Allelotype of Human Malignant Astrocytoma

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ABSTRACT

Astrocytoma, the most common brain tumor in humans, is usually malignant and virtually incurable. Two types of malignant astrocytomas can be distinguished histopathologically: anaplastic astrocytoma and glioblastoma multiforme. Studies using DNA markers that detect restriction fragment length polymorphisms have shown that loci on chromosomes 10 and 17p are lost frequently in tumor DNA from malignant astrocytoma patients, suggesting that tumor suppressor genes important in astrocytoma tumorigenesis may be present on 2 different chromosomes. To identify additional regions of chromosome loss, we carried out an allelotype analysis of 41 malignant astrocytoma patients using restriction fragment length polymorphism markers for each arm of every human autosome. Loss of heterozygosity was found for every autosome except chromosome 21, indicating an even greater complexity of genomic alterations than reported previously. Many tumors showed loss of heterozygosity for multiple chromosomes and the number of chromosomes involved correlated with tumor histopathology. A high-resolution restriction fragment length polymorphism study of chromosome 10 loci in these patients showed that loss of broad regions of chromosome 10 was a common event, particularly in glioblastoma multiforme. An allelotype analysis has been carried out on only one other tumor, human colorectal carcinoma. Different profiles of allele loss were observed in malignant astrocytoma and colorectal carcinoma, suggesting that the genetic events leading to these 2 human cancers may proceed along different pathways.

INTRODUCTION

Astrocytoma, the most common brain tumor in humans, is usually malignant. Despite treatment, malignant astrocytomas tend to recur and recurrent tumors are often more aggressive. One classification scheme based on histopathology recognizes 2 grades of malignant astrocytomas: anaplastic astrocytoma and glioblastoma multiforme (1). Glioblastoma, the most malignant form of astrocytoma, is uniformly fatal. Although the less malignant anaplastic astrocytomas may show a favorable response to treatment initially, they often progress to glioblastoma.

Tumor progression is widely regarded as a multistep process that begins with a single altered cell whose clonal descendants are forced to undertake a program of increasingly deregulated growth (2, 3). Tumor-suppressor genes (recessive oncogenes) comprise a class of genes that is a target for mutations that can be distinguished histopathologically: anaplastic astrocytoma and glioblastoma multiforme. Studies using DNA markers that detect restriction fragment length polymorphism markers for each arm of every human autosome. Loss of heterozygosity was found for every autosome except chromosome 21, indicating an even greater complexity of genomic alterations than reported previously. Many tumors showed loss of heterozygosity for multiple chromosomes and the number of chromosomes involved correlated with tumor histopathology. A high-resolution restriction fragment length polymorphism study of chromosome 10 loci in these patients showed that loss of broad regions of chromosome 10 was a common event, particularly in glioblastoma multiforme. An allelotype analysis has been carried out on only one other tumor, human colorectal carcinoma. Different profiles of allele loss were observed in malignant astrocytoma and colorectal carcinoma, suggesting that the genetic events leading to these 2 human cancers may proceed along different pathways.

MATERIALS AND METHODS

Human Tumor Samples. All tumor samples were removed surgically from the brain prior to radiation or chemotherapy except in 8 cases of tumors that recurred following treatment (A1, A3, U7, U16, U18, U24, U26, U31). Histopathological grading of astrocytomas was based on the classification scheme of Burger et al. (1). A single neuropathologist reviewed all tumor samples.

DNA Extraction and Southern Transfer Analysis. Extraction of genomic DNA from human tumor samples and from peripheral blood...
ers was carried out as described previously (13). In some cases, DNA "allelotype analysis of 41 patients with malignant astrocytoma. In cases where tumor DNA showed deleted allele due to non-tumor cell DNA contamination. All cases of DNA (surgical specimens) from 26 patients with glioblastoma pairs of somatic DNA (peripheral blood leukocytes) and tumor DNA. The small acrocentric chromosome arms (13p, 14p, 15p, 21q, and 22p) were not examined. Original references for all chromosome arms for which the marker was informative in the leukocyte DNA (Table 2). The median FAL for the glioblastoma patients (0.224) was more than twice that for the anaplastic astrocytoma patients (0.108), indicating a direct correlation between the number of chromosomal alterations occurring in these tumors and their malignancy grade as determined by histopathology.

To determine the effect of prior radiation and chemotherapy on the number of chromosomal alterations occurring in tumor DNA, we calculated the median FAL for the treated patient group (0.151) was less than that for the untreated group (0.198), indicating that radiation and chemotherapy did not contribute significantly to the observed chromosomal alterations.

Loss of One Copy of Chromosome 10 is a Common Event in Glioblastoma Multiforme. We carried out a high-resolution RFLP analysis of 32 patients with glioblastoma and 13 patients with anaplastic astrocytoma using 13 markers that spanned both arms of chromosome 10. Loss of heterozygosity was found in 17 of the 32 patients with glioblastoma (53%) but in only 2 of the 13 patients with anaplastic astrocytoma (15%). The relative specificity of chromosome 10 loss for glioblastoma multiforme is consistent with studies reported previously (9, 10).

To determine whether a specific region of chromosome 10 was examined with a panel of DNA markers for loci on every nonacrocentric autosomal arm. The number of patients showing loss of heterozygosity with these markers is shown in Table 1. Fig. 1 shows the frequency of allele loss for each chromosome arm. Loss of heterozygosity was found for loci on every autosome except chromosome 21, where no loss was found in 14 informative patients.

To identify chromosomes most likely to contain tumor suppressor genes, we divided the chromosome arms into 3 groups according to the percentage of patients showing loss of heterozygosity. In group 1 (10p, 10q, 17p), >38% of informative patients showed loss of heterozygosity; in group 2 (5q, 7p, 11p, 14q, 15q), 16–21% showed loss; and in group 3 (the remaining chromosome arms), <14% showed loss. When viewed in this way, the data suggest that chromosomes in group 1 (10 and 17) probably contain tumor suppressor genes important in astrocytoma tumorigenesis and that chromosomes in group 2 are possible sites for such genes. The much lower frequency of allele loss observed in group 3 most likely reflects random chromosome loss.

When the RFLP data from individual patients were analyzed, two results became apparent. First, many tumors showed loss of heterozygosity for multiple chromosomes and second, tumor histopathology correlated with the number of chromosomes involved. Among the 41 malignant astrocytoma patients, 32 showed loss of heterozygosity for loci on one or more chromosomes (78%) and 18 patients showed loss on 2 or more chromosomes (44%). Fig. 2 shows representative autoradiograms from 2 glioblastoma patients (U21 and U3) with loss of heterozygosity for loci on multiple chromosomes.

In the previously reported allelotype analysis of human colorectal carcinoma, a parameter termed fractional allelic loss (FAL) was defined to compare the extent of chromosomal alterations among tumor patients (15). We calculated the FAL for each of the 32 patients that showed loss of heterozygosity by dividing the number of chromosome arms on which loss of heterozygosity was observed in the tumor DNA by the number of chromosome arms for which the marker was informative in the leukocyte DNA (Table 2). The median FAL for the glioblastoma patients (0.224) was more than twice that for the anaplastic astrocytoma patients (0.108), indicating a direct correlation between the number of chromosomal alterations occurring in these tumors and their malignancy grade as determined by histopathology.

RESULTS

Loss of Heterozygosity for Loci on Multiple Chromosomes is Found in Human Malignant Astrocytoma. We carried out an allelotype analysis of 41 patients with malignant astrocytoma. Pairs of somatic DNA (peripheral blood leukocytes) and tumor DNA (surgical specimens) from 26 patients with glioblastoma multiforme and 15 patients with anaplastic astrocytoma were...
was commonly lost in malignant astrocytomas (a candidate region for a tumor suppressor gene), we examined the patterns of allele loss among the 19 patients that showed loss of heterozygosity for loci on chromosome 10 (Fig. 3). Three patients (U1, U18, U22) had allele loss patterns indicative of subchromosomal deletions in the tumor genome. That is, heterozygosity was lost with some chromosome 10 markers and maintained with others. In each of these patients, heterozygosity was maintained in tumor DNA with only a single marker (THH54 in U1, CMM17.1 in U18, and TB14.16 in U22). However, heterozygosity was lost with every other informative marker indicating that the deleted sequences spanned broad areas of chromosome 10. In the remaining 16 patients, heterozygosity was lost with every informative marker spanning both p and q arms of chromosome 10 indicating that one entire copy of chromosome 10 had probably been lost from the tumor cells.

**DISCUSSION**

We report here the results of an allelotype analysis of a series of patients with malignant astrocytoma. Loss of heterozygosity was found for loci on almost every chromosome. Many patients showed loss of heterozygosity on multiple chromosomes and the number of chromosomes involved correlated with tumor histopathology. In addition, a high-resolution RFLP analysis of chromosome 10 loci in these patients showed that loss of broad regions of chromosome 10 was a common event in patients with glioblastoma multiforme, the most malignant form of astrocytoma.

RFLP studies reported previously by several laboratories showed that loci on chromosomes 10 and 17 were lost frequently in patients with malignant astrocytomas (9-13). The results of this allelotype analysis extend these observations and...
Allelotype of Human Malignant Astrocytoma

Fig. 2. RFLP analysis showing loss of heterozygosity for loci on multiple chromosomes in 2 patients with glioblastoma multiforme: patient U21 (A) and patient U3 (B). Genomic DNA (5 μg) from peripheral blood leukocytes (L) and tumor tissue (T) obtained from each patient was digested with restriction enzyme, electrophoresed through 1% agarose gels, transferred to nylon filters, and hybridized to 32P-labeled DNA probes specific for loci on the chromosomal arms indicated. Dried filters were exposed to X-ray film at ~70°C. for 16–144 h to generate the autoradiogram shown. On each autoradiogram, the length of each restriction fragment is expressed in kilobase pairs. The restriction enzymes used were TaqI (EFD49.2, YNH37.3), MspI (EFD122), PvuII (TB10.171), and Rsal (TB14.16, MLJ14, 79-2-23).

Comparison of the allelotype data from colorectal carcinoma and malignant astrocytoma revealed certain differences in the profiles of allele loss in these 2 human cancers. Common to both tumor types was the frequent loss of sequences from chromosome 17p, suggesting that inactivation of a tumor suppressor gene on 17p, perhaps p53, may be a central event in the pathogenesis of both tumor types. Unlike malignant astrocytomas, colorectal carcinomas seldom showed loss of heterozygosity on chromosome 10, suggesting that a tumor suppressor gene on chromosome 10 is involved uniquely in astrocytoma tumorigenesis. Since the frequency of allele loss on chromosome 18q in the malignant astrocytoma patients was low compared with that for colorectal carcinoma patients, the DCC gene implicated in colorectal carcinogenesis probably does not

A systematic allelotype analysis has been carried on only one other tumor type, human colorectal carcinoma (15). The results of that study showed loss of heterozygosity on every chromosomal arm with 17p and 18q affected most frequently. Subsequent investigations led to the identification of 2 candidate tumor suppressor genes important in colorectal carcinogenesis, namely, the p53 gene on chromosome 17p and a novel gene termed DCC on chromosome 18q (20, 21). The results of the allelotype analysis reported here are similar to those reported with colorectal cancer and raise the possibility that multiple tumor suppressor genes on many different chromosomes might be involved in astrocytoma tumorigenesis.

reveal an even greater complexity of genomic alternations in these tumors than reported previously.
#### Fig. 3. Allele loss patterns among the 19 patients with malignant astrocytoma (glioblastoma multiforme and anaplastic astrocytoma) that showed loss of heterozygosity for loci on chromosome 10. The physical location of certain markers on chromosome 10 is indicated on the karyogram (left). Hybridization results for each patient are shown (right) with patient identifiers (top). •, Loss of one allele in tumor DNA (loss of heterozygosity). ○, Presence of both alleles in tumor DNA. A hyphen indicates that the marker was uninformative (somatic heterozygosity); nd, results not determined.

play a significant role in astrocytoma tumorigenesis. These different profiles of allele loss suggest that the genetic events leading to malignant astrocytoma and to colorectal carcinoma may proceed along different pathways.

Although loss of heterozygosity for loci on chromosome 10 was found frequently in glioblastoma patients, the high-resolution RFLP analysis that we carried out did not reveal a small subchromosomal deletion that might help locate a tumor suppressor gene. On the contrary, loss of one entire copy of chromosome 10 was a much more common event. Although the molecular mechanisms that bring about chromosome loss in transformed cells are not well understood, biochemical rearrangements that occur during astrocytoma progression may preclude orderly segregation of chromosomes and lead to loss of whole chromosomes. For example, decreased cytosine methylation, which is found in the DNA of many types of tumors, is associated with impaired chromosome pairing during mitosis (22, 23). Nevertheless, one-half of the glioblastoma patients had no apparent loss of chromosome 10 sequences. It is possible that these patients have small deletions or other mutations in tumor DNA that are beyond detection with the markers currently available.

Our RFLP data from individual astrocytoma patients gave witness to an increased number of chromosomal aberrations occurring in patients with histopathologically more malignant tumors. These findings are consistent with a model for astrocytoma progression wherein inactivation of tumor suppressor genes on different chromosomes leads to increasingly deregulated cell growth.

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#### REFERENCES


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