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Comparative genomic hybridization analysis of genomic alterations in benign, atypical and anaplastic meningiomas

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Abstract

Background : Meningiomas are common tumors of the central nervous system. Although most are benign tumors, approximately 10% show a histologic progression to a higher malignancy grade similar to atypical (GII) and anaplastic (GIII) meningiomas. Monosomy 22q12 is the most frequent genetic alteration detected in these tumors, but failure of detection of 22q mutations in about 40% of tumors which are indistinguishable from meningiomas with 22q deletions with respect to clinical and histopathologic features, makes it apparent that an alternative mechanism is responsible for the initiation of meningioma. Moreover, little is known about genetic alterations during malignant progression of meningioma.

Purpose : In order to determine the genetic pathways underlying the development of meningioma, 15 benign (WHO grade I), 7 atypical (WHO grade II) and 3 anaplastic (WHO grade III), sporadic meningiomas were screened by Comparative Genomic Hybridization (CGH).

Results : Statistical analysis revealed a significant correlation between the number of chromosomal imbalances and the tumor grade ; the numbers of total alterations detected per tumor were 2.20 (2.24 for GI, 10.00 (1.17 for GII and 14.66 (1.15 for GIII. The most frequent abnormality seen in benign tumors was loss on 22q (47%). The second alteration was 1p deletion (33%) and this abnormality was also the common aberration in three tumors without CGH detected 22q deletion. In GII, aberrations most commonly identified were losses on 1p (6/7 cases), 22q (5/7 cases), 10q (4/7 cases), 14q and 18q (3/7 cases) as well as gains on 15q and 17q (3/7 cases). In GIII, genomic loss on 1p was the most commonly observed abnormality (3/3). Losses on 9p,10q,14q,15q, 18q and 22q as well as gains on 12q, 15q and 18p were the other genomic alterations detected by CGH. Combined 1p/14q deletions were encountered in 2/15 benign, 3/7 atypical and 2/3 anaplastic meningiomas. By CGH, DNA sequences on 17q21-qter were seen to be amplified in 1/7 GII and 2/3 GIII, whereas highly amplified DNA sequences on 12q13-qter, 20q and 22q11-q12 were seen in one GII, two GII/one GIII, and one GIII, respectively.

Conclusion : It was concluded that chromosomal deletion from 1p could play a major role in the initiation and progression of meningiomas and that 1p/14q deletions could be a primary focus of further detailed assessment of tumour genesis.

Key words **:** Meningiomas ; Comparative Genomic Hybridization (CGH) ; deletions ; amplifications ; tumor progression.

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Introduction

Meningiomas are common tumors of the central nervous system. They account for 15 to 25% of all intracranial and intraspinal tumors and their annual incidence has been estimated to be about 6 per 100.000 population (4, 16, 31). Most meningiomas are slowly-growing benign tumors and correspond histologically to grade I of the World Health Organization (WHO) classification of tumors of the central nervous system (4, 18, 28, 41). Increased cellularity, increased mitotic activity, high nuclear to cytoplasmic ratio and foci of necrosis are histologically characterized features of about 6-8% of meningiomas (WHO grade II) and are associated with a high likelihood of recurrence. Anaplastic (malignant) meningiomas of WHO grade III are rare tumors (2-3% of meningiomas), characterized by histological signs of frank malignancy, and are associated with a high risk for local recurrence and a poor diagnosis (4, 9, 18, 36, 41).

Meningiomas were among the first solid tumors studied by cytogenetics. Of the cytogenetically analyzed meningiomas, about 75% had chromosomal abnormalities. Loss of chromosome 22 is the most commonly encountered aberration and has been detected in between 40% and 70% of cases (4, 8, 14, 24, 37, 40, 43, 44). Recent studies have showed that most sporadic meningiomas with 22q deletion carry mutations in the neurofibromatosis type 2 gene (NF2), a tumor suppressor gene located at 22q12 locus (33, 39). The presence of mutations and/or loss of NF2 gene in meningiomas of all malignancy grades indicates that inactivation of this tumor suppressor gene represents an early genetic event in the pathogenesis of meningiomas (34, 42). However, cytogenetic and molecular

investigations have failed to detect 22q12 mutations in about 40% of all tumors. These tumors are indistinguishable, with respect to clinical and histopathologic features, from meningiomas with 22q mutations, but the presence of nonrandom chromosome aberrations in these tumors makes it apparent that an alternative mechanism (or mechanisms) is responsible for the development of the part without 22q mutation (4, 7, 16). The molecular alterations associated with the progression to GII and GIII are poorly understood. Cytogenetic studies strongly suggest that GII and GIII frequently show complex numerical and structural aberrations (17, 29, 36, 40, 41). In a comprehensive overview of the genomic alterations in benign, atypical and anaplastic meningiomas, Weber *et al*. (41) report a marked accumulation of genomic alterations with increasing malignancy grade and they propose a model for the genetic alterations associated with meningioma progression. In addition to chromosome 22q mutations, the results of a few other reports show losses and/or gains and/or amplifications of chromosomes 1, 2, 6, 9, 10, 12, 14, 15, 17, 19 and 20. These chromosomes also carry cancer-related genes, genetic changes in which are implicated in the malignant progression of meningioma (9, 16, 20, 29, 41).

In the present study, we used comparative genomic hybridization (CGH) to investigate the genomic alterations in sporadic meningiomas of all malignancy grades on a series of 25 tumors.

Materials and methods

Tumor samples : We studied 25 meningiomas from 14 females and 11 males. The ratio of females to males was of 1.27/1. The median age at diagnosis was 49.84 ± 10.60 years (23-65 years. Histopathologic evaluation of these samples showed an estimated tumor cell content of 80% of the tissue volume. The tumors were classified previously according to the World Health Organisation (WHO) histological classification ; 15 tumors were in grade I, 7 were classified as atypical (grade II) and 3 were malignant (grade III). All tumor samples were available as formalin-fixed and paraffin embedded material. A 15 µm section from each formalin-fixed paraffin embedded tissue was collected into eppendorf tubes and resuspended in 100 µl of extraction buffer (100 mM/l NaCl, 100 mM/l Tris-HCl, pH 7.6, 25 mM/l EDTA, 0.5% SDS) and 2 ml of Proteinase K (20 mg/ml) and incubated at 50°C for 24 h. At the end of this period, an additional 2 ml of Proteinase K (20 mg/ml) was added and incubated at the same temperature for up to 3 days. The digested samples were then incubated at 95°C for 10 min to inactivate the Proteinase K. High molecular weight DNA was then extracted with phenol-chloroform and precipitated with ethanol.

A paired blood sample was drawn from each patient and used as a control. The blood samples of the cases and the reference DNA used in CGH analysis were isolated from peripheral blood of a karyotypically normal male. High-molecular weight DNA from all lymphocytes was extracted using standard methods.

Degenerated Oligo-nucleotide Primed –PCR (DOP-PCR)

DOP-PCR was used to amplify the DNA uniformly as previously described (38) by using the primer 6MW (5' CCGACTCGAGNNNNNNATGTGG-3'). The DOP-PCR products were labelled through a secondary DOP-PCR reaction by incorporation of biotin-16-dUTP (Sigma) into tumor DNA and digoxigenin – 11- dUTP (Roche) into reference DNA. The PCR conditions in the second reaction was as follows : The mixture was heated to 93°C for 8 minutes, followed by 25 cycles of 93°C 1 min, 56°C 1 min, 72°C 2 minutes and a final incubation at 72°C for 7 minutes. After PCR labelling, DNA size was brought to below 500bp using a controlled DNase I digestion for 10 minutes at room temperature and the reaction was stopped by 2-3 min incubation at 95°C. The test and reference DNA samples, labelled in different colors, were precipitated together in the presence of 40mg unlabelled Cot1 DNA (Gibco BRL), 1/10 volume of 3M Na-acetate (Sigma) and 2 volumes of ice-cold ethanol. The resultant DNA pellet was dried before being resuspended in 12 µl hybridization mixture (50% formamide/ 2SSC), denaturated at 70°C for 10 minutes and preannealed for 1 hr at 37°C.

Preperation of Metaphase Slides and Denaturation

Diploid metaphase spreads were obtained from peripheral blood lymphocyte cultures of normal male donors. Slide preparation was optimized to minimize residual cytoplasm and generate well-spread metaphases as described by Henegariu *et al*. (15). Metaphase slides were denaturated in 70% formamide/2 × SSC pH 7 at 73°C for 3-5 minutes, dehydrated in ice-cold 70%, 90% and 100% ethanol and air dried.

Hybridization of the resultant probe mixture with the normal male metaphase spreads proceeded for 72 hrs at 37°C in a humidified chamber. After post-hybridization stringent washes, biotin and digoxigenin labelled DNAs were treated with FITC conjugated avidin (Vector) and rhodamine conjugated antidigoxigenin (Roche) mixture (antibodies were stored as 1mg/ml stock solution and diluted 1:100 in 4 × SSC for use). The slides were stained with DAPI and mounted with antifade solution.

Slides were viewed on a Zeiss Axiophot fluorescence microscope and images were captured and stored using a Photometrics CCD camera with MacProbe version 4.11 software (Perceptive Scientific International, UK). A minimum of 10 high quality metaphase spreads were analysed from each hybridization, and average green-to-red fluorescence intensity ratio profiles were generated for each chromosome. CGH ratio values of 1.25 and 0.85 were used as the upper and lower thresholds, respectively, for the identification of chromosomal imbalances. Any shifts in the average green-to-red ratio value above 1.25 were considered as an indication of gains of chromosomal material and any shifts below 0.85 as an indication of losses.

Results

The sex, age, clinical data, histopathological diagnosis and genomic alterations detected by CGH analysis in the 25 sporadic meningiomas are given in Table 1. The mean age of the patients with benign meningiomas was 46.06 ± 9.98 years, of patients with atypical meningiomas, 54.28 ± 11.08, and for patients with malignant meningiomas, 58.33 ± 0.57 years.

In 12 patients (48%) the tumors were located over the cerebral convexities, while in 6 (24%) they were parasagittal, and in 2 (8%) they were located along the sphenoid wing and pontocerebellar angle. Most of the malignant tumors (66%) were located over the cerebral convexities. Meningotheliomatous and transitional meningiomas accounted for 66% of the tumors, followed by the psammomatous (20%) and the fibroblastic (14%) subtypes.

The patients with benign meningioma were followed up for 3 months to 7 years (mean follow up 2.4 years) and no cases of recurrence were encountered. Clinical and radiological recurrence was observed in 2 of 7 patients with atypical meningiomas. By contrast, all patients with malignant

Table 1

Summary of histopathologic and CGH results of 25 meningiomas

							Comparative genomic hybridization		
Case No	Sex	Age	Location	Recurrence	Pathologic Diagnosis	Grade	Losses	Gains	Amplifications
MN-1	F	32	Parasagittal	-	Transitional	I	1p, 12p,18q, 22q	2, 5q	
MN-2	M	59	Convexity	-	Meningotheliomatous	I	22		
MN- 3	F	46	Tentorium	-	Transitional	I	1p, 14q	3, 12, 20	
MN-4	F	45	Sphenoid	-	Psommomatous	I	-		
MN-5	F	46	Parasagittal	-	Meningotheliomatous	I	1p, 3p, 5p		
MN-6	F	23	Parasagittal	-	Meningotheliomatous	I	14q, 18q, 22	3p21-pter, 5, 13q	
MN-7	M	58	Sphenenoid	-	Transitional	I	22q	2, 20	
MN-8	F	44	Cerebello-pontin angle	-	Fibroblastic	I	22q		
MN-9	F	47	Convexity	-	Meningotheliomatous	I	22q		
MN-10	F	37	Convexity	-	Transitional	I	-		
MN-11	F	49	Parasagittal	-	Psommomatous	I	1p, 22q, 14q	17q	
MN-12	F	56	Convexity	-	Fibroblastic	I	1p	6, 12q	
MN-13	F	41	Olfactory groove	-	Meningotheliomatous	I	-		
MN-14	M	52	Convexity	-	Psommomatous	I	-		
MN-15	M	56	Convexity	-	Transitional	I	-		
MN-16	M	65	Convexity		Atypical	II	1p, 10q, 14q, 18q, 22q	2p, 6p, 15q, 16p	
MN-17	M	49	Cerebello-pontin angle	1	Atypical	II	1p, 2p16-pter, 3p12-p21, 10q, 14q, 22q	1q, 9q34, 12q, 15q22-25, 16p, 17q21-qter	20q
MN-18	M	47	Convexity	-	Atypical	II	1p22-pter, 2, 5, 7, 8, X	2p, 3, 4, 9p, 15q, 17q21-qter, 21q	
MN-19	F	59	Parasagittal	-	Atypical	II	1p, 10q, 14, 18q,22q		12q13-qter, 17q21-qter
MN-20	M	60	Cerebellar convexity	-	Atypical	II	1p, 2p, 3q, 9p, 11q, 13q, 14q	6, 10, 17q21-qter, Y	20q
MN-21	M	35	Convexity	-	Atypical	II	9p,10q,22q	5p,17q	-
MN-22	M	65	Convexity	1	Atypical	II	1p, 5q, 8q, 10q, 11p, 16p, 18q, 22q	1,11q, 12q	
MN-23	F	59	Convexity	2	Anaplastic	III	1p, 3q, 4p15-pter, 9p24, 11p, 14q12-q21, 15q, 16q, 22q	12q22-qter, 13q31-qter, 17q21-qter, 18p	22q11-q12
MN-24	M	58	Parasagittal	3	Anaplastic	III	1p, 4q21-q31, 6q16-qter, 7p, 10q, 14q12-q21, 15q11-q21, 18q	2, 3, 9q, 10p, 15q23-qter, 18p	17q21-qter, 20q
MN-25	F	58	Parasagittal	1	Anaplastic	III	1p, 9p22-p23, 10q, 18q, 22q	1q, 6p, 11q, 12q, 15q, 17p, 20q, 21q	17q21-qter

meningioma had a recurrence. However, no relation was seen between the genomic abnormality and tumor location and/or tumor size.

The CGH profiles of the cases MN-1 (GI), MN-17 (GII) and MN-24 (GIII) are given in Figures 1, 2 and 3, respectively.

Benign Meningiomas : Comparative Genomic Hybridization identified genomic abnormalities in 10 (67%) of 15 benign tumors. The lymphocytes of each case were also analysed by the CGH as controls and no chromosomal abnormality was seen. Complete or partial loss of chromosome 22 was the most commonly seen abnormality with the 47% prevalence (7 of 15 tumors). Complete or partial monosomy 22 as the sole abnormality was present in 20% (3 of 15 samples) of the GI tumors, whereas the remaining 5 tumors with chromosome 22 abnormality had other chromosome aberrations. Of these 7 tumors, 2 had complete chromosome 22 loss, but chromosome 22q12 region deletion was detected in 5 samples. Other nonrandom genomic alteration (> 2 cases) was loss on 1p, observed in 5 cases (33%). In three tumors no chromosome 22 aberration was detected but the common abnormality in these samples was loss on 1p.

Atypical Meningiomas : Genomic alterations were seen in all tumors. The most commonly found aberration (6 tumors) was loss of 1p. Other changes detected by CGH in more than two cases were losses on 22q and 10q in 5 cases, 14q and 18q (3 cases) as well as gains on 17q in 4 and 15q in 3 cases. The prevalence of chromosome 22 abnormality was lower than chromosome 1p aberration. Two tumors without CGH-detected 22q loss demonstrated complex abnormalities, including gains on chromosomes 3,4,6,10, and Y.

By CGH, high level amplifications were identified on 20q (MN-17 and MN-20) as well as 17q21-qter (MN- 19) and 12q13-qter (MN-19) in one case each.

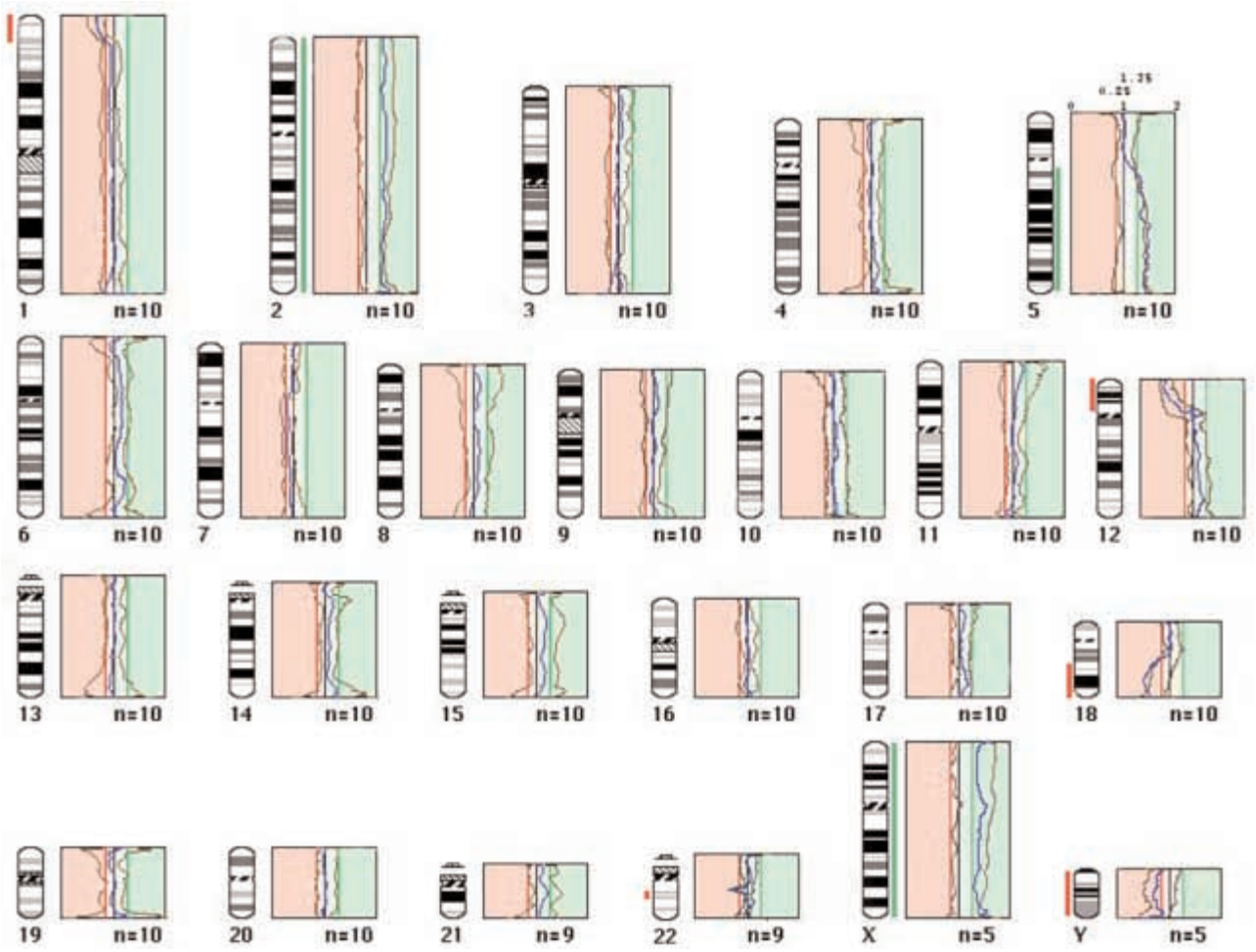

FIG. 1. — CGH profiles of the case MN-1 WHO Grade I.

Ratio profiles represent the average fluorescence ratio obtained from at least ten metaphase spreads. Right and left lines indicate the 1.25 and 0.85 ratio values, respectively. Blue line indicates balanced state of chromosome material in tumor compared to reference DNA. Deviations below 0.85 indicate loss of chromosomal segments and those above 1.25 indicate gains. A vertical red line on the left side of a chromosome represents regions of lost genetic material whereas green line on the right side indicate increased genetic material. The patient was female. CGH with female tumor DNA (XX) and normal male DNA (XY) on normal male metaphase preperations (46,XY) should indicate gain of chromosome X in the tumor DNA.

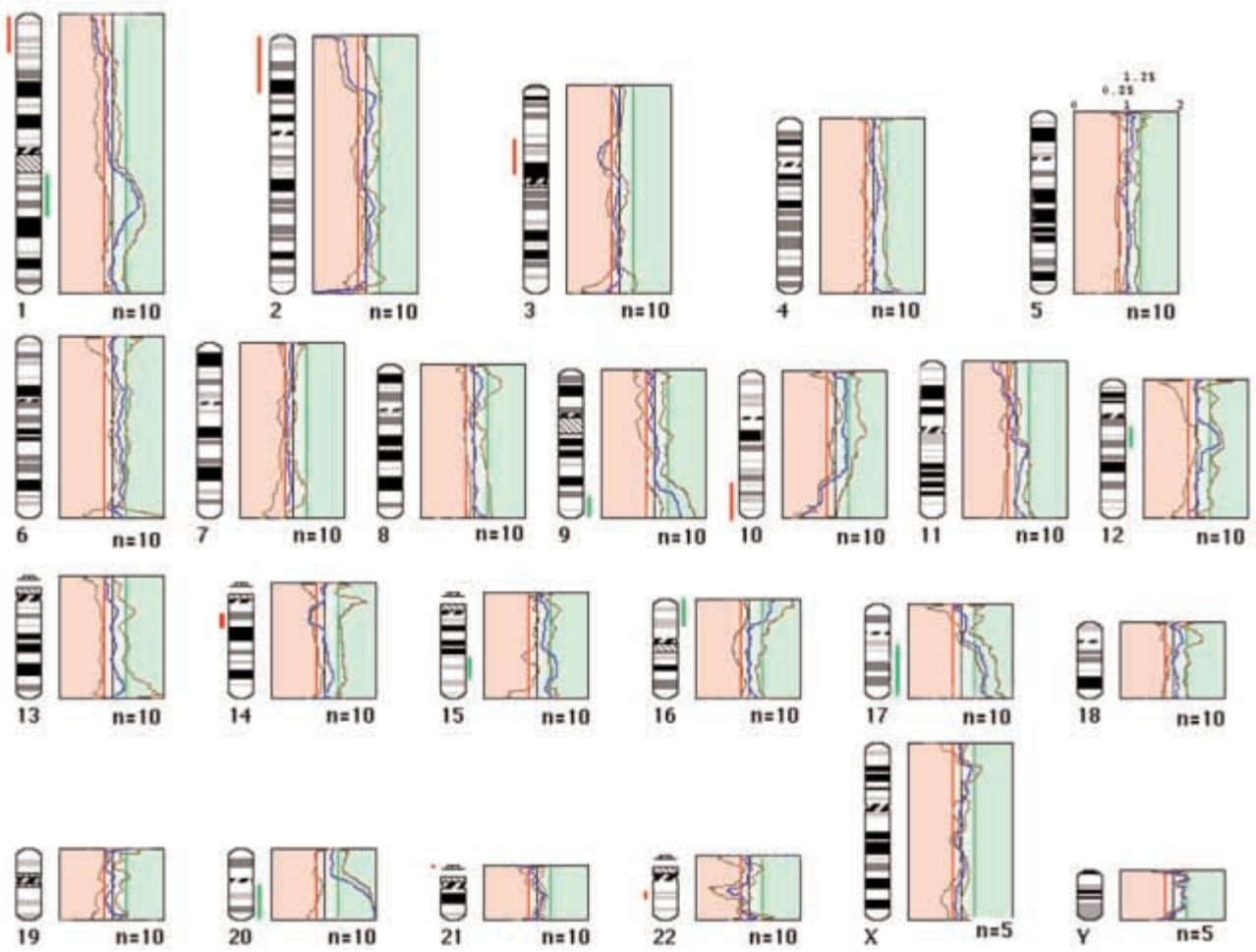

FIG. 2. — CGH profiles of the case MN-17 WHO Grade II

Anaplastic Meningiomas : Comparative Genomic Hybridization identified genomic alterations in all GIII malignant tumors (100%). The mean abnormality per tumor of this group (14.66 ± 1.15) was significantly higher than the means of GI (2.20 ± 2.24) and GII (10.00 ± 1.17) tumor groups. The most frequently seen CGH aberration was loss of 1p, which was detected in all cases. Other abnormalities occuring in more than two cases were losses of 9p, 10q, 14q and 15q, 18q and 22q as well as gains on 12q, 15q, and 18p .

By CGH, high level amplification of chromosomal bands 17q21-qter was identified in two cases (MN-24 and MN-25), but 20q and 22q11-q12 were identified in one case each (MN-24 and MN-23, respectively).

Common Gain/Amplification Regions : Comparative Genomic Hybridization analysis indicated that high level amplification regions were located at 17q21-qter and 20q in GII and GIII tumors. Amplification on 12q13-qter and 22q11-q12 subband was seen in one case each of atypic and anaplastic meningioma groups. No high level amplification region was detected in benign tumor samples.

Chromosome 1q21-q31, 12q13-q15, 15q24-qter, 17q21-qter and 20q were common gain regions in GII and GIII tumors. No high level amplification was seen among the benign tumors.

Commonly Deleted Regions : Chromosome 22 was the most commonly deleted region in all grades of meningiomas, although the prevalence of this abnormality decreased with increase of malignancy grade. Chromosome 1p was the common deleted region in all meningioma types, found in >33% of GI, GII and GIII tumors. Losses on 10q and 14q were seen frequently in atypical and anaplastic meningioma groups. The regions of common loss were located at 1p34-pter, 10q24-qter, 14q21.

Number of genomic alterations detected per tumor : The mean numbers of genomic aberrations detected per tumor were 2.20 + 2.24 in benign, 10.00 ± 1.17 in atypical and 14.66 + 1.15 in anaplastic meningioma groups and there were signifi-

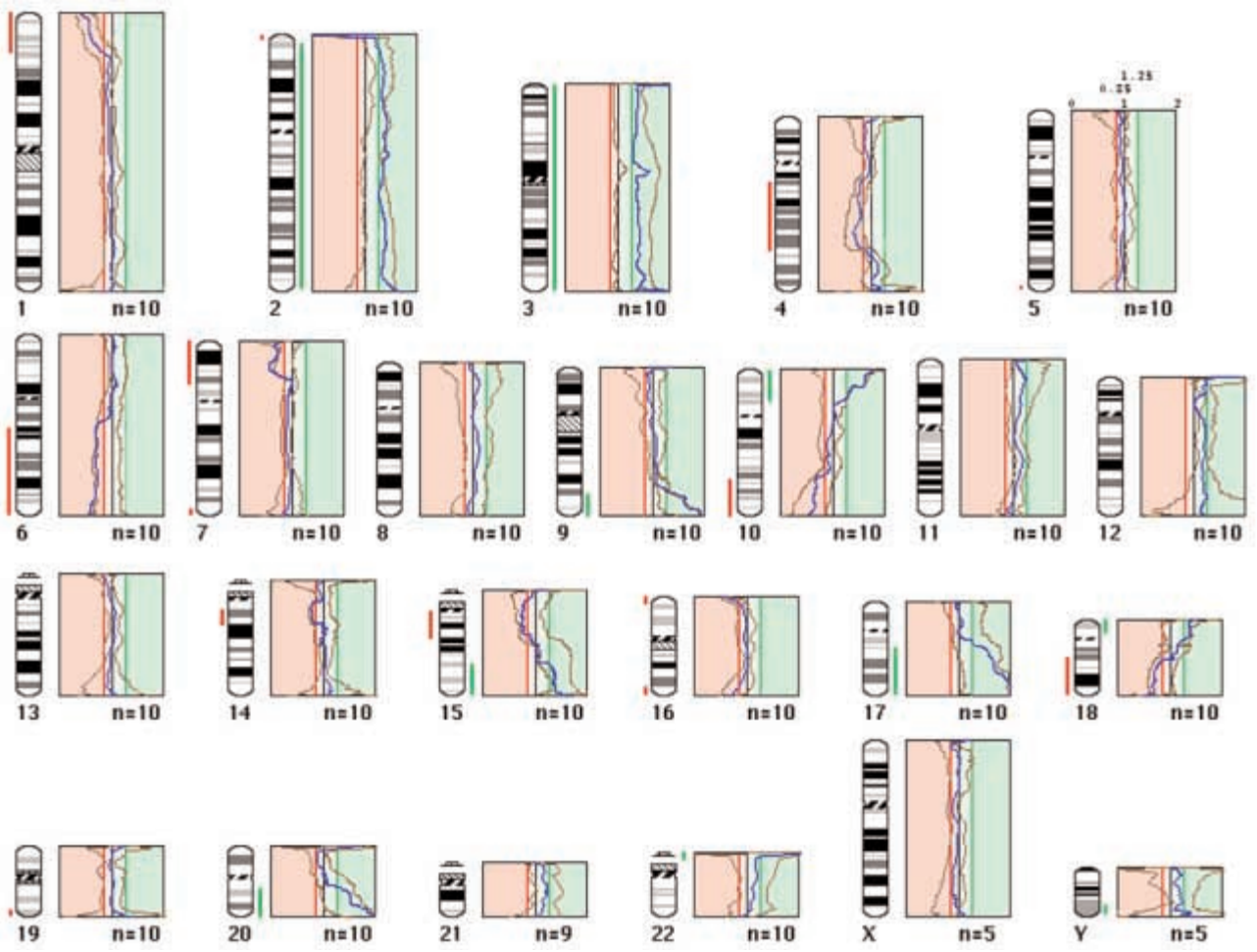

FIG. 3. — CGH profiles of the case MN-24 WHO Grade III

cant differences between the groups (F:46.46, $p < 0.01$).

Discussion

Meningiomas are common nervous system tumors and little is known about their molecular pathogenesis. Although they are usually benign tumors, approximately 10% show histological progression to a higher malignancy grade which are classified as atypical and anaplastic (= malignant) and they have a worse prognosis (13, 27, 36). To date, meningiomas are cytogenetically characterized by complete or partial loss of chromosome 22 as a typical primary abnormality. Numerous cytogenetic and molecular genetic studies are in agreement with the view that loss of 22q is the main genetic change associated with tumor initiation. The genomic differences between benign and higher grade tumors are not well understood, although there is a marked accumulation of genomic aberrations with increasing malignancy (4, 7, 11, 12, 13, 17, 26, 40).

Benign Meningiomas : Loss of chromosome 22 was seen in 47% of benign meningiomas and was the most frequently detected abnormality in this group. The prevalence of this abnormality is in the range (40-70%). It is now clear that chromosome 22 abnormality is the most commonly occuring initial event and NF2 tumor suppressor gene located at 22q12 is the main target for chromosome 22q12 deletions. However, MN1, BAM22 and INI1 are the other genes located on chromosome 22q and they have also been reported in meningiomas, but the roles of these genes in the genesis of meningiomas are not clear yet (20, 35, 41).

Cytogenetic and molecular genetic analyses have failed to detect either aberrations of chromosome 22 or mutations in the NF2 gene in approximately 40% of all tumors and therefore there may be an alternative mechanism(s) responsible for the development of a part of meningiomas. The genetic changes associated with tumor initiation in meningiomas without chromosome 22 abnormality are not yet clear. In our series, different genomic alterations were detected in three tumors that demonstrated no loss of chromosome 22, but loss of 1p was significant since it was the common abnormality seen in all these samples. Loss of chromosome 1p has been reported as the most commonly detected abnormality in atypical and anaplastic meningiomas whilst seen in only a low

fraction in benign tumors (1, 6, 25, 41). Our results, with respect to the prevalence of 1p aberration in tumor groups are in aggrement with these results. The results in our series also showed the role of 1p deletion in atypical and malignant tumor progression. However, being a consistent genetic abnormality in all benign tumors without loss of 22q may be associated with tumor initiation other than chromosome 22q deletion. Recently, high-resolution analysis of chromosome arm 1p alteration in meningiomas have been performed and 1p36, 1p34-p32 and 1p21-p22 main target regions have been reported as having several putative tumor suppressor genes involved in meningioma (1, 3, 21, 26). The p73 gene at 1p36.3, tissue non-specific alkaline phosphatase Alpl gene at 1p36.1-p34 and CDKN2C at 1p32 have been reported as candidate tumor suppressor genes in meningiomas (3, 22, 26). Since the common deleted region on 1p in our series was located at 1p34-pter, we suggest that 1p could also be a primary focus of detailed assessment of tumorigenesis and not only these candidate tumor suppressor genes may be involved in tumor progression, but also they may take place in initiation of meningioma.

Losses on chromosome 3p,14q, and 18q were the other genomic alterations detected in benign tumor samples. Previous studies have suggested that chromosome 1p and 3p deletions may contribute to meningioma tumorigenesis and these abnormalities have been reported in a low fraction of benign tumors (7). Loss on chromosome 14q has been reported as the other abnormality detected in benign tumors and recently, it has been suggested that there was a trend for anaplastic meningiomas with 14q deletions and atypical meningiomas with combined 1p/14q losses (5). In agreement with previous studies (20, 41), there was an increase in the frequency of both chromosome losses in our GII series and we also suggested that genetic losses from 1p and /or 14q are effective changes on early progression of meningioma.

Atypical Meningioma : According to reported cytogenetic studies (17, 40, 41) there is a significant association between chromosomal aberrations and aggressiveness in meningiomas and this relation was also detected in our series.There was a significant difference between the number of genomic alterations per tumor in benign and anaplastic meningiomas. Monosomy 1p has been reported as the most common progression-associated chromosome change (1, 19, 21, 23, 26, 41) and this anomaly has been proposed to be associated with the development of atypical and anaplastic meningiomas. In our atypical meningioma series, loss on 1p was frequently found genomic alteration. Being a secondary chromosome change in typical meningiomas and being the most frequently detected abnormality in anaplastic meningiomas supported our suggestion that loss of genetic material from 1p34-pter could play a major role for initiation and progression of meningiomas. Recently, high-resolution analysis of chromosome arm 1p alteration in meningiomas have been performed and 1p36 and 1p34-p32 main target regions have been reported as having several putative tumor suppressor genes involved in meningioma (1, 3, 21, 26).

In our series, combined 1p/14q deletions were encountered in 2/15 benign, 3/7 atypical and 2/3 anaplastic meningiomas. Our results are in agreement with the previous studies that 1p and 14q deletions are highly associated with increasing histologic grade and play an important role in meningioma tumor progression (5, 41).

Monosomy 10q24-qter was the other frequently found genomic alteration in our atypical meningioma series. Chromosome 10q mutations are frequently seen genomic alterations in grade II meningiomas with the prevalence of 27-50% (9, 19, 29, 41). Chromosome 10q25-qter region has been identified as a commonly deleted region in glioblastomas (32). The PTEN (MMAC1) gene cloned at 10q23.3 is a tumor suppressor gene and it was shown to be inactivated by deletion and mutation in some glioblastomas. Also, germ-line PTEN mutations were found to be responsible for Cowden disease, which is a hereditary syndrome predisposing to multiple tumors, including meningiomas (2, 30). Chromosome 10q25-qter might be an important region for the progression of meningiomas because of the inactivation of the PTEN gene or another tumor suppressor gene, as yet unknown.

It has been reported that losses of genetic material from 18q are frequent in atypical and anaplastic meningiomas but rare in benign meningiomas (9,20,41). Monosomy 18q21-qter was identified in atypical meningioma samples in our study. MADH2, MADH4, APM-1 and DCC are tumor suppressor genes located at 18q21, although no significant role has been found for these gene alterations in the pathogenesis of meningiomas (4).

Amplification in 17q23 has been reported as a commonly found genomic alteration in the meningiomas Grade II and III and it seems to be an important molecular mechanism for tumor progression from grade II to grade III (6,41). No 17q23 amplifications were detected in benign (grade I) and atypical (Grade II) meningiomas in the study by Cai *et al*. (6), although amplification on 17q in GII meningiomas with a prevalence of 5% was reported by Weber et al (41). In agreement with previous studies, we found an increase in the frequency of 17q amplification in GIII , but 5 of 7 atypical meningiomas investigated herein had also shown gain/amplification on 17q21-qter. PS6K a putative oncogene mapped to chromosome 17q23 encodes a serine/threonine kinase that takes a role in the phosphorylation of ribosomal subunit 6 and

is part of the insulin receptor signal transduction pathway (6).

We conclude that the presence of copy number changes on 17q in GII in high fraction could be an informative indicator of the important roles of putative cancer-related genes located at this region for tumor progression.

Anaplastic meningiomas : In our series, anaplastic meningiomas showed the chromosomal changes seen in atypical meningiomas together with other aberrations. The most frequently seen CGH aberrations were loss of 1p and gain/amplification on 17q. In addition to an increased frequency of these alterations, GIII samples were found to have frequent loss on 9p. Deletion of 9p and amplification on 17q have been reported as frequently seen abnormalities in GIII tumors but rarely detected in GI and GII (41). CDKN2A, p14, CDKN2B are tumor suppressor genes located at 9p21. The majority of anaplastic meningiomas either show homozygous deletions of all these genes, mutations in CDKN2A and p14, or lack of expression of one or more of these genes. These results show that inactivation of the G/S phase cell cycle checkpoint is an important aberration in anaplastic meningiomas (3). Although cancer-related genes, located on chromosomes 1p, 9p, 14q, and 17q are mainly involved in late tumor progression, other genomic alterations detected in anaplastic meningiomas would also play roles in malignant meningioma progression. It is necessary, therefore, to perform further molecular studies.

Putative Model of Tumor Progression of Meningioma

One of the fundamental features of cancer is tumor clonality, the development of tumors from single cells that begin to proliferate abnormally. The clonal origin of tumors does not imply that the original progenitor cell that gives rise to a tumor has initially acquired all of the characteristics of a cancer cell. On the contrary, at the cellular level, the development of cancer is viewed as a multistep process involving mutation and selection for cells with progressively increasing capacity for proliferation, survival, invasion and metastasis. The first step in the process, tumor initiation , is the result of a mutation leading to abnormal proliferation of a single cell. During the tumor progression, additional mutations occur within the cells of the tumor population. Some of these mutations confer a growth advantage to the cell. Colorectal carcinomas, prostate cancer, renal cell tumors are some of the clear examples of tumor initiation and progression during the development of a common human malignancy (10). For meningiomas, the model of genomic alterations in meningioma progression has been proposed by Weber *et al*. (41). This model has been mainly supported by the genomic alterations detected by CGH in our series. However, we propose that loss of chromosome 1p might be the genetic change associated with tumor initiation in the meningiomas that do not show chromosome 22 abnormality. The tumor suppressor genes located at 1p34-pter might be the putative cancer-related genes for the initiation of meningiomas. The other difference of our results from the proposed model by Weber *et al*. (41) is the presence of chromosome 17q gains and amplifications in grade II meningiomas. The amplification of the putative oncogene PS6K mapped to chromosome 17q23 has also been reported in breast cancer, another hormonally driven neoplasm (6). This gene encodes a serine/threonine kinase and is part of the insulin receptor signal transducing pathway. Protein-serine/threonine kinases are ubiquitous regulators of cell proliferation and differentiation (10). Therefore, mutations in such kinases may confer a selective advantage to the cell, such as more rapid growth. Either the putative oncogene PS6K itself or additional genes located at 17q23-qter could an play important role during the tumor progression in meningiomas.

In conclusion, further studies are needed to clerify the mechanism of initiation and progression of meningioma by mapping the deleted and amplified chromosomal regions to identify the genes involved in meningioma.

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