Prognostic value of allelic losses and telomerase activity in meningiomas

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Object. The goal of this study was to examine allelic losses and telomerase activity in meningiomas to determine whether they could be used to predict disease recurrence.

Methods. To identify predictive markers of recurrence, a cohort of high-grade (24 World Health Organization [WHO] Grade II and six WHO Grade III) and low-grade (21 WHO Grade I) meningiomas was investigated for losses of heterozygosity (LOHs) on chromosomes 1p, 9p, 10q, 14q, and 22q, a deletion of CDKN2A, and telomerase activity. Results of molecular analyses were compared with radiological and histological findings and progression-free survival (PFS). Losses of heterozygosity on chromosomes 22q, 1p, and 10q, as well as telomerase activity were related to the WHO histological grades of the lesions (p < 0.01, p < 10^{-3}, p < 10^{-4}, and p = 0.002, respectively). In the absence of an LOH on 22q, the other alterations were found infrequently. Overall, the number of molecular alterations was closely related to the histological grades of the lesions (p < 10^{-5}). An LOH on 22q occurred much more frequently in convexity or falx (33 [87%] of 38 lesions) than in skull base or spinal meningiomas (four [31%] of 13 lesions) (p < 0.001). The histological grade; Simpson grade; an LOH on chromosome 1p, 9p, or 10q; and telomerase activity were correlated with a shorter PFS time (p < 10^{-4}, p = 0.02, p = 0.000365, p = 0.022, p = 0.00027, and p = 0.000512, respectively).

Conclusions. On the basis of these data the authors suggest that LOH analysis and a telomerase activity assay could be useful to determine molecular predictors of outcome in patients with meningiomas.

KEY WORDS • meningioma • NF2 gene • chromosome • telomerase • prognostic marker

MENINGIOMAS are predominantly slow-growing tumors arising from arachnoidal cells. More than 90% of these lesions correspond to the WHO classification of Grade I and have an excellent prognosis when the tumor is completely removed. Atypical (Grade II) and anaplastic (Grade III) meningiomas represent 6 to 9% of these tumors and are characterized by their tendency to recur, even after complete resection.16 Because histological criteria are subjective, the WHO classification remains controversial,24 and there is a need to identify additional prognostic markers. On the basis of recent data one can infer that molecular predictors of outcome could help the clinician select the most appropriate treatment for individual cases.5

Materials and Methods

Tumors and Blood Samples

All WHO Grade II and III tumors that were consecutively surgically treated between 1996 and 1997 at the Pitié–Salpêtrière Hospital (Paris, France) were collected as well as a subset of WHO Grade I meningiomas, which were surgically treated during the same period and used as a control group. Overall, 59 samples were obtained from the 53 patients enrolled in the study, including five tumors that recurred during the follow-up period. Blood samples were available in 46 patients. The median age of the patients was 58.6 years (range 20–89 years) and the male/female ratio in this group of patients was

Abbreviations used in this paper: LOH = loss of heterozygosity; PFS = progression-free survival; TRAP = telomeric repeat amplification protocol; WHO = World Health Organization.
Forty-five tumors were located at the convexity or falx, 11 at the skull base, and three in the spine. Meningiomas were classified according to current WHO criteria and included 21 Grade I (seven meningothelial, nine mixed, two fibroblastic, two psammomatous, and one angiomatous) tumors; 32 Grade II (29 atypical, two clear cell, one chordoid) tumors; and six Grade III (anaplastic) tumors. Genomic DNA was extracted from both the tumor and the blood of the patient by using a QIAamp tissue kit (Qiagen, Hilden, Germany) and a Nucleon BACC3 DNA extraction kit (Amersham Pharmacia Biotech, Little Chalfont, England), respectively.

Blood and tumor DNA were screened for LOH by using the following polymorphic markers: 1) for chromosome 1 (1pter–1qter) D1S450, D1S2667, D1S234, D1S255, D1S2797, D1S2890, D1S206, D1S2726, D1S484, D1S2878, D1S196, D1S249, D1S213, D1S2800, D1S2785, D1S2842, and D1S2836; 2) for chromosome 9p (9pter–centromere) D9S286, D9S168, D9S1687, D9S156, and D9S1870; 3) for chromosome 10q (centromere–10qter) D10S537, D10S219, D10S1744, D10S541, D10S579, D10S1755, D10S1671, D10S597, D10S209, D10S587, D10S1723, and D10S212; 4) for chromosome 14 (14pter–14qter) D14S261, D14S283, D14S275, D14S70, D14S288, D14S276, D14S63, D14S258, D14S74, D14S68, D14S280, D14S65, D14S985, and D14S292; and 5) for chromosome 22q (centromere–telomere) D22S420, D22S1167, D22S282, D22S283, D22S1123, and D22S1169; and six intragenic microsatellite markers—GaI, CaI, CaII, CaIV, CaV, and GaII—that spanned the NF2 locus.

One of the primers was labeled with Hex, 6-Fam, or Ned fluorochrome (Perkin-Elmer, Norwalk, CT). The samples were examined using an automatic sequencer (Abi Prism 377 DNA sequencer; Perkin-Elmer) and analyzed with the aid of a Gene Scan program (Abi Prism; Perkin-Elmer).

**Search for a Homozygous Deletion of the CDKN2A/ARF Gene**

Tumor DNA was screened for homozygous deletions of the CDKN2A/ARF gene (also known as the p16 gene) multiplex PCR. The D9S196 marker was coamplified with CDKN2A/ARF exon 2, as previously described. The PCR ethidium bromide fluorescence signal response was quantified by scanning.

**Telomerase Activity**

Tumor samples were screened for telomerase activity while using the TRAPeze Telomerase Detection Kit (Intergen Co., Purchase, NY) in the manner described by the manufacturer. The TRAP products were loaded onto a 12.5% polyacrylamide gel. The gels were stained for 20 minutes with SYBR Green I and analyzed under ultraviolet light.

**Statistical Analysis**

The Fisher exact test was used to examine possible associations between molecular and histological characteristics of the tumors. We investigated the prognostic value of the variables for PFS. Progression-free survival times in patients were calculated from surgery until relapse. Survival curves were derived from Kaplan–Meier estimates. The log-rank test was used to examine equality of survival distributions. A Cox proportional hazard regression model analysis was used for quantitative variables. The following variables were
investigated: histological grade, Simpson grade; 32 location; LOHs on chromosomes 22q, 1p, 9p, 10q, and 14q; homozygous deletions of the CDKN2A/ARF gene; and telomerase activity. Two-sided probability values lower than 0.05 were considered significant.

Results

Loss of Heterozygosity

The analysis of LOH reported in Fig. 1 was performed on 51 tumors (46 initial tumors and five recurrent lesions). All recurrent tumors (M42-2, M42-3, M36-2, M36-3, and M75-2) displayed a genetic profile identical to the initial tumor.

An LOH on chromosome 22q was found in 37 tumors. It was significantly related to the histological grades of the lesions (p < 0.01, Fisher exact test) and was present in 10 (48%) of 21 Grade I, 21 (87.5%) of 24 Grade II, and six (100%) of six Grade III lesions (Fig. 2a). In 20 cases, the LOH was restricted to several markers, indicating a partial deletion. One tumor (M88) displayed an LOH limited to two markers of the NF2 region (CaIV and CaV), which was suggestive of an interstitial deletion of the NF2 locus. On the other hand, six tumors (M42, M33, M36, M51, M48, and M35) displayed an LOH of the chromosome 22 markers except for one or several markers of the NF2 locus (Fig. 1). This pattern is suggestive of a homozygous deletion in the NF2 locus, because the retention of heterozygosity is probably explained by the presence of contaminating normal tissue.

An LOH on chromosome 1p was found in 29 tumors. It was highly correlated with the histological grades of the lesions (p < 10^-4, Fisher exact test) and was present in three (14%) of 21 Grade I, 20 (83.3%) of 24 Grade II, and six (100%) of six Grade III meningiomas (Fig. 2b). In 26 (90%) of these tumors there was also an LOH on chromosome 22q (p = 0.0013, Fisher exact test).

An LOH on chromosome 9p was found in 10 tumors: one (7%) of 15 Grade I, seven (29%) of 24 Grade II, and two (33%) of six Grade III (p = 0.19, Fisher exact test; Fig. 2c). Nine of these tumors had an associated LOH on chromosome 22q.

An LOH on chromosome 10q was found in 14 tumors: two (9.5%) of 21 Grade I, six (24%) of 24 Grade II, and six (100%) of six Grade III lesions (p = 0.0001, Fisher exact test; Fig. 2d). In 13 (93%) of these tumors there was an associated LOH on chromosome 22q.

An LOH on chromosome 14q was found in 15 tumors: six (29%) of 21 Grade I, six (25%) of 24 Grade II, and three (50%) of six Grade III lesions (p = 0.05, Fisher exact test; Fig. 2e).

The loss of chromosome 22 was tightly related to the anatomical location of the tumor. Thirty-three (87%) of 38 genotyped convexity–falx meningiomas displayed an LOH compared with four (31%) of 13 genotyped skull base–spinal meningiomas (p < 0.001, Fisher exact test). Similarly, an LOH on 1p was associated with convexity–falx meningiomas (p < 0.01, Fisher exact test), but not an LOH on 9p, 10q, or 14q, or telomerase activity.

Homozygous Deletion of the CDKN2A/ARF Gene

Homozygous deletion of the CDKN2A/ARF gene was detected in six (60%) of 10 tumors with an LOH on 9p and only three (8.5%) of 35 tumors without an LOH on 9p (p < 0.002, Fisher exact test). It was found in zero of 15 Grade I, seven (29%) of 24 Grade II, and two (33%) of six Grade III (p = 0.03, Fisher exact test; Fig. 3).

Telomerase Activity

Because the enzymatic assay was not reliable for testing several old frozen tissues, only 43 samples were available for telomerase activity (Fig. 4). Telomerase was present in two (15%) of 13 benign meningiomas, nine (37.5%) of 24 atypical meningiomas, and six (100%) of six anaplastic meningiomas; the findings were tightly correlated to the histological grades of the lesions (p = 0.002, Fisher exact test; Figs. 2f and 5). Telomerase activity was significantly correlated to an LOH on 1p (p = 0.0003) and 10q (p = 0.0006), but not with LOHs on 22q (p = 0.7), 9p (p = 0.09), or 14q (p = 0.056).

Fig. 2. Bar graphs demonstrating the frequency of chromosome deletions and telomerase activity observed in Grade I, II, and III meningiomas: LOH on 22q (a), LOH on 1p (b), LOH on 9p (c), LOH on 10q (d), LOH on 14q (e), and telomerase activity (f). All alterations, except for LOH on 14q, are correlated to the WHO histological grading system.

Fig. 3. Results of a competitive multiplex PCR of CDKN2A and D9S196 (control). No CDKN2A/ARF is detected in the M33, M36-1, M48, and M87 samples, indicating an homozygous deletion of the locus. In contrast, the M16, M35, and M39 samples display no CDKN2A/ARF deletion.
Correlation Between Molecular Profile and PFS

For all patients, the median PFS was 76 months. Progression-free survival was significantly related to the extent of surgery (76 months for Simpson Grades I and II, and 39 months for Simpson Grades III through V; \( p = 0.02 \), log-rank test) and the histological grade (Grade I, no recurrences observed; Grade II, median PFS 61 months; Grade III, median PFS 20 months; \( p < 10^{-4} \), log-rank test; Fig. 6a and b). Progression-free survival was not related to tumor location (median PFS 64 months for skull base–spine and 76 months for convexity–falx regions), patient sex, or age.

The median PFS was 61 months for patients with tumors with an LOH on 1p, whereas no recurrence was observed when patients were without this particular LOH (\( p = 0.000365 \), log rank test; Fig. 7a). The median PFS was 26.5 months for patients with tumors with an LOH on 9p and greater than 65 months for patients without this particular LOH (\( p = 0.022 \), log-rank test; Fig. 7b); however, PFS was not related to the homozygous deletion of \( CDKN2A/ARF \).

The median PFS was 30 months for patients with tumors with an LOH on 10q and greater than 70 months for patients without this particular LOH (\( p = 0.00027 \), log-rank test; Fig. 7c). The median PFS was not significantly related to an LOH on 14q and another at 22q (\( p = 0.24 \) and 0.15, respectively, log-rank test; Fig. 7d and e). The median PFS was 30 months for patients with tumors with telomerase activity and 64 months for those without it (\( p = 0.000512 \), log-rank test; Fig. 7f).

As shown on Figs. 2 and 5, the number of molecular alterations increased with the grade of meningioma. Two or more alterations were found in only five of 21 Grade I tumors, but in 21 of 24 Grade II and six of six Grade III meningiomas (\( p < 10^{-4} \), chi-square test). The number of alterations was also inversely correlated with the duration of PFS (\( p = 0.0012 \), Cox proportional hazard regression model).

Discussion

The loss of chromosome 22, which we found in 37 (73%)
of 51 cases, is the most frequent genetic alteration in meningiomas. It is usually associated with NF2 inactivation.28,37 No mutation screen was performed in the present study, but the involvement of NF2 gene is supported by the fact that five of the tumors with an LOH on chromosome 22 exhibited a pattern suggestive of homozygous deletion within the NF2 locus, and another tumor displayed an interstitial heterozygous deletion limited to the NF2 region (Fig. 1). The LOH on 22q is common in Grade I meningiomas and is, therefore, considered to be an early event that is not involved in progression to malignancy. Nevertheless, we found a statistical relationship between an LOH on 22q and histological grading, in agreement with two earlier studies.10,29 Although it did not reach significance, the LOH on 22q was found more frequently in tumors that recurred during the follow-up period. Moreover, there was a correlation between the LOH on 22q and the presence of other genetic alterations. Thus, when no alteration of chromosome 22 was found, other genetic alterations were uncommon. These results indicate that the LOH on 22q and NF2 inactivation may facilitate the occurrence of additional chromosomal aberrations involved in tumor progression.36

The tumorigenesis of meningiomas without an LOH on 22q is still unclear and does not involve NF2 inactivation.7,28,37 These meningiomas probably involve another still unknown mechanism. In this perspective, it is interesting to note that skull base–spinal meningiomas and convexity–falx tumors present distinct molecular patterns. Indeed, a tight correlation was found between an LOH on 22q and the convexity–falx location, whereas most skull base or spinal meningiomas did not involve an LOH on 22q or NF2 inactivation, indicating distinct molecular pathways depending on the tumor location.

Deletion mapping showed two or more deleted regions on 1p, 9p, 10q, and 14q (Fig. 1). The tumor suppressor

![Figure 6](image.png)

**Fig. 6.** Graphs demonstrating PFS as a function of histological grading (a) and the extent of tumor removal (Simpson Grades I or II compared with Simpson Grade > II). p < 10^{-4} (a) and p = 0.02 (b).

![Figure 7](image.png)

**Fig. 7.** Graphs showing PFS as a function of LOHs on chromosomes 1p (a: p = 0.0003), 9p (b: p = 0.02), 10q (c: p = 0.0003) 14q (d: p = 0.24), and 22q (e: p = 0.15); and as a function of telomerase activity (f: p = 0.0005).
genes involved in these regions are not known, except for CDKN1A/ARF, which is located on 9p21 and encodes both p16, an inhibitor of the cycline-dependant kinase, and p14, an inhibitor of p53 degradation. Both the p16 and p14 genes are frequently inactivated by homozygous deletion. Therefore, the close association between 9p loss and CDKN1A/ARF homozygous deletion is not surprising. The 10q23.3 contains the PTEN/MMAC1 gene, which is unlikely to be a critical gene in meningioma progression because only four mutations have been reported so far.2,8,25 On the 1p chromosome, deletion mapping studies have identified at least three different regions1,4,22,33 and several candidate genes such as p73 on 1p36 and p18 and hRad54 on 1p32 have been excluded.6,16,17,19 Loss of heterozygosity studies of chromosome 14q have identified several partial deletions but there is still no clearly identified consensus region.15,20,31,34

Our data confirm that LOHs on chromosomes 1p and 10q are related to the histological grade, whereas we could not demonstrate a role for LOHs on chromosomes 9p and 14q. This may possibly be due to a lack of statistical power because a recent compilation of the available literature by our group showed that losses of 22q, 1p, 9p, 10q, and 14q appeared to be involved in tumor progression.6 Data concerning the recurrence rate of meningiomas are scarce and, except for the 1p deletion, molecular profiles have not been correlated to PFS.9 It is therefore of interest to note that LOHs on 1p, 10q, and 9p were significantly related to the recurrence rate. We also found a very strong correlation between telomerase reactivation and the risk of recurrence (p = 0.00027), in agreement with findings of previous reports.13,30

Taken together, these results indicate that molecular screening for telomerase activity and LOHs on chromosomes 1p, 9p, and 10q could be useful in the clinical setting to complete the WHO grading of meningiomas and to determine the most appropriate treatment of these lesions.

References

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