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Analysis of genomic alterations in benign, atypical, and anaplastic meningiomas: Toward a genetic model of meningioma progression

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ABSTRACT **Nineteen benign [World Health Organization (WHO) grade I; MI], 21 atypical (WHO grade II; MII), and 19 anaplastic (WHO grade III; MIII) sporadic meningiomas were screened for chromosomal imbalances by comparative genomic hybridization (CGH). These data were supplemented by molecular genetic analyses of selected chromosomal regions and genes. With increasing malignancy grade, a marked accumulation of genomic aberrations was observed; i.e., the numbers (mean ± SEM) of total alterations detected per tumor were 2.9 ± 0.7 for MI, 9.2 ± 1.2 for MII, and 13.3 ± 1.9 for MIII. The most frequent alteration detected in MI was loss on 22q (58%). In MII, aberrations most commonly identified were losses on 1p (76%), 22q (71%), 14q (43%), 18q (43%), 10 (38%), and 6q (33%), as well as gains on 20q (48%), 12q (43%), 15q (43%), 1q (33%), 9q (33%), and 17q (33%). In MIII, most of these alterations were found at similar frequencies. However, an increase in losses on 6q (53%), 10 (68%), and 14q (63%) was observed. In addition, 32% of MIII demonstrated loss on 9p. Homozygous deletions in the *CDKN2A* gene at 9p21 were found in 4 of 16 MIII (25%). Highly amplified DNA sequences were mapped to 12q13–q15 by CGH in 1 MII. Southern blot analysis of this tumor revealed amplification of *CDK4* and *MDM2*. By CGH, DNA sequences from 17q were found to be amplified in 1 MII and 8 MIII, involving 17q23 in all cases. Despite the high frequency of chromosomal aberrations in the MII and MIII investigated, none of these tumors showed mutations in exons 5–8 of the *TP53* gene. On the basis of the most common aberrations identified in the various malignancy grades, a model for the genomic alterations associated with meningioma progression is proposed.**

Meningiomas are tumors arising from cells of the meningeal coverings of the brain and spinal cord. They account for 15–25% of primary intracranial and intraspinal neoplasms and their annual incidence has been estimated to be about 6 per 100,000 individuals (1). Approximately 90% of meningiomas are slowly growing benign tumors (MI) that histologically correspond to grade I according to the World Health Organization (WHO) classification of central nervous system tumors (2). About 6–8% of meningiomas are histologically characterized by several of the following features: increased cellularity, high nuclear to cytoplasmatic ratio, increased mitotic activity, uninterrupted patternless or sheetlike growth, and foci of necrosis (2). These tumors are designated as atypical meningiomas (WHO grade II, MII) (2) and show a tendency for local tumor recurrence even after complete resection (3). Approximately 2–3% of meningiomas exhibit histological signs of frank malignancy far in excess of the abnormalities in MII. These tumors are classified as anaplastic (malignant) meningiomas of WHO grade III (MIII) (2) and are associated with a high risk for local recurrence and metastasis (1).

Meningiomas were among the first solid neoplasms studied by cytogenetics. A missing G group chromosome was detected as a consistent chromosomal change as early as 1967 (4) and identified as chromosome 22 in 1972 (5, 6). These original reports have been corroborated by numerous other cytogenetic and molecular genetic studies showing loss of chromosome 22 in 40–70% of all meningiomas (7–10). More recent studies have demonstrated that the majority of sporadic meningiomas with loss on 22q carries mutations in the neurofibromatosis type 2 gene (*NF2*) on 22q12.2 (9, 11). Mutation and/or loss of *NF2* were found in meningiomas of all malignancy grades, indicating that inactivation of this gene represents an early genetic event in the pathogenesis of meningiomas (9, 11). The molecular alterations associated with the progression to MII and MIII, however, are poorly understood at present. Cytogenetic studies have indicated that MII and MIII frequently show complex numerical and structural aberrations (8, 10, 12). Loss of heterozygosity (LOH) analyses have identified three chromosomal regions that are rarely altered in MI but often lost in MII and MIII. These are located on 1p, 10, and 14q and have been suspected to contain tumor suppressor genes associated with meningioma progression (13–15).

In the present study, the genomic alterations in sporadic meningiomas of all malignancy grades were investigated on a series of 62 tumors by comparative genomic hybridization (CGH) (16, 17). The CGH data were supplemented by molecular genetic analyses of selected genes and chromosomal regions. This combined approach provided a comprehensive overview of the genomic alterations in benign, atypical, and anaplastic meningiomas and allowed us to propose a model for the genetic alterations associated with meningioma progression.

MATERIALS AND METHODS

Tumor Samples. Sporadic meningiomas from 62 patients were investigated (Fig. 1). The tumors were classified according to the WHO classification of tumors of the central nervous system (2). Unfixed frozen samples were used for DNA extraction in all tumors. Histological evaluation of these pieces revealed an estimated tumor cell content of >90% for all cases except MN83 (80%) and MN91A (70%).

Immunocytochemistry. All tumors were evaluated on formalin-fixed paraffin sections for the expression of the proliferation-associated nuclear antigen Ki-67 (MIB1; Dianova, Hamburg, F.R.G.) as described (18) (Fig. 1).

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Abbreviations: CGH, comparative genomic hybridization; LOH, loss of heterozygosity; WHO, World Health Organization; MI, benign meningioma, WHO grade I; MII, atypical meningioma, WHO grade II; MIII, anaplastic meningioma, WHO grade III.
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Case-No.	Sex	Age	Sub-type	WHO grade	Note	Ki-67 (%)	*TP53* mut	*CDK4* amp	*MDM2* amp	*CDKN2A* copy no.	LOH detected on chromosome arms	Losses detected by CGH	Gains detected by CGH
MN34	m	55	anapl	III	3. rec	22	-	-	-	2	1p, 9p, 10q, 19p, 19q, 22q	1p, 3q, 4p15-pter, 5q, 7p, 9p24, 10q, 11q, 14q, 15q, 16q, 22q, Y	9q, 12q22-qter, 16p, 17q11.2-q12, **17q21-qter**, 18p, 20q, 21q
MN49A	f	55	anapl	III	1. rec	8.9	-	-	-	2	1p, 10q, 18q, 22q	1p, 4, 10, 14q, 18, X	1q, 17pter-q12, **17q21-qter**
MN63B	f	78	anapl	III	3. rec	10.5	-	-	-	2	1p, 18q, 22q	1p, 10p, 14q, 18, 22q, X	1q, 8
MN64	m	6	anapl	III	2. rec	4.5	-	-	-	1	ND	6, 10, 18, 22q	2p14-qter, 3, 7, 9q, 15q, **17q21-qter**
MN67	f	70	anapl	III	1. rec	4	-	-	-	2	ND	1p, 6q, 10, 11p14-pter, 14q, 18, X	12q24, 17, 20q
MN70	f	41	anapl	III		1	-	-	-	0	1p, 9p, 10q, 22q	1p, 6q15-qter, 9p21-pter, 10q21-qter	1q, 15q23-qter, 17q, **17q23**, 20q
MN71	f	76	anapl	III		3.6	-	-	-	1	1p, 18q, 22q	1p, 10, 14q, 18, 22q, X	8, 20
MN72	f	76	anapl	III	3. rec	3.5	-	-	-	2	ND	1p, 2p, 3p, 4, 6, X	8, 10, 11q, 12, 14q24-qter, 15q24-qter, 16, 17, **20**, 21q22
MN73	f	66	anapl	III	5. rec	11.9	-	-	-	2	9p22-p23, 22q	14q21	17, 20
MN74	f	67	anapl	III	3. rec	6	-	-	-	1	1p, 9p, 10q, 18q	1p, 6, 9p21-pter, 10, 11p, 14q, 18	11q, 12q24, 13q31-qter, 16, 17, 20q
MN77	m	66	anapl	III	2. rec	10.6	-	-	-	ND	ND	1p, 1q32-qter, 4q21-q31, 10p, 13q, 14q12-q21, 15q11.2-q21, 20p, Y	1q21-q31, 2p23-pter, 6p21-p22, 8p22-pter, 8q, 9q22-qter, 12pter-q23, 16p, 17p, **17q22-qter**, 20q, 21q22, 22q, X
MN83	m	79	anapl	III		10.1	-	-	-	0	1p, 9p, 18q, 19q, 22q	1p, 6p, 9p21-pter, 11p14-pter, 14q, 18, 21q	-
MN84	m	63	anapl	III		6	-	-	-	ND	ND	1p, 5q13-qter, 6q16-qter, 7q31-qter, 10q, 11p, 12p, 16p, 22q13	1q, 2, 3, 6p, 9q, 10p, 11q, 12q, 13q, 14q, 15q, 16q, 17, 18q, 20, 21q, **22q11.2-q12**, X
MN85	f	42	anapl	III	2. rec	22	-	-	-	ND	ND	-	15q23-qter, **17q22-q23**, 17q24-qter
MN86	f	79	anapl	III	2. rec	3.7	-	-	-	2	ND	1p, 6q, 14q, 22q	-
MN88B	m	51	anapl	III	spin met	16.8	-	-	-	2	ND	1p, 3p, 4, 6q, 10q, 12p, 12q21-qter, 16q23-qter, 17p, 18q, 22q	1q, 2, 3q, 5, 6p, 7, 8, 9, 13q, 15q, 17q, 18p, 20, 21q, X
MN89	m	78	anapl	III	2. rec	4.4	-	-	-	2	ND	-	-
MN90	m	58	anapl	III		8.7	-	-	-	0	ND	1p, 4q, 6, 9p, 10, 14q, 22q, Y	1q, 2, 5, 7, 9q, 11, 12, 13q, 15q, 16, 17pter-q12, **17q21-qter**, 20, 21q22
MN91A	m	66	anapl	III	5. rec, irr	22.7	-	-	-	0	1p, 9p, 10q, 18q, 19p, 22q	1p22-pter, 3p, 6q, 7p, 9p, 10, 12p, 14q, 18q	1q, 2, 5, 7q, 9q, 12q, 13q, 15q, 16p, 17pter-q21, **17q22-qter**, 18p, 20
MN4	m	70	atyp	II	1. rec	5.1	-	-	-	2	1p, 18q, 22q	1p, 10, 13q, 14q, 18, 22q, Y	1q, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 15q
MN14	f	66	atyp	II		8.5	-	-	-	2	1p, 18q, 22q	1p22-pter, 6q, 18q, 22q	1q, 2, 5, 6p, 11q, 13q22-qter, 15q, 16p, 20, 21q
MN16	f	78	atyp	II		9.9	-	AMP	AMP	2	1p, 18q, 19q, 22q	1p, 5p, 6q, 7q, 18q21-qter, 22q	1q, 4, 5q, 9p, 11q13-qter, **12q13-q15**, 13q, 15q24-qter
MN20	m	44	atyp	II		3.1	-	-	-	2	1p, 10q, 22q	1p, 10, 14q, 22q	1q, 2, 3, 5, 6, 9, 12, 13q
MN22	f	71	atyp	II		16	-	-	-	2	-	-	3, 5, 8, 9, 12, 15q, 20
MN40	f	71	atyp	II		13	-	-	-	2	1p, 10q, 22q	1p, 2p16-pter, 10q, 22q	**17q21-qter**
MN42	f	58	atyp	II		3.4	-	-	-	2	-	-	3, 4, 5, 9, 12, 15q, 16, 17, 20
MN47	f	81	atyp	II		10.5	-	-	-	2	-	-	-
MN56B	m	47	atyp	II	4. rec	10	-	-	-	1	1p, 9p, 18q, 22q	1p, 6q, 9p21-pter, 14q, 18	15q22-q25, 16p
MN59A	m	68	atyp	II		1.9	-	-	-	2	1p, 18q, 22q	1p, 5q14-qter, 6q, 10p15, 14q13-q24, 18, 22q	-
MN60	f	72	atyp	II		4.8	-	-	-	2	22q	14q, 22q	-
MN61	f	74	atyp	II		6	-	-	-	2	1p	1p31-pter, 11q14-qter	3, 5, 8, 9, 12, 15q, 20
MN62	f	74	atyp	II	1. rec	4.1	-	-	-	2	1p, 10q	1p, 10, 14q, 22q, X	1q, 2, 5, 7, 17, 20, 21q22
MN65	f	76	atyp	II	2. rec	5.3	-	-	-	1	ND	1p31-pter, 4p, 6, 8pter-q22, 9p21-pter, 10p, 12p, 13q12-q22, 17p, 18	2, 3, 9q, 10q, 11, 12q, 14q, 15q, 16q, 17q, 20
MN66	f	90	atyp	II		2.3	-	-	-	2	ND	1p22-pter, 2q23-qter, 3p12-p21, 4, 5q, 22q13	1q, 5p, 8q, 9q34, 10p, 12q24, 17, 20q
MN68	f	43	atyp	II		4.1	-	ND	ND	2	ND	1p, 10, 14q, 18, 22q	1q, 17q21-qter
MN69	m	38	atyp	II	2. rec	11	-	-	-	2	ND	1p, 4p, 6, 14q, 18q, 22q13	2q, 18p, Yq
MN78	f	61	atyp	II		<1	-	-	-	2	22q	-	-
MN80	f	55	atyp	II		1.7	-	-	-	2	1p, 10q, 18q	1, 6, 10q25-qter, 14q11.2-q31, 18	12q24, 15q22-q25, 16, 17, 20q
MN81	m	51	atyp	II		1.2	-	-	-	2	1p, 22q	1p, 2p13-pter, 22q, Y	20
MN82	m	40	atyp	II		<1	-	-	-	2	1p, 22q	1p, 4p	12q24, 16p, 17, 20q
MN1	m	65	men	I	2. rec	2	-	-	-	2	22q	14q, 22q	-
MN2	m	42	fib	I		<1	-	-	-	2	-	-	-
MN7	f	50	fib	I		<1	-	-	-	2	1p, 22q	1p35-pter*, 16q, 22q	3p21-pter, 7q21-qter, 17q23-qter
MN10	f	38	trans	I		6	-	-	-	2	22q	22q	7
MN11	m	49	trans	I		<1	-	-	-	2	22q	22q, Y	-
MN12	m	74	men	I		<1	-	-	-	2	1p, 10q, 22q	1p, 10q, 14q, 22q, Y	16p
MN15	f	69	men	I	1. rec	<1	-	-	-	2	18q, 22q	14q, 18, 22q	-
MN17	f	55	fib	I		1.3	-	-	-	2	1p, 18q, 22q	1p, 8, 12p, 12q21-qter, 18q22-qter, 22q12	12q13-q15, 13q, 15q
MN19	f	52	fib	I		<1	-	-	-	1	1p, 9p, 22q	1p34-pter*, 2q31-q35, 9p21-pter, 22q	-
MN27	f	73	trans	I		<1	-	-	-	2	1p, 18q, 22q	1p31-pter, 14q, 16, 18	1q, 7q, 17
MN31	m	47	ang	I		<1	-	-	-	2	9p22-p23	Y	5, 6, 12, 17, 20, 21
MN36	f	53	fib	I		<1	-	-	-	2	-	-	-
MN37	f	71	fib	I		<1	-	-	-	2	-	-	16p
MN38	m	53	trans	I		2.1	-	-	-	2	-	-	-
MN41	f	55	fib	I		<1	-	-	-	2	-	-	-
MN45	f	65	trans	I		5.5	-	-	-	2	22q	22q, X	-
MN51	m	63	psam	I		1.1	-	-	-	2	-	-	-
MN53	m	73	secr	I		<1	-	-	-	2	-	-	-
MN58	f	59	men	I		<1	-	-	-	1	22q	22q	-
MN30**	f	57	trans			<1	-	-	-	2	-	-	-
MN79**	m	35	men			<1	-	-	-	2	-	-	-
MN87**	f	71	men			1	-	-	-	2	-	-	-

FIG. 1. Summary of selected clinical data, histopathological characteristics, and genomic alterations identified by CGH and other molecular genetic techniques in all meningioma groups investigated. m, Male; f, female; anapl, anaplastic; atyp, atypical; men, meningothelial; fib, fibrous; trans, transitional; ang, angiomatous; psam, psammomatous; secr, secretory; rec, recurrence; spin met, spinal metastasis; irr, irradiated prior to operation; mut, mutation; amp, amplification; ND, no data. High-level amplifications are given in boldface type in the section "gains detected by CGH." *, Losses of 1p34–p36 determined by CGH were only included if confirmed by LOH data; **, meningiomas with brain invasion but no other signs of anaplasia.

DNA Extraction and CGH. The preparation of high molecular weight DNA from tumor tissue and peripheral blood leukocytes was carried out as described (19). CGH was performed as described (20).

For CGH analysis, image acquisition and processing was performed by using the CYTOVISION system version 3.1 (Applied Imaging, Sunderland, Tyne and Wear, U.K.). Mean ratio profiles were determined from the analysis of 12 metaphase spreads. The threshold values used to distinguish between the balanced and imbalanced state of the average ratio profiles were 0.75 (lower threshold) and 1.25 (upper threshold) in accordance with reported CGH analysis protocols (21). High-level amplifications were defined as gains of chromosomal material that led to either a very bright and distinct fluorescent band in the hybridization pattern of the tumor DNA and/or a deviation of the average ratio profile to the right beyond the 2.0 threshold (see Fig. 3).

Negative control experiments in which differently labeled normal DNAs were hybridized to normal metaphase chromosomes were regularly performed in parallel with the tumor hybridizations. Because gains of chromosomal region 1p34–p36 and of chromosome 19 were occasionally observed in these control experiments distal 1p and chromosome 19 were excluded from CGH evaluation. To obtain data on 1p34–p36 and 19, these regions were analyzed for LOH by microsatellite analysis.

Microsatellite Analysis. PCR primers were selected from the Généthon microsatellite map (22). The following 17 loci were studied: *D1S224* (1p22), *D1S496* (1p34), *D1S468* (1p36), *D9S171* (9p21), *D9S157* (9p21), *D9S168* (9p), *D10S185* (10q22), *D10S212* (10q26), *D18S69* (18q), *D18S70* (18qter), *D19S216* (19p13), *D19S217* (19q13), *D19S210* (19q13), *D22S264* (22q11), *D22S929* (22q12), *D22S430* (22q12), and *D22S304* (22q12). In addition, two highly polymorphic loci within the *MXI1* locus at 10q25 were investigated by using primers reported by Wechsler *et al.* (23). Assessment for allelic loss was performed as described (18).

Single-Strand Conformation Polymorphism Analysis. Mutational screening of exons 5–8 of the *TP53* gene was carried out by single-strand conformation polymorphism analysis as described (24).

Fluorescent Differential PCR. The gene dosages of *MDM2*, *CDK4*, and *CDKN2A* were analyzed by differential PCR with fluorescein-labeled primers. Two reference loci were used, *GAPDH* (12p) and a sequence-tagged site on 9q (*9q-STS*). Primers for the *9q-STS* and *MDM2* were taken from Ueki *et al.* (25) and Hunter *et al.* (26). Primer sequences for the other loci were as follows (primers with the prefix 5′-F- carried a 5′-fluorescein label): 5′-F-AACGTGTCAGTGGTGGACCTG-3′ and 5′-AGTGGGTGTCGCTGTTGAAGT-3′ for *GAPDH*, 5′-F-CATGTAGACCAGGACCTAAGG-3′ and 5′-AACTG-GCGCATCAGATCCTAG-3′ for *CDK4*, and 5′-F-CCAACG-CACCGAATAGTTACGG-3′ and 5′-AAACTTCGTCCTC-CAGAGTCGC-3′ for exon 1 of *CDKN2A*. Controls included the glioblastoma cell lines TP365MG (*MDM2* and *CDK4* amplification) (27) and U118MG (homozygous deletion of *CDKN2A*) (28). The PCR products were analyzed with an automated fluorescent DNA sequencer (A.L.F., Pharmacia) as described (24). Only increases in the target gene/reference locus ratios of more than 3 were considered as evidence of gene amplification. *CDKN2A*/*9q-STS* ratios of less than 0.3 relative to the constitutional control were considered as homozygous deletion, and ratios between 0.3 and 0.7 were regarded as hemizygous deletion.

Southern Blot Analysis. DNA from tumors (2.5 μg) and corresponding leukocyte DNA (2.5 μg) were digested with the restriction enzyme *Taq*I, separated on 0.8% agarose gels, and blotted. The membranes were sequentially hybridized with probes for *MDM2*, *CDK4*, and the control locus *ERBB3* as described (29). Densitometric evaluation was performed after scanning the autoradiograms with a GS-700 imaging densitometer (Bio-Rad).

Statistics. Given are mean ± SEM, if not indicated otherwise. Data were assessed for normal distribution by the Shapiro Wilks test. Comparisons were performed by analysis of variance (ANOVA) followed by Duncan's multiple range test or ANOVA on ranks (Kruskal–Wallis test) followed by pairwise multiple comparisons (Dunn's method), as appropriate. Associations between variables were evaluated by univariate regression analysis using the Pearson correlation coefficient. $P < 0.05$ was considered statistically significant.

RESULTS

A summary of selected clinical data, the histopathological diagnoses, and all genomic alterations identified by CGH and/or molecular genetic techniques in the 62 sporadic meningiomas investigated is given in Fig. 1.

Benign Meningiomas. CGH and/or molecular genetic investigations detected genomic abnormalities in 13 of 19 MI (68%). The most common alteration was loss on 22q, which was found by CGH in 10 of 19 tumors (53%). Other aberrations found in more than three cases were losses on 1p (26%) and 14q (21%) (Fig. 2). In two tumors (MN31 and MN37), genomic alterations were identified by CGH in the absence of loss on 22q. MN31, an angiomatous meningioma, demonstrated gains of multiple chromosomes (5, 6, 12, 17, 20, 21) and loss of the Y chromosome. MN37, a fibroblastic variant, showed gain of 16p as the only detectable aberration.

Microsatellite analysis showed LOH at all informative loci from 22q in each of the tumors with loss on 22q demonstrated by CGH. One tumor demonstrated LOH at *D22S430* (the only informative 22q locus in this case) but no loss on 22q by CGH. Differential PCR revealed evidence for hemizygous deletion of *CDKN2A* in two MI.

Meningiomas with Brain Invasion but no Further Signs of Anaplasia. All of the three meningiomas (MN30, MN79, and MN87) that showed tumor invasion into the adjacent brain tissue in the absence of further cytological and histological signs of anaplasia showed low Ki-67 indices of ≤1%. Neither CGH nor the molecular genetic analyses carried out revealed any abnormalities in these cases.

Atypical Meningiomas. Genomic alterations were detected in 20 of 21 MII (94%). By CGH, the aberration most commonly found was loss on 1p in 76%. Other changes detected by CGH in more than three cases were losses on 22q in 57%; 14q and 18q in 43%; 6q in 33%; 10p, 10q, and 18p in 29%; 4p in 19%; 6p in 14% as well as gains on 20q in 48%; 12q and 15q in 43%; 5p and 5q in 38%; 1q, 9q, 17q, and 20p in 33%; 2q, 3, and 9p in 29%; 2p, 12p, 16p, and 17p in 24%; and 8q and 11q in 19% (Fig. 2).

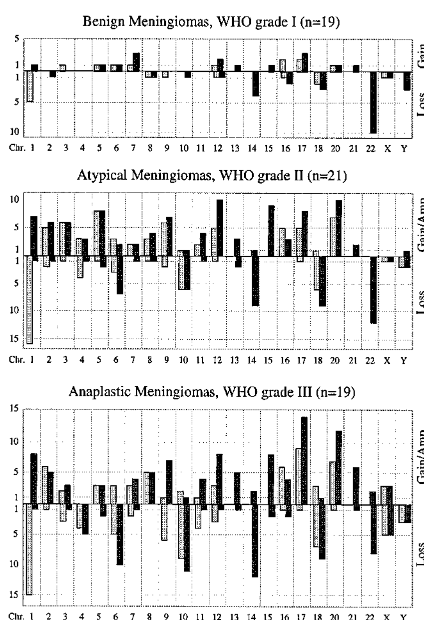

FIG. 2. Frequency of all CGH results obtained in the different meningioma groups. *x* axis, chromosomes 1–22, X, Y; *y* axis, number of cases. The bars showing upwards represent gains or amplifications on the short arms (shaded bars) or long arms (solid bars), whereas the bars pointing downwards stand for losses on the short arms (shaded bars) or long arms (solid bars) of the respective chromosomes.

LOH at all informative 22q loci studied was found in 11 of 17 MII. Eight of these tumors also showed loss on 22q by CGH and 3 tumors showed no CGH abnormality on 22q. When combining the CGH and LOH results, losses and/or LOH on 22q were found in 15 MII (71%).

Five MII without 22q loss and/or LOH on 22q demonstrated complex abnormalities including gains on chromosomes 3, 9, 12, 15, and 20 in four cases.

CGH identified 2 MII with high-level amplifications of chromosomal bands 12q13–q15 and 17q21–qter, respectively. The tumor with amplification on 12q demonstrated coamplification of *CDK4* and *MDM2* by Southern blot analysis (Fig. 3). No further case with *CDK4* or *MDM2* amplification was found in the other 61 meningiomas. The *CDKN2A* gene was hemizygously deleted in 2 MII.

Anaplastic Meningiomas. Genomic alterations were detected in 18 of 19 MIII (95%). The CGH aberrations occurring in more than 3 cases were losses on 1p in 79%; 14q in 63%; 10q in 58%; 6q in 53%; 10p and 18q in 47%; 22q in 42%; 18p in 37%; 9p in 32%; 4q, 6p, and X in 26%; 4p and 11p in 21% as well as gains on 17q in 63%; 20q in 58%; 17p in 47%; 1q, 12q, and 15q in 42%; 9q in 37%; 2p, 16p, 20p, and 21q in 32%; 2q, 8, and 13q in 26%; 7q, 11q, and 16q in 21% (Fig. 2). High-level amplifications were identified on 17q in 42%, as well as on 20 and 22q11.2–q12 in one case each (Fig. 3).

Five MIII showed LOH at all informative markers from 22q but no loss on 22q by CGH. Thus, the total percentage of MIII with abnormalities detected on 22q by CGH and/or LOH analysis was 68%. LOH on 19 that was found in 3 of 8 informative MIII was restricted to loci from 19q in one case.

Differential PCR revealed evidence for homozygous deletions of *CDKN2A* exon 1 in 4 of 16 MIII (25%). Three additional tumors showed evidence of hemizygous *CDKN2A* loss. Neither

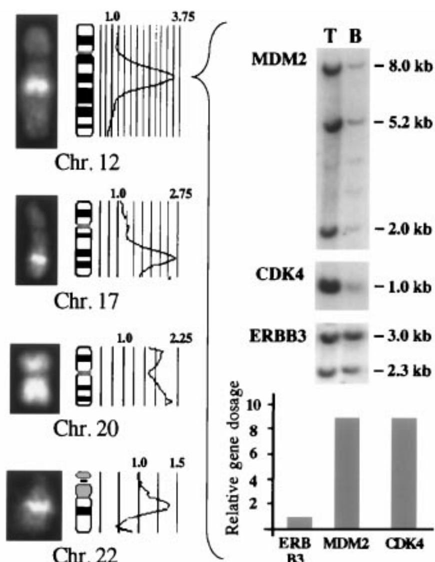

FIG. 3. Amplified DNA sequences were identified in MII and MIII and mapped to four different chromosomes by CGH. Shown are the images revealing the hybridization pattern of the tumor DNA (*Left*) next to the average ratio profile (*Center*). The value for the balanced threshold (1.0) and the highest threshold depicted are given for each profile. (*Right*) Southern blot analysis demonstrates that the genes found to be amplified on 12q were *MDM2* and *CDK4*, which were amplified 9-fold as compared with the control locus *ERBB3* (determined by densitometry; *Lower Right*).

MIII nor MI or MII showed single-strand conformation polymorphism abnormalities of exons 5–8 of the *TP53* gene.

Commonly Deleted Regions. Losses on 1p, 6q, 10p and q, 14q, and 18p and q were found in 29% or more of MII and MIII. The regions of common loss were mapped to 1p34–pter, 6q16–qter, 10p15, 10q25–qter, 14q21, and 18q22–qter. The area demonstrating loss on 9p by CGH included 9p21, the cytogenetic band containing *CDKN2A*, in eight tumors. Three tumors showed LOH at the *D9S168* locus (9p22–p23) but retention of heterozygosity at *D9S157* (9p21) and no *CDKN2A* loss by differential PCR.

Regions of Common Gain/Amplification. 1q, 9q, 12q, 15q, 17q, 20 showed gains or amplifications in >30% of MII and MIII. CGH indicated that regions of common gain/amplification were located at 1q21–q31, 9q34, 12q13–q15, 12q24, 15q24–q25, 17q23, and 20q.

Comparative Analyses. The mean numbers of genomic alterations detected per tumor were significantly higher for MII (9.2 ± 1.2) and MIII (13.3 ± 1.9) than for MI (2.9 ± 0.7) ($P < 0.05$). The mean Ki-67 indices determined for MII (5.9 ± 1.0%) and MIII (9.5 ± 1.6%) were significantly higher than the mean Ki-67 index obtained for MI (1.3 ± 0.4%) ($P < 0.05$) (Fig. 4). Regression analysis of the entire group of 62 tumors showed a positive linear relationship ($r = 0.48$; $P < 0.0001$) between the number of genetic alterations detected per tumor and the Ki-67 index.

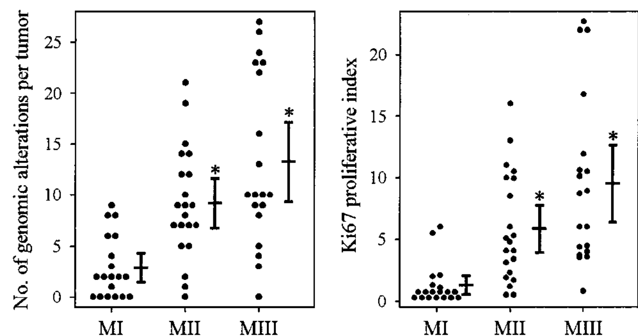

FIG. 4. (*Left*) Accumulation of genetic alterations during meningioma progression. All distinct genomic alterations detected in an individual tumor by the different methods used were added and are shown in their respective groups. (*Right*) Comparable increase of the Ki-67 proliferative index in MII and MIII versus MI. Beside the column of data points, the mean ± 2 SEM is given. *, Significant difference to values for MI ($P < 0.05$).

When comparing the CGH results with those obtained by microsatellite analysis of 17 loci from five chromosome arms, loss by CGH was paralleled by LOH in 95% of 223 informative events. In the remaining cases, LOH was found in regions that showed no loss by CGH. This could be due to uniparental disomy or small losses below the resolution limit of CGH. The presence of uniparental disomy has not been investigated in meningiomas to date. However, it was previously identified in other primary brain tumors. In one study, the majority of astrocytomas in which LOH on chromosome 17 had been detected were shown to have no net loss of chromosomal material but rather a duplication of uniparental alleles on chromosome 17 (30).

DISCUSSION

Genomic Alterations in Benign Meningiomas. Losses on chromosome 22 were found in 58% of MI and thus represented the most frequent alteration identified in this tumor group. This finding is in line with numerous other cytogenetic and molecular genetic studies, in which losses or LOH on chromosome 22 have been found at similar percentages (7–10). It is now well established that the main target for deletions on 22q in meningiomas is the *NF2* gene from 22q12 (9, 11). Alterations of other genes from 22q, e.g., *MN1* (10) and *BAM22* (31), have also been reported in meningiomas but are restricted to individual cases.

The genetic changes associated with tumor initiation in those meningiomas that do not show chromosome 22 involvement are as yet unclear. Among the eight MI in our series that demonstrated no loss on 22q, only two tumors showed other genomic changes, including gains on several autosomes and loss of one sex chromosome. Thus, no single consistent genetic alteration associated with tumor initiation other than loss on 22q was found in this study.

Several MI of our series showed genomic changes in addition to loss on 22q, including most commonly losses on 1p and 14q. These findings are in line with previous studies reporting allelic loss on 1p in a low fraction of MI (15, 18). Occasional MI with alterations on chromosome 14 have also been reported (10, 32, 33). In agreement with previous LOH studies (15, 18), we also found an increase in the frequency of both loss on 1p and on 14q in MII as opposed to MI. Thus, it appears that losses of genetic information from 1p and/or 14q represent early progression associated changes, i.e., are involved in the transition from MI to MII.

We have recently reported that a commonly deleted region on 1p in meningiomas is located distally to *D1S496* (i.e., 1p34–pter) (18). The additional tumors analyzed in the present study did not allow a further narrowing of this region, which overlaps with the chromosomal segment frequently deleted in neuroblastoma, malignant melanoma, and different types of carcinoma (34).

The region of common deletion on 14q determined by CGH in our series was located at 14q21 and defined by tumor MN73. This location is proximal to the region 14q24–q32 previously implicated to contain a locus for a putative tumor suppressor gene in meningiomas (15, 33). Thus, there may be two candidate regions for further investigation on 14q.

Genomic Alterations in Meningiomas with Brain Invasion but no Other Signs of Anaplasia. Three meningiomas of our series showed unequivocal brain invasion but no other signs of anaplasia. These tumors were treated as a separate group because it is debated whether such tumors should be classified as benign or anaplastic (1, 35). No alterations were detected by CGH or molecular genetic techniques in any of these tumors. This finding extends the observation of Rempel *et al.* (13) who reported no

LOH on chromosomes 10 and 22 in five tumors classified as malignant by invasive characteristics only. Similarly, Simon *et al.* (15) found no LOH on 1p, 10q, and 14q in two meningiomas designated as nonanaplastic tumors with brain invasion.

Genomic Alterations in Atypical Meningiomas. In addition to an increased frequency of losses on 1p and 14q, MII were found to have frequent losses on 6, 10, and 18q. Cytogenetic studies have described structural abnormalities or monosomy of chromosome 6 in small series of MII from adult patients (10) and young children or adolescents (36). This latter study identified one tumor with a translocation breakpoint at 6q14–q16 that overlaps the commonly deleted region determined for our tumor series (6q16–qter).

Complete or partial losses of chromosome 10 were found in 38% of MII investigated herein. Likewise, previous studies have reported LOH on 10q in 27–50% of MII (13, 15). We found evidence for at least two distinct regions involved on chromosome 10, one on the short and the second on the long arm (commonly deleted regions at 10p15 and 10q25–qter, respectively). The latter region has also been identified as a commonly deleted region in glioblastomas (37). Recently, the *PTEN* (*MMAC1*) tumor suppressor gene at 10q23.3 was cloned and shown to be inactivated by deletion and mutation in a subset of glioblastomas and other advanced tumors (38, 39). In addition, germ-line *PTEN* mutations were found to be responsible for Cowden disease (40), a hereditary syndrome predisposing to multiple hamartomas and tumors, including meningiomas (41). However, it remains to be shown whether *PTEN* or a yet unknown tumor suppressor gene on 10q25–qter represents the target of 10q deletions in sporadic meningiomas.

Partial or complete losses of chromosome 18 were identified in 43% of MII in our study. This finding contrasts with cytogenetic studies that had rarely detected 18q alterations in meningiomas (10, 32). At least three tumor suppressor genes were identified on 18q and found to be mutated and/or deleted in various tumors including colorectal carcinomas, pancreatic carcinomas, and lung cancers. These are *DCC* (42), *DPC4* (43), and *JV18–1* (44). In meningiomas, our CGH data would argue in favor of a novel gene because the region of common deletion determined for our tumor series mapped to 18q22–qter and thus was distal to 18q21, where *DCC*, *DPC4*, and *JV18–1* are located.

In addition to chromosomal losses, CGH identified a number of MII and MIII with multiple chromosomal gains. The individual target genes for these gains are not known in most cases. Likely targets for gains on 12q13–q15 observed in 13 meningiomas are the protooncogenes *CDK4* and *MDM2*. Amplification of *CDK4* and/or *MDM2*, however, appears to be rare in meningiomas. We identified only one MII in which *CDK4* and *MDM2* were amplified among 62 tumors investigated. Similarly, elevated expression of the mdm2 protein was not seen in 16 primary meningiomas (45), and a study on 22 meningiomas failed to detect *CDK4* amplification (46).

Genomic Alterations in Anaplastic Meningiomas. Two genomic alterations were frequently found in MIII but rarely detected in MI and MII. These were loss on 9p and amplification on 17q. An important target for 9p deletions in human tumors has been identified as the *CDKN2A* [*MTS1, p16*(*INK4A*)] tumor suppressor gene at 9p21 (47, 48). We, therefore, investigated whether deletions of the *CDKN2A* gene could be detected in our series of tumors. Evidence for homozygous *CDKN2A* deletions was found in 4 of 16 MIII but none of the MI or MII. In contrast to our data, two previous reports did not find homozygous deletions or mutations of *CDKN2A* in studies of 22 and 9 meningiomas, respectively (46, 49). Our results, however, provide evidence that inactivation of *CDKN2A* does represent a molecular mechanism involved in the progression of an appreciable fraction of meningiomas. The finding of three tumors in our series showing LOH on 9p restricted to markers located distally to *CDKN2A* suggests the possibility of a further tumor suppressor gene locus on 9p.

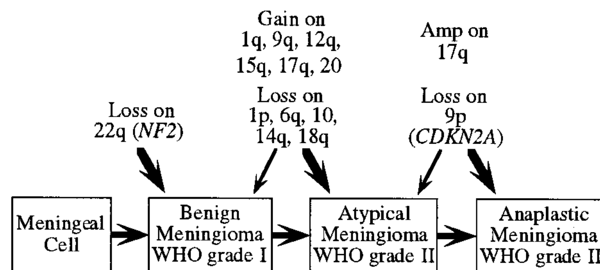

FIG. 5. Proposed model of genomic alterations in meningioma progression. Gains and losses are given before the tumor grade in which they are first detected at a frequency of more than 30%. However, the changes may already have occurred in a lower grade in a smaller percentage of tumors. To illustrate this fact thin arrows are pointing toward the lower tumor grade.

High-level amplification on 17q was identified by CGH in 8 of 19 MIII (48%). In contrast, 17q amplification was found in only 1 of 21 MII and 0 of 19 MI investigated. Amplification of 17q sequences thus appears to be an important molecular mechanism for progression from MII to MIII. The 17q amplification was restricted to chromosomal bands 17q22–q23 in one and to 17q23 in another tumor. In the remaining cases, the amplified sequences involved a larger region, 17q22–qter or 17q21–qter. Amplification on 17q is also frequent in breast cancers (50, 51). The protooncogene *ERBB2*, which maps to 17q11.2–q12 and is amplified in 25–30% of breast carcinomas (52), was not found to be amplified in our series of meningiomas (data not shown).

Although the vast majority of MII and MIII demonstrated complex genomic aberrations by CGH, these tumors lacked mutations in the *TP53* tumor suppressor gene, an alteration thought to predispose to genomic instability (53). Absence of *TP53* gene mutations in meningiomas has also been reported by Ohgaki *et al.* (54) and single meningiomas with *TP53* mutation have been found by other authors (55). Unless there are mutations in the rarely affected regions of *TP53*, alterations of this gene appear to be of minor significance in meningiomas.

A Model of the Genomic Alterations Associated with Meningioma Progression. According to the concept of clonal evolution, the development of a tumor is thought to be initiated by the clonal expansion of a single cell carrying a mutation that leads to a growth advantage (56). Subsequently, any cell of this original clone may acquire additional genetic alterations giving rise to more rapidly growing subclones. Tumors thus progress in a multistep process by the cumulative acquisition of genetic changes. Models in line with this concept have been proposed for the genetic alterations associated with tumor initiation and progression in various human tumor types including colorectal carcinomas (57), gliomas (58, 59), renal cell tumors (60), prostate cancer (61), and head and neck squamous cell carcinomas (62). For meningiomas, a model of the most likely sequence of chromosomal and genetic alterations based on the genomic changes identified in more than 30% of MI, MII, and/or MIII investigated in our study is depicted in Fig. 5. So far, the individual genes targeted by the chromosomal aberrations shown are not known except for 22q (*NF2*) and 9p (*CDKN2A*). The model proposed herein will certainly need refinement by further molecular studies aimed at the identification of the genes representing the targets of the chromosomal deletions, gains, and amplifications identified so far.

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