 mutations had LOH of 17p, while four cases had LOH of 17p without detectable p53 mutations. These data reveal that p53 mutations are not confined to the grade IV GBM but are found in the lower-grade astrocytic tumors as well.
well and show an association between p53 mutations and LOH of 17p.

The finding of p53 mutations in lower-grade astrocytic tumors suggest that p53 mutations occur during the initial stages of tumor formation. This is supported by the observation of similar proportions of p53 mutations in grade II (3 of 8) and grade III (4 of 14) tumors in our study and in grade IV GBM previously reported from our laboratory (5 of 11) (22) and by Fults et al. (7 of 25) (23). Recently, Sidransky et al. (26) detected varying populations of mutant p53 cells in three of three grade II astrocytomas that progressed to GBM by clonal expansion (26). This emphasizes that p53 mutations occur early in astrocytoma tumorigenesis, and is in agreement with our case 88, which showed p53 mutation in the original grade II tumor and in the recurrent GBM (data not shown). The discovery of germline p53 mutations in the Li-Fraumeni syndrome, in which affected members develop a variety of malignancies early in life (including malignant gliomas), further supports a role for p53 early in tumorigenesis. Chung et al. (22) have also described a germline p53 mutation in a patient with neurofibromatosis type I and a GBM.

The association of p53 mutations and LOH 17p in our series suggests that p53 acts as a recessive tumor suppressor gene in those lower-grade astrocytomas with a mutant p53 and loss of the other allele. Because we did not observe cases with p53 mutations and maintenance of both 17p alleles, our study does not provide evidence that p53 acts in a “dominant negative” fashion in these tumors. It is possible, however, that initial p53 mutations occurring before loss of the second allele provide a growth advantage and act at that stage in a “dominant negative” manner (13, 15, 27). Four of our cases, however, had LOH 17p without p53 mutations; this can be explained in several ways. (a) It is possible that a second tumor suppressor gene exists on chromosome 17p, which is involved in astrocytoma tumorigenesis. A telomeric 17p tumor suppressor gene has been implicated in breast (28) and hepatocellular (29) carcinomas. To address this possibility, we used multiple highly informative variable number of tandem repeat markers telomeric to the p53 gene on 17p but were unable to find any astrocytomas that had lost only telomeric portions of 17p (data not shown). (b) Cases with LOH 17p that did not have detectable p53 mutations in our study may carry mutations outside of the examined regions. Because SSCP has been shown to be highly effective in detecting p53 mutations (30), it is unlikely that we have missed mutations in exons 5–8. p53 mutations outside of exons 5–8, however, are uncommon in astrocytomas (23), but intronic regions have not been systematically examined. Recent work from our laboratory and others have shown that p53 mutations may lie

Table 1  Genetic alterations on chromosome 17p in astrocytomas

<table>
<thead>
<tr>
<th>Case</th>
<th>WHO</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>LOH 17p</th>
<th>SSCP shift</th>
<th>Codon</th>
<th>Mutation</th>
<th>Resulting change</th>
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<tr>
<td>88</td>
<td>All</td>
<td>F</td>
<td>40</td>
<td>LOH</td>
<td>ex7</td>
<td>256</td>
<td>ACA -&gt; #CA</td>
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<td>114</td>
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<td>F</td>
<td>31</td>
<td>n</td>
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<td>CGA -&gt; CGG</td>
<td>Polymorphism</td>
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<td>178</td>
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<tr>
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<td>F</td>
<td>32</td>
<td>n</td>
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<td>Tyr -&gt; Cys</td>
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<td>6</td>
<td>n</td>
<td>ex5-ex 8 wild type</td>
<td>262</td>
<td>GGT -&gt; G'T</td>
<td>Frame-shift</td>
</tr>
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<td>ex8</td>
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<td>Cys -&gt; Tyr</td>
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Fig. 2. SSCP analysis of exon 6 of the p53 gene. Wild type pattern in patient 50, abnormal migration pattern in case 24, and polymorphism in case 156. Case 156 reveals loss of heterozygosity in the SSCP polymorphism. B, blood DNA; T, tumor DNA; nd, nondenatured.
within introns (14, 22). We cannot exclude that such mutations are present in our three cases with LOH 17p in which we did not detect SSCP abnormalities.

SSCP is an effective means of detecting single base alterations in the p53 gene but does not distinguish between mutations and polymorphisms. In case 156, SSCP suggested a mutation in exon 6, and sequencing of this exon revealed a CGG at codon 213 instead of CGA. The same SSCP abnormality and sequence change were detected in the constitutional blood DNA and have been previously identified as a DNA polymorphism (25). Such polymorphisms represent potential pitfalls in SSCP analysis and stress the importance of confirming SSCP migration abnormalities by sequence analysis.

References

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