Somatic Mutations of PTEN in Glioblastoma Multiforme

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Abstract

Altered PTEN expression in glioblastoma multiforme. To determine the frequency of PTEN alteration, 34 consecutive glioblastomas were studied in detail. Sequencing each of the nine exons amplified from tumor DNA revealed 11 mutations. Analysis of polymorphic markers within and surrounding the PTEN gene identified an additional four homozygous deletion mutations. Loss of heterozygosity (LOH) was observed in 25 of 34 (74%) cases. All mutations occurred in the presence of LOH. PTEN was mutated in 44% (15 of 34) of all glioblastomas studied and 60% (15 of 25) of tumors with LOH on 10q. Thus, PTEN appears to be the major target of inactivation on chromosome 10q in glioblastoma multiforme.

Introduction

Glioblastoma multiforme is the most aggressive form of glioma, and patients diagnosed with glioblastoma typically survive less than 2 years (1). Genetic analysis of glioblastoma tumorigenesis has identified alterations of p15/p16/Rb, CDK4, p53/MDM2, and EGFR (Ref. 2 and references therein). Mutations affecting cyclin D activity are present in nearly all glioblastomas. The majority of tumors have deletions of p15 and p16 (3, 4). Tumors lacking mutations in these genes frequently harbor inactivating mutations of Rb or an amplification of CDK4 (5). p53 and MDM2 are altered in approximately 30% and 10% of tumors, respectively (Ref. 6 and references therein). Gene amplification of EGFR concomitant with augmented phosphatase activity (13-15). Mutations of this gene have been detected in some 10q23, contains a phosphatase domain, and its protein product has demonstrated LOH at the PTEN locus, and 60% (15 of 25) of these cases contained somatic PTEN mutations of both alleles, implicating the PTEN gene in tumor development.

Materials and Methods

Tumor Samples. Thirty-four normal-tumor pairs of glioblastoma multiforme were kindly provided by the Columbia Comprehensive Cancer Center Tumor Bank (Department of Pathology, Columbia University). Preparation of genomic DNA from blood and tumor tissues has been described previously (17).

LOH Analysis. Four highly polymorphic dinucleotide-repeat markers flanking the PTEN gene, D10S532, D10S1687, D10S541, and D10S583 (Research Genetics, Huntsville, AL), which have been mapped on the sequence tag site (STS)-based map of the human genome (18), were used to determine allelic imbalance in this locus. Another polymorphic dinucleotide-repeat marker, PTENCA (forward primer, 5’-GGTTGATGATAGTGACGCTC-3’; reverse primer, 5’-TTAAGAGACTAGTGAGGAG-3’) derived from a bacterial artificial chromosome (BAC) clone D that contains the 5’ end of the gene was also included in the analysis (13). Each forward primer was labeled with [32P]ATP by T4 polynucleotide kinase (New England Biolabs), and the PCR product was analyzed by 6% PAGE. In addition, a missense polymorphism was found 32 bp from the splice donor site of intron 8, T70G, and the PCR product was used to test for LOH by sequencing normal-tumor paired DNA. LOH was defined as a reduction of band intensity of more than 90% relative to the second allele for at least two markers. Only one case (case 18) demonstrated loss of only one marker and clearly displayed microsatellite instability at another loci.

Mutational Analysis. Intronic primer pairs were designed to amplify and sequence each exon, including the splice junctions (16). Forward and reverse primer sequences for exon 2 are 5’-GGTTGATGATAGTGACGCTC-3’ and 5’-GGCTTGAAGATCCTTTGAAATG-3’, respectively; for exon 3, 5’-AAGCATGATAGTGACGCTC-3’ and 5’-TTAAGAGACTAGTGAGGAG-3’, respectively; for exon 4, 5’-CATTATAAGAGTGACGGCAGAAGT-3’ and 5’-GACGATAGTACGCTTC3’, respectively; and for exon 5, 5’-GATTACGGCAATGTTTGTG-3’. The reverse PCR primer for exon 3 was used as a sequencing primer for the same exon. Primers for each exon were synthesized and purified by Alderfer, and then incorporated into the sequencing PCR reaction. In addition, the PCR product was sequenced using the same primer set, and the DNA sequence was subjected to computer analysis.

Results

Somatic PTEN Mutations. Glioblastoma multiforme samples from 34 tumor cases were sequenced throughout all 9 exons of the PTEN gene, and mutations were found in 11 cases (Table 1). Frame-shift mutations (Fig. 1A) that resulted in premature translational terminations were detected in three cases (cases 2, 19, and 21). In case 2, an insertion at cysteine 136 resulted in a subsequent termination at codon 146; in case 19, an AT deletion at nucleotide positions 227-228 converted tyrosine 76 to a stop codon; in case 21, a 4-bp deletion from nucleotide positions 1011-1014 at codon 337 resulted in a subsequent termination at codon 343. Nonsense mutations were detected at tyrosine 225, arginine 335, and tyrosine 336 in cases 10, 6, and 8, respectively (Fig. 1B). Five missense mutations were found...
Table 1 Summary of PTEN mutations in primary glioblastoma multiforme

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Codon changea</th>
<th>Mutation</th>
<th>Exon</th>
<th>Predicted alterationb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CGA1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>C136fs</td>
</tr>
<tr>
<td>6</td>
<td>TAT1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>R335Opal</td>
</tr>
<tr>
<td>8</td>
<td>TAT1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>Y336Amber</td>
</tr>
<tr>
<td>10</td>
<td>TAT1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>Y225Amber</td>
</tr>
<tr>
<td>15</td>
<td>GGA387 → AGA187</td>
<td>Nonsense</td>
<td>8</td>
<td>P1337fs</td>
</tr>
<tr>
<td>19</td>
<td>TAT1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>Y76fs</td>
</tr>
<tr>
<td>20</td>
<td>GGA387 → AGA187</td>
<td>Nonsense</td>
<td>8</td>
<td>F337fs</td>
</tr>
<tr>
<td>21</td>
<td>TAT1005 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>D107Y</td>
</tr>
<tr>
<td>26</td>
<td>GAT321 → ATGT468</td>
<td>Frameshift</td>
<td>5</td>
<td>L345Q</td>
</tr>
<tr>
<td>29</td>
<td>CTO1053 → CAG1053</td>
<td>Frameshift</td>
<td>5</td>
<td>A121P</td>
</tr>
<tr>
<td>30</td>
<td>GCA163 → CCA163</td>
<td>Frameshift</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

a Mutated nucleotides are in boldface type.
b Positions refer to the deduced PTEN protein sequence (13); fs, frameshift.

leucine 345 was mutated to glutamine. No mutations were detected in
the blood-derived DNA, indicating that all mutations were somatic.

LOH at the PTEN Locus. Four microsatellite markers flanking
the PTEN gene, D10S532, D10S1687, D10S541, and D10S583, were
initially used to evaluate allelic loss in this locus (Fig. 2). Individually,
the frequency of LOH was 83% (19 of 23) for D10S532, 81% (17 of
21) for D10S1687, 57% (12 of 21) for D10S541, and 64% (18 of 28)
for D10S583. Tumors with microsatellite instability were detected in

Fig. 1. Illustration of codon mutations found in the glioblastoma cases. A, frameshift
mutations; B, nonsense mutations; C, missense mutations. Case numbers and mutated
positions refer to Table 1. Arrows, mutated codons. Lanes were loaded in the normal
tumor order. All cases were sequenced with sense primers except for cases 15 and 29, for
which antisense primers were used. Nucleotide sequences shown for each case: case 2,
393–447; case 6, 1001–1019; case 8, 1007–1025; case 10, 668–686; case 15, partial
intron 5 and 493–507; case 19, 220–241; case 20, 374–397; case 21, 1007–1025; case 26,
308–331; case 29, partial intron 8 and 1050–1027; and case 30, 346–381.

(Fig. 1C). A G to T mutation in case 26 resulted in a glutamate 107
to tyrosine alteration, and a G to C change in case 30 resulted in a
change of alanine 121 to proline. Glycine 129 and glycine 165 were
mutated to arginine in cases 20 and 15, respectively. In case 29,
Homozygous Deletions of PTEN. Four cases (cases 4, 12, 25, and 33) were identified which contained homozygous deletions in the PTEN locus (Fig. 3), as determined by apparent retention of heterozygosity flanked by LOH (19). The results revealed that the retention was present between D10S1687 and D10S583 in cases 25 and 33 and between PTENCA and D10S583 in cases 4 and 12 (Figs. 2 and 3). In cases 25 and 33, the homozygous deletions appear to target PTENCA, whereas in cases 4 and 12, they appear to target the 3' end of the gene and include D10S541.

Discussion

Overall, 15 cases were mutated for both alleles of PTEN (Fig. 1). Mutations were found in exons 4–9; no mutations could be found in exons 1–3. Exon 5, which codes for a phosphatase domain, and exon 8 were the most frequently mutated. In addition, we have demonstrated that one copy of PTEN is lost through LOH for 74% (25 of 34) of glioblastomas (Fig. 2). Moreover, 60% (15 of 25) of the cases with LOH carried somatic mutations of the second copy of PTEN, including 11 codon mutations (Fig. 1) and 4 homozygous deletions (Figs. 2 and 3). Interestingly, our previous study has shown that 63% (5 of 8) of glioblastoma cell lines contain mutations (13), but in this study only 44% (15 of 34) of primary glioblastoma cases were mutated (Fig. 2). The mutation rate of PTEN in this study could be underestimated due to our inability to detect all homozygous deletions or alterations of the promoter.

Previous glioblastoma studies have shown that the LOH frequency on chromosome 10q ranges from 83–95%, with the epicenter of loss located at 10q24–25 (9–11). The epicenter of LOH on 10q24–25 may be a summation of deletions targeting PTEN and random partial chromosomal losses distal to the PTEN locus. Consistent with the thought that PTEN is the major target of deletion, all examples of LOH indicate chromosomal break points that are centromeric to PTEN (Fig. 2). These data indicate that LOH in this region typically includes the PTEN gene. Alternatively, another tumor suppressor may exist distal to PTEN.

The genetic analysis of glial tumor development clearly implicates chromosome 10q and therefore PTEN in the transition from anaplastic astrocytoma to glioblastoma multiforme (2). Of the genetic alterations identified in gliomas, only p53 is altered in all grades of astrocytoma-derived tumors, suggesting that p53 inactivation is an early step in glial tumor formation (6). Progression to anaplastic astrocytoma is associated with mutations of p15/p16/Rb/CDK4 (4, 5). The transition to glioblastoma correlates with loss of chromosome 10q and an increase in the frequency of alterations affecting the cyclin D pathway (4, 5). Many glioblastomas are diagnosed in the absence of a prior, lower-grade glial tumor, however. In these cases, p53 mutations are not commonly observed; rather, EGFR amplifications are seen at high frequency along with alterations of 10q and the cyclin D pathway. Interestingly, p53 and EGFR alterations are not found in the same tumor (8). Thus, at a minimum, individual tumors accumulate alterations in the cyclin D regulatory pathway, chromosome 10q, for which PTEN is the likely target, and either p53 or EGFR.

Note Added in Proof


References


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