Advances in Brief

Analysis of the Neurofibromatosis 2 Gene in Human Ependymomas and Astrocytomas

Mari-Paz Rubio, Katia M. Correa, Vijaya Ramesh, Mia M. MacCollin, Lee B. Jacoby, Andreas von Deimling, James F. Gusella, and David N. Louis

Molecular Neuro-Oncology Laboratory [M-P. R., K. M. C., D. N. L.], Department of Pathology (Neuropathology) [D. N. L.], Neurosurgical Service [L. B. J., D. N. L.], and Molecular Neurogenetics Unit [M. M. M., V. R., L. B. J., J. F. G.], Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02129, and Institute for Neuropathology, University of Bonn, Bonn, Germany [A. v. D.]

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 oligodendroglial 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 identified 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.

Materials and Methods

All tumors were classified by a neuropathologist according to the WHO criteria (1). Of the eight ependymomas, four were WHO grade II and four had features of anaplastic ependymoma, WHO grade III; none of the tumors was of the myxopapillary type. Five of the ependymomas were fourth ventricular tumors; two were intramedullary spinal cord lesions; and one was located in the frontal lobe. All of the 30 astrocytic tumors were diffuse, fibrillary astrocytomas from the cerebral hemispheres of adult patients; two were WHO grade II; eight were anaplastic astrocytoma, WHO grade III; and 20 were glioblastoma multiforme, WHO grade IV. None of the patients had clinical or radiological evidence of NF2. DNA was extracted from fresh tumor (all astrocytomas and four ependymomas) and blood specimens according to standard phenol-chloroform procedures. DNA was extracted from formalin-fixed paraffin-embedded tissues of four ependymomas using a published protocol (9).

SSCP was performed as described (6) using published polymerase chain reaction conditions and oligonucleotides. All 17 published exons of the NF2 gene and portions of the 3’ untranslated region were screened. Amplification products larger than 200 base pairs were cleaved with appropriate restriction enzymes to yield fragments less than 200 base pairs in size. The amplification products were separated on 6–8% nondenaturing polyacrylamide gels with 10% glycerol overnight at 3–5 watts. Assays included positive control DNA from NF2 patients or schwannomas with known NF2 mutations. Cases with mobility shifts on SSCP were directly sequenced with Vent (exo-) DNA polymerase and the CircumVent Thermal Cycle Sequencing kit (New England BioLabs, Beverly, MA) using the SSCP or internal primers.

Results and Discussion

SSCP screening of the entire coding sequence of the NF2 gene in 8 ependymomas and 30 fibrillary astrocytic tumors 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).

Received 10/26/93; accepted 11/22/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by American Cancer Society CB-31A [D. N. L.], NIH CA 57683 [J. F. G., D. N. L.], CA 51410 [L. B. J.], and NS 24279 [J. F. G.].

2 To whom requests for reprints should be addressed, at Molecular Neuro-Oncology Laboratory, CNY6, Massachusetts General Hospital, Charlestown, MA 02129.

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, 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 D22S71 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


Analysis of the Neurofibromatosis 2 Gene in Human Ependymomas and Astrocytomas


Cancer Res 1994;54:45-47.