Human Cancer Biology

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Genomic Profiling Reveals Alternative Genetic Pathways of Meningioma Malignant Progression Dependent on the Underlying *NF2* Status

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Abstract

Purpose: Meningiomas are the most common central nervous system tumors in the population of age 35 and older. WHO defines three grades predictive of the risk of recurrence. Clinical data supporting histologic malignant progression of meningiomas are sparse and underlying molecular mechanisms are not clearly depicted.

Experimental Design: We identified genetic alterations associated with histologic progression of 36 paired meningioma samples in 18 patients using 500K SNP genotyping arrays and *NF2* gene sequencing.

Results: The most frequent chromosome alterations observed in progressing meningioma samples are early alterations (i.e., present both in lower- and higher-grade samples of a single patient). In our series, *NF2* gene inactivation was an early and frequent event in progressing meningioma samples (73%). Chromosome alterations acquired during progression from grade I to grade II meningioma were not recurrent. Progression to grade III was characterized by recurrent genomic alterations, the most frequent being *CDKN2A*/*CDKN2B* locus loss on 9p.

Conclusion: Meningiomas displayed different patterns of genetic alterations during progression according to their *NF2* status: *NF2*-mutated meningiomas showed higher chromosome instability during progression than *NF2*-nonmutated meningiomas, which had very few imbalanced chromosome segments. This pattern of alterations could thus be used as markers in clinical practice to identify tumors prone to progress among grade I meningiomas. *Clin Cancer Res; 16(16); 4155–64. ©2010 AACR.*

Meningiomas are the most common central nervous system tumors in the population of age 35 and older (1). They originate from the meningeal coverings of brain and spinal cord. The only well-established gene in meningioma genesis is *NF2* (Neurofibromatosis 2 OMIM 101000). Individuals with neurofibromatosis type 2 (NF2) are at significantly elevated risk for developing meningiomas, suggesting that the *NF2* gene might play a central role in regulating leptomeningeal cell proliferation (2). Biallelic inactivation of the *NF2* gene on chromosome 22 has been found in 30% to 70% of sporadic meningiomas, leading to the loss of the *NF2* gene product, merlin/schwannomin (3). In addition, *NF2* inactivation is thought to be an early event in sporadic meningioma pathogenesis and is observed as frequently in grade I tumors as in high-grade tumors (4). NF2 mouse modeling also supports a strong involvement of this gene in meningioma tumorigenesis. *Nf2* gene inactivation in meningeal cells using conditional *Nf2* knockout mice induces meningioma development (5).

According to the 2000 WHO classification (6), about 80% of meningiomas are slow-growing grade I benign tumors. Atypical grade II meningiomas constitute 15% to 20% of meningiomas, and their incidence has been increasing since the WH0 2000 classification (7). One to three percent of meningiomas are grade III tumors and behave as true malignant neoplasms. Histologic progression of meningiomas has been recently depicted (8, 9): 17% to 38% of grade II meningiomas derive from grade I tumors and 54% to 70% of grade III meningiomas derive from grade I or II tumors. Several studies have been done to identify genes involved in meningioma progression using

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Translational Relevance

Up to 25% of meningiomas show aggressive histologic features and are characterized as grade II or III according to the WHO classification. A subset of these tumors originate from grade I tumors that undergo malignant progression. This study focuses on a unique series of 36 paired meningioma samples (18 patients) that showed tumor recurrence with histologic malignant progression. High-density 500K SNP array genotyping allowed the fine cartography of genomic alterations by comparing samples and their subsequent progressing counterparts in the same patient. The main finding of this study is that genomic alterations associated to date with grade II or III meningiomas, such as 1p, 6q, and 14q losses, are also observed in grade I meningiomas with *NF2* mutations that undergo malignant progression. This chromosome instability phenotype associated with *NF2* status could be used as a marker in clinical practice to identify grade I meningiomas prone to progress.

cDNA microarray and low-density comparative genomic hybridization (10–14). A comparison of independent cases from grade I, II, and III tumors identified homozygous *CDKN2A*/*CDKN2B* deletions in almost half of grade III meningiomas (15). In grade III meningiomas, 9p21 deletions encompassing *CDKN2A*/*CDKN2B* are associated with poorer survival (16). Very few studies including successive, recurrent specimens from the same patient whose tumor had progressed to a more malignant histologic phenotype have been conducted (17–20). They showed losses on chromosome 22q in samples of all grades, associated with 1p, 9q, 10q, and 14q losses, sometimes solely observed in high-grade tumors. These studies were limited by the small numbers of tumors involved (one to four progressing cases) and by the fact that analyses were conducted at the cytogenetic level.

High-density SNP arrays are new powerful tools, enabling the fine mapping of the tumor genome. Genomic and transcriptional studies have been successfully conducted in a variety of cancers, such as lung cancer (21), prostate cancer (22), and melanoma (23). In particular, high-density SNP arrays, such as 500K Single Nucleotide Polymorphism array (500K SNP), enable detailed and genome-wide identification of both loss of heterozygosity (LOH) events and copy number alterations in cancer (24).

In this study, we identified genetic alterations associated with histologic progression of 36 paired meningioma samples in 18 patients using 500K SNP genotyping arrays. A major event associated with progression to grade III was loss of the *CDKN2A*/*CDKN2B* locus on 9p. Meningiomas displayed different patterns of alteration during progression according to their *NF2* status; *NF2*-mutated meningiomas showed higher chromosome instability during progression than *NF2*-nonmutated meningiomas.

Materials and Methods

Patients and tumor samples

A total of 18 progressing meningioma cases, corresponding to 37 tumor samples, were selected (17 patients with 2 samples and 1 patient with 3 samples). Clinical data are depicted in Table 1. Each sample had frozen material and paraffin-embedded blocks available to be included in this study. All the patients were recruited in accordance with French law and consented under the Institutional Review Board of Partners Healthcare. Patients with multiple meningiomas or NF2 were excluded from the study. Two independent pathologic reviews by two neuropathologists were conducted on each case. A neuropathologist at the hospital where the surgery occurred reviewed each tumor specimen, and a neuropathologist at Brigham and Women's Hospital (J.C.) reviewed all paraffin slides and confirmed the diagnosis, grade, and histologic subtype (Supplementary Table S1). All tumors were graded according to the WHO 2000 grading scheme (6). Twenty 20-μm frozen sections were allocated for purification of genomic DNA with AllPrep DNA/RNA minikit (QIAGEN GmbH) following the manufacturer's protocol for each specimen. The tumor cell content was quantified by H&E staining of the frozen section. Only samples with a tumor cell content >90% were retained for subsequent analysis.

NF2 characterization

The 17 *NF2* exons were amplified by PCR and sequenced using Big Dye terminator chemistry on an ABI3110 (Applied Biosystems) following the manufacturer's recommendations. PCR protocols and primers sequence are available on request. Sequences were analyzed using Sequencher 4.7 software (Gene Codes). When no *NF2* mutation was identified, DNA samples were further examined for large alterations using multiplex ligation-dependent probe amplification (MRC Holland) following the manufacturer's protocol.

Search for alterations in *NF1* and *CDH7* genes

Molecular investigation of *NF1* gene was done at the cDNA level. Briefly, after reverse transcription of 1 μg of total RNA, eight overlapping PCR products were sequenced (BDT Cycle Sequencing Ready Reaction Kit, Applied Biosystems) using a panel of 25 sequencing primers covering the entire 8,520-bp NF1 coding sequence. *CDH7* mutations were investigated by direct sequencing of tumor DNA. Primer sequences and reaction conditions are available on request.

LOH and copy number analysis

High-resolution genome-wide copy number variation and LOH analyses were carried out using GeneChip Human Mapping 500K Array Set (Affymetrix, Inc.) following the manufacturer's instructions. Eight HapMap samples

(data provided by Affymetrix) and normal blood DNA from two patients (mh117 and mh179) were used for normalization. LOH was inferred by paired comparison of samples in the same patient. SNP data were analyzed using dChip software (25). Genomic alterations were classified as loss, homozygous deletion, gains, and hemizygosity. Deletions and gains were deduced following a hidden Markov model with a smoothing window of five-SNP probes using dChip. LOH regions were determined in dChip by paired analysis of the two (or three) samples of each patient. When blood was available, it was compared with each sample of the case. We then used two strategies to define regions of gain and loss. Raw data output from dChip was analyzed using customized program written in R (version 2.0.1, http://www.R-project.org). In brief, SNP clusters were searched (10 consecutive SNP, 8 of them corresponding to the search criteria) for homozygous deletion (raw copy number <0.5), loss (raw copy number <1 and LOH), gain (raw copy number >3), or neutral copy number LOH regions (raw copy number >1 and <3, and LOH). Each region was then manually checked to confirm the alteration and define borders. The physical positions of the detected deletions were determined based on the human hg17 assembly (National Center for Biotechnology Information Build 35). Imbalanced chromosome segments (ICS) were defined as the sum of gains, losses, neutral copy number LOH, and homozygous deletion.

Fractional allele loss (FAL; ref. 26) in a meningioma sample was defined as the number of chromosome arms on which allele loss was observed, divided by the number of chromosome arms studied. Unpaired *t* tests were used to compare values in GraphPad Prism 4.0 software. Microsatellite instability was ruled out as described in ref. 27.

Results

NF2 inactivation is an early and frequent event in progressing meningioma samples

Tumor DNA sequencing identified a *NF2* gene mutation in DNA from 26 of 37 progressing meningioma samples, and one additional whole exon deletion was found in one sample by multiplex ligation-dependent probe amplification (Table 2). Twenty mutations were predicted to lead to a premature stop codon (direct stop or frameshift); six were splice site mutations. When a patient harbored a *NF2* gene mutation in a more advanced stage, the mutation was always present in the lower-grade tumor, suggesting that *NF2* loss is an early event in meningioma genesis. These 27 samples (14 patients) are hereafter referred to as *NF2*-mutated. No *NF2* mutation was identified in 10 samples (6 patients), hereafter referred to as *NF2*-nonmutated. On SNP analysis, 100%

Table 1. Main clinical and pathologic features of 18 progressing meningioma cases

Case ID	Gender	Localization	Size (cm)	WHO grade I				WHO grade II			WHO grade III		
				Sample ID	Age (y)	ttt	Histologic subtype	Sample ID	Age (y)	ttt	Sample ID	Age (y)	ttt
93	F	Falx	NA	93.1	48	—	Fibroblastic				93.3	49	S
117	M	Tentorium	6	117.1	58	—	Meningothelial				117.3	60	S
86	M	Occipital	8	86.1	48	—	Fibroblastic	86.2	54	Sx2	86.3	55	Sx3
91	M	Temporal	NA	91.1	57	—	Transitional	91.2	61	—			
95	F	Tentorium	5	95.1	37	—	Meningothelial	95.2	39	—			
177	M	Sphenoid	5	177.1	56	—	Meningothelial	177.2	63	Sx2, Rs, I			
170	M	Parasagittal	3	170.1	56	Rs	Transitional	170.2	59	Rs, S, Rt, G			
295	M	Sphenoid	NA	295.1	72	—	Meningothelial	295.2	74	—			
89	F	Frontal	6	—	53	—	Meningothelial	89.2	68	Sx2, Rt, H	89.3	70	Sx3, Rt, H
100	F	Parasagittal	8					100.2	63	—	100.3	66	Sx2
103	M	Parasagittal						103.2	31	—	103.3	36	Sx8, Ptx2, H, Rt
111	M	CP angle	3					111.2	54	S	111.3	55	Sx2
115	M	Frontal	5					115.2	67	—	115.3	68	—
119	M	Frontal	4	—	50	—	Meningothelial	119.2	54	Sx2	119.3	57	Sx5, Rt, H
146	M	Parasagittal	3					146.2	66	—	146.3	75	
175	F	Frontal	NA					175.2	70	S	175.3	71	Sx2, Fl
179	M	Parasagittal	NA					179.2	51	Sx2	179.3	53	Sx3, Rt, Rs
207	F	Frontal	7					207.2	50	—	207.3	52	—

Abbreviations: ttt, treatment before surgery; S, surgery; Rs, radiosurgery; Rt, radiotherapy; Pt, proton therapy. Chemotherapy: F, flutamide; G, gefitinib; H, hydroxycarbamide; I, imatinib mesylate.

of *NF2*-mutated samples harbored 22q loss, whereas 2 of 10 (20%) of samples without a *NF2* mutation had a loss of 22q ($P < 0.001$), suggesting biallelic *NF2* inactivation in *NF2*-mutated samples.

Chromosome alterations are frequent even in grade I progressing meningioma samples

Of the 37 meningioma samples examined, 35 exhibited various ICS (losses, gains, homozygous deletion, and neutral copy number LOH), with only 2 samples (1 patient) free of any chromosome aberrations. The majority of aberrations encompassed a large segment of a chromosome arm or even the whole chromosome. There were a median number of 13 ICS per meningioma sample (range, 0–39). Losses were more frequently detected than gains at the chromosome arm level (total number of aberrations, 317 versus 90). The most common chromosome arm loss was at 22q [30 meningioma samples (81%)], encompassing the *NF2* tumor suppressor gene. Frequent losses of chromosome arms 1p (78%), 6q (62%), 14q (49%), 3p (43%), 10q (43%), and 18q (43%) were observed. Losses involving the whole chromosome arm were seen in 29% of cases and were <5 Mb in 36.6%. The frequencies of chromosome arm

Table 2. *NF2* status of 37 progressing meningioma samples

Case	Sample	DNA	Exon	Protein (predicted)	Result	*NF2* LOH (SNP array)
86	86.1	c.493del C	5	p.Gly165fs	Frameshift	Yes
	86.2	c.493del C	5	p.Gly165fs	Frameshift	Yes
	86.3	c.493del C	5	p.Gly165fs	Frameshift	Yes
89	89.2	c.955C>T	10	P.Gln319X	Stop	Yes
	89.3	No mutation				No
91	91.1	c.743del C	8	p.Asn248fs	Frameshift	Yes
	91.2	c.743del C	8	p.Asn248fs	Frameshift	Yes
93	93.1	c.448−1G>A	5		Splice	Yes
	93.3	c.448−1G>A	5		Splice	Yes
95	95.1	No mutation				No
	95.2	No mutation				No
100	100.2	c.1219C>T	12	p.Gln407X	Stop	Yes
	100.3	c.1219C>T	12	p.Gln407X	Stop	Yes
103	103.2	IVS12_IVS13del*	13			Yes
	103.3	No mutation*				No
111	111.2	No mutation				No
	111.3	No mutation*				No
115	115.2	c.225_226insA	2	p.Leu75fs	Frameshift	Yes
	115.3	c.225_226insA	2	p.Leu75fs	Frameshift	Yes
117	117.1	c.552G>A	6	p.Trp184X	Stop	Yes
	117.3	c.552G>A	6	p.Trp184X	Stop	Yes
119	119.2	c.1263G>T	12	p.Glu422X	Stop	Yes
	119.3	c.1263G>T	12	p.Glu422X	Stop	Yes
146	146.2	c.1058_1065del7	11	p.Arg353fs	Frameshift	Yes
	146.3	c.1058_1065del7	11	p.Arg353fs	Frameshift	Yes
170	170.1	c.602_603del AT	7	p.Asp201fs	Frameshift	Yes
	170.2	c.602_603del AT	7	p.Asp201fs	Frameshift	Yes
175	175.2	c.1340+1G>A	12		Splice	Yes
	175.3	c.1340+1G>A	12		Splice	Yes
177	177.1	c.517−1G>A	6		Splice	Yes
	177.2	c.517−1G>A	6		Splice	Yes
179	179.2	No mutation*				Yes
	179.3	No mutation*				Yes
207	207.2	c.203_204insA	2	p.Ileu68fs	Frameshift	Yes
	207.3	c.203_204insA	2	p.Ileu68fs	Frameshift	Yes
295	295.1	No mutation*				No
	295.2	No mutation				No

*Sample tested by multiplex ligation-dependent probe amplification.

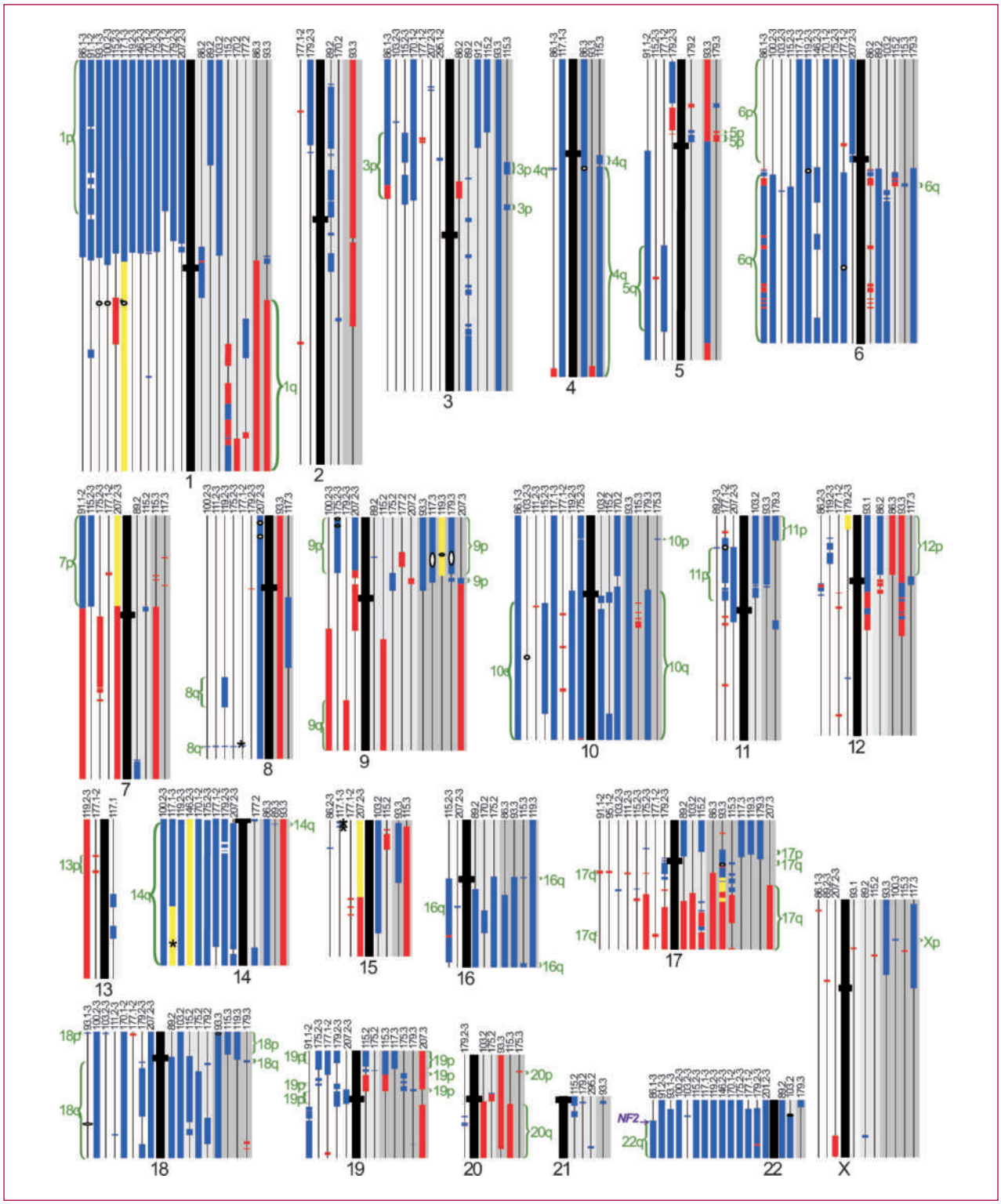

Fig. 1. Overview of all ICS observed in 37 progressing meningioma samples. Each chromosome is depicted as a vertical black bar with the position of the centromere. All alterations in a defined tumor are depicted as a vertical bar with sample ID indicated on top. Early alterations (i.e., present in each samples for one patient) are indicated on the left side of the chromosome. Specific grade I, II, or III alterations (present only in the grade I, II, or III sample of the patient) are depicted on the right side of the chromosome on a gray scale background representing the three grades. Losses are in blue, gains in red, and neutral copy number LOH regions in yellow. Black circles, homozygous deletions. *, the alteration is also observed in blood DNA, when available. Green vertical braces highlight recurrent alterations (early on the left side and specific grade I, II, or III on the right side of the chromosome).

amplification were much lower. The most frequent chromosome arm gains were on 17q (27%), 7p (19%), and Xp (19%). The extent of gains on chromosome arms was smaller than that of losses: gains involving the whole chromosome arm were seen in 16.7% and were smaller than 1 Mb in 54.4%. Twenty-two homozygous deletions were observed in 17 meningioma samples, spanning 0.003 to 7.5 Mb. One homozygous deletion on 1q21.1 was observed in 6 samples (3 patients: 93, 100, and 117). This deletion was also present in matched DNA from blood lymphocytes of patient 117 and is likely to be a polymorphism. Neutral copy number LOH were observed in 11 samples (5 patients). They encompassed large chromosome regions or whole chromosome arm in 9 samples and were limited to a 1-Mb region in 2 samples (1 patient). Interestingly, in patient 146, neutral copy number LOH involving 14q was observed in the 2 meningioma samples and in matched DNA from blood lymphocytes. Although not significant, the mean number of ICS per sample increased with an increase in tumor grade: 8.6, 16.8, and 18.2 in grades I, II, and III, respectively.

The most frequent chromosome alterations observed in progressing meningioma samples are early alterations (i.e., present both in lower- and higher-grade samples of a single patient)

Along with histologic progression, there were no additional ICS in three patients (cases 95.1/2, 111.2/3, and 146.2/3). The median percentage of early ICS was 84.6%, ranging from 100% in grade I samples to 83.3% in grade II and 85.8% in grade III. Figure 1 summarizes the entire panel of ICS (gains, losses, homozygous deletions, neutral copy number LOH) observed in our tumor sample set. Early alterations, present both in lower- and higher-grade samples

Table 3. New alterations acquired during progression from grade I to grade II meningioma

Case ID	Chr	Alteration	Cytoband coordinate (hg17 assembly)	Size (Mb)	No. of genes	Selected RefSeq genes
170.2	1p	Gain	1q42.13/1q44 225.099/245.378	20.3	>100	*AKT3*
177.2	1q	Loss	1q23.1/1q25.3 153.769/178.485	24.7	>100	
177.2	1q	Loss	1q42.12/1q42.13 221.463/225.844	4.4	49	
170.2	2q	Loss	2q23.3/2q24.1 153.510/155.171	1.7	4	*RPRM, GALNT13, AX746678, KCNJ3*
91.2	3p	Loss	0/3p21.1 0/53.174	53.2	>100	
177.2	9p	Loss	9p21.3/9p21.3 21.398/30.006	4.7	7	*CDKN2A, 2B, MTAP, ELAVL2, TUSC1*
170.2	10p	Loss	0/10p11.21 0/37.088	37.1	>100	
170.2	10q	Loss	10q11.21/10q26.3 43.055/135.323	92.3	>100	
86.2	12p	Loss	12p13.33/12p11.1 0.037/36.216	36.2	>100	*CDKN1B, LRMP*
86.2	12q	Gain	12q12/12q12 39.079/40.868	2.1	8	*LRRK2*
86.2	12q	Gain	12q12/12q12 41.098/41.852	0.7	4	*PPHLN1, HSPC232, PRICKLE1*
86.2	12q	Gain	12q13.11/12q13.12 45.569/47.807	2.2	38	*WNT1*
177.2	14q	Loss	14q11.2/14q11.2 19.273/22.590	3.3	73	
177.2	14q	Loss	14q32.2/14q32.33 96.284/106.356	10	>100	
170.2	16q	Loss	16q12.2/16q22.1 54.739/68.253	13.5	>100	
295.2	21q	Loss	21q22.2/21q22.2 40.873/40.888	0.01	1	*DSCAM*

Table 4. Recurrent new alterations observed during progression from grade I or II to grade III meningiomas

No. of cases	Case ID	Chr	Alteration	Cytoband (coordinate hg17 assembly)	Size (Mb)	Selected RefSeq genes
4	93, 117, 119, 179	9p	Loss	9p24.3/9p21.1 (0.031/28.683)	44.1	>100 genes
4	93, 117, 179, 207	9p	Loss	9p13.1/9p13.1 (38.664/38.758)	0.01	None
4	86, 93, 115, 119	16q	Loss	16q24.2/16q24.3 (86.060/88.691)	2.1	87 genes
4	86, 93, 115, 207	17q	Gain	17q21.31/17q25.3 (39.015/78.605)	39.6	>100 genes
3	93, 115, 119	16q	Loss	16p11.2/16p11.1 (34.067/34.615)	0.6	None
3	93, 117, 119	17p	Loss	17p11.2/17p11.2 (17.612/19.959)	61	>100 genes
3	93, 115, 119	18p	Loss	18p11.32/18p11.21 (0.151/13.597)	13.6	57 genes
3	93, 100, 117	Xp	Loss	Xp22.11/Xp22.11 (23.951/23.994)	0.04	*KLHL15, IEF2S3*
2	93, 86	1q	Gain	1q21.1/1q44 (142.856/245.378)	103.9	>100 genes
2	115, 93	3p	Loss	3p14.2/3p14.1 (61.903/69.309)	7.4	45 genes
2	115, 93	3p	Loss	3p11.2/3p11.1 (87.347/90.308)	2.9	*CHMP2B, POU1F1, HTR1F, CGGBP1, ZNF654, C3ORF38, EPHA3*
2	115, 86	4q	Loss	4q12/4q13.1 (57.547/63.350)	133.8	>100 genes
2	115, 86	4q	Loss	4q13.1/4q35.2 (64.500/191.306)	126.8	>100 genes
2	179, 93	5p	Gain	5p12/5q11.1 (45.135/49.609)	4.5	*HCN1*
2	179, 93	5p	Gain	5p12/5p12 (42.958/44.418)	3.5	26 genes
2	115, 179	6q	Loss	6q13/6q13 (74.377/76.363)	2	*SLC17A5, AK124950, CD109, AF086303, COL12A1, COX7A2, TMEM30A, FILIP1*
2	93, 175	10p	Loss	10p13/10p13 (13.925/14.426)	0.5	*FRMD4A*
2	93, 179	10q	Loss	10q11.21/10q26.3 (44.144/135.323)	91.1	>100 genes
2	93, 179	11p	Loss	11p15.5/11p15.2 (0.197/14.956)	16	>100 genes
2	86, 93	12p	Gain	12p13.33/12p11.1 (0.037/36.216)	34.1	>100 genes
2	86, 89	14q	Loss	14q11.2/14q11.2 (21.632/22.046)	0.7	*TRAC*
2	93, 115	17q	Loss + HD	17q11.2/17q11.2 (24.909/26.239)	1.3	17 genes
2	93, 179	18q	Loss	18p11.21/18q11.2 (15.097/18.414)	1.5	*ROCK1, ESCO1, SNRPD1, ABHD3, MIB1, GATA6, CTAGE1*
2	115, 117	19p	Loss	19p13.3/19p13.2 (0/10.965)	11	>100 genes
2	115, 175	19p	Loss	19p13.12/19p13.11 (14.485/16.655)	3.3	52 genes including *notch3*
2	175, 179	19p	Loss	19p12/19p12 (23.070/24.165)	1.2	*ZNF91, 675, 681, 254*
2	93, 175	20p	Gain	20p12.2/20p12.2 (9.577/9.580)	0.01	*PAK7*
2	93, 115	20q	Gain	20q11.21/20q13.32 (29.696/56.479)	56.5	>100 genes

Abbreviation: HD, homozygous deletion.

of a single patient, are depicted on the left side of each chromosome. Of note, we observed clusters of chromosome alterations: 43% (16 of 37) of meningioma samples harbored simultaneous 22q, 1p, 6q, and 14q deletions.

Chromosome alterations acquired during progression from grade I to grade II meningioma are not recurrent

Six patients had progression from grade I to grade II meningioma. A mean of 3.7 ICS observed in grade II samples were not observed in the corresponding grade I specimens (Table 3). None of these alterations were found in more than two individuals. Thus, in this series, each case had a unique genetic progression pathway and did not point to a specific gene or chromosome region.

Progression to grade III is characterized by recurrent genomic alterations, the most frequent being *CDKN2A/CDKN2B* loss

Thirteen meningioma samples showed progression to grade III tumors: 2 patients from grade I to III, 1 patient from grade I to II to III, and 10 patients from grade II to III. A mean of 4.5 *de novo* grade III alterations per sample were identified. Twenty-eight alterations were recurrent (Table 4). Chromosome regions are depicted in green in Fig. 1. Most of these regions were larger than 2 Mb and encompassed several genes. Three overlapping homozygous deletions were found on chromosome 9p21.3 (patients 117, 119, and 179). The smallest homozygous deletion spanned a 0.2-Mb region overlapping with the other two deletions and contained only the *CDKN2A/CDKN2B* and methylthioadenosine

phosphorylase (*MTAP*) genes. These deletions were not observed in lower-grade counterpart tumors, suggesting that these recurrent alterations are directly associated with the grade III histomorphologic transition.

A homozygous microdeletion including the *NF1* gene was observed during progression to grade III meningioma

A 918.9-kb homozygous deletion was observed in case 93 on 17q11.2, between SNPs rs7225461 and rs8074383. This deletion included *NF1* and neighboring genes (*CENTA2*, *RNF135*, *OMG*, *EVI2B*, *EVI2A*, *RABFIP4*, and *C17orf79*) and is quite similar to the deletion described in "*NF1* microdeletion syndrome," characterized by a more severe phenotype than the majority of NF1 patients (28). The incidence of meningioma in the NF1 population does not seem to be increased (2 of 158 in ref. 29 and 1 of 523 in ref. 30), but one case of NF1-related grade II meningioma has been published (31) and sporadic *NF1* mutations have been recently identified in glioblastoma multiforme (32). Based on this observation, we screened eight grade III samples (86.3, 89.3, 100.3, 103.3, 111.3, 115.3, 117.3, and 119.3) for *NF1* gene mutations. No mutation was identified, suggesting that *NF1* alteration is not a major event in meningioma malignant progression.

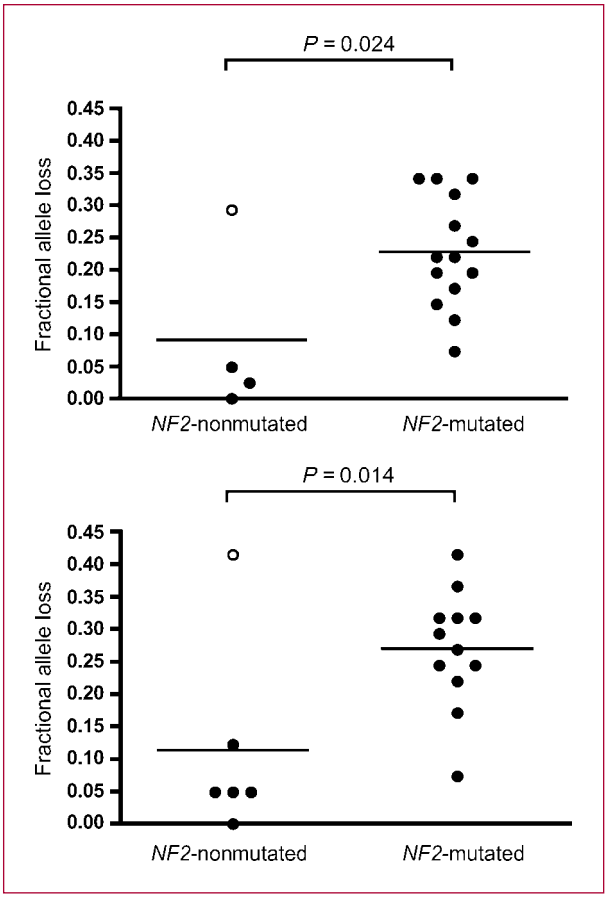

Fig. 2. FAL is significantly different between *NF2*-mutated meningiomas and *NF2*-nonmutated meningiomas. Top, lower-grade sample of paired progressing meningiomas. Bottom, higher-grade sample of paired progressing meningiomas. The empty dots refer to case 179 that showed 22q loss but no *NF2* mutation.

The *NF2* status is associated with the pattern of genomic alterations in progressing meningiomas

To rule out factors associated with the accumulation of chromosome alterations, FAL was calculated for each sample. There was a tendency toward increase of FAL according to histologic grade ($P = 0.17$); there was no difference according to sex or previous radiation therapy (data not shown). When divided according to their *NF2* status, meningioma samples showed significantly different FAL. Whether we take into account lower- or higher-grade samples of each pair, *NF2*-nonmutated meningioma samples showed very few chromosome alterations (mean FAL, 0.09 and 0.11 in lower- and higher-grade samples of each case, respectively). In contrast, mean FAL in *NF2*-mutated tumors was 0.23 and 0.27 in lower- and higher-grade samples, respectively [$P = 0.024$, lower-grade; $P = 0.014$, higher-grade (unpaired *t* test); Fig. 2]. To further rule out a genetic unstable phenotype, microsatellite instability was assessed in these tumors. No microsatellite instability was found in 29 samples distributed in the three grades, independent of the presence of an *NF2* mutation (data not shown).

NF2-nonmutated samples showed very few ICS

A 0.6-Mb interstitial deletion in 18q22, encompassing solely the *CADHERIN 7* gene (*CDH7*), was shown in case 111. *CDH7* is a cadherin expressed in the nervous system and has been linked to carcinogenesis (33). Thus, *CDH7* was a potential candidate gene for meningioma development. We searched for mutations in the 11 coding exons of *CDH7* in 16 meningioma samples, including case 111. No mutation was found, suggesting that *CDH7* is not inactivated by biallelic events in meningioma genesis.

Discussion

The strength of this work is the direct comparison between a meningioma and its subsequent recurrence with histologic malignant progression in the same patient. To our knowledge, this series is the largest in the literature analyzing such tumors. In addition, the use of 500K SNP mapping arrays allowed high-density genome-wide analysis.

A revisited meningioma genesis scheme based on the *NF2* status of tumors

Meningioma tumorigenesis should be divided into two main arms according to the *NF2* status of the tumor sample. About one third of meningiomas arise in the absence of *NF2* loss. These tumors are characterized by the scarcity of chromosome alterations. The underlying genetic events involved in initiation of *NF2*-nonmutated meningiomas remain to be elucidated. On the other hand, about two thirds of meningiomas arise through an *NF2*-dependent pathway. For these cases, most of grade I meningiomas

do not progress to a higher grade and are characterized by very few chromosome alterations, mainly isolated 22q loss (34, 35). A subset of grade I tumors are able to progress to higher grades and are characterized by a pattern of chromosome alterations including 1p, 6q, and 14q losses. These alterations have been associated with grade II and III meningiomas (14, 20, 35–37), but they are also present in lower-grade samples (grade I or II) in our series. This observation suggests that these chromosome alterations are not late events involved in meningioma malignant progression (18) because they exist in grade I meningiomas. On condition that the *NF2* status of samples is determined, these chromosome alterations could certainly be used as markers in clinical practice to identify tumors prone to progress among grade I meningiomas. In case of aggressive genotypic signature, partially resected grade I meningiomas could benefit from adjuvant therapies (surgery, radiosurgery, radiotherapy) before any tumor regrowth. This hypothesis must be evaluated in appropriate clinical trials.

NF2 loss is associated with chromosome instability in progressing meningioma samples

This raises the question whether *NF2* drives chromosome instability in meningioma precursor cells, or whether *NF2* loss is the consequence of an earlier event responsible for chromosome instability. Of note, the effect of *NF2* loss on chromosome instability and malignant transformation seems to be tissue specific because different situations occur in various tumor types having *NF2* gene mutations as common denominator. In schwannoma, a benign tumor of the peripheral nervous system, *NF2* inactivation is universal, necessary, and sufficient for tumor development in the absence of chromosome instability (38, 39). Rare cases of schwannomas undergo malignant transformation, generally after radiation therapy, which possibly induces *p53* mutations (40). Cases of "somatic" *NF2* instability have been described in schwannomas: different *NF2* mutations have been found in seven different tumors from one patient with sporadic schwannomatosis (41), suggesting additional underlying genetic events predisposing to *NF2* loss. *INI1/SMARCB1* has recently been implicated in familial schwannomatosis (42) but this role does not seem to be related to induction of genomic instability. In malignant mesothelioma, a highly malignant tumor of the pleura, half of the cases harbor *NF2* gene mutations and 22q losses (43) and they show chromosome instability (44). Similarly to malignant meningiomas, loss of *CDKN2A/CDKN2B* on 9p is another major event (80%) observed in mesothelioma genesis (45).

A single patient (case 179) in the *NF2*-nonmutated group showed a high FAL, in the range of *NF2*-mutated samples. Of note, this sample is the only one in the *NF2*-nonmutated group that harbors 22q loss; thus, this sample might indeed have *NF2* inactivation through, for example, epigenetic mechanisms. In the literature, the *NF2* status of meningioma samples is seldom determined, but review of literature clustering samples according to their 22q status also finds an association between 22q loss and FAL in the meningiomas studied (14, 35, 36, 46–48). These studies are in agreement with our finding that *NF2* inactivation in meningiomas is associated with greater chromosome instability.

Clonality

A paired analysis, comparing progressing samples in the same patient, showed a clonal evolution during progression in 16 of 18 patients: the two (or three) samples of the same patient sharing common alterations, and few alteration subsets being found only in one of the samples. Two patients (89 and 103) did not fit in this model of clonal evolution. These grade II samples showed a pattern of genetic alterations including 22q loss and a *NF2* gene mutation. The corresponding grade III tumors, arising from the remnant of the first surgery, had none of the alterations of the parental grade II tumor, including the *NF2* mutation, suggesting that these two grade III meningiomas originated from a different clone. Interestingly, these two patients both received radiotherapy before their second surgery. Thus, radiotherapy could have been the impetus for this new clone to arise.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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