DMBT1 Polymorphisms: Relationship to Malignant Glioma Tumorigenesis

Hikaru Sasaki, Rebecca A. Betensky, J. Gregory Cairncross, and David N. Louis

ABSTRACT

The deleted in malignant brain tumors 1 (DMBT1) gene on 10q25-26 is a candidate tumor suppressor gene in malignant gliomas, but its role is controversial, e.g., some DMBT1 homozygous deletions reflect unmasking of constitutional deletion polymorphisms by 10q loss. To clarify the role of DMBT1 in gliomagenesis, we investigated three reported deletion hot spots. Homozygous deletions at DMBT1 repeat 2-4 to 2-7 were found in 10 of 73 gliomas with 10q loss, but all 10 deletions reflected unmasking of constitutional hemizygous deletions. Alleles bearing deletion 2-4/2-7 were not selected significantly for by 10q loss, with retention of only 10 of 16 deleted alleles. No homozygous deletion was detected at locus 74k in the 5′ upstream region of DMBT1, and four tightly linked polymorphisms were found around this region; chromosome 10q loss randomly affected alleles with or without the variant sequences around locus 74k. Moreover, no significant selection pressure was detected for the haplotype with both deletion 2-4/2-7 and 5′ polymorphisms. There was no segregation of deletion 2-4/2-7 in glioma patients compared with unrelated individuals from reference families but a suggestion of a difference in the distribution of the 5′ polymorphisms between the reference individuals and glioma patients. Constitutional polymorphisms at DMBT1 repeat 2-9/2-10 appeared common in patients with both benign brain tumors and gliomas. A homozygote for both the 2-4/2-7 deletion and the 5′ polymorphisms had a glioma arise at a typical age and without an apparent family cancer predisposition. These data suggest that DMBT1 polymorphisms are not likely primary targets of 10q loss in malignant gliomas and do not support a major role for DMBT1 in gliomagenesis.

INTRODUCTION

Allelic loss of the long arm of chromosome 10 is one of the most frequent genetic alterations in malignant gliomas, commonly affecting all or most of the chromosomal arm. Although the PTEN gene, the sole established tumor suppressor on chromosome 10q at the present time, has been identified on 10q23 based on allelic deletion analysis in advanced prostate carcinoma (1) and by representational difference analysis (2), 10q25-26 has been suggested as the primary target region of 10q losses in gliomas (3–7). Moreover, PTEN gene alterations have only been found rarely in some tumors associated with 10q loss, such as lung cancer and pancreatic cancer (8), indicating another possible target for 10q loss.

The DMBT1 gene is located between D10S209 and D10S587, an interval included in some of the putative suppressor regions for gliomas and other cancers (4, 6, 7, 9–11). Although only a few potential point mutations have been identified to date (12–14), frequent intragenic homozygous deletions and lack of expression have suggested DMBT1 as a candidate tumor suppressor in glioblastoma, medulloblastoma, lung cancer, and gastrointestinal cancer (12, 15–17). DMBT1, however, is composed of highly homologous repeating units (DMBT1 repeats; Ref. 18), and recent findings of intragenic deletion polymorphisms in a subset of normal individuals have given pause to accepting DMBT1 as a tumor suppressor (19). Indeed, some of the homozygous deletions in tumors simply reflect allelic loss unmasking a constitutional deletion polymorphism (20).

DMBT1 encodes a secreted or membrane-linked protein belonging to the SRCR superfamily. DMBT1 binds to surfactant proteins (collectins), suggesting a role in mucosal immune defense (21, 22). Mollenhauer et al. (19, 22, 23) have proposed a possible role for DMBT1 in cancer immune surveillance based on its stimulatory effect on macrophages, as suggested for another SRCR protein, Mac-2-bp/90K. Specific binding and potent growth-inhibitory activity of mann-binding lectin, a collectin, to colorectal carcinoma cells also raises the possibility of an antitumor activity for DMBT1 (19, 22, 24). Three deletion hot spots have been suggested in the DMBT1 gene, from centromeric to telomeric, at the 74k locus in the 5′ upstream region of the gene; at DMBT1 repeat 2-4 to 2-7 (deletion 2-4/2-7); and at DMBT1 repeat 2-9 and 2-10 (deletion 2-9/2-10; Fig. 1). Because point mutations are only found rarely, such intragenic deletion appears the predominant mechanism of DMBT1 alteration. In the present study, we investigated these deletion hot spots using a large panel of malignant gliomas to clarify the role of DMBT1 in glioma tumorigenesis. Specifically, we evaluated whether tumor-specific deletions occur during tumorigenesis, if selective pressure favors loss of the wild-type allele in the presence of constitutional deletion polymorphisms, and if the deletion polymorphisms are related to glioma predisposition.

MATERIALS AND METHODS

Tissues and DNA. A total of 126 malignant gliomas, all from different patients and all with paired constitutional-tumor DNA samples, were initially included. Of these cases, 99 had sufficient constitutional DNA quality for successful amplification of long-range PCR products and were therefore studied further: 78 glioblastomas, 9 anaplastic astrocytomas, 5 anaplastic oligoden-drogliomas, 2 anaplastic oligoastrocytomas, and 5 high-grade gliomas that were not easily classified into the above common groups. To evaluate DMBT1 alterations in a control population, constitutional DNA from 64 apparently unrelated and unaffected individuals4 (25, 26) in the CEPH reference families was obtained from Coriell Cell Repositories.2 For polymorphisms at DMBT1 2-9/2-10, constitutional DNA was studied by Southern blotting from 17 patients with benign intracranial tumors (9 meningiomas, 7 schwannomas, and 1 hemangioblastoma) and 16 patients with gliomas (3 glioblastomas, 2 anaplastic astrocytomas, 8 anaplastic oligodendrogliomas, and 3 low-grade oligo-denrogliomas; no overlap with 126 cases mentioned above); these were selected randomly from the anonymous DNA bank in our laboratory. Tumor DNA was extracted from either frozen tumor tissue or formalin-fixed, paraffin-embedded sections, and constitutional DNA was extracted from blood leukocytes (27). The present investigations have been approved by the Massachusetts General Hospital Subcommittee on Human Studies and the Review Board for Health Science Research Involving Human Subjects at the University of Western Ontario.

Received 9/28/01; accepted 1/14/02.

1 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 Supported by NIH Grants CA57683 and MRC-MOP-37849.

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Fig. 1. The DMBTI gene. Top: exon-intron structure of DMBTI. Except for the first and the last exons, only exons encoding SRCR domains are numbered (reference sequence: GenBank AJ243211). RsaI fragments that contain any of the first 13 SRCR domains and hybridize with Southern blot probe DMBTI/sr1sid2 are also described. Middle: distribution of the highly homologous DMBTI repeats. Bottom: the reported three deletion hot spots. Long-range PCR primers for deletion 2-4/2-7, the expected size of long-range PCR products, and the position of the 74k locus primers are depicted. STS positions, which had reportedly undergone frequent homozygous deletions, are also shown.

LOH Studies. Allelic loss of chromosome 10q was assessed by LOH assays using microsatellite markers at 10q25-26 (D10S187, D10S221, D10S587, and D10S1723) as described previously (20, 27).

Multiplex PCR. Three sets of multiplex PCR with different target/reference sequence pairs were used to detect deletion 2-4/2-7 (see Fig. 1): (a) g14 (150 bp, intron 18/c12 (190 bp; Ref. 15); (b) intron 20 unique sequence (150 bp)/desmin sequence (171 bp, chromosome 2q); and (c) intron 20 unique sequence (150 bp)/desmin, and for 32 cycles with gradually decreasing annealing temperature from 63°C to 56°C.

Southern Blotting. Southern blotting for the 2-4/2-7 and 2-9/2-10 deletions was performed as described previously with minor modification (18). Briefly, 12–15 μg of genomic DNA from either peripheral blood leukocytes or frozen tumor tissues were digested with 200 units of RsaI. After ethanol precipitation, the digested DNA solubilized in 25 μl of Tris-EDTA-agarose gel at 45 V and transferred with 0.4M NaOH to Hybond N+ (Amersham Pharmacia Biotech, Little Chalfont, United Kingdom). The hybridization was carried out for 20 h at 65°C in 25 ml of modified Church and Gilbert buffer [0.5 M phosphate buffer (pH 7.2) containing 7% SDS; Ref. 28], with a cDNA probe DMBTI/sr1sid2 covering exons 2-12 (2-gene gift from Dr. Jan Mollenhauer; referenced sequence: GenBank AJ243211) labeled with the Megaprime DNA labeling system (Amersham Pharmacia Biotech) using [α-32P]dATP and [α-32P]dCTP. Because of the high homology of the first 13 SRCR domains, RsaI fragments that contain any of these SRCR domains hybridize with DMBTI/sr1sid2, which contains SRCR domains 1 and 2 (exons 7 and 10; Fig. 1). The total copy numbers of each fragment representing DMBTI were estimated by visual inspection. For the 4.1-kb fragment, only cases with estimated copy numbers clearly less than or more than the reported wild-type configuration were judged normal (19).

For the 74k locus, a 414-bp PCR probe (nt2698–13111 in GenBank AJ243211) was generated with primer L1975 (5′-CATGCGCTCGTTTCAGGAGAATG-3′) and L1936: 5′-AAGGTGTTCCTGAATACAGG-3′) for 5′ end of the locus (15). PCR was performed for 30 cycles with annealing temperatures of 63°C to 56°C for amplification of the DMBTI/sr1sid2, which contains SRCR domains 1 and 2 (exons 7 and 10; Fig. 1). The total copy numbers of each fragment representing DMBTI were estimated by visual inspection. For the 4.1-kb fragment, only cases with estimated copy numbers clearly less than or more than the reported wild-type configuration were judged normal (19).

RT-PCR. Gli 13, a glioma cell line that is homozygous for the four variant sequences in the 5′ upstream region of the DMBTI gene, was investigated by RT-PCR for expression of DMBTI. Total RNA extraction and reverse transcription was performed as described (20). The published DMBTI-specific primers were used to amplify a 947-bp DMBTI cDNA sequence spanning exons 45–51 (GenBank AJ243211; Ref. 15).

SSCP Analysis. The presence or absence of the variant sequences in the 5′ upstream region of the DMBTI gene was assessed using two overlapping SSCP analyses (primers: L1952: 5′-CTCGTCTTCTCCAAGGAGTGA-3′; L2045: 5′-GGGAGGTCCAAATCTGCTTG-3′; 74kf2; Ref. 15; and L1936: 5′-GAGGACTCCAAGTGTTC-3′) as described (29).
Absence of Selection Pressure for Constitutional Deletion 2-4/2-7. In 99 malignant gliomas with good quality constitutional DNA, 73 had allelic loss at 10q25-26 and were included in an analysis to determine whether there is selection pressure for loss of the intact versus the deleted DMBT1 allele. All 73 tumors were analyzed successfully by both multiplex PCR g14/c12 and intron 20 sequence/desmin assays for homozgyous deletion at DMBT1 2-4/2-7. Homozygous deletion was found in 10 of the 73 malignant gliomas with LOH at 10q25-26. The possibility of constitutional hemizygous deletion was investigated by two long-range PCR assays, using primer pairs L1595/L1629 and L1595/L1591 flanking the deletion, in which the presence of truncated 5.5- and 4.2-kb products would indicate a constitutional deletion (Figs. 1 and 2). Blood DNA quality was verified by successful amplification of an 8-kb product in a control long-range PCR, L1595/L1864, which was performed at the same time as PCR L1595/L1629 (Figs. 1 and 2). These strategies eliminated the possibility that constitutional deletions would be missed because of poor DNA quality or rare polymorphisms in primer target sequences. Long-range PCRs L1595/L1629 and L1595/L1591 demonstrated constitutional hemizygous deletion at 2-4/2-7 in 17 of the 73 cases whose tumors had 10q LOH. These 17 cases included all 10 cases whose tumors showed homozgyous deletion, indicating that all homozgyous deletions at DMBT1 repeat 2-4/2-7 in tumors occurred as a result of 10q loss, unmasking the constitutional deletion polymorphism.

The possibility of selection pressure for the deleted versus intact allele was then assessed by comparing the DMBT1/control ratio of tumor DNA to those of paired constitutional DNA using multiplex PCR g14/c12, intron 20 sequence/desmin, and g14/APEX. Of the 17 cases with constitutional hemizygous deletions, chromosome 10q loss affected alleles bearing the wild-type DMBT1 gene in 10 cases, all of which had homozzygous deletion (Fig. 3). On the other hand, alleles bearing the deletion polymorphism were lost in 6 cases. The identity of the lost allele was indeterminate in a single case. Southern blotting with DMBT1/sr1sid2 as a probe confirmed constitutional deletions in 7 of the 17 cases and lost alleles by 10q loss in 4 of the 7 cases. In all 17 tumors, allelic loss at DMBT1 locus was confirmed by LOH at flanking markers. Alleles with constitutional deletions at DMBT1 2-4/2-7 were not significantly selected for by 10q loss in malignant gliomas (the rate of wild-type allele loss is 62.5%; CI: 35 and 85%, \( P = 0.45 \)). In addition, there was no significant difference in the frequency of constitutional 2-4/2-7 deletions between malignant glioma patients with (17 of 73) and without LOH (5 of 26, including 1 homozygote; \( P = 0.79 \); Table 1).

Because Mollenhauer et al. (19) found the constitutional hemizygous deletion at 2-4/2-7 in only 1 of 36 normal individuals (3%), we next investigated the frequency of constitutional DMBT1 deletions in 64 unrelated individuals from CEPH reference families to compare an unaffected population with a group of glioma patients. Constitutional hemizygous deletions at 2-4/2-7 were found in 17 of the 64 CEPH individuals, with a frequency (26.5%; CI: 16 and 39%) not significantly different from the 22.2% (22 of 99; CI: 14 and 32%) in malignant glioma patients (\( P = 0.57 \); Table 1). Moreover, the age at onset of primary glioblastoma patients with constitutional deletions and those homozzygous for the wild-type copy at 2-4/2-7 was not significantly different (\( P = 0.96 \)). Therefore, the data do not support the possibility that carriers of the constitutional DMBT1 2-4/2-7 deletions are predisposed to glioma.

Common Polymorphisms in the 5’ Upstream Region of the DMBT1 Gene. The 74k locus, which had been noted to undergo homozygous deletion in 4/21 glioblastomas by PCR (16), was investigated by two multiplex PCR assays covering the entire original 74k locus (Fig. 1; Ref. 15). The 74k-1/APEX multiplex PCR assay did not show any tumor with 10q LOH that had an under representation of the target sequence, which would have been compatible with homozzygous deletion. On the other hand, the 74k-5/desmin multiplex PCR demonstrated under representation of the target sequence in 21 of the 73 gliomas and reference individuals (\( P = 0.45 \)). In addition, there was no significant difference in the frequency of constitutional 5’ polymorphisms between patients with (17 of 73) and without LOH (5 of 26, including 1 homozygote; \( P = 0.79 \); Table 1).

<table>
<thead>
<tr>
<th>Cases</th>
<th>Cases with deletion 2-4/2-7(^{ab})</th>
<th>Cases with 5’ polymorphisms(^c)</th>
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<tr>
<td>Patients with malignant glioma (99 cases)</td>
<td></td>
<td></td>
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<tr>
<td>73 cases with LOH</td>
<td>17 (0)(^d)</td>
<td>38 (7)</td>
</tr>
<tr>
<td>26’ cases without LOH</td>
<td>5 (1)</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Reference individuals(^e) (64 cases)</td>
<td>17 (0)</td>
<td>36 (2)</td>
</tr>
</tbody>
</table>

\(^a\) No significant difference between malignant gloma patients with and without LOH (\( P = 0.79 \)).

\(^b\) No significant difference between patients with malignant gliomas and reference individuals (\( P = 0.57 \)).

\(^c\) Suggestion of a difference in the distribution between patients with malignant gliomas and reference individuals (\( P = 0.08 \)).

\(^d\) Number of homozygotes are indicated in parentheses.

\(^e\) Twenty-five cases were examined for 5’ polymorphisms.

\(^f\) Apparently unrelated and unaffected individuals from the CEPH reference families.

Table 1 DMBT1 constitutional polymorphisms in patients with malignant gliomas and reference individuals.
tumors with 10q LOH, and the 74k-5 sequence was not amplified even in normal leukocyte DNA, as illustrated by loss of the wild-type allele. In tumor 1670, the allele with the variant sequence retained. Cases 6368 and 6320, the wild-type alleles were selectively retained after 10q loss. In tumors 6368 and 6320, the wild-type sequences was selected for by 10q loss, as illustrated by loss of the wild-type allele. The possibility of allelic loss with the variant sequences by 10q loss. The possibility of selection pressure to retain the polymorphic allele (the rate of wild-type allele loss is 45%; CI: 27 and 64%, P = 0.72). In addition, DMBT1 was shown by RT-PCR to be expressed in a glioma cell line (Gli 13) homozygous for the four polymorphisms, in contrast to glioma cell lines that lacked DMBT1 expression, such as IN157, NHG, and U343 (data not shown).

In 64 unrelated CEPH individuals, two were homozygous, and 34 were heterozygous for the polymorphisms by SSCP, with a suggested difference in the distribution of genotypes between glioma patients and CEPH individuals (P = 0.08; Table 1). The age at onset of primary glioblastoma patients homozygous for the polymorphisms was not significantly different from heterozygotes and those homozygous for the wild-type copy at the 74k locus (P = 0.10). The haplotype with both deletion 2-4/2-7 and the two 5' polymorphisms within the region analyzed by SSCP (2-bp deletion and G to T missense) were not significantly selected for by 10q loss, with 10 of 14 alleles bearing the haplotype selected (the rate of wild-type allele loss is 71%; CI: 42 and 92%, P = 0.18).

**Insertion/Deletion Polymorphisms at DMBT1 Repeat 2-9/2-10.** Deletion polymorphisms at DMBT1 repeat 2-9/2-10 have been found in 9 of 36 (25%) normal individuals by Southern blotting (19). Because it was not possible to design a unique PCR assay within the putative deleted region at 2-9/2-10 to assess the possibility of selection pressure, to address the possibility of predisposition of deletion carriers to gliomas, we compared the frequency of these polymorphisms in patients with benign intracranial tumors and patients with malignant gliomas. Southern blot analysis with DMBT1/ sr1sid2 as a probe demonstrated aberrations in the DMBT1 gene in 10 of 17 patients with benign intracranial tumors, relative to the reportedly wild-type G1 configuration (19). These included 8 patients with a gain (n = 2) or loss (n = 6) at DMBT1 repeat 2-9/2-10 and 2 with a deletion at 2-4/2-7 (Fig. 5). In 16 patients with gliomas, 6 had a gain (n = 1) or loss (n = 5) at DMBT1 repeat 2-9/2-10, 1 had a deletion at DMBT1 2-4/2-7, and 2 had both deletions at 2-9/2-10 and 2-4/2-7.

**Deletion at 74k-5 locus had at least one allele with the polymorphisms.** In 9 of 36 (25%) normal individuals by Southern blotting (19). Because it was not possible to design a unique PCR assay within the putative deleted region at 2-9/2-10 to assess the possibility of selection pressure, to address the possibility of predisposition of deletion carriers to gliomas, we compared the frequency of these polymorphisms in patients with benign intracranial tumors and patients with malignant gliomas. Southern blot analysis with DMBT1/ sr1sid2 as a probe demonstrated aberrations in the DMBT1 gene in 10 of 17 patients with benign intracranial tumors, relative to the reportedly wild-type G1 configuration (19). These included 8 patients with a gain (n = 2) or loss (n = 6) at DMBT1 repeat 2-9/2-10 and 2 with a deletion at 2-4/2-7 (Fig. 5). In 16 patients with gliomas, 6 had a gain (n = 1) or loss (n = 5) at DMBT1 repeat 2-9/2-10, 1 had a deletion at DMBT1 2-4/2-7, and 2 had both deletions at 2-9/2-10 and 2-4/2-7.

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parents are heterozygous, and the patient is homozygous for the polymorphisms. Bars, bands specific for wild-type and variant sequences. Bottom band, missense) in the 5'H11032 analysis flanking two of the four polymorphism sites (G to T missense and T to C DMBT1 and 3.9-kb fragments, representing a single copy of a 4.1-kb fragment, indicating deletion polymorphism at DMBT1.

Fig. 6. A, Southern blot analysis of the family members of a glioma patient homozygous for constitutional deletion 2-4/2-7. a, a glioma patient with probable wild-type DMBT1 configuration for direct comparison; b, a patient with an anaplastic oligodendroglioma who is constitutionally homozygous for deletion 2-4/2-7. This patient also has only a single copy of a 4.1-kb fragment, indicating deletion polymorphism at DMBT1 2-9/2-10. c, father of the patient; d, mother of the patient. Arrows, hemizygous losses of 1.8-, 2.1-, and 3.9-kb fragments, representing DMBT1 repeats 2-4/2-7, in both parents. B, SSCP analysis flanking two of the four polymorphism sites (G to T missense and T to C missense) in the 5'H11032 upstream region of DMBT1. a, leukocyte DNA homozygous for the wild-type sequence; b, father of the patient; c, mother of the patient; d, patient. Both parents are heterozygous, and the patient is homozygous for the polymorphisms. Bars, bands specific for wild-type and variant sequences. Bottom band, non-denatured, double-strand DNA.

(Fig. 5). Three patients with benign tumors (2 meningiomas and 1 schwannoma) and a patient with glioblastoma lacked the whole DMBT1 repeat 2-9/2-10 in both alleles (Fig. 5). The age of tumor presentation in these 4 patients was typical (47–52 years of age for benign tumors and 46 years of age for glioblastoma).

Constitutional Homozygotes for DMBT1 Deletion 2-4/2-7. In the course of our previous (20) and present studies, which have included 190 patients with gliomas, 17 with benign intracranial tumors, and 64 CEPH individuals, we have encountered 2 persons with constitutional homozygous deletions at DMBT1 2-4/2-7. These 2 persons were also constitutionally homozygous for the three 5'H11032 polymorphisms within SSCP region. Both were patients with malignant glioma: (a) a 32-year-old woman with a glioblastoma; and (b) a 35-year-old man with an anaplastic oligodendroglioma. The family history was available from the latter patient; although both parents are healthy, his brother has Crohn’s disease, and his sister had multiple sclerosis. Southern blotting with DMBT1 srs1sid2 as a probe and SSCP analysis demonstrated that both parents are heterozygous for both the 2-4/2-7 deletion and the 5'H11032 polymorphisms, suggesting Mendelian inheritance of these polymorphisms (Fig. 6).

DISCUSSION

Homozygous deletion at the g14 locus is present in a significant fraction of various cancers and has suggested DMBT1 as a candidate tumor suppressor (12, 13, 15–17, 30). However, only rare somatic point mutations of uncertain significance have been found in human tumors (12–14). Furthermore, Mollenhauer et al. reported constitutional hemizygous deletion 2-4/2-7 involving the g14 locus in 1 of 36 normal individuals (3%), indicating that some homozygous deletions in tumors may reflect allelic loss unmasking preexistent, constitutional deletions. In malignant gliomas, we have shown that at least some homozygous DMBT1 deletions indeed reflect constitutional deletions and allelic loss (20). In the present study, we identified 10 tumors with homozygous deletion at DMBT1 2-4/2-7 in a series of 73 malignant gliomas with 10q loss; significantly, all of these deletions represented constitutional hemizygous deletions coupled with allelic loss. Taken together, the above data argue against DMBT1 as a classical tumor suppressor gene after the somatic “two-hit” model.

Nonetheless, there is increasing evidence of associations between common polymorphisms and diseases, including cancer (31, 32). It remains possible that constitutional DMBT1 deletion polymorphisms are tumorigenic once loss of the wild-type allele takes place. To address this possibility, we determined whether allelic loss preferentially affected the wild-type versus the deleted DMBT1 copy. We hypothesized that, if these polymorphisms contributed to tumorigenesis, there would be selection to delete the wild-type allele. However, no significant selection pressure was apparent for alleles with either the 2-4/2-7 deletion, polymorphisms in the 5'H11032 upstream region of the gene, or the haplotype with both. If these polymorphisms play a major role in tumorigenesis, alleles with the polymorphisms should be selected exclusively for when 10q is lost. Therefore, these data suggest that DMBT1 polymorphisms are not likely primary targets of 10q loss in gliomas. Nonetheless, we cannot exclude the possibility that a specific haplotype, such as a combination of three polymorphisms (5'H11032 upstream, 2-4/2-7, and 2-9/2-10) or polymorphisms with a particular parental inheritance, might be selected for by 10q loss.

Because polymorphic sequences in putative cancer susceptibility genes may be associated with an increased risk of cancer (33, 34), it remained possible that constitutional DMBT1 polymorphisms predispose to glioma formation. Although there was a suggestion of a difference in the distribution of the 5'H11032 upstream polymorphisms between patients with malignant gliomas and CEPH individuals, a constitutional 2-4/2-7 deletion was found at a similar frequency in CEPH individuals (26.5%) and in patients with malignant gliomas (22.2%). In patients with benign brain tumors, aberrations at 2-9/2-10 appeared as common as in patients with gliomas. Furthermore, the age of glioblastoma presentation for patients who had the constitutional 2-4/2-7 deletion and for patients who were homozygous for the 5'H11032 polymorphisms was not significantly different from those of patients with two wild-type copies at each of the polymorphic sites. All of these results, except for the possible difference in the distribution of the 5'H11032 polymorphisms between glioma patients and CEPH individuals, argue against DMBT1 as a potential glioma susceptibility gene.

Strikingly, two patients were homozygous for the constitutional 2-4/2-7 deletion, as well as for the 5'H11032 polymorphisms. These patients apparently had no history of developmental abnormalities, arguing against an essential role for DMBT1 in development. In addition, the lack of other known tumors in these individuals, and their relatively typical age of clinical presentation, as well as the lack of family cancer predisposition in the patient for whom family history was available, all also argue against a role for these polymorphisms in tumorigenesis. Interestingly, however, this family history raises the intriguing possibility that DMBT1 alterations could relate to other diseases, because the patient’s siblings suffered from inflammatory bowel disease and multiple sclerosis, both considered autoimmune diseases. Because

\[ \text{A. von Deimling, personal communication.} \]

\[ \text{7 The 95% upper confidence limits for the rates of wild-type allele loss are 85, 64, and 92% for deletion 2-4/2-7, the 5'H11032 polymorphisms, and the haplotype, respectively.} \]
DMBT1 has a putative role in mucosal immune defense as an opsonin receptor for surfactant proteins (22) and DMBT1 might be involved in the regulation of inflammation (21), DMBT1 could be involved in the pathogenesis of such diseases.

The STS 74k locus is located 22-bp upstream of DMBT1 exon 1 and was homozygously deleted in 4 of 21 glioblastomas in another study (16), suggesting that this deletional hot spot could partly account for the lack of DMBT1 expression frequently found in cancers (18). In the present study, however, we found no malignant gliomas with homozygous deletion at the STS 74k locus. Rather, the proximal 5’ upstream region of the DMBT1 gene is highly polymorphic, containing at least four polymorphisms that appear tightly linked. In our studies, failure to PCR amplify at the 74k locus resulted exclusively from primer mismatches occasioned by the polymorphisms. Furthermore, a glioma cell line homozygous for the four polymorphisms expressed the DMBT1 transcript; this haplotype is therefore not likely associated with silencing of DMBT1 expression. However, because these polymorphic sites are within regulatory regions of DMBT1 (35), haplotypes involving additional polymorphic sites could be associated with differential transcriptional activity, as has been reported for the platelet-derived growth factor α-receptor gene in neural tube defects (36).

The expression of DMBT1 is reduced in a significant fraction of different cancer types, leaving open the possibility that DMBT1 inactivation could be involved in tumorigenesis (12, 15, 17). The majority of the present data do not support such a role, and the lack of DMBT1 expression in these tumors could be a result of transformation, rather than a causative factor. Nonetheless, it remains possible that selection pressure by 10q loss could occur for specific severely altered than a causative factor. Nonetheless, it remains possible that selection pressure by 10q loss could occur for specific severely altered DMBT1 haplotypes and that segregation of DMBT1 polymorphism homozygosity could occur in glioma patients. These possibilities and the presence of diverse polymorphisms encourage additional work on their functional significance and the biological effects of DMBT1 inactivation.

ACKNOWLEDGMENTS

We thank Magdalena Zlatescu for help with clinical specimens and John Matthew Esposito and Jennifer Roy for their technical assistance.

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DMBT1 Polymorphisms: Relationship to Malignant Glioma Tumorigenesis

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