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Deletion Mapping of Chromosome 10 in Human Glioma

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We analyzed DNAs from 35 gliomas (27 malignant, grades III and IV; 8 less malignant, grades I and II) for loss of heterozygosity (LOH) using microsatellite sequences on chromosome 10 as polymorphic markers. An LOH was found in 8 of 11 (73%) glioblastomas (grade IV) and 4 of 16 (25%) grade III gliomas, but not in the less malignant types. We detected three commonly deleted regions. One was located in a telomeric region of 10p and the others were relatively large regions of 10q. Our results suggested that three putative tumor suppressor genes on chromosome 10 are involved in the malignant progression of gliomas.

Key words: Human glioma — Deletion map — LOH on chromosome 10 — Microsatellite sequences — Tumor suppressor gene

The most common brain tumors in humans are gliomas, which are divided into four stages according to the WHO classification scheme.[1)] Glioblastoma (grade IV) is the most malignant brain tumor and is almost always fatal. Frequent deletions of specific chromosomal regions in gliomas suggest the presence of glioma tumor suppressor genes. Studies of loss of heterozygosity (LOH) in gliomas have suggested that tumor suppressor genes are located on chromosomes 9p,[2,3)] 10,[4–6)] 11p,[7,8)] 13q,[9)] 17p,[10,11)] 19q,[12)] and 22q.[13)] However, only the *p53* gene on 17p[14,15)] and the *MTS1* and *MTS2* genes on 9p[16,17)] have been identified as the responsible genes. The most frequently reported genetic alterations are mutations or deletions of the *p53* gene,[14,15)] LOH at chromosomes 17p[10,11)] and 10,[4–6)] and amplification and rearrangement of the epidermal growth factor receptor (*EGFR*) gene.[18)] The *p53* gene is mutated in all grades of glioma. Thus, alterations of the *p53* gene may play a role in early glioma formation.[18)] On the other hand, LOH on chromosome 10 and amplification and rearrangement of *EGFR* gene have been detected frequently in malignant glioma, especially in grade IV tumors, suggesting these are late events during glioma progression.[18)] Deletions at loci on chromosome 10 have been found in more than 50% of glioblastomas. However, a putative tumor suppressor gene on chromosome 10 has not yet been identified. To isolate putative suppressor genes on chromosome 10, a commonly deleted region has been narrowed down by restriction fragment length polymorphism analysis (RFLP).[4,19–22)] Microsatellite markers are highly polymorphic and are useful for LOH study. We therefore constructed a deletion map of chromosome 10 in human glioma using 20 microsatellite markers.

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MATERIALS AND METHODS

Human tissue samples All glioma samples were obtained surgically from 35 patients at the Department of Neurosurgery, Tohoku University School of Medicine, Sendai. The tumors were classified according to the WHO brain tumor scheme.[1)] The gliomas examined consisted of 6 astrocytomas (grade II), 2 oligodendrogliomas (grade II), 13 anaplastic astrocytomas (grade III), 3 anaplastic ependymomas (grade III) and 11 glioblastomas (grade IV). Control DNA for each tumor was obtained from leukocytes isolated from frozen peripheral blood, or normal brain tissue of the same individual.

PCR analysis of microsatellite markers The polymorphic markers analyzed here are summarized in Table I. LOH was detected in 35 pairs of DNA samples using 12 markers (at the *D10S526, D10S506, D10S509, D10S507, D10S524, D10S1146, D10S516, D10S1136, D10S520, D10S521, D10S528* and *D10S1134* loci) from the Genome Data Base (GDB). Microsatellite sequences of the Généthon group were used to characterize further the regions of chromosome 10p showing allelic losses.[23)] Polymerase chain reaction (PCR) was performed using 50 ng of template DNA in a volume of 5 μl with 30 cycles at 94°C for 20 s and at 60°C for 2 min in a Gene Amp PCR system 9600 (Perkin-Elmer Cetus, Norwalk, CT). The reaction mixture was diluted 5-fold with a solution containing 95% formamide, 20 m*M* EDTA, 0.05% xylene cyanol and 0.05% bromphenol blue, then denatured by heating at 80°C for 3 min. Electrophoresis proceeded in 5% polyacrylamide gels containing 7 *M* urea.

RESULTS

Eight of 11 (74%) glioblastomas and 4 of 16 (25%) grade III gliomas (3 anaplastic astrocytomas and one

Table I. Polymorphic Markers on Chromosome 10 Used for LOH Analysis

Probe name	Locus symbol	Chromosomal localization	GDB No.
UT1699	*D10S526*	10pter-p13	G00-140-218
UT538	*D10S506*	10pter-p13	G00-139-720
UT541	*D10S509*	10pter-q11.2	G00-139-723
UT539	*D10S507*	10pter-q11.2	G00-139-721
UT1466	*D10S524*	10pter-q25	G00-140-156
UT5043	*D10S1146*	10q11.2-q25	G00-316-351
UT919	*D10S516*	10q11.2-q25	G00-139-903
UT635	*D10S1136*	10q21-q25	G00-316-266
UT925	*D10S520*	10q21-q25.1	G00-139-907
UT5027	*D10S521*	10q23-q25	G00-139-951
UT5419	*D10S528*	10q23-qter	G00-140-233
UT464	*D10S1134*	10q23-qter	G00-316-235
AFM207wd12	*D10S249*	10p15	G00-134-786
AFM317zd9	*D10S594*	10p15	G00-141-291
AFM248vb9	*D10S558*	10p15	G00-140-703
AFM343vd9	*D10S602*	10p15	G00-141-418
AFM309yd9	*D10S591*	10p15.3-p15.2	G00-141-238
AFM063xf4	*D10S189*	10pter-p13	G00-133-007
AFM260zc5	*D10S226*	10pter-p13	G00-133-682
AFM214yc9	*D10S547*	10p15-p11.2	G00-140-538

anaplastic ependymoma) showed LOH at at least one locus on chromosome 10, but none of the less malignant types showed LOH at any locus examined on chromosome 10.

Representative results of the LOH analysis are shown in Fig. 1. For example, a comparison of tumor and normal DNA from patient 7 revealed LOH at the *D10S226, D10S507* and *D10S520* loci. Although the signals for the *D10S594* locus of this patient were not informative, the results, together with those of other loci, indicated the total loss of chromosome 10. LOH at loci on chromosome 10 in 12 malignant gliomas including tumor 7 are summarized in Fig. 2. The tumors with allelic losses were divided into 3 groups. The first group included tumors with LOH at almost all loci on chromosome 10 (Fig. 2A). The second and third groups were tumors with LOH exclusively at loci on the short and long arms, respectively (Figs. 2B, C), although these two groups contained only one or two tumors. Only the *D10S526* locus (10p) was deleted from tumor 10. Two separate regions on 10q were deleted from tumor 1, whereas in tumor 3, only the *D10S1146* (10q) locus was lost. These results suggested two separate regions at which a deletion was involved in the tumorigenesis of gliomas.

For further characterization of the commonly deleted region on 10p, 8 additional markers mapped to the telomeric region on 10p (Table I) were similarly analyzed. Based on available information about the markers,[23] the region commonly deleted from the *D10S602* locus to a locus on 10pter may be about 4 centimorgans (Fig. 3).

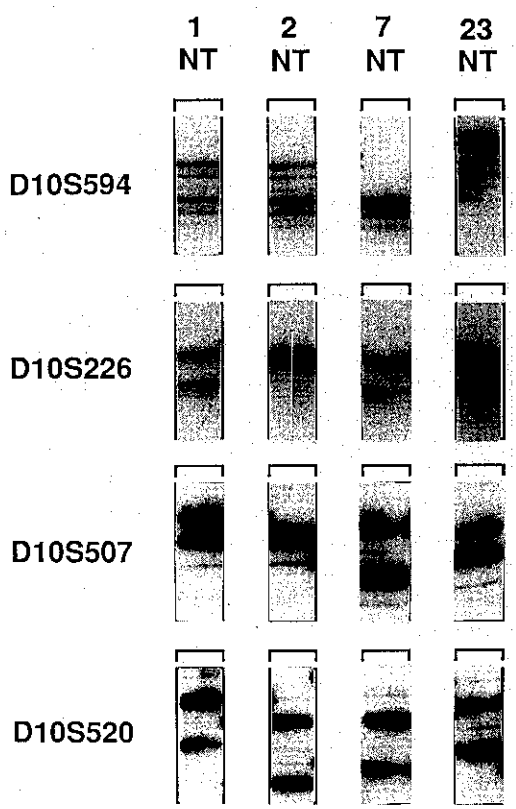

Fig. 1. LOH at loci on chromosome 10 in malignant gliomas. N and T indicate normal and tumor DNA, respectively, from individual patients. LOH in tumor samples was detected as a loss of signal intensity of polymorphic markers amplified from tumor DNA, compared with those from normal DNA.

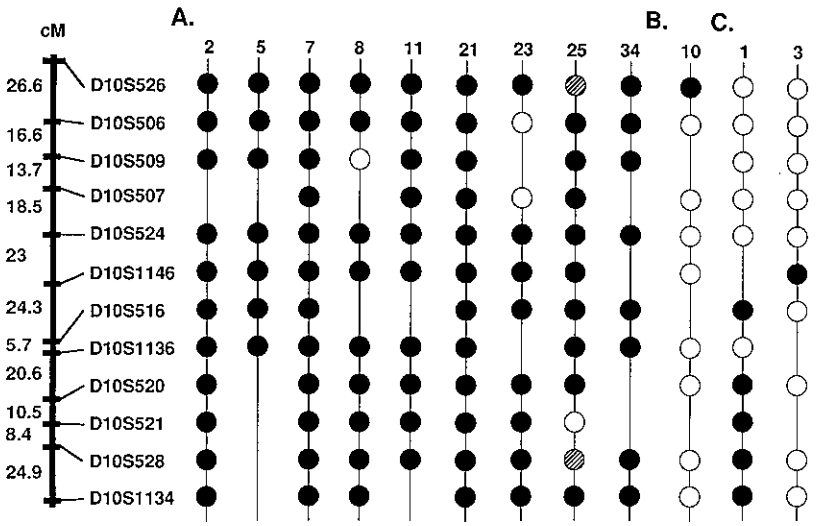

Fig. 2. Allelic losses in 12 gliomas with LOH at loci on chromosome 10. Allelic losses were found at loci on almost all of the entire chromosome (A), the short arm of chromosome 10 (B) and the long arm of chromosome 10 (C). Tumor numbers are indicated on the top of the figure. Solid and unfilled circles indicate loss and retention of heterozygosity, respectively. Striped circles indicate samples in which the results were inconclusive or unclear. Non-informative samples are indicated by vertical lines. The region commonly deleted is indicated by a vertical bar. Information about the order and the genetic distance of the markers was obtained from Drs. Hans Albertsen and Raymond L. White of the University of Utah (personal communications).

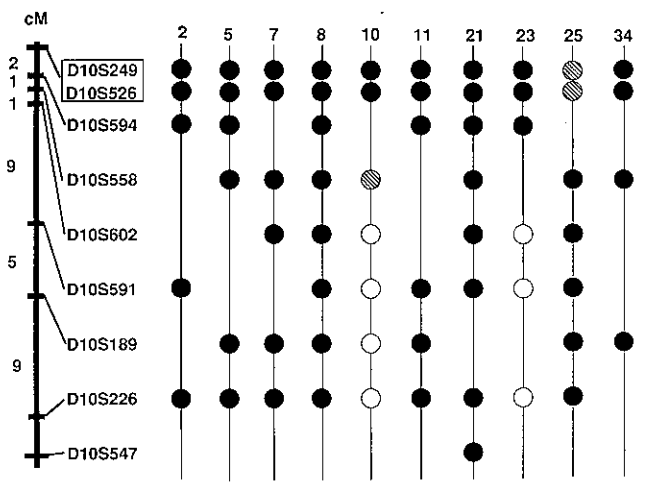

Fig. 3. Allelic losses at telomeric loci on 10p in gliomas. Symbols are the same as in Fig. 2. The orders of the loci in the box are unknown.

DISCUSSION

Twelve tumors that showed LOH at loci on chromosome 10 were all malignant gliomas, 8 being glioblastomas and 4 being grade III gliomas. On the other hand, gliomas with a low grade of malignancy did not show any LOH at loci on chromosome 10. These results were consistent with other observations.[4, 20, 24] Chromosome 10 was almost totally deleted from 9 of 12 tumors showing LOH. However, using many highly polymorphic markers, we found that some of these tumors (8, 23 and 25) had apparent retention at loci on either 10p or 10q. We found three commonly deleted regions, one in 10p and two in 10q.

LOHs on chromosome 10p and 10q have been identified in glioblastomas.[5, 24] Functional analysis has shown that chromosome fragments containing these regions have tumor-suppressive activity.[22, 25] These reports suggested that independent suppressor genes are located on both 10p and 10q in glioma.

Allelic losses of the 10p region have also been found in prostate cancers.[26, 27] Our results indicated that the region between the *D10S602* and *D10S249* loci was deleted. This region of about 4 centimorgans overlapped the reported regions and was narrower than they were. Allelic losses on chromosome 10q have also been found in several tumors.[19–21, 27, 28] In this study, two deleted regions were mapped on chromosome 10q, although the numbers of informative tumors are quite limited. One region was mapped between the *D10S524* and *D10S516* loci, and the other was located between the *D10S521* and a locus on 10qter. Thus, the large deleted region found on 10q could be divided into two independent regions.

These results indicated that three tumor suppressor genes are located on chromosome 10 and that they are involved in the malignant progression of gliomas.

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