Identification of a Consistent Region of Allelic Loss on 1p32 in Meningiomas: Correlation with Increased Morbidity

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Abstract

Meningioma is a common tumor of the central nervous system. Deletions of the short arm of chromosome 1 (1p) are the second most commonly observed chromosomal abnormality in these tumors. Here, we analyzed tumor and normal DNAs from 157 meningioma patients using PCR-based polymorphic loci. Loss of heterozygosity (LOH) for at least one informative marker on 1p was observed in 54 cases (34%), whereas LOH on 1q occurred in only 9 cases (6%). High-resolution deletion mapping defined a consensus region of deletion flanked distally by DIS1S2713 and proximally by DIS1S2134, which spans 1.5 cm within 1p32. LOH in this region has also been observed in several other malignancies, suggesting the presence of a tumor suppressor gene or genes that are important for several types of cancer. Statistical analysis revealed that 1p LOH was associated with chromosome 22 deletions and with abnormalities of the NF2 gene in meningioma. In addition, unlike other clinical and molecular characteristics, only 1p LOH was shown to be significantly associated with recurrence-free survival.

Introduction

Meningioma is a typically benign tumor of the meninges that surround the central nervous system. It is one of the most common intracranial tumors, occurring at a frequency of ~2.3/100,000 people per year and accounting for 13–20% of adult brain tumors (1). Meningiomas are twice as common in females as in males, and although there is a peak occurrence during the fifth to sixth decade of life, asymptomatic tumors have been found at autopsy in older patients (2). Although the majority of meningiomas are benign, between 5 and 10% are malignant (WHO grade III), and ~20% recur (3, 4).

Multiple primary meningiomas occur in up to half of patients with NF2, a disorder involving central nervous system tumors (5). Early cytogenetic analysis identified monosomy or deletion of chromosome 22 in ~70% of cases (6). Subsequently, LOH studies on a panel of 170 meningiomas identified a region of consistent deletion on the long arm of chromosome 22 (22q) in ~70% of tumors (7–10). Inactivating mutations in the NF2 tumor suppressor gene (11), located on 22q12, were later identified in a majority of cases with distal 22q LOH (12, 13).

Deletion of several other chromosomal regions has been observed by cytogenetic analysis in meningiomas, often in conjunction with chromosome 22 deletions (14–17), and deletion or monosomy of the short arm of chromosome 1 (1p) occurs most frequently (18). Preliminary molecular studies using a small cohort of tumors identified LOH of 1p in ~25–30% of cases, and 1p LOH was found to be correlated with a more malignant tumor phenotype (19, 20).

Here, we analyzed tumor and normal DNAs from 157 meningioma patients using PCR-based STRP loci. Nearly all patients in the study had been examined previously for chromosome 22 deletions or rearrangements and NF2 mutations (7, 8, 10, 13). A region of consistent allelic loss was identified at 1p32 in 34% of these cases. In addition, a subset of patients from this cohort was analyzed for correlations between 1p LOH and other clinical and molecular genetic characteristics of meningioma.

Materials and Methods

Tumor and Blood Samples. Samples used in this study represent a subset of those used in earlier reports. Surgical specimens of tumors were collected over a 5-year period and were frozen at ~135°C for up to 3 years prior to extraction of DNA. Tumors were classified according to the 1979 WHO scheme (3). Peripheral blood DNA was used as the normal control. Clinical data for the 157 patients have been described previously (8, 10, 21).

STRP Analysis. Details on the STRP loci used are available from the Genome Database. PCR was performed in 20-µl volumes containing 40 ng of human genomic DNA as a template, 0.2 µM each primer, 0.2 mM each dNTP, 1.5 mM MgCl2, 1 × PCR buffer, 0.5% (w/v) Ficoll, 0.0025% (w/v) bromphenol blue, and 1 unit of AmpliTaq Gold (Perkin-Elmer Corp., Branchburg, NJ) or Taq DNA polymerase (Promega, Madison, WI). Prior to PCR, the sense primer in each reaction was end-labeled with [γ-32P]dATP (Amersham, Arlington Heights, IL) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Amplification was performed as described previously (22). Following amplification, 5 µl of 0.02% bromphenol blue in formamide were added to each product. Samples were denatured at 94°C for 4 min and left standing at 80°C while they were loaded into an 8% polyacrylamide/7 M urea sequencing gel. Gels were analyzed by autoradiography for 4–48 h at room temperature.

Statistical Analysis. Analysis was performed using Stata 5.0 (Stata Corp., College Station, TX). The Fisher's exact test was used to examine possible associations between 1p LOH and other molecular and clinical characteristics of meningioma. Kaplan-Meier survival curves (23) were calculated, and log-rank tests (24) were used to compare differences between recurrence-free survival based on sex, WHO tumor grade, histological subtype, 1p LOH, chromosome 22 deletions, and NF2 mutations. Recurrence-free survival analysis was conducted using only the first recurrences of meningioma as the individual events.

Results

We analyzed 157 sporadic meningiomas for 1p LOH on chromosome 1. Initial deletion mapping of these tumors was conducted using six highly polymorphic STRP loci distributed equidistantly along 1p (Ref. 25; Fig. 1A). A seventh locus, DIS1632, located on 1q32, was used to detect whole chromosome deletions and background LOH. The number of tumors with LOH of all informative tumors for each of the

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3 The abbreviations used are: NF2, neurofibromatosis type 2; LOH, loss of heterozygosity; STRP, short tandem repeat polymorphism; SRO, smallest region of overlap.
seven loci, listed from telomere to centromere, were as follows: D1S548 (31 of 95, 33%); D1S552 (37 of 110, 34%); D1S1591 (22 of 75, 29%); D1S1596 (38 of 121, 31%); D1S551 (34 of 116, 29%); D1S1623 (27 of 122, 22%); and D1S1632 (9 of 118, 8%). All patients were informative for at least two of the 1p loci. Overall, 54 (34%) of the tumors exhibited LOH for at least one of the six 1p loci, whereas only nine tumors (8%) demonstrated loss on 1q.

The initial analysis using six 1p loci defined a SRO that was consistently deleted in all informative tumors with 1p LOH. This region was bounded distally by D1S552 and proximally by D1S1596 and spanned ~32 cM. The 54 tumors with 1p LOH were then analyzed to more precisely define the SRO. Eight additional STRP loci were selected that spanned or flanked this region (Fig. 1A). Screening of the tumors with 1p LOH narrowed the consistently deleted SRO to ~6 cM. The distal boundary of the SRO (at D1S1598) was defined by tumor 79 and the proximal boundary (at D1S2134) by tumor 96 (Fig. 1A).

Next, we selected the four tumors with deletion breakpoints that closely bounded the defined SRO to perform more precise LOH analysis with an additional set of 11 STRP loci. The SRO could be narrowed further to a <1.5-cM distance within 1p32, defined distally by D1S2713 and proximally by D1S2134 (Fig. 1B). Representative examples of LOH around this region are shown in Fig. 2. This region was deleted in all but one of the tumors exhibiting 1p LOH. Tumor 117, previously uninformative in this region, retained heterozygosity within the narrow SRO and defined a more distal deletion, bounded proximally by D1S2743 and extending toward the telomere. Because of its location at the boundary of the most common SRO, D1S2134 was used to reexamine all tumors previously scored as showing no 1p LOH, but no new cases of 1p LOH were identified with this locus. No microsatellite instability was observed in any of the 157 tumors analyzed.

A subset of patients used for the molecular analysis of 1p LOH was subjected to statistical analysis for possible associations with other molecular genetic and clinical characteristics of meningioma. For this analysis, 138 patients with clinical follow-up times of more than 1 month from their primary operation were included. The mean age at the time of the primary surgery to remove the tumor was 55 years (range, 18–84 years). Of the 138 patients, 134 had been previously analyzed for chromosome 22 abnormalities, and 131 had been exam-
Table 1

<table>
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<tr>
<th>Variable</th>
<th>n</th>
<th>No. recurrences observed (%)</th>
<th>No. recurrences expected (%)</th>
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<td>43</td>
<td>12 (27.9)</td>
<td>9.8 (22.7)</td>
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* Patients 40 and 168 were classified as WHO grade I but could not be classified to a specific histological subtype.

* Normal chromosome 22 indicates no deletion detected; deleted indicates either monosomy or partial deletion of 22q.

* Normal NF2 indicates no detectable mutation or deletion; aberrant indicates the detection of an inactivating point mutation, rearrangement, or homozygous deletion.

The presence or absence of 1p LOH was examined for possible association with deletions, rearrangements, or monosomy of all or part of chromosome 22; with inactivating mutations, homozygous deletions, or rearrangements of the NF2 tumor suppressor gene; and with other clinical and demographic characteristics of meningiomas. No significant association was seen between 1p LOH and patient sex, age at diagnosis, or histopathological subtype. Five of seven (71%) grade III (anaplastic) tumors exhibited 1p LOH, but the sample size was too small for statistical analysis.

A strong correlation was observed between chromosome 22 aberrations and 1p LOH (P < 0.0005). Of those cases with normal chromosomes 22, 90% also retained both copies of 1p. Conversely, 50% of tumors with chromosome 22 aberrations exhibited 1p LOH. A similarly strong association between NF2 abnormalities and 1p LOH was observed (P = 0.003). Only 24% of cases with no NF2 mutations had 1p LOH. In contrast, 50% of cases with NF2 mutations also exhibited 1p LOH. As expected, nearly all cases with normal chromosomes 22 (95%) had normal NF2 genes. NF2 abnormalities were observed in ~50% of patients with chromosome 22 deletions. Finally, only five patients showed LOH on 1p with no apparent involvement of chromosome 22 or NF2.

The significance of 1p LOH was assessed for predicting recurrence-free survival. The median follow-up time for the 138 patients used for this statistical analysis was 5.1 years (range, 1 month to 42.5 years). Seven patients (5%) had died from meningioma. Fourteen of the 138 patients used for statistical analysis had follow-up times that were longer than 10 years and were censored at 10 years of follow-up. Of these 14, 8 had recurrences of meningioma after the 10-year follow-up time. Table 1 shows the log-rank test results analyzing the predictive value of 1p LOH, along with age, sex, WHO tumor grade, histological subtype, and chromosome 22 and NF2 anomalies. Significant predictive value was only observed for 1p LOH (P = 0.0061). Kaplan-Meier survival curve analysis confirmed the predictive importance of 1p LOH (Fig. 3). It should be noted that 60% of the samples used for LOH analysis of the recurrent patients came from the recurrent tumor, as opposed to the primary tumors. However, no statistically significant association was observed between 1p LOH and whether primary or recurrent tumor tissue was used for analysis (P = 0.3). None of these patients had received chemotherapy or radiation therapy.

Fig. 3. Kaplan-Meier curve of recurrence-free survival. The predictive importance of 1p LOH for recurrence-free survival is shown for a 10 year follow-up period. Curves are shown for the absence and presence of 1p LOH (n = 90 and n = 48, respectively; P = 0.0061). Included are all patients with follow-up times of >1 month. Patients with follow-up times of >10 years were censored. Seven patients died of meningioma. Thirty-five recurrent events are shown. Overall frequency of recurrence for the 1p LOH group was 42% compared to 17% for the group without 1p LOH.

4 J. P. Dumanski, unpublished data.
Discussion

Previous cytogenetic studies of meningioma have implicated 1p as the most frequent site of deletion after 22q (18). In addition, earlier hybridization-based studies observed LOH for 1p in >25% of cases (19, 20). Using a large set of high-resolution STRP loci, we observed 1p LOH in 34% of 157 tumors. 1p LOH was shown to be associated with chromosome 22 and NF2 alterations and to predict more frequent tumor recurrence following surgery.

Although 1p LOH was not as frequent as chromosome 22 deletions in this series of tumors (.34 versus .57%), it is the second most common genetic aberration reported in meningiomas (14–18). All meningiomas with 1p LOH in this series contained deletions of at least 20–30 cM, suggesting that it is unlikely that a tumor with a small deletion was overlooked. Also, the results of this study contradicted previous reports of microsatellite instability in meningioma (26), suggesting that this form of genetic instability may not be involved in tumor development.

The location of the commonly deleted region at 1p32 is consistent with earlier reports. Cytogenetic studies by Jiménez-Lara and coworkers (18) identified 19 cases (in a series of 125) with deletions from 1p32 to 1pter. Preliminary studies by Bello and colleagues (19) identified allelic loss from 1p32–p36. In addition, a case has been reported with the sole observed cytogenetic anomaly of a deletion from 1p32–1pter (27). However, our report is the first to identify a narrow region of consistent deletion (<1.5 cM) using a large battery of PCR-based polymorphisms specific for 1p. The identification of additional regions of deletion in this study is consistent with the possible existence of multiple tumor suppressor loci on 1p. The identification of additional regions of deletion in this study is consistent with the possible existence of multiple tumor suppressor loci on 1p, as hypothesized in breast carcinoma and neuroblastoma (25, 29). Finally, a recent study using CGH with 25 meningiomas (23 of which were part of the current study) identified two regions of deletion on 1p which correspond to the regions identified in this study (28). Both tumors with deletions identified by comparative genomic hybridization exhibited corresponding LOH in this study.

The SRO identified from D1S2713 to D1S2134 lies within SROs defined in several other malignancies, which is consistent with deletion of a tumor suppressor gene in this region. Specifically, studies have reported 1p32 deletions in melanomas, breast carcinoma, neuroblastoma, oligodendroglioma, and pheochromocytoma (25, 29). A second region of allelic loss in all but two of the tumors with loss in the study (tumors 31 and 79), located within 1p35–36, has been particularly well defined in neuroblastoma and is also commonly deleted in melanoma, hepatoma, germ cell tumors, Merkel cell cancer, breast cancer, and colon carcinoma (30).

Candidate tumor suppressor genes located within the D1S2713 to D1S2134 region include the T-cell leukemia-related genes TAL1 and SIL and the tyrosine kinase JAK1, as well as CDKN2C (p18), a member of the family of cyclin-dependent kinase inhibitors (31–34). Further analysis of these candidate genes in meningioma will be prohibited calculations of statistical significance, an association between 1p LOH and a malignant phenotype may exist.

In a retrospective study of 215 meningioma karyotypes, Steudel and colleagues (37) identified 6 cases with deletions of 1p. These patients had a 60% rate of recurrence, compared to an overall rate of 15% in their study. This is consistent with our study in which 42% of 1p-deleted tumors recurred, compared to 17% of nondeleted tumors. Thus, 1p LOH may serve as an important prognostic indicator based on its ability to predict a greater likelihood of recurrence, which may have implications for treatment. A study using a large series of recurrent patients analyzed for 1p LOH would confirm the prognostic significance.

References


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