DMBT1 Polymorphisms: Relationship to Malignant Glioma Tumorigenesis

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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 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.

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 oligodendrogliomas, 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 individuals (4, 25, 26) in the CEPH reference families was obtained from Coriell Cell Repositories. 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 oligodendrogliomas; 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.
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) g14/APEX nuclease sequence (187 bp, chromosome 14q). All primer sequences have been published previously except primers for intron 20 sequence (L1865: 5'-GCCCTGGTCTTTTTCATC-3' and L1866: 5'-GAAGGAGCTCTTTACTGAT-3'; Refs. 15 and 20). PCR was performed for 29 cycles with an annealing temperature of 58°C to amplify g14/c12, for 30 cycles at 50°C to amplify intron 20 sequence/desmin, and for 32 cycles with gradually decreasing annealing temperature from 63°C to 56°C to amplify g14/APEX. The products were separated on 3% Tris-borate EDTA agarose gel and visualized by ethidium bromide staining.

The 74k locus was investigated using two sets of multiplex PCR assays covering the entire original 74k locus (293 bp; Fig. 1; Ref. 15). Novel target sequences were designed to allow amplification of DNA extracted from archival materials, 74k-1 (165 bp, L1930: 5'-TGACCAAGACCTTGGGAGTC-3', 74krl; Ref. 15) for 3' end of the locus and 74k-5 (156 bp, L1935: 5'-AAAACCATCCCAAGAGATG-3' and L1936: 5'-AAGTTTCTCTGAATACAGG-3') for 5' end of the locus (15). PCR was performed for 30 cycles with annealing temperatures of 60°C and 50°C for 74k-1/APEX and 74k-5/desmin, respectively.

Long-range PCR. The possibility of a constitutional 2-4/2-7 deletion, spanning 12.7 kb from intron 14 to exon 14 (reference sequence: GenBank AJ243211), was assessed by long-range PCR (primers: L1595 in intron 10 and L1629 in intron 20) flanking the deletion as described previously (20). With this long-range PCR, the wild-type product would be >18 kb, and, therefore, only alleles with intragenic deletions are amplified (5.5 kb; Fig. 1). Blood DNA quality was verified by successful amplification of an 8-kb, long-range PCR product with primers L1595 and L1684 (intron 17, 5'-GAGGCTCGAAGTAGCTTCCATC-3') and L1865: 5'-GCCCTGGTGTTTTTCATC-3' (Fig. 1); this control long-range PCR was performed at the same time as PCR L1595/L1629 using common PCR reagents, such as deoxynucleotide triphosphates and Taq DNA polymerase. The presence of a constitutional deletion polymorphism was next confirmed by another long-range PCR with a different antisense primer (L1591 in intron 20, 4.2 kb; Ref. 20). Because reliability of the selection pressure analysis depends largely on the accuracy of this assay, the sensitivity and specificity of the long-range PCR assay were verified by comparison with Southern blot data in 48 cases (see below) using the probe DMBT1/sr1sid2.

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 Rsal. After ethanol precipitation, the digested DNA solubilized in 25 μl of 0.5 M NaOH to Hybridon 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 μl of mature DNA, 25 μl of 0.5 M NaOH, 0.2 μl of 5 M NaCl, 2 μl of 25% dextran sulfate, 2 μl of 0.5 M Tris-HCl, pH 7.2, 2 μl of 0.05 M EDTA, and 23.7 μl of 1x Church and Gilbert buffer] and then transferred with 0.4M NaOH to Hybond N+ (Amersham Pharmacia Biotech) using [α-32P]dATP and [α-32P]dCTP. Because of the high homology of the first 13 SRCR domains, Rsal fragments that contain any of these SRCR domains hybridize with DMBT1/sr1sid2, which contains SRCR domains 1 and 2 (exons 7 and 10; Fig. 1). The total copy numbers of each fragment representing DMBT1 were 2-4/2-7 (1.8-, 2.1-, and 3.9-kb fragments) or 2-9/2-10 (4.1-kb fragment) 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 (nt12698–13111 in GenBank AJ243211) was generated with primer L1975 (5'-CATGCCTTCTGCTCCCTGA-3') and the published antisense primer for the original 74k locus (74krl), using genomic DNA from glioma cell line IN157, which is homozygous for the wild-type sequence, as a template. Genomic DNA (12–15 μg) from glioma cell lines IN157 and NHG was digested with 200 units of KpnI or EcoRI and separated on a 1.2% Tris acetate-EDTA agarose gel at 45 V. Hybridization was carried out as described above.

RT-PCR. Gli 13, a glioma cell line that is homozygous for the four variant sequences in the 5' upstream region of the DMBT1 gene, was investigated by RT-PCR for expression of DMBT1. Total RNA extraction and reverse transcription was performed as described (20). The published DMBT1-specific primers were used to amplify a 947-bp DMBT1 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 DMBT1 gene was assessed using two overlapping SSCP analyses (primers: L1852: 5'-CTCTTGTCTCCACCCCGG-3', L2045: 5'-GGGTGTTTATCCATTCTG-3', 74krl; Ref. 15; and L1935: 5'-GAGACTCCCAAGAGATG-3') as described (29).

DNA Sequencing. The sequence of the proximal 5' upstream region of the DMBT1 gene was investigated by direct sequencing of PCR products (primers: DMBT1/repetitions of DMBT1/MHYB/DMBT1 polymorphisms in malignant gliomas

Fig. 1. The DMBT1 gene. Top: exon-intron structure of DMBT1. Except for the first and the last exons, only exons encoding SRCR domains are numbered (reference sequence:GenBank AJ243211). Rsal fragments that contain any of the first 13 SRCR domains and hybridize with Southern blot probe DMBT1/sr1sid2 are also described. Middle: distribution of the highly homologous DMBT1 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.
which had homozygous 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 homozygous 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 homozgyous 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 homozygous 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 (73 cases with LOH 17 (0); Table 1)...

Table 1 DMBT1 constitutional polymorphisms in patients with malignant gliomas and reference individuals

<table>
<thead>
<tr>
<th>Cases</th>
<th>Cases with deletion 2-4/2-7</th>
<th>5’ polymorphisms</th>
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<tr>
<td>Cases with deletion 2-4/2-7</td>
<td>26 cases without LOH</td>
<td>Reference individuals (64 cases)</td>
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<tr>
<td>17 (0)</td>
<td>5 (1)</td>
<td>17 (0)</td>
</tr>
<tr>
<td>38 (7)</td>
<td>9 (3)</td>
<td>6 (2)</td>
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- No significant difference between malignant glioma patients with and without LOH (P = 0.79).
- No significant difference between patients with malignant gliomas and reference individuals (P = 0.57).
- Suggestion of a difference in the distribution between patients with malignant gliomas and reference individuals (P = 0.08).
- Number of homozygotes are indicated in parentheses.
- Twenty-five cases were examined for 5’ polymorphisms.
- Apparently unrelated and unaffected individuals from the CEPH reference families.
tumors with 10q LOH, and the 74k-5 sequence was not amplified even in corresponding blood DNA in 7 of the 21 cases. However, on Southern blotting with a probe covering the 74k locus, fragments containing exon 1 sequence were detected in both cell lines: (a) IN157, which had amplification of 74k-5; and (b) NHG, which lacked 74k-5 amplification (data not shown). This suggested that the 74k locus 5' upstream of DMBT1 exon 1 was present in both cell lines. Sequencing of the proximal 5' region in 9 samples (3 cell line DNA, 2 blood DNA, and 4 tumor DNA) demonstrated at least four polymorphic sites (2-bp deletion and G to T missense), which had amplification of 74k-5; and (c) 12816 morphic sites: (w) 1670, and 4 tumor DNA) demonstrated at least four poly-

of chromosome 10q. The primer target sequences are unique to the wild-type sequence, and 1672 are heterozygotes for the linked polymorphisms whose tumors had allelic loss. Southern blot analysis with a probe covering the 74k locus, fragments of chromosome 10q-5; and (b) 12816 morphic sites: (w) 1670, and 4 tumor DNA) demonstrated at least four poly-

Fig. 4. A multiplex PCR 74k-5/desmin. N4, leukocyte DNA homozygous for the wild-type sequence at 5' upstream of DMBT1. Cases 1576, 8086, 6368, 6320, 6140, 1670, and 1672 are heterozygotes for the linked polymorphisms whose tumors had allelic loss of chromosome 10q. The primer target sequences are unique to the wild-type sequence, and 1672 are heterozygotes for the linked polymorphisms whose tumors had allelic loss. Southern blot analysis with a probe covering the 74k locus, fragments of chromosome 10q-5; and (b) 12816 morphic sites: (w) 1670, and 4 tumor DNA) demonstrated at least four poly-

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 selec-

tion pressure, to address the possibility of predisposition of deletion carriers to gliomas, we compared the frequency of these polymor-

phisms 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.

Fig. 5. Southern blot analysis with DMBT1 cDNA probe. RsaI-digested constitutional DNA was hybridized with DMBT1/sr1sid2, and fragments containing SRCR domains are shown. a, a glioma patient with probable wild-type DMBT1 configuration. The total copy numbers of each fragment are depicted on the right (see Fig. 1). About 2-fold increased intensities are shown for the 4.1- and 3.9-kb fragment, because of duplication. DMBT1 repeats 2-9/2-10 are represented by 1.8-, 2.1-, and 3.9-kb fragments, and DMBT1 repeats 2-9 and 2-10 are represented by 4.1-kb fragments. For 4.1-kb fragments, the distinction between three and four copies was difficult and not attempted. b, a meningioma patient who lacks the entire DMBT1 repeat 2-9/2-10 in both alleles; c, a glioma patient who lacks three of the four copies at 2-9/2-10 and one of the two copies at 2-4/2-7; d, a glioma patient who lacks two of the four copies at 2-9/2-10. An extra band ~1.6 kb with a single copy intensity was noted occasionally. Arrows, aberrations, with the copy numbers of the remaining fragments noted.
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′ analysis flanking two of the four polymorphism sites (G to T missense and T to C). DMBT1 and 3.9-kb fragments, representing strand DNA. A single copy of a 4.1-kb fragment, indicating deletion polymorphism at DMBT1. This patient also has no constitutional homozygous deletions at DMBT1. Constitutional homozygous deletions at the g14 locus is present in a significant fraction of various cancers and has suggested DMBT1 as a candidate tumor suppressor (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′ 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′ 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′ 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′ 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′ 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′ 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...
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 glial 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 DMBT1 haplotypes and that segregation of 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.

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