The Putative Glioma Tumor Suppressor Gene on Chromosome 19q Maps between APOC2 and HRC

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ABSTRACT

The frequent allelic loss of chromosome 19q in human gliomas suggests that 19q harbors a tumor suppressor gene that is integral to glioma tumorigenesis. Our initial deletion mapping of this gene localized the common region of deletion to the distal long arm, 19ql3.2-13.4. To bracket the putative tumor suppressor gene further, we have studied this region in 55 gliomas, using loss of heterozygosity studies for 11 well mapped, highly informative microsatellite polymorphisms that cover this area: D19S178; BCL3; APOC2; ERCC1; DM1; D19S112; HRC; D19S246; KLM; D19S180; and D19S254 (from centromeric to telomeric). Twenty astrocytic, oligodendrogial, and mixed gliomas had deletions affecting this region. Of nine partial deletions, two cases maintained heterozygosity at APOC2 while showing allelic loss at the more telomeric markers, ERCC1 and DM1, while five cases maintained heterozygosity at HRC but lost the more centromeric markers, D19S112 and DM1. Nine cases lost the entire D19S178 to D19S254 region. Three astrocytic gliomas, including one with an interstitial deletion, had terminal deletions of 19q13.4. The minimum area of overlap shared by the interstitial deletions is between APOC2 and HRC, including ERCC1, DM1, and D19S112. These findings suggest that the glioma tumor suppressor gene maps to an approximately 8-cM/megabase region on 19q13.2-13.3 between the proximal marker APOC2 and the distal marker HRC. Among the DNA repair/DNA metabolism genes on chromosome 19q, ERCC1, LIG1, and perhaps ERCC2 are within the common area of deletion; XRCC1 is centromeric and is therefore excluded as a candidate.

INTRODUCTION

Gliomas are the most common primary human brain tumors of adults and can be divided primarily into astrocytomas (including GBM1), oligodendrogliomas, and mixed gliomas (oligastrocytomas). Loss of heterozygosity studies in human astrocytomas have suggested the presence of tumor suppressor genes on chromosomes 9p, 10p, 10q, 11p, 13q, 17p, 19q, and 22q, while analyses of oligodendrogliomas and mixed gliomas have suggested tumor suppressor genes on chromosomes 1p and 19q (1). However, only the p53 gene on chromosome 17p and the retinoblastoma susceptibility gene on chromosome 13q have been implicated as the responsible genes at these chromosomal loci (2, 3). For the remaining chromosomes, ongoing studies are attempting to narrow the locations of these putative tumor suppressor genes.

The chromosome 19q locus is of particular interest since it is frequently involved in astrocytomas, mixed gliomas, and oligodendrogliomas. In our experience, allelic loss of chromosome 19q occurs in 57% of oligodendrogliomas, 67% of mixed gliomas, and 27% of astrocytomas (4). Ransom et al. (5) detected allelic losses of chromosome 19q in four of four informative oligodendrogliomas, and Bigner et al. (6) have shown that structural abnormalities of chromosome 19q are common in malignant gliomas. Within fibrillary astrocytic tumors, chromosome 19q is rarely lost in low-grade tumors but is lost in approximately 50% of anaplastic astrocytomas, implying that this locus may be important in anaplastic progression of astrocytomas (7).

To localize this putative tumor suppressor gene, we have recently used polymorphic markers spanning chromosome 19 to search for interstitial deletions using loss of heterozygosity studies. A common, overlapping region of deletion was demonstrated on the distal portion of the long arm, between the proximal marker D19S178 and the distal marker D19S180 (8), suggesting that the gene maps to 19q13.2-13.4. However, this region remains a large portion of the chromosome, measuring between 21 and 29 cM (9, 10). To bracket this tumor suppressor gene further, we have performed fine deletion mapping of the distal 19q13.2-13.4 region using 11 well mapped, highly informative microsatellite polymorphisms.

MATERIALS AND METHODS

Tumor tissues and blood samples were obtained from patients operated on at the Massachusetts General Hospital (Boston, MA), the University Hospital (Bonn, Germany), and the University Hospital (Zurich, Switzerland). All tumors were examined by a neuropathologist and graded according to WHO criteria (11). Tumors were excluded from the present study if they had previously shown loss of the entire long arm of chromosome 19. The 55 studied gliomas consisted of 13 WHO grade II astrocytomas, 15 grade III anaplastic astrocytomas, 8 grade IV GBM, 1 grade IV gliosarcoma, 6 grade II oligodendrogliomas, 3 grade III anaplastic oligodendrogliomas, 4 grade II mixed gliomas, and 5 grade III anaplastic mixed gliomas. DNA was extracted from frozen tissue tumors and blood samples according to standard phenol-chloroform procedures (12).

For each blood-tumor DNA pair, PCR was performed on a Programmable Thermal Controller (M. J. Research) according to a published protocol (13). PCR was performed in 20-μl volumes with one of the oligonucleotide primers previously end-labeled using T4 polynucleotide kinase and [γ-32P]ATP. The appropriate annealing temperatures, optimum magnesium concentrations, and references for oligonucleotide primer sequences and allele frequencies are listed for each locus in Table 1. PCR products were diluted in 10 μl of formamide buffer, denatured, and separated on 6% polyacrylamide denaturing gels at 80 W for 1–3 h. Dried gels were exposed to X-OMAT (Kodak) autoradiographic film for 2–24 h. Alleles were scored as suggested previously (13). The polymorphic loci, their locations on chromosome 19q, and genetic distances between the loci are indicated in Fig. 1.

RESULTS

Allelic losses in the 19q13.2-13.4 region, from D19S178 to D19S254, occurred in 20 of 55 gliomas. Losses were noted in 2 of 13 grade II astrocytomas, 6 of 15 anaplastic astrocytomas, 5 of 8 GBM, 3 of 6 oligodendrogliomas, 2 of 4 mixed gliomas, and 2 of 5 anaplastic mixed gliomas but were not detected in the 1 gliosarcoma or the 3 anaplastic oligodendrogliomas. As discussed below, however, these...
numbers do not reflect the true frequency of chromosome 19q losses in an unselected group of gliomas.

Nine cases had interstitial deletions that affected part of the D19S178 to D19S254 region (Fig. 2, left). Of the tumors with partial losses, two were oligodendrogliomas, one was a grade II astrocytoma, three were anaplastic astrocytomas, and three were GBMs. Two gliomas (2506 and 354) maintained heterozygosity at APOC2 on 19q13.2 but lost distal loci at ERCC1 or DM on 19q13.3 (Fig. 3). Five cases (354, 262, 420, 2012, and 438) maintained heterozygosity at HRC on 19q13.3 but lost alleles at proximal loci at D19S112 or DM on 19q13.3 (Fig. 4). The remaining cases with partial deletions (534, 332, and 308) showed losses of larger areas within the D19S178 to D19S254 region. The common region of overlap for these partial deletions involves 19q13.2-13.3, telomeric to APOC2 and centromeric to HRC, and encompasses the loci ERCC1, DM, and D19S112 (Fig. 2).

Nine cases showed loss of the entire area extending from D19S178 to D19S254 (Fig. 2, middle). These tumors included one oligodendroglioma, two mixed gliomas, two anaplastic mixed gliomas, one grade II astrocytoma, one anaplastic astrocytoma, and two GBMs. Two gliomas (both anaplastic astrocytomas: 62, 2754) had terminal deletions only, while one case (a GBM, 438) had a terminal deletion in addition to an interstitial loss. These distal losses involved 19q13.4, either D19S180 and D19S254 or D19S254 alone (Fig. 2, right; Fig. 5).

DISCUSSION

By blanketing the 19q13.2-13.4 region distal to D19S178 with numerous polymorphic markers, we have demonstrated that interstitial losses in human gliomas target the 19q13.2-13.3 area between the loci APOC2 and HRC. These data agree with and extend our earlier mapping of chromosome 19q deletions in gliomas, which suggested that deletions preferentially occurred in 19q13.2-13.4, distal to the marker D19S178 and proximal to the marker D19S180 (see Fig. 1) (4, 8). The data also support previous cytogenetic studies of malignant gliomas, which have shown a clustering of breakpoints at 19q13 (6).

In a comprehensive allelotype of oligodendrogliomas, Ransom et al. (5) analyzed five oligodendrogliomas for chromosome 19q deletions using Southern blotting with probes to markers D19S7 (19q12) and D19S8 (19q 13.2), both centromeric to D19S178. These authors detected a "possible homozygous deletion" involving D19S8. However, since 4% of the population exhibits a null allele at the D19S8 SsrI polymorphism recognized by probe p17.1 (14), it is possible that their finding represents loss of a single allele rather than a homozygous deletion. Furthermore, the lack of any markers distal to D19S8 in the study by Ransom et al. (5) precludes comparison of their findings with the present data.

Our current study greatly narrows the common region of deletion and suggests that a glioma tumor suppressor gene resides between APOC2 and HRC. On the basis of recent maps of chromosome 19q, the genetic distance between these two markers is approximately 8 cM (9, 10, 15, 16) (see Fig. 1). Given that the genetic map of chromosome 19q is approximately twice the size of the physical map, however, the entire region between APOC2 and HRC could be less than 5 megabases.

At least four DNA repair/DNA metabolism genes reside at 19q13.2-13.3: XRCC1, ERCC1, ERCC2; and LIG1. Since DNA repair genes are emerging as an important class of tumor suppressors in human cancer (17, 18), these four genes could be candidates for the chromosome 19q glioma tumor suppressor gene. XRCC1 is the most centromeric of these genes and maps between CEA and D19S178 (19). Our present data therefore exclude XRCC1 as a candidate, since its location is proximal to the common region of deletion. ERCC2 maps approximately 250 kilobases centromeric to ERCC1, approximately halfway between APOC2 and ERCC1 (see Fig. 1) (19). Because no PCR-based polymorphism was available for ERCC2, we could not determine whether ERCC2 itself falls within the common area of deletion in cases such as 2506 and 354 in which the deletion ends centromeric of these genes and maps between CEA and D19S178 (19).

Our present data therefore exclude XRCC1 as a candidate, since its location is proximal to the common region of deletion. ERCC2 maps approximately 250 kilobases centromeric to ERCC1, approximately halfway between APOC2 and ERCC1 (see Fig. 1) (19). Because no PCR-based polymorphism was available for ERCC2, we could not determine whether ERCC2 itself falls within the common area of deletion in cases such as 2506 and 354 in which the deletion ends centromeric of these genes and maps between CEA and D19S178 (19).

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The 19q13.2-13.3 tumor suppressor locus appears to be involved in tumorigenesis of astrocytic, mixed, and oligodendroglial gliomas, since all three glioma types suffered deletions in this region. Because the cases in this study were selected by excluding tumors which had previously shown loss of the entire long arm of chromosome 19, the present analysis underestimates the frequency of 19q losses in gliomas.

Table 1. Locus name, type of microsatellite repeat polymorphism, reference for oligonucleotide primer sequences and allele frequencies, and cycle conditions.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat</th>
<th>Ref.</th>
<th>T°(C)</th>
<th>Mg(%)</th>
<th>Cycles</th>
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* T°, annealing temperature; Mg, optimum magnesium concentration; Cycles, number of PCR cycles; dinuc, dinucleotide, trinuc, trinucleotide; tetranuc, tetranucleotide.
Fig. 2. Deletion map of allelic losses of chromosome 19q13.2–13.4 in 20 gliomas. The two bold double line pairs on the left indicate the cases which define the common region of deletion between APOC2 and HRC. The loci are in order from centromeric (top) to telomeric (bottom). □, loss of heterozygosity; ■, noninformative; □, maintenance of heterozygosity; A, astrocytoma; AA, anaplastic astrocytoma; GM, glioblastoma multiforme; O, oligodendrogliaoma; OA, mixed glioma (oligoastrocytoma); AOA, anaplastic mixed glioma (anaplastic oligoastrocytoma).

Fig. 3. Representative data that define the centromeric limit of the common region of deletion. Case 354 shows maintenance of heterozygosity at APOC2 with loss of heterozygosity at ERCC1. N, constitutional DNA; T, tumor DNA; bars, alleles; arrow, loss of the upper allele at ERCC1.

Fig. 4. Representative data that define the telomeric limit of the common region of deletion. Cases 438, 262, and 2012 all show loss of heterozygosity at D19S112 with maintenance of heterozygosity at HRC. N, constitutional DNA; T, tumor DNA; bars, alleles; arrows, lost alleles.

Fig. 5. DNA markers and glioma grades for cases of chromosome 19q deletion. The numbers represent cases with deletions at the indicated loci. APOC2 and ERCC1 define the centromeric limit of the common deletion region. HRC and D19S112 define the telomeric limit of the common deletion region. O, oligodendrogliaoma; A, astrocytoma; AA, anaplastic astrocytoma; GM, glioblastoma multiforme; OA, mixed glioma (oligoastrocytoma); AOA, anaplastic mixed glioma (anaplastic oligoastrocytoma).

Our previous mapping study of chromosome 19q losses in gliomas did not include any markers telomeric to D19S180 (8). To assay the terminal region of 19q for deletions, we therefore included the marker D19S254, which lies substantially telomeric to D19S180 (see Fig. 1). Three cases displayed telomeric deletions that involved D19S254 (Fig. 2); nine cases had losses of the entire region, including D19S254. The finding of some terminal deletions raises the possibility that a second tumor suppressor locus exists at distal 19q13.4. Candidate genes that map to distal 19q13.4 include a number of genes encoding zinc finger proteins (16). While these appear to cluster near the q terminus, their exact locations relative to D19S180 and D19S254 remain unclear. Protein kinase C-γ, which maps to 19q13.4 (21), is not a likely tumor suppressor candidate since this protein is expressed in most GBM (22). If distal 19q13.4 harbors a second glioma tumor suppressor gene, it is tempting to speculate that this gene is involved in the progression of astrocytic tumors, since distal telomeric losses...
were restricted to high-grade astrocytic tumors (anaplastic astrocytoma and GBM). On the other hand, while multiple tumor suppressor genes can occur on the same chromosome arm, these terminal deletions may be random and nonspecific, since similar telomeric losses have been noted in deletion mapping studies that have definitively implicated more proximal genes (3). Further study of more astrocytomas with additional distal markers is necessary to clarify the significance of the telomeric losses.

REFERENCES


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