Meningiomas: Analysis of Loss of Heterozygosity on Chromosome 10 in Tumor Progression and the Delineation of Four Regions of Chromosomal Deletion in Common with Other Cancers

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ABSTRACT

Purpose: Loss of heterozygosity (LOH) of alleles on chromosome 10 has been reported in many cancers, leading to the identification of tumor suppressor genes on this chromosome. Several reports implicate LOH of chromosome 10 alleles in meningioma progression, but the frequency and complexity of the loss have not been well characterized. Furthermore, the location and identity of the putative tumor suppressor genes on this chromosome that contribute to meningioma progression are unknown because the currently characterized tumor suppressor genes do not appear to be involved. Therefore, this study was undertaken to (a) assess the frequency and complexity of LOH in meningioma progression, (b) map the LOH patterns of individual meningiomas to define the smallest regions of shared chromosomal deletion, and (c) compare the identified regions with chromosome 10 deletions in other cancers, and thereby initiate the localization of the putative tumor suppressor genes.

Experimental Design: We examined 11 microsatellite dinucleotide repeat loci in 208 meningiomas of all grades using laser capture microdissection and fluorescence-based detection of PCR products.

Results: For all markers examined, the incidence of LOH was much higher in all grades than that previously reported, with incidence and complexity of LOH increasing with tumor grade. LOH mapping identified four regions of chromosomal deletion: 10pter-D10S89, D10S109-D10S215, D10S187-D10S209, and D10S169-10qter. These deletions on chromosome 10 are shared with other cancer types.

Conclusions: These results delineate chromosomal locations of putative tumor suppressor genes on chromosome 10 that likely play an early role in meningioma tumorigenesis as well as tumor progression.

INTRODUCTION

Despite the fact that meningiomas (1, 2) are the most frequent type of brain tumor (3) and were among the first solid neoplasms to be studied cytogenetically (4–6), little is known about their molecular genetic profile, with the exception of the identification of the NF2 locus on chromosome 22 (7–10). To date, allelic losses on chromosomes 1, 9, 10, 14, 18, and 22 have been implicated in the pathogenesis of meningiomas (11–16). Several studies have associated loss of alleles on chromosome 10 with malignant meningioma progression. In the first study, we have demonstrated LOH in 1 of 2 (50%) atypical and 4 of 13 (30.8%) malignant meningiomas using two chromosome 10p (D10S89 and D10S111) and two chromosome 10q (D10S109 and D10S169) loci (11). LOH was observed for D10S89 and D10S169. In a second study, Simon et al. (12) expanded the number of loci to seven, including three 10p markers (D10S179, D10S89, and D10S111) and four 10q markers (D10S109, D10S169, D10S187, and D10S209). Their study did not report results obtained at individual loci, but combined results for chromosomal arms. LOH was observed on 10q in a small percentage of benign tumors (12%), with increasing LOH in atypical (27%) and malignant (40%) tumors. The smallest region of allelic deletion using these loci was found to be 10q24–10qter. In a third study, Weber et al. (13), using two chromosome 10q markers (D10S185 and D10S212), identified LOH in 1 of 19 (5.3%) benign, 4 of 21 (19.0%) atypical, and 5 of 9 (55.6%) malignant tumors. Similarly, in a fourth study, von Deimling et al. (14), using two chromosome 10p markers (D10S674 and D10S89) and four chromosome 10q markers (ZNF22, D10S676, D10S678, and D10S169), found LOH for combined 10q loci in 3 of 56 (5.4%) benign, 4 of 10 (40%) atypical, and 5 of 9 (55.6%) malignant meningiomas. Lamszuz et al. (15) examined four 10q loci (D10S469, D10S169, D10S187, and D10S209). Reporting results for individual loci, they found LOH in two of five patients at D10S187 and D10S209. These five studies examining tumors derived from different patients demonstrate early LOH in a subset of benign
meningiomas with increasing frequency of LOH during tumor progression.

However, it is difficult to assess the frequency or complexity of LOH occurring in the different grades by comparing the chromosome 10 LOH data in these reports because different loci were used, and the results for individual loci were not always reported separately. Furthermore, because meningiomas do not tend to lose the entire chromosome 10 (17), as is the case with the majority of astrocytic gliomas (18), detailed LOH studies may well be useful in defining candidate tumor suppressor loci. Therefore, we examined 11 microsatellite dinucleotide repeat loci in 208 meningiomas of all grades using LCM and fluorescence-based detection of PCR products (a) to assess the frequency and complexity of LOH throughout tumor progression, (b) to map the LOH patterns of individual tumors to define regions of chromosomal deletion, and (c) to compare these chromosomal deletions with those found in other cancers.

MATERIALS AND METHODS

Specimens. A total of 208 paraffin-embedded meningioma specimens were collected. Tumors were derived from patients surgically treated at Henry Ford Hospital between 1968 and 2001. Institutional review board-approved informed consent was obtained from all patients or from the patient’s guardian for use of tumor tissue collected at the time of tumor resection. Since 1993, informed consent included the collection of blood for the extraction of lymphocyte DNA. For specimens collected before 1993, normal DNA was extracted from microdissected adjacent brain tissue present on the paraffin-embedded tumor specimens. Two 5-μm serial sections having diagnostic tumor were obtained for each specimen; one was stained with H&E, whereas the other remained unstained. The H&E-stained sections were used for grading. All tumors were graded as either benign, atypical, or anaplastic/malignant, which correspond to WHO grades I, II, and III, respectively (2). In addition, the neuropathologist circled the area representative of diagnostic tumor or normal brain on the H&E-stained section.

LCM and DNA Extraction. All tumors were microdissected. LCM (PixCell II; Arcturus Engineering Inc., Mountain View, CA) was used to minimize the contamination of tumor DNA by normal cell DNA, and vice versa. The H&E-stained slides were used as reference to localize the homogeneous regions of diagnostic tumor or normal brain tissue on the unstained sections that had been deparaffinized in xylene for 1–2 h, and air dried. To ensure adequate DNA copy number for subsequent PCR reactions, a minimum of 1000 cells were captured using between 1000 and 1500 pulses of the focused 30-μm-diameter laser. The transfer of the cells to capture film was verified by examining the LCM caps at ×40 magnification before DNA extraction. A 5-μl droplet of the digestion buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 1% Tween 20, and 0.1% proteinase K] was applied to coat the cells attached to the capture film on the LCM cap. After 1 min, the LCM caps were transferred to 0.5-ml microfuge tubes (Perkin-Elmer, Norwalk, CT) containing 45 μl of extraction buffer and warmed at 52°C for 5 min. The tubes were then inverted and incubated overnight (16–18 h) at 52°C. After incubation, the tubes were up-righted and centrifuged at 13,000 rpm for 5 min. Proteinase K was inactivated at 96°C for 8 min, and the extracts were used directly as templates for PCR. Lymphocyte DNA was extracted using the Stratagene DNA extraction kit (La Jolla, CA).

PCR. Matched tumor and normal DNAs were PCR amplified at 11 polymorphic microsatellite markers mapping to chromosome 10: D10S179; D10S189; D10S89; D10S580; D10S109; D10S215; D10S574; D10S187; D10S209; D10S217; and D10S169. One primer from each primer pair was fluorescently labeled with either D2, D3, or D4 Beckman WellRED dyes (Research Genetics, Huntsville, AL), so that LOH analyses could be multiplexed. PCR amplifications (PTC-200 Thermal Cycler; MJ Research, Watertown, MA) were performed using 2 μl of DNA template, 0.5 unit of platinum Taq polymerase (Invitrogen, Carlsbad, CA), 1.25 mM deoxynucleotides, 1.5 mM MgCl2, and 4 μM of each forward and reverse primer, in a total volume of 10 μl. Cycling conditions consisted of 95°C for 5 min, followed by 45 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min according to published reports (19, 20). The final elongation was at 72°C for 10 min.

LOH Analysis. Loci analyzed were selected based on previously reported loci that were found to be informative for LOH (11–15). Not all primers amplified well in our hands. Eleven primer sets were finally selected. LOH was assessed using the CEQ 2000 XL fragment analyzer (Beckman Coulter, Fullerton, CA) as reported previously (21). Using 96-well plates, each well was loaded with 3 μl of amplified DNA, 0.57 μl of Beckman WellRED D1-labeled size standard, and 37 μl of deionized formamide; topped with mineral oil; and mixed. Specimens were scored as: (a) having maintenance of both alleles; (b) having LOH; (c) being NI; (d) having microsatellite instability; or (e) not amplified. LOH was assigned when the peak area of one allele in tumor tissue was <40% of the other tumor allele in comparison with the ratio of peak areas of the two alleles in the corresponding constitutional DNA, as reported previously (16, 22).

Smallest Regions of Allelic Deletion. For each specimen, the regions of LOH were mapped along the chromosome. Each LOH region was limited to the first maintained locus on either side of the lost allele (except for the telomeric alleles that were limited to one side only). A composite map indicating the type and frequency of the LOH patterns for all tumors was used to generate the smallest regions of allelic deletion (22).

Data Analyses. For data analyses, the following calculations were performed. The number of informative tumors = the number of tumors maintaining both alleles + the number of tumors with LOH. The percentage of LOH = (the number of tumors with LOH)/(the number of informative tumors) × 100.

RESULTS

Tumor Specimens. Of the 208 tumors collected, 173 (83.2%) of the tumors were benign (grade I), 20 (9.6%) were atypical (grade II), and 15 (7.2%) were malignant (grade III) tumors. Matched lymphocyte DNA was available for 148 tumors. For 60 tumors, matched normal DNA was obtained from microdissected adjacent normal brain tissue.

LOH Analyses. Table 1 lists the dinucleotide repeat loci and the primers used for the PCR reactions. Fig. 1 demonstrates
typical profiles obtained using these primers for the maintenance of both alleles, the LOH on the shorter allele, the LOH on the longer allele, and a NI locus.

Overall, of the 208 meningiomas, 202 (97.1%) were heterogeneous in their normal tissue for at least one polymorphic marker, and 156 of 202 informative cases (77.2%) displayed LOH. The summary of the PCR results for each chromosome 10 marker, and 156 of 202 informative cases (77.2%) displayed LOH. The summary of the PCR results for each chromosome 10 marker, and 156 of 202 informative cases (77.2%) displayed LOH. The summary of the PCR results for each chromosome 10 marker, and 156 of 202 informative cases (77.2%) displayed LOH. The summary of the PCR results for each chromosome 10 marker, and 156 of 202 informative cases (77.2%) displayed LOH. The summary of the PCR results for each chromosome 10 marker, and 156 of 202 informative cases (77.2%) displayed LOH.

LOH and Meningioma Progression. By grade, there was LOH for at least one locus in 73.4% (127 of 173) of the benign tumors, 80.0% (16 of 20) of the atypical tumors, and 86.7% (13 of 15) of the malignant tumors. The data indicate that a large percentage of benign tumors have LOH for at least one chromosome 10 allele and that the percentage of tumors having LOH increases slightly during progression. Furthermore, a significant percentage of benign (75 of 173, 43.3%) and atypical (9 of 20, 45%) tumors have LOH at more than one locus, and a further increase was observed in the percentage of malignant (10 of 15, 66.7%) tumors with complex loss.

Four Regions of Chromosomal Deletion. Fig. 2 illustrates the patterns of LOH observed for the 156 informative tumors with LOH. The number of tumors in each grade having each pattern is indicated. The majority of atypical and malignant tumors had LOH at all informative alleles, and therefore they did not contribute to the mapping of the smallest regions of deletion. This observation was not unexpected because the percentage loss increased with increasing grade. The regions of deletion were therefore defined, for the most part, by the benign tumors, and these were the 10p region pter-D10S179 and the 10q regions D10S109-D10S215, D10S187-D10S209, and D10S169-10qter.

It should be noted that mapping was performed placing D10S179 more telomeric than D10S189 on chromosome 10p. Such positioning would, in fact, define a smaller region of deletion from D10pter-D10S179 than we indicate. However, although both markers have been mapped to the same location, the precise distance of D10S179 from the telomere in Mb has not been reported, and it is possible that D10S179 is centromeric to D10D189. Therefore, we have reported a more conservative, broader region of deletion in this region of 10p. It should also be noted that overlapping patterns of LOH suggest that another small region of deletion may exist from D10S179 to D10S215 and D10S189-D10S209. However, because only a small number of tumors defined this region, and none had independent loss, this region is noted but is not called a region of deletion.

Patterns of LOH. Based on these criteria, several deletion patterns were noted. Of the 127 tumors having LOH, 38 (29.9%) had loss restricted to the q arm, 8 (6.3%) had loss limited to the p arm, and 81 (63.8%) had LOH on both arms. In contrast, the majority (15 of 16) of the atypical tumors (93.8%) and 100% of malignant tumors with LOH had loss on both chromosomal arms.

Comparison of Chromosome 10 Deletions in Meningiomas and Other Cancers. Fig. 3 illustrates the four regions of deletion characterized by this study and the regions of deletion reported for glioblastoma (18), prostate (23–25), endome-
and overlaps with the deletion described for bladder cancer. The region D10S187-D10S209 overlaps the location of DMBT1 gene and q arm deletions described for small cell lung carcinoma and endometrial cancer. Finally, the telomeric deletion D10S169-qter overlaps with the large q telomeric deletion described for endometrial cancer.

**DISCUSSION**

In this study we examined the LOH of 11 dinucleotide repeat loci on chromosome 10 in 208 meningiomas of all grades. We investigated the incidence and complexity of LOH relative to tumor progression. For all alleles examined, the incidence of LOH was much higher in all grades than that reported previously, with incidence and complexity of LOH increasing with tumor grade. Mapping of the regions of LOH of all of the tumors defined four regions of chromosomal deletion. These deletions coincide with those found in other cancers, supporting the hypothesis that candidate tumor suppressor genes in these regions contribute to meningeal tumorigenesis and progression.

To expand on previous studies, we examined LOH of alleles used in previous meningioma reports (11–15), as well as additional loci mapping near known and candidate tumor suppressor genes on 10q. The present data are in good agreement with our reported data (11) that LOH occurs at markers D10S89 and D10S169 and with the 10q LOH reported by Simon et al. (12) and von Deimling et al. (14). Our results also agree with those of Lamszus et al. (15), confirming LOH on loci D10S187 and D10S209. In further agreement with published results, we observed LOH in benign tumors, and the frequency of LOH increased slightly with increasing grade. Of interest was the high percentage of benign and atypical tumors having LOH on multiple loci with further increase in complex loss to malignant tumors. This large increase between atypical and malignant tumors may indicate a discontinuous change in genome stability with the onset of malignancy.

In contrast with previous reports, however, we observed LOH at much higher percentages. We suggest that this increase in detection of LOH may have resulted from a combination of effects including the examination of polymorphic loci that had

**Table 2** Percentage of tumors with LOH by locus

For each locus, the total = number of tumors that were informative + number that were NI. The total number of informative loci (I) = # maintained (M) + # having LOH. The number of tumors at each locus having LOH or M alleles is indicated. The percentage of tumors with LOH (%LOH) = (# of tumors with LOH/# of I tumors) × 100.

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<th># M</th>
<th># LOH</th>
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**Fig. 1** Examples of CEQ 2000 XL allelic profiles. Panels represent normal (N) and matched tumor (T) DNA profiles for samples demonstrating maintenance of both alleles (M), LOH for the first (short) allele, LOH for the second (long) allele, or NI amplification (NI).
previously been shown to have LOH in meningiomas and increased odds of detecting LOH by examining a larger number of loci. We do not believe the increase in observed LOH was due to less stringent data calculations because LOH was assigned using a conservative lower limit of 40% loss. This is more stringent than previous reports, where 30% loss was considered indicative of LOH (11), and loss between 30% and 40% was considered suggestive of LOH (22). Furthermore, although the loss ratios for individual loci ranged from 40% to 100% in this study, 77% of the loci had a loss ratio greater than 50%, with almost a quarter of the loci (23.2%) having 100% loss of an allele (data not shown), even under PCR conditions set up to detect small amounts of a given allele. Therefore, the majority of tumors had LOH with a loss ratio much higher than our designated cutoff.

In an attempt to characterize regions where the putative tumor suppressor genes inferred by the LOH analyses reside, we mapped and compared the LOH patterns for each meningioma to define the smallest regions of deletion occurring in this tumor population. Mapping resulted in the identification of one region of chromosomal deletion on 10p (pter-D10S89[p12.1]) and three on 10q (D10S109[q22.3]-D10S215[q24.1], D10S187[q25.3]-D10S209[qq26.11], and D10S169[q26.3]-qter). It is interesting to note that there were three major patterns of LOH for the benign tumors. Because the majority of atypical tumors and all of the malignant tumors had loss on both arms, it is tempting to speculate that benign tumors with loss only on the q arm may be less aggressive than those having LOH patterns more similar to the higher-grade tumors with loss on both arms, and this study is under way.

An examination of the patterns also indicated diverse patterns of loss for the benign meningiomas, with a given pattern often occurring for a single meningioma. The diversity of patterns might result from instability at fragile sites (31). On chromosome 10, 7 fragile sites (FRA10A-Fra10G) have been localized to the q arm. Of these, FRA10D is in the neighborhood of D10S580 and D10S109, whereas FRA10E and FRA10B are near D10S187, and FRA10F is near D10S209. Further investigation will determine whether these fragile sites impact LOH at the loci examined in this study. In addition, many tumors showed retention for either D10S109 or D10S580. It is possible that the apparent retention between these two markers really represents homozygous deletions. This would result in a slightly larger region of deletion from D10S580 to D10S215.

Of the three deletions on the q arm, one (D10S187-D10S209) overlaps with the location of DMBT1 (deleted in malignant brain tumors 1 [10q26.11–26.12]). Although considered a candidate tumor suppressor gene due to its frequent intragenic homozygous deletions and lack of expression in other cancers including glioblastomas (32), its role as a tumor sus-

Fig. 2 For each specimen, the regions of LOH were mapped along the chromosome. Each LOH region was limited to the first maintained locus (vertical bar) on either side of the lost allele (except for the telomeric alleles). The locations of the smallest regions of chromosomal deletion are indicated by a line under the chromosome. The number and grade of tumors having the individual patterns are indicated. B, benign; A, atypical; M, anaplastic/malignant.
pressor gene in malignant gliomas remains controversial (33). This gene has not yet been studied extensively in meningiomas, although one report found no DBMT1 mutations in atypical (0 of 7) and malignant (0 of 7) meningiomas (14). Therefore, for meningiomas, DBMT1 may not be the candidate gene for this region, but further analysis of a larger number of tumors is required.

Other known or candidate tumor suppressor genes on 10q, including PTEN/MMAC1 [10q23.3 (34, 35)], MXII [q24–25 (36, 37)], LGII [10q (24)], and LAPSER1 [10q24.3 (38)], do not colocalize with the chromosomal deletions in meningiomas. Bostrom et al. (39), Wellenreuther et al. (40), and Joachim et al. (41) have reported that mutation of PTEN is not common in meningiomas. MXII, LGII, and LAPSER1 have also not been extensively studied in meningiomas. Although these candidate tumor suppressor genes do not reside in any of the smallest regions of deletion detected by these analyses, some tumors did have LOH at D10S574, the locus closest to these genes. Therefore, without further investigation, we cannot rule out the possibility that loss or mutation by other mechanisms of one of these genes may contribute to the tumorigenesis or progression for a subset of meningiomas.

Our results do suggest that other genes located in the chromosomal deletion regions may be important. To further substantiate this hypothesis, we compared the regions of deletion described in these meningiomas with those reported for other cancers. All four chromosomal deletions described for the meningiomas overlapped or encompassed chromosomal deletions described in one or more other cancers. These commonalities support the hypothesis that candidate tumor suppressor genes contributing to meningiomas likely reside in these regions. If the putative tumor suppressor genes in these regions are the same in meningiomas as these other malignancies, the occurrence of LOH and the loss of these genes may impact meningeal cells earlier in tumor progression than cancer derived from the other cell types.

Our observations further suggest that LOH is an early event in a significant number of benign meningiomas, and therefore, the LOH of individual loci themselves may serve as markers to identify subsets of benign meningiomas or identify those patients having more aggressive tumors and/or those at risk for shorter time to survival or tumor recurrence.

In conclusion, we observed frequent chromosome 10 allelic deletions early in the development of meningiomas, with increased incidence and complexity of allelic loss with progression to higher grade. Finally, a comparison of chromosome 10 allelic deletions found in this study with those identified in other cancer types suggests that continued investigation of these re-
genons is warranted to identify those genes that contribute to meningioma initiation and progression.

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