Somatic Mutations of PTEN in Glioblastoma Multiforme

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Abstract

Alterations of the PTEN gene occur 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 mutations deleting 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 expression of the receptor occurs in 40–50% of glioblastomas (7), and alterations of EGFR and p53 are mutually exclusive in the tumor (8). Loss of chromosome 10q occurs in the vast majority of glioblastomas (9–11) and is associated with alterations of both EGFR and p53 (8, 12).

The PTEN gene, a tumor suppressor recently discovered on chromosome 10q23, contains a phosphatase domain, and its protein product has phosphatase activity (1–15). Mutations of this gene have been detected in glioblastoma cell lines and tumors (13, 14). In addition, other types of cancer and the inherited predisposition to cancer, Cowden disease, are associated with PTEN mutations (13, 14, 16). To determine the frequency of PTEN alterations in glioblastomas, 34 normal-tumor pairs were investigated. The results showed that 74% (25 of 34) of glioblastomas demonstrated LOH in 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, PENTCA (forward primer, 5'-GTTGATGATGATCCGACTC-3'; reverse primer, 5'-TTAAAGGATCTAGTGGAGGAG-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 [γ-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, 5'TTATCGCTTCTAGAAGA-3', and we 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 1 are 5'-GCTGATGGTGCATTCTGCTG-3' and 5'-GGCTTAGAAATCCCCAAGTCTTCTT-3', respectively; for exon 2, 5'-AATGACATGATTCTCCTCTCA-3' and 5'-TCTAACGGTCAGAGGAACTCAA-3', respectively; and for exon 3, 5'-CATTATAAGGTCAGGCAATG-3' and 5'-GACGAGTAGAGATCTCCTAC-3', respectively. The sequencing primer for exon 2 is 5'-TCTGAGATGGAAAACACATGAAA-3' (antisense); for exon 4, 5'-GATTCAGCCGAATTTTCTTAT-3' (sense). The reverse PCR primer for exon 3 was used as a sequencing primer for the same exon. All primers used in this study were purchased from DNAa (Malvern, PA). Ten to 50 ng of genomic DNA were amplified at 94°C for 30 s, 54°C for 1 min, and 72°C for 1 min for 35 cycles. Prior to sequencing reactions, PCR products were treated with 10 units of exonuclease I and 2 units of shrimp alkaline phosphatase for 25 min at 37°C and incubated at 80°C for 15 min. Cycle sequencing (Amersham Life Science) was performed for 25 cycles. Sequencing reactions were resolved on 5% polyacrylamide gels buffered with taurine. Each mutation was verified independently at least twice.

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 A 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 9-15.

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2 The abbreviation used is: LOH, loss of heterozygosity.
Table 1 Summary of PTEN mutations in primary glioblastoma multiforme

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Codon change</th>
<th>Mutation</th>
<th>Exon</th>
<th>Predicted alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>TGT→ATGT</td>
<td>Frameshift</td>
<td>5</td>
<td>C136fs</td>
</tr>
<tr>
<td>6</td>
<td>CGA→TGA</td>
<td>Nonsense</td>
<td>8</td>
<td>R335Opal</td>
</tr>
<tr>
<td>8</td>
<td>TAC→TAG</td>
<td>Nonsense</td>
<td>10</td>
<td>Y336Amber</td>
</tr>
<tr>
<td>10</td>
<td>TAT→TAG</td>
<td>Nonsense</td>
<td>15</td>
<td>Y225Amber</td>
</tr>
<tr>
<td>15</td>
<td>GGA→GAG</td>
<td>Missense</td>
<td>19</td>
<td>G165R</td>
</tr>
<tr>
<td>19</td>
<td>TAT→ΔAT</td>
<td>Frameshift</td>
<td>20</td>
<td>Y76fs</td>
</tr>
<tr>
<td>21</td>
<td>GGA→AGA</td>
<td>Missense</td>
<td>26</td>
<td>G129R</td>
</tr>
<tr>
<td>26</td>
<td>TTG→ΔTTC</td>
<td>Frameshift</td>
<td>29</td>
<td>F337fs</td>
</tr>
<tr>
<td>30</td>
<td>GCA→CCA</td>
<td>Missense</td>
<td>30</td>
<td>L345Q</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. 2.** LOH analysis for 34 glioblastoma cases. ■, LOH; □, no allelic loss; △, not informative; R, replication error. FS, frameshift; HD, homozygous deletion. Cases with codon mutations are indicated (Table 1).
Cases 25 and 33 are presented. Dinucleotide repeat D10S532, respectively, missing allele.

Cases 25 and 33 are not informative at D10S541 and retention of heterozygosity at PTENCA. Cases 25 and 33 are not informative at D10S541 and PTENCA. The potential homozygous deletion region was proposed based on the retention of heterozygosity at PTENCA. Cases 25 and 33 are not informative at D10S541 and D10S583, respectively. C, control, shown from case 22, which is not informative at PTENCA.

A.

![Fig. 3. Potential homozygous deletion at PTEN. Cases 25 and 33 are presented. Dinucleotide repeat markers are indicated with the prefix “D10S” left off. C, control, shown from case 22, which is not informative at PTENCA. The potential homozygous deletion region was proposed based on the retention of heterozygosity at PTENCA. Cases 25 and 33 are not informative at D10S541 and D10S583, respectively. C, control, shown from case 22, which is not informative at PTENCA. The potential homozygous deletion region was proposed based on the retention of heterozygosity at PTENCA. Cases 25 and 33 are not informative at D10S541 and D10S583, respectively.](image)

B.

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 associated with alterations of 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 glioblastoma 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 glioblastoma 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

PTEN GENE MUTATIONS IN GBM


Somatic Mutations of *PTEN* in Glioblastoma Multiforme

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