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Analysis of the Neurofibromatosis 2 Gene in Human Ependymomas and Astrocytomas

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Abstract

Ependymomas and astrocytomas commonly have allelic losses of chromosome 22q, which suggests the presence of a glioma tumor suppressor gene on 22q. A candidate tumor suppressor gene on 22q is the neurofibromatosis 2 (NF2) gene since NF2 patients have an increased susceptibility to ependymomas and astrocytomas. Using single strand conformation polymorphism analysis and direct DNA sequencing, we screened 8 ependymomas and 30 fibrillary astrocytomas from non-NF2 patients for mutations in the coding sequence and portions of the 3' untranslated region of the NF2 gene. Only one mutation was detected, a single base deletion in NF2 exon 7 from a spinal ependymoma, which had also lost the wild-type allele. These results suggest that the NF2 gene may be important in the formation of some ependymomas but the NF2 gene is probably not the critical chromosome 22q tumor suppressor gene involved in astrocytoma tumorigenesis.

Introduction

Gliomas are the most common primary human brain tumors. These neoplasms are a diverse group that can be histopathologically divided into astrocytomas, oligodendrogliomas, and ependymomas (1). Molecular genetic analyses have identified patterns of allelic chromosomal loss in these tumors, suggesting a role for tumor suppressor genes in glioma tumorigenesis. In astrocytomas, frequent allelic loss has been noted for chromosomes 9p, 10q, 13q, 17p, 19q, and 22q (2). Oligodendrogliomas and ependymomas have been studied less extensively, but allelic loss of chromosome 19q is common in oligodendrogial tumors (3), and loss of chromosome 22q is frequent in ependymomas (4, 5). With the exception of the p53 gene on chromosome 17p (6), however, distinct glioma tumor suppressor genes have not been implicated on these chromosomes.

A candidate glioma tumor suppressor gene on chromosome 22q is the recently cloned neurofibromatosis 2 (NF2) gene (7). NF2 is an autosomal dominant syndrome in which patients develop bilateral vestibular schwannomas (acoustic neuromas), schwannomas in other sites, and multiple meningiomas. In addition, NF2 patients have a higher incidence of gliomas, particularly ependymomas and, to a lesser extent, astrocytomas (8). We therefore evaluated the NF2 gene in 8 sporadic ependymomas and 30 sporadic astrocytomas to determine whether the NF2 gene is a glioma tumor suppressor gene on chromosome 22q.

Results and Discussion

SSCP screening of the entire coding sequence of the NF2 gene in 8 ependymomas and 30 fibrillary astrocytomas revealed a single migration shift which occurred in exon 7 of an ependymoma (Fig. 1, Lane 5). Only aberrantly migrating bands were noted in the tumor, implying loss of the remaining wild-type allele. DNA sequencing of this exon revealed a deletion of a single thymidine nucleotide (base 840) in codon 207 (Fig. 2, left). Only faint bands were present from the wild-type sequence, again implying loss of the remaining wild-type allele. The frameshift mutation resulted in a stop in codon 208, thus leading to a severely truncated protein product. To date, similar frameshift and nonsense mutations have been the most common types of mutations detected in the NF2 gene in schwannomas and in NF2 patients. The combination of mutation of one NF2 allele and chromosomal loss of the second 22q allele thus fulfills the classic paradigm of a recessively-acting tumor suppressor gene.

The tumor with an NF2 gene mutation was a recurrent, intramedullary cervical ependymoma, WHO grade II, from a 45-year-old man without family history or stigmata of NF2. Sequencing of the patient's constitutional DNA revealed the wild-type sequence (Fig. 2, right).

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3 The abbreviations used are: WHO, World Health Organization; SSCP, single strand conformation polymorphism analysis.

confirming the somatic nature of the mutation. Histological examination of the original tumor revealed no atypical or anaplastic features. Although formalin-fixed, paraffin-embedded tissue was available from the first tumor, adequate DNA could not be extracted.

Molecular genetic and cytogenetic studies have demonstrated that chromosome 22q loss is common in ependymomas (4, 5, 10). While such analyses have suggested that chromosome 22q harbors an ependymoma tumor suppressor gene, they have not narrowed down the location of this putative tumor suppressor gene. Our data implicate the NF2 gene as the target of 22q allelic loss in at least some ependymomas. It is tempting to speculate that NF2 mutations may be particular to intramedullary spinal ependymomas since these are the types of ependymomas characterizedly associated with NF2 (11) and we did not detect NF2 mutations in any of the six intracranial ependymomas. Alternatively, the lack of mutations in the remainder of cases may imply that the presence of a second chromosome 22q ependymoma tumor suppressor gene or that NF2 mutations may occur in nonexonic portions of the gene, such as in promoters or introns, or in additional, alternatively spliced exons. Our recent screening of the NF2 gene in NF2 patients and schwannomas has revealed a considerable number (approximately 50%) of cases without detectable mutations in the same regions assayed in the present study,4, 5 supporting the possibility that mutations may occur in other regions of the gene. Finally, the lack of mutations in the remainder of the ependymomas may be due to problems in the screening method since SSCP may not detect all point mutations and is not an adequate means of identifying larger genomic deletions. Such larger genomic deletions have already been noted in several NF2 patients (7).

None of the thirty astrocytomas had detectable mutations in the NF2 gene (Fig. 3). These findings make it improbable that the NF2 gene is the critical astrocytoma tumor suppressor gene on chromosome 22q, unless mutations occur exclusively in other regions of the gene. Three astrocytomas have been reported with loss of heterozygosity at D22S171 on distal 22q, but with maintenance of heterozygosity at the more proximal locus D22S80 (12). Since D22S80 is distal to NF2, these findings support our conclusion that the NF2 gene is not the chromosome 22q astrocytoma tumor suppressor gene and that the putative gene lies distal to NF2. Further detailed deletion mapping of chromosome 22q in astrocytomas may help to narrow down the location of this distal culprit.

References


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