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ARTICLE

Chromosome 1p36 and 22qter Deletions in Paraffin Block Sections of Intracranial Meningiomas

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Meningiomas are the most frequent benign tumors of the intracranial cavity. The classification and underlying pathogenetic mechanisms have been reported to be investigated by both pathological and genetic methods. In this study, we aimed to detect 1p36 and 22qter deletions by fluorescence in situ hybridization (FISH) in archival materials of 50 intracranial meningioma patients. The clinical material consisted of paraffin-embedded tissue sections from 50 patients who were surgically treated and had histopathologic diagnosis of an intracranial meningioma. We observed 1p36 deletion in 23/50 (46%) and 22qter deletion in 33/50 (66%) patients. In addition, we observed 22qter deletion in 26/36 (72.2%) patients with meningothelial meningioma. This finding implies that 22qter deletion might play an important role in the pathogenesis of meningothelial meningioma. On the other hand, no alterations were documented in the frequency of these chromosomal alterations according to the grade of meningiomas, suggesting that malignant progression of these tumors depends on other, more relevant, genetic changes. (Pathology Oncology Research Vol 11, No 4, 224–228)

Key words: meningioma, 22q, 1p36, fluorescence in situ hybridization, grading

Introduction

Meningiomas are tumors that arise from cells of the meningeal coverings of the brain and spinal cord. They account for 15-25% of all central nervous system tumors and their annual incidence has been estimated to be about 6 per 100,000 individuals.[22] Although the majority of these tumors are histologically benign, in studies with long-term follow-up, meningiomas have shown significant rates of recurrence, morbidity and mortality. Clinical progress is difficult to predict exactly.[22] The World Health Organization (WHO) classifies meningiomas into three histologic grades, grade I (benign), grade II (atypical), grade III (anaplastic) in accordance with the clinical prognosis. Recurrence is a complication that influences the patient's clinical course.[9]

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Meningiomas are among the first cytogenetically studied solid neoplasms. Although the molecular alterations associated with progression to grade II and grade III are still poorly understood at present, the most common chromosome aberration in benign meningiomas is the loss of chromosome 22. Previous reports demonstrated loss of chromosome 22 in 40 to 70% of all meningiomas.[1,4,22] The second most common aberration in meningioma, the deletion of 1p (partial or complete loss of the short arm) appears to characterize an early step in the progressive loss of chromosomal material in histologically atypical and anaplastic meningiomas.[18,22]

Numerical deviations of chromosome pairs 1, 6, 7, 8, 9, 10, 13, 14, 15, 17, 22, X and Y comprise other common chromosomal abnormalities found in meningiomas. Molecular genetic studies suggested that allelic loss at 1p, 9q, 10q and 14q appear to be associated with meningioma progression.[6-8,10,11,13,15,18-20,22]

In this study, we aimed to investigate the incidence of deletion of chromosome 22qter and 1p36 regions by FISH in paraffin block samples from 50 patients with different types of sporadic meningiomas.

Materials and Methods

Patients

Paraffin-embedded tissue sections of meningiomas from 50 patients who had undergone surgery at the Department of Neurosurgery between 1999 and 2002 were included in the study. The study was approved by the Institutional Clinical Research Ethics Committee. Of the patients, 37 were female and 13 were male with a mean age of 54.78 (SD 13.02). All cases were diagnosed to have intracranial tumors. In each case, the histopathological diagnosis of meningioma had been established by standard light-microscopic evaluation of sections stained with hematoxylin and eosin. The sections of each case were re-evaluated using the World Health Organization (WHO) 2000 criteria for brain tumor classification (ÖÖ, BD).[14]

FISH studies

Interphase FISH was performed on 3 to 4 μm thick paraffin-embedded tissue sections obtained from all patients for the detection of 1p36 and 22qter deletions. The tissue sections were placed on poly-L-lysine-coated slides, and deparaffinized with a slightly modified procedure from previously described.[21] After deparaffinization at 56°C overnight, xylene dehydration in alcohol, pepsin digestion and fixation of slides, denaturation and hybridization were carried out according to the manufacturer's information for each probe in HyBrite denaturation/hybridization system for FISH (Vysis Inc., IL). Chromosome 1p36 midisatellite probe spectrum green (Q-BIOgene) and chromosome 22qter spectrum red (Q-BIOgene ptel 22q-R) telomere-specific DNA probes were used. Signals were counted in at least 200 cells for both 1p36 and 22qter using the recommended filters (Nikon E600, Kingston, UK), and imaging was performed with an image analysis system (Cytovision, Applied Imaging, UK). Cut-off scores for the two probes were determined as 1% and 2.5% for 1p36 and 22qter regions respectively. For this, we analyzed tissue sections of 5 gastric ulcer patients that were hybridized for H. pylori detection.

Results

Paraffin block sections from 50 patients with different types of sporadic meningiomas were included in our study. According to the WHO classification,[9] 80% of the tumors were classified as grade I (including 2 fibroblastic, 1 psammomatous, 1 transitional and 36 meningothelial), 12% as grade II (or atypical), and 8% as grade III (or anaplastic) meningiomas. Tumor localizations and histological grades are shown in *Table 1*.

Thirty-six (72%) samples displayed deletions for at least one of the two chromosome regions analyzed. Deletion of 22qter was observed in 33 (66%) and deletion of 1p36 was observed in 23 (46%) of the patients (*Table 2*). Neither any signal augmentation nor decrease suggesting structural abnormalities of the relevant regions was observed during the examinations.

Of the 40 patients with grade I meningiomas, 22qter deletions were observed in 28 (70%), 1p36 deletions were observed in 18 (45%), and 10 (25%) resulted in normal signal for each of the probes (*Table 2*). 22qter deletion was observed in transitional and psammomatous meningioma (1 case each), however, we found that the 2 fibroblastic meningioma cases were normal (*Table 1*). 1p36 deletion was present in the psammomatous and fibroblastic meningioma cases, however, we did not observe 1p36 deletions in transitional meningioma (1 case).

We observed recurrence in two patients with meningothelial meningiomas. Recurrence occurred in one patient two years later, and in the other one year later (*Table 1*). Both of them were normal for 22qter and 1p36 deletions (*Table 1*, Patient no. 15 and 30).

Of the 6 atypical meningioma patients, 22qter deletion was observed in 4 and 1p36 deletion was observed in 3, while in 3 patients there was deletion of both regions. Both probes resulted in normal signal in 2 patients (*Table 1*).

When we have stratified our tumor samples according to their grade to low- (grade I) or high-grade (II-III) tumors, we have found that the frequency of neither of the chromosomal alterations (1p36 and/or 22qter) was different (*Table 2*), suggesting that the studied genetic alterations may be irrelevant concerning the malignant progression.

Discussion

Although generally considered to be benign, meningiomas may recur and often invade the adjacent skull or soft tissue. Both atypical and malignant meningiomas are associated with significant morbidity and mortality. Ki-67 has been reported to be correlated with aggressive histology and/or biologic behavior in meningiomas.[17] However, in our study the standard deviations of Ki-67 positivity in the three groups seemed to be overlapping (data not shown).

Tumorigenesis of meningioma has been associated with changes affecting chromosome 22, but other genetic alterations have also been implicated. Molecular cytogenetic and molecular genetic studies have investigated the association between the allelic losses at genomic regions other than chromosome 22 and tumor progression, suggesting that loss of heterozygosity (LOH) at 1p, 9q, 10q and 14q appears repeatedly associated with atypical and anaplastic tumors. However, specific chromosomal aberrations have not been found for the different clinical characteristics or the wide variety of histologic subtypes. [3,8,10,15,18,22]

Cytogenetic studies have shown monosomy or deletion of chromosome 22 as the most common chromosomal

Table 1. Clinical findings, pathological classification, and FISH results of the patients included in the study

Case no.	*Age/ sex*	*Pathology*	*Grade*	*Region*	*1p36*	*22q*	*Recurrence*
1	40/F	Transitional	I	Frontoparietal	N	Del	-
2	65/F	Psammomatous	I	Temporal	Del	Del	-
3	50/F	Fibroblastic	I	Cerebellopontin	Del	N	-
4	59/F	Fibroblastic	I	Frontal	Del	N	-
5	33/F	Meningothelial	I	Frontal	N	N	-
6	62/F	Meningothelial	I	Frontal	N	Del	-
7	53/F	Meningothelial	I	Frontal	Del	Del	-
8	38/F	Meningothelial	I	Frontal	Del	Del	-
9	52/F	Meningothelial	I	Frontal	Del	Del	-
10	35/M	Meningothelial	I	Frontal	Del	Del	-
11	59/F	Meningothelial	I	Frontal	Del	Del	-
12	61/F	Meningothelial	I	Frontal	N	Del	-
13	60/F	Meningothelial	I	Frontal	N	Del	-
14	68/F	Meningothelial	I	Frontal	N	Del	-
15	57/F	Meningothelial	I	Frontal	N	N	+ (2 years later)
16	72/M	Meningothelial	I	Occipital	Del	Del	Exitus
17	66/F	Meningothelial	I	Temporal	N	N	-
18	66/F	Meningothelial	I	Temporal	N	N	-
19	35/F	Meningothelial	I	Temporal	N	Del	-
20	72/M	Meningothelial	I	Temporal	N	Del	-
21	51/F	Meningothelial	I	Temporal	Del	Del	-
22	48/F	Meningothelial	I	Parietal	N	Del	-
23	70/M	Meningothelial	I	Parietal	N	Del	-
24	30/M	Meningothelial	I	Parietal	N	N	-
25	69/F	Meningothelial	I	Falx	Del	Del	-
26	41/F	Meningothelial	I	Falx	N	Del	-
27	68/F	Meningothelial	I	Parafalx	Del	Del	-
28	41/M	Meningothelial	I	Cerebellum	N	N	-
29	43/F	Meningothelial	I	Cerebellum	Del	Del	-
30	40/F	Meningothelial	I	Cerebellum	N	N	+ (1 year later)
31	60/F	Meningothelial	I	Orbital	Del	Del	-
32	50/F	Meningothelial	I	Tuberculum sellae	N	Del	-
33	76/F	Meningothelial	I	Parasellar	N	N	-
34	66/F	Meningothelial	I	Sphenoid	Del	Del	-
35	72/F	Meningothelial	I	Sphenoid	N	N	-
36	37/F	Meningothelial	I	Sphenoid	Del	Del	-
37	49/F	Meningothelial	I	Parasagittal	N	Del	-
38	41/F	Meningothelial	I	Parasagittal	Del	Del	-
39	62/M	Meningothelial	I	Lateral ventricle	Del	Del	-
40	36/M	Meningothelial	I	Lateral ventricle	N	N	-
41	65/F	Atypical	II	Frontotemporal	Del	Del	-
42	45/F	Atypical	II	Frontal	Del	Del	-
43	60/F	Atypical	II	Frontal	N	N	-
44	70/F	Atypical	II	Frontotemporal	N	N	-
45	72/M	Atypical	II	Frontal	Del	Del	-
46	50/M	Atypical	II	Temporal	N	Del	-
47	40/F	Anaplastic	III	Anterior fossa	Del	Del	-
48	63/M	Anaplastic	III	Frontoparietal	N	N	-
49	50/M	Anaplastic	III	Frontal	N	N	-
50	71/M	Anaplastic	III	Frontal	Del	N	-

N: no deletion, Del: deletion

abnormality in meningiomas.[3-5,10,20,22] In the development of multiple meningiomas, the main target for deletion of 22q is the NF2 gene located at 22q12 and 22q13 locus. A putative meningioma locus was proposed between 22q12.3-qter in previous reports.[3-5] In a study by Baström et al., NF2 heterozygosity has been found to be particularly frequent in fibroblastic and transitional but less frequent in meningothelial type among grade I meningiomas.[2] In our study evaluating 22qter deletion frequency among grade I meningiomas, we saw that 22qter deletion was highly frequent in meningothelial meningioma (72.2%), indicating that 22qter deletion might have an important role in the pathogenesis of this tumor type. We also detected the deletion in psammomatous and transitional type meningioma cases. On the other hand, fibroblastic meningiomas seemed to result in normal signals.

Table 2. Frequency of chromosomal alterations in meningiomas according to the grade

	N/N (%)	*1p36 (%)*	*22q (%)*	*1p36/22q (%)*
Total (n=50)	14/50 (28)	23/50 (46)	33/50 (66)	20/50 (40)
Grade I (n=40)	10/40 (25)	18/40 (45)	28/40 (70)	16/40 (40)
Grade II-III (n=10)	4/10 (40)	5/10 (50)	5/10 (50)	4/10 (40)

N/N: neither 1p36 nor 22q alteration

The deletion rate of 22qter was higher in benign and atypical meningiomas than in anaplastic meningioma (70% and 66.7% respectively, vs. 25%). Although there was discrepancy between the numbers of patients examined, when they are grouped according to tumor grades, this finding suggested the association of the benign process with 22qter deletion.

The short arm of chromosome 1 represents the second most frequently deleted chromosomal arm after 22q in meningiomas. Although 22q deletion was found frequently in benign meningiomas, allelic loss on 1p was found predominantly in atypical and anaplastic meningiomas. According to data in the literature, the frequency of 1p loss in meningiomas significantly increases with advancing grade of malignancy, indicating that 1p contains a tumor suppressor gene involved in meningioma progression.[2]

We observed 1p36 deletion in 45% of our grade I patients and, although the number of cases was low, 50% in grade II and III samples. Our results do not seem to support previous reports.[2,7,8,10,11,13,16,18,19,22] The significant association between LOH on 1p and atypical or anaplastic meningiomas also has a possible role for this aberration as a prognostic parameter for meningioma patients. [2,12,16,17] Previous reports have demonstrated a high risk of recurrence of 1p-deleted tumors compared to nondeleted ones.[12,13,16] A small fraction of tumors in our series showed recurrence with the lack 1p36 deletion. This situation may be explained by deletion of another region on chromosome 1p or genetic aberrations other than 1p36 deletion. Our study suggested that investigations of only 22q and 1p deletions are insufficient as follow-up for patients with meningiomas.

Murakami et al. suggested that 1p analysis is needed in meningioma cases and FISH is the more suitable method, since it is less time consuming and requires neither normal individual nor tumor as LOH and comparative genomic hybridization analysis.[16] We applied FISH on paraffin block sections for previously diagnosed patients. The method is also useful for fresh frozen sections obtained during surgery. It could be used to be beneficial to the surgeon and the patient since the operation could be directed according to the FISH result.

Another point to be stated about the association of our findings on meningiomas is that we studied fixed tissue samples. Association of FISH with conventional tissue culture methods and cytogenetic analysis of fresh samples will enable the whole chromosomes to be screened and will not be limited to the single region. Molecular methods could be used in detecting survival following cytogenetic methods, and they could be more patient-specific.

The above findings do not let us draw a direct conclusion about a positive correlation between the biologic nature of meningioma and chromosomal aberrations, although we cannot exclude such correlation either. A conclusion drawn from our study could be that 22qter and 1p36 deletion screening alone is insufficient to assume future follow-up for patients with meningiomas. The high frequency of high-grade meningioma cases without the studied 1p/22q alterations suggests that other genetic alterations could be relevant concerning the malignant progression of meningiomas.

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