Common Regions of Deletion on Chromosome 22q12.3-q13.1 and 22q13.2 In Human Astrocytomas Appear Related To Malignancy Grade

YASUSHI INO, MD, JONATHAN S. SILVER, LISA BLAZEJEWSKI, RYO NISHIKAWA, MD, MASAO MATSUTANI, MD, ANDREAS VON DEIMLING, MD, AND DAVID N. LOUIS, MD

Abstract. Approximately 30% of human astrocytomas have been reported to display allelic loss of the long arm of chromosome 22, suggesting the presence of a chromosome 22q astrocytoma suppressor gene. To define the most likely location for this putative tumor suppressor, we performed deletion mapping on 141 tumors using 16 chromosome 22q microsatellite markers. Allelic loss of 22q was observed in 2/12 (17%) of astrocytomas, 9/29 (31%) of anaplastic astrocytomas, and 38/100 (38%) of glioblastomas, consistent with a role for chromosome 22q loss in astrocytoma progression as well as formation. Twenty-two tumors exhibited allelic loss at every informative locus, consistent with loss of the entire arm of 22q. Twenty-seven tumors showed partial deletions, with one common region of deletion at 22q12.3–q13.1 between markers D22S380 and D22S282, and a second candidate region at 22q13.2 near the marker D22S1170. For the proximal candidate region, the incidence of allelic loss was similar between grades; for the distal locus, the incidence increased with grade, raising the possibility that the distal locus is involved in a later stage of astrocytoma tumorigenesis.

Key Words: Astrocytoma; Chromosome 22q; Glioblastoma; Tumor suppressor gene.

INTRODUCTION

Astrocytomas, including the highly malignant glioblastoma, are the most common primary brain tumors of adults. Characteristic allelic chromosomal losses, reflecting inactivation of tumor suppressor genes, occur in these tumors and are generally associated with specific malignancy grades. For instance, losses of chromosomes 17p and 22q are reportedly early changes, occurring in World Health Organization (WHO) grade II astrocytomas. Losses of chromosomes 9p, 13q, and 19q, however, are more commonly found in higher-grade tumors, such as WHO grade III anaplastic astrocytomas and WHO grade IV glioblastomas. Finally, allelic loss of all of chromosome 10 is typically associated with the highest-grade tumor, glioblastoma (1). For some of these chromosomal regions, specific tumor suppressor genes have been implicated in astrocytoma tumorigenesis: the CDKN2A/p16 gene on chromosome 9p, the PTEN/MMAC1 gene on chromosome 10q, the RB gene on chromosome 13q and the TP53 gene on chromosome 17p. Astrocytoma tumor suppressors on chromosomes 10p, distal 10q, 19q, and 22q, however, remain to be identified.

The NF2 gene on 22q12.1 is a tumor suppressor gene responsible for the neurofibromatosis 2 (NF2) syndrome, in which patients are predisposed to meningiomas, schwannomas, and ependymomas. Since NF2 patients also have a tendency to develop astrocytic glial hamartomas and astrocytomas, the NF2 gene was a good candidate for being a chromosome 22q astrocytoma gene. However, the NF2 gene is not altered in sporadic astrocytomas (2–4), suggesting the presence of another chromosome 22q suppressor gene. The most likely location for this putative astrocytoma suppressor gene remains ill defined, with some studies indicating possible locations either proximal or distal to the NF2 locus. Allelic loss of chromosome 22q has also been detected in other types of human tumors, including pheochromocytomas, rhabdoid tumors and hepatocellular, colorectal, ovarian, breast, and oral squamous cell carcinomas (5–10). The tumor suppressor gene recently implicated in renal rhabdoid tumor maps to 22q11.2 (11), far centromeric of the NF2 gene, but the other tumors have common regions of deletion distal to the NF2 gene. To define the common region of chromosome 22 allelic loss in astrocytic tumors of different grades, we performed deletion mapping using multiple chromosome 22 loci in a large series of astrocytomas.

MATERIALS AND METHODS

Tissue Specimens and Histopathology

Tumor tissues and blood samples were obtained from 141 patients operated on at Massachusetts General Hospital (Boston, MA), University Hospital (Bonn, Germany), University Hospital (Zurich, Switzerland), and Saitama Medical College Hospital (Saitama, Japan). All tumors were examined by a neuropathologist and graded according to WHO criteria (12). The series consisted of 12 WHO grade II astrocytomas, 29 WHO grade III anaplastic astrocytomas, and 100 grade IV glioblastomas. Prior to DNA extraction, all tumors were examined by frozen section to avoid contaminating normal tissue. DNA was extracted from frozen tumor tissue and blood samples according to standard phenol chloroform procedures.
higher percentage could reflect our use of greater numbers of 22q markers, more informative markers, and/or markers fortuitously closer to the tumor suppressor loci. By grade, LOH occurred in 2/12 WHO grade II astrocytomas (16.7%), 9/29 WHO grade III anaplastic astrocytomas (31.0%), and 38/100 WHO grade IV glioblastomas (38%). For individual markers, the percentages of cases showing LOH were 11.9% at D22S420, 19.5% at D22S301, 17.7% at D22S345, 25.0% at D22S421, 22.2% at D22S300, 19.8% at D22S275, 24.0% at D22S268, 25.2% at D22S280, 24.1% at D22S283, 22.1% at D22S282, 27.1% at D22S1171, 25.2% at D22S274, 26.4% at D22S1153, 27.0% at D22S1160, 33.3% at D22S1170, and 23.9% at D22S1169; the D22S1170 locus was therefore the most frequently affected (Table).

Twenty-two tumors exhibited allelic loss at all informative loci, consistent with loss of the entire long arm. Twenty-seven tumors, however, had partial deletions that enabled common regions of deletion to be defined (Fig. 2). Of these 27 partial deletions, 16 extended to the telomere and 11 cases were interstitial. Both grade II astrocytomas with chromosome 22q loss had interstitial deletions with a common region of deletion at 22q12.3–q13.1 between D22S280 and D22S282. Since nonspecific chromosomal loss is uncommon in lower grade tumors, these particular interstitial deletions strongly suggest the location of the tumor suppressor near the D22S280 and D22S282 markers. In addition, the patterns of allelic loss in the D22S280–D22S282 region for these 2 low-grade cases are complex, suggesting either multiple breakpoints in the region or homozygous deletions (markers displaying apparent maintenance of heterozygosity between markers displaying allelic loss); either of these possibilities make the D22S280–D22S282 interval even more attractive as a candidate tumor suppressor region. Significantly, the D22S280–D22S282 region was affected in 5/6 (83.3%) anaplastic astrocytomas and 27/36 (75.0%) glioblastomas that had chromosome 22q loss and were informative for the region. These data support the hypothesis that an astrocytoma tumor suppressor locus maps to 22q12.3–q13.1 between D22S280 and D22S282.

A second common area of deletion was detected more distally, at the D22S1160–D22S1170 region on band 22q13.2. In anaplastic astrocytomas, the D22S1160–D22S1170 region had the highest frequency of allelic loss (40.0%), and 4 glioblastomas had interstitial deletions affecting this area. Significantly, the D22S1160–D22S1170 interval was lost in 6/6 (100%) of anaplastic astrocytomas and 26/31 (83.9%) of glioblastomas that had chromosome 22q loss and were informative for the region. Therefore, a second astrocytoma suppressor candidate region maps near D22S1170 at 22q13.2. The presence of multiple suppressor loci on a single chromosome is not
**TABLE**

<table>
<thead>
<tr>
<th>Marker</th>
<th>AS</th>
<th>AA</th>
<th>GBM</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>D22S420</td>
<td>*0%</td>
<td>**(0/8)</td>
<td>5.9%</td>
<td>(1/17)</td>
</tr>
<tr>
<td>D22S301</td>
<td>0.0%</td>
<td>(0/9)</td>
<td>12.5%</td>
<td>(2/16)</td>
</tr>
<tr>
<td>D22S345</td>
<td>0.0%</td>
<td>(0/8)</td>
<td>15.0%</td>
<td>(3/20)</td>
</tr>
<tr>
<td>D22S421</td>
<td>0.0%</td>
<td>(0/7)</td>
<td>20.0%</td>
<td>(4/20)</td>
</tr>
<tr>
<td>D22S300</td>
<td>0.0%</td>
<td>(0/8)</td>
<td>22.2%</td>
<td>(4/18)</td>
</tr>
<tr>
<td>D22S275</td>
<td>0.0%</td>
<td>(0/11)</td>
<td>14.3%</td>
<td>(3/21)</td>
</tr>
<tr>
<td>D22S268</td>
<td>0.0%</td>
<td>(0/9)</td>
<td>6.3%</td>
<td>(1/16)</td>
</tr>
<tr>
<td>D22S280</td>
<td>11.1%</td>
<td>(1/9)</td>
<td>16.8%</td>
<td>(4/24)</td>
</tr>
<tr>
<td>D22S283</td>
<td>8.3%</td>
<td>(1/12)</td>
<td>15.0%</td>
<td>(3/20)</td>
</tr>
<tr>
<td>D22S282</td>
<td>11.1%</td>
<td>(1/9)</td>
<td>15.0%</td>
<td>(3/20)</td>
</tr>
<tr>
<td>D22S171</td>
<td>12.5%</td>
<td>(1/8)</td>
<td>38.5%</td>
<td>(5/13)</td>
</tr>
<tr>
<td>D22S274</td>
<td>11.1%</td>
<td>(1/8)</td>
<td>21.7%</td>
<td>(5/23)</td>
</tr>
<tr>
<td>D22S153</td>
<td>0.0%</td>
<td>(0/9)</td>
<td>26.7%</td>
<td>(4/15)</td>
</tr>
<tr>
<td>D22S160</td>
<td>0.0%</td>
<td>(0/10)</td>
<td>36.4%</td>
<td>(4/11)</td>
</tr>
<tr>
<td>D22S170</td>
<td>0.0%</td>
<td>(0/8)</td>
<td>40.0%</td>
<td>(6/15)</td>
</tr>
<tr>
<td>D22S169</td>
<td>0.0%</td>
<td>(0/11)</td>
<td>30.4%</td>
<td>(7/23)</td>
</tr>
</tbody>
</table>

* % relative to number of informative cases.
** Number of cases with loss of heterozygosity/number of informative cases.

---

Fig. 2. Summary of loss of heterozygosity (LOH) results from 27 gliomas with partial deletion of chromosome arm 22q. Cytogenetic location of the markers used in this study and of the NF2 gene are determined according to the Southampton Integrated Map (http://www.sanger.ac.uk). Squares represent LOH data. White squares represent maintenance of heterozygosity on that locus, while black solid squares represent loss of heterozygosity. Bars without squares represent the constitutional homozygosity on those markers or inadequate PCR. Rectangular area I indicates the cases showing LOH within the common region on 22q12.3–q13.1, and area II indicates cases showing LOH in the common region on 22q13.2. The broken lines in area I show potentially involved cases, depending on the status of other informative markers within the region.
unique, even in gliomas, with chromosome 10 suspected to harbor 3 such loci for high-grade astrocytomas (16).

DISCUSSION

The observed increase in allelic loss with higher grade tumors contrasts with the common assumption that chromosome 22q loss is primarily an early change in astrocytoma tumorigenesis (17, 18). Interestingly, however, the 2 candidate regions differ with respect to tumor grade. Loss of the D22S280-D22S283 region was noted at relatively similar frequencies in grade II astrocytomas (16.7%), anaplastic astrocytomas (19.2%), and glioblastomas (27.5%). On the other hand, loss of the D22S1170 was not observed in grade II tumors, but was detected in 40.0% of anaplastic astrocytomas and 35.1% of glioblastomas. While the number of lower grade tumors in our study is considerably smaller than that of glioblastomas, the results raise the possibility that the D22S1170 region is related to tumor progression rather than formation.

Two particular chromosome 22q loci, the hSNF5/D11 gene and D22S300, warrant discussion relative to the present deletion mapping data. hSNF5/D11, the gene responsible for malignant rhadoblast tumors, maps to 22q11.2 between the markers D22S301 and D22S345 (11), centromeric to the NF2 gene. Since malignant rhadoblast tumors may occur as primary brain tumors and may demonstrate astrocytic differentiation (19, 20), hSNF5/D11 was a potential astrocytoma suppressor gene as well. We therefore selected both D22S301 and D22S345 for our study. Allelic loss at these loci was observed in 22/120 (18.3%) of our informative cases, but all but 2 of those cases had lost the entire long arm of chromosome 22. Thus, there were no deletions to implicate this region in astrocytoma tumorigenesis.

The D22S300 marker at 22q12.2, 0.2 cM centromeric to the NF2 gene, has been reported in one series to be deleted in 80% of glioblastomas (21). However, in our study, only 22% of informative cases (24/108) had LOH at D22S300, and 20 of those 24 (83%) cases showed loss of the entire long arm. One glioblastoma, however, had an interstitial deletion that included the D22S300 locus, leaving the possibility that D22S300 lies near an important gene.

Loss of heterozygosity on 22q has been reported in other types of tumors. In ovarian cancers, the common region of deletion is at 22q13.1-q13.2, between D22S284 and CYP2D (6). For breast carcinomas, the most likely candidate region is also at 22q13.1, between D22S272 and D22S279. In oral squamous cell carcinoma, deletions preferentially occur near D22S274 at 22q13.2 (8). The candidate region remains larger for colorectal cancers, at 22q12-q13 between D22S90 and D22S94, but is still distant to the NF2 locus (10). Thus, deletions at 22q13.1 occur in tumors from a host of human organs. The more centromeric common region of deletion in this report, at 22q12.3-q13.1 between D22S280 and D22S282, is consistent with candidate regions proposed for many of these other human cancers, suggesting that chromosome 22q harbors a tumor suppressor gene relevant to many human neoplasms. The present deletion mapping may contribute to narrowing this locus. Construction of a comprehensive physical map, as well as ongoing genomic sequencing of chromosome 22, will hopefully soon lead to the identification of these important gene or genes.

REFERENCES

6. Byun EJ, Watson RH, Davis M, Hitchcock A, Foulkes WD, Campbell IG. Localization of an ovarian cancer tumor suppressor gene to a 0.5-cM region between D22S284 and CYP2D, on chromosome 22q. Cancer Res 1995;56:719-21

J Neuropathol Exp Neurol, Vol 58, August, 1999


Received March 3, 1999
Revision received April 20, 1999
Accepted April 20, 1999