Oncogene (1999) 18, 2231–2239

http://www.stockton-press.co.uk/onc

NF2 gene mutations and allelic status of 1p, 14q and 22q in sporadic meningiomas

Paola E Leone[1,5], M Josefa Bello[1], Jose M de Campos[2], Jesus Vaquero[3], Jose L Sarasa[4], Angel Pestaña[1] and Juan A Rey*[,1]

[1]*Instituto de Investigaciones Biomédicas (CSIC), Arturo Duperier, 4, 28029-Madrid, Spain;* [2]*Department of Neurosurgery of Hospital del Rio Hortega, Valladolid Madrid, Spain;* [3]*Department of Neurosurgery, Clínica Puerta de Hierro, Madrid, Spain and* [4]*Department of Anatomical Pathology, Fundación Jiménez Diaz, Madrid, Spain*

Formation of meningiomas and their progression to malignancy may be a multi-step process, implying accumulation of genetic mutations at specific loci. To determine the relationship between early *NF2* gene inactivation and the molecular mechanisms that may contribute to meningioma tumor progression, we have performed deletion mapping analysis at chromosomes 1, 14 and 22 in a series of 81 sporadic meningiomas (54 grade I (typical), 25 grade II (atypical) and two grade III (anaplastic)), which were also studied for *NF2* gene mutations. Single-strand conformational polymorphism analysis was used to identify 11 mutations in five of the eight exons of the *NF2* gene studied. All 11 tumors displayed loss of heterozygosity (LOH) for chromosome 22 markers; this anomaly was also detected in 33 additional tumors. Twenty-nine and 23 cases were characterized by LOH at 1p and 14q, respectively, mostly corresponding to aggressive tumors that also generally displayed LOH 22. All three alterations were detected in association in seven grade II and two grade III meningiomas, corroborating the hypothesis that the formation of aggressive meningiomas follows a multi-step tumor progression model.

Keywords: meningiomas; *NF2*; allelic losses; tumor progression; 1p and 14q deletion mapping

Introduction

Meningiomas are neoplasms that arise from the leptomeningeal covering of the brain and spinal cord, accounting for 15–20% of all central nervous system tumors. The current WHO grading system comprises three grades as follows: most meningiomas are slow growing, are generally considered benign tumors, and correspond to grade I (typical meningiomas); about 10% of cases are classified as grade II (atypical) or anaplastic/malignant (grade III), exhibiting more aggressive clinical behaviour, with a higher risk of recurrence when compared to typical grade I meningiomas (Kleihues *et al*., 1993). These neoplasms are usually sporadic, but a few families have been described with multiple tumors inherited in an autosomal dominant fashion (Memon, 1980; Battersby *et al*., 1986; Butti *et al*., 1989; Domenicucci *et al*., 1989), and they also occur in as many as half of the patients with the dominantly inherited familial neurofibromatosis type 2 syndrome (NF2) (Martuza and Eldridge, 1988). Using positional cloning approaches, the candidate gene for NF2 has been isolated from chromosome 22q12 region (Rouleau *et al*., 1993; Trofatter *et al*., 1993), and mutations have been observed in both germ-line of NF2 patients and in about 30% of sporadic meningiomas (Ruttledge *et al*., 1994; Lekanne-Deprez *et al*., 1994; Merel *et al*., 1995; Papi *et al*., 1995; De Vitis *et al*., 1996; Harada *et al*., 1996). These findings indicate that inactivation of the *NF2* gene is important in the development of a significant number of sporadic cases that also generally display LOH for markers located on chromosome 22. This provides conclusive evidence that *NF2* acts as a tumor suppressor gene in at least a subgroup of sporadic tumors. Cytogenetic analysis of meningiomas previously provided evidences of the non-random involvement of chromosome 22 in about 60% of tumors, suggesting that inactivation of tumor suppressor loci (the *NF2* gene is now identified as one of them) located in this chromosome might represent an early step in meningioma tumorigenesis (Zang, 1982; Al Saadi *et al*., 1987; Maltby *et al*., 1988; Rey *et al*., 1988; Casalone *et al*., 1990). Non-random loss of chromosome 14 and structural rearrangements of chromosome 1, generally leading to the loss of short arm regions, were also identified as characteristic cytogenetic features of meningiomas; to a lesser degree, abnormalities of chromosomes 7, 8, 10, 18, 19 and 20 have also been found (Rey *et al*., 1988; Casalone *et al*., 1990; Casartelli *et al*., 1989; Poulsgard *et al*., 1993; Vagner-Capodano *et al*., 1993; Lekanne-Deprez *et al*., 1995; Lopez Gines *et al*., 1995; Schneider *et al*., 1995; Perry *et al*., 1996). The accumulation of chromosomal abnormalities secondary to chromosome 22 loss, frequently in parallel to the genesis of grade II and III tumors, allowed the proposal that meningiomas might display clonal progression through a pattern, implying the accumulation of several genetic anomalies in which monosomy of chromosome 22 would represent an early step (Rey *et al*., 1988; Poulsgard *et al*., 1993). Molecular genetic studies have also recently investigated the association between the allelic loss at genomic regions other than chromosome 22 and tumor progression of meningiomas, suggesting that LOH at 1p, 14q and chromosome 10 appear recurrently

*Correspondence: JA Rey
[5]Current address: Laboratorio de Genetica Molecular y Citogenetica Humana, Facultad de Medicina, Pontificia Universidad Católica de Ecuador, Apartado 17-1-2184 Quito, Ecuador
Received 3 March 1998; revised 27 October 1998; accepted 27 October 1998

associated with grade II and grade III meningiomas (Rempel *et al*., 1993; Bello *et al*., 1994; Lindblom *et al*., 1994; Simon *et al*., 1995; Menon *et al*., 1997). A multistep mechanism similar to that described for other neoplasms (Fearon and Vogelstein, 1990) might thus explain the origin and evolution of aggressive meningiomas. To determine the relationship between *NF2* gene inactivation and the genomic regions harboring 'progression loci' potentially involved in the neoplastic evolution of meningiomas, we examined *NF2* gene mutations and the allelic constitution of 1p, 14q and 22q in a series of 81 sporadic meningiomas. Our data confirmed the association between losses of 1p and/or 14q and the genesis of grade II–III tumors, and provide insights into their relationship with some specific inactivating *NF2* gene mutations.

Results

LOH studies

Twenty-nine tumors were characterized by allelic losses for loci of chromosome 1 (Figures 1 and 2). Two of these 29 tumors lost alleles at loci spanning the entire short arm of chromosome 1 and the loss extended into the long arm in both cases, suggesting that the entire chromosome 1 had probably been lost in these meningiomas (tumors M-22 and M-60). Interstitial and/or terminal deletions were observed in the remaining 27 cases. Twelve meningiomas displayed terminal deletion of 1p, which in one sample (M-29) was restricted to the most distaly analysed locus *D1Z2*. Three tumors were characterized by loss of the 1p32-pter region, with break points distal to *D1S17* (M-65), *D1S57* (M-3) and *D1S7* (M-14). Five cases displayed break points at 1p13, distal to *D1S440* (M-13, M-15 and M-74) and distal to *D1S189* in cases M-72 and M-23. Complex chromosome rearrangements occurred in tumors M-16, M-17 and M-64; LOH was observed for loci at pter-p32 and at p13, with retention of alleles at *D1S10, D1S73* and *D1S17*, respectively, which would define two regions of chromosomal losses on the short arm of chromosome 1. Interstitial 1p deletions were evidenced in 15 meningiomas. In five tumors, locus *D1Z2* limited the losses distally, with the proximal border of the deletions defined by break points in the p13-cen region (tumors M-18, M-53, M-76, M-11 and M-75). This was also the proximal deletion border in

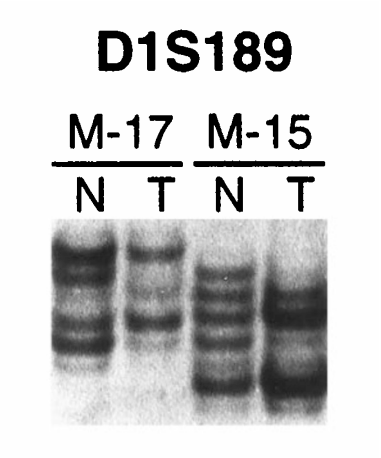

Figure 2 Examples of loss of heterozygosity detection with PCR-amplified microsatellites. Data for marker D1S189 of cases M-17 and M-15 are shown. N, T: normal, tumoral DNA

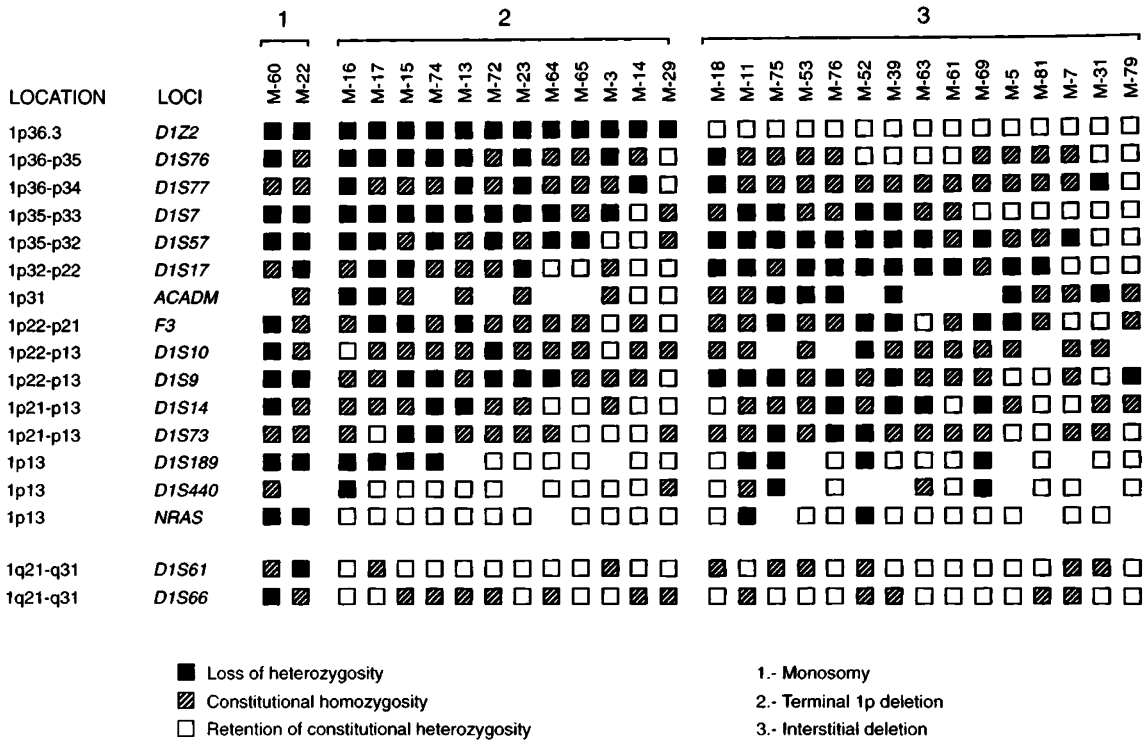

Figure 1 Summary of chromosome 1 deletion mapping in sporadic meningioma. The loci analysed and their location is shown to the left (blank: no data available)

three samples characterized by retention of heterozygosity at D1S76 (meningiomas M-52, M-39 and M-61). Four additional tumors presented the 1p distal border of the deletion limited by the retention of heterozygosity at locus *D1S7*, but with a varying length of deletion, as marked by the proximal border according to the loci displaying retention of heterozygosity: *NRAS* (M-69), *D1S9* (M-5 and M-81) and *D1S17* (M-7). The break points in this meningioma delimited a small loss involving only locus *D1S57*. In tumor M-79, LOH was restricted to locus *D1S9* and boundaries delimited by retention of heterozygosity at *D1S73* and *D1S17*. Finally, tumors M-31 and M-63 presented LOH at two distinct 1p regions, as evidenced by losses involving *D1S77* and *ACADM* in M-31, and deletion of the *D1S7-D1S57* and *D1S14* regions in tumor M-63.

According to these findings, up to three distinct regions of deletion might be proposed. The frequent loss of *D1Z2* and *D1S76* suggests that 1p36-p35 might be a critical region. A subgroup of tumors with interstitial deletions was defined by the frequent loss of loci *D1S57* and *D1S17* (located at 1p35-p32 and 1p32-p22, respectively), with case M-7 displaying marker *D1S57* as the sole loss. This locus was also frequently deleted in most samples with terminal 1p deletions but, as shown in Figure 3, this group of meningiomas displayed a slightly more telomeric deletion, as indicated by the frequent loss of marker *D1S7* instead of *D1S17*. A third region of interest might be defined by tumors losing marker *D1S9* at 1p22-p13. In M-79, the 1p deletion was restricted to this marker, although the loss of this 1p region commonly appeared in both meningioma subgroups, with terminal or interstitial deletions.

Of the 23 tumors with LOH for chromosome 14 loci, 11 displayed loss of the entire chromosome, ten were characterized by partial 14q deletions and two samples had complex patterns of allelic loss and retention consistent with complex chromosomal rearrangement. Two deletion regions might be defined, corresponding to 14q22-q24 and 14q32-qter. Tumor M-77 displayed LOH involving only marker *D14S13*, whereas tumors M-46 and M-62 only showed loss at *D14S20*. These findings might indicate the existence of two critical distinct subregions within 14q32 (Figure 4).

Unambiguous evidence of LOH at one or more chromosome 22 markers was observed in 44 of 81 (54%) meningiomas (Table 1). In 38 tumors, the results were compatible with the loss of the whole chromosome, because loss of alleles occurred at all informative loci along chromosome 22. In the other six instances, partial chromosome 22 deletions were evidenced with break points located at q11 in five tumors, distal to *D22S9* (in two instances), distal to IGLV (in one instance), and distal to *D22S20* (in two samples). One

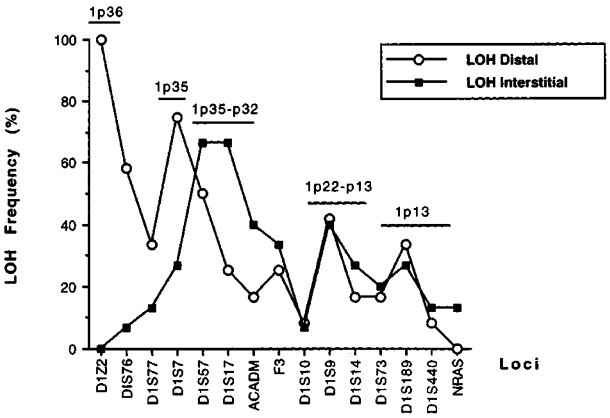

Figure 3 Graphic representation of the LOH frequency for each locus analysed in meningiomas displaying terminal or interstitial 1p deletions. Region 1p36 (locus *D1Z2*) was lost in the group of tumors with terminal deletion, whereas loss at the 1p22-p13 region (loci *D1S9* and *D1S189*) occurred in a similar frequency in both groups of meningiomas. In region 1p35-p32 (loci *D1S7*, *D1S57* and *D1S17*), some differences can be observed, as allelic loss is more proximal in the group of meningiomas with interstitial 1p deletion

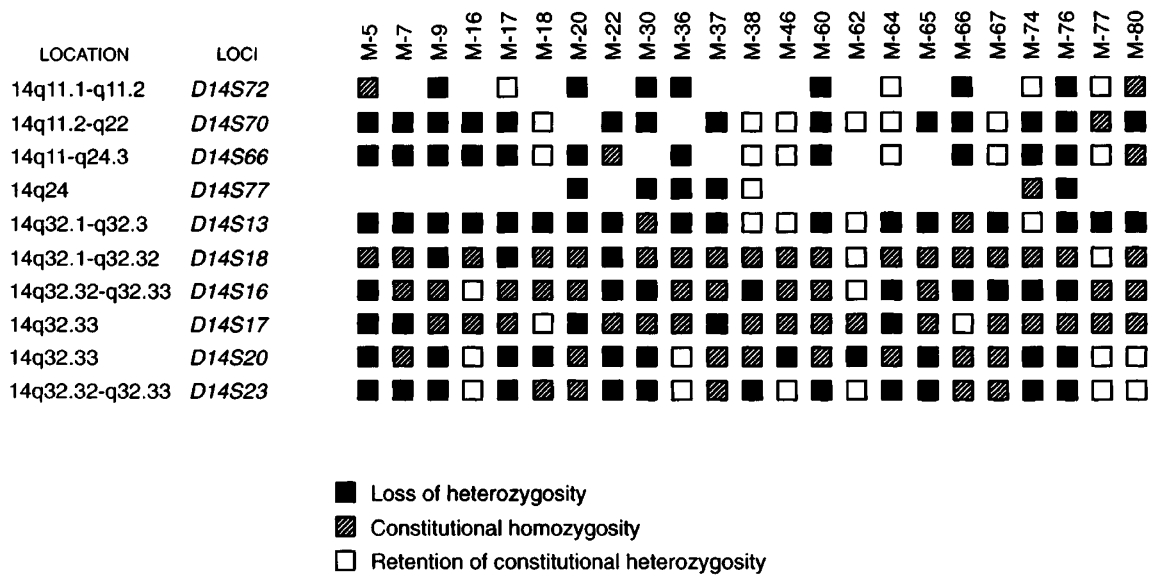

Figure 4 Summary of chromosome 14q deletion mapping in sporadic meningioma. The loci analysed and their location is shown to the left (blank: no data available)

Table 1 Clinical features, allelic losses and *NF2* gene mutations in 81 meningiomas

				LOH			*NF2 mutations*			
Tumor	*Sex/age*	*Grade and classification*[a]	*Location*	*1p*	*14q*	*22q*	*Exon*	*DNA sequence alt.*	*Nucl. change (codon)*	*Type of mutation*
M1	F/67	I, t	Sphenoid	–	–	+				
M2	M/40	I, t	Convexity	–	–	–				
M3	M/37	II, m, rec	Parasagital	+	–	+	E2	Transition	GGA→GGG (63)	Gly→Gly
M4	F/57	I, t	Spinal	–	–	+				
M5	F/47	II, m	Parasagital	+	+	+	E7	2-bp deletion	GAA TAT→GAAT (206–207)	Frameshift
M6	F/68	I, t	Parasagital	–	–	+	E11	Transition	CGA→TGA (341)	Nonsense (Arg→Stop)
M7	F/54	III, m, mult	Parasagital	+	+	+	E11	Transition	CGA→TGA (341)	Nonsense (Arg→Stop)
M8	M/64	I, t	Convexity	–	–	–				
M9	M/65	I, m	Convexity	–	+	+	E11	Transition	CAG→TAG (337)	Nonsense (Gln→Stop)
M10	F/44	I, t	Posterior fossa	–	–	–				
M11	F/60	II, f	Skull base	+	–	–				
M12	F/68	I, m, rec	Convexity	–	–	+	E11	Transition	CAG→TAG (362)	Nonsense (Gln→Stop)
M13	F/71	II, m	Convexity	+	–	+				
M14	F/69	I, ps	Spinal	+	–	–				
M15	M/54	II, m	Convexity	+	–	–				
M16	F/68	II, m	Convexity	+	+	+				
M17	F/71	III	N/A[b]	+	+	+	E2	Transition	CGA→TGA (57)	Nonsense (Arg→Stop)
M18	F/69	III	Skull base	+	+	+				
M19	F/36	I, t	Skull base	–	–	–				
M20	F/43	I, t	N/A	–	+	–				
M21	F/43	I, t	Convexity	–	–	–				
M22	F/71	II, f	N/A	+	+	+				
M23	M/35	II, N/A	Convexity	+	–	+				
M24	M/54	I, t	Convexity	–	–	–				
M25	M/58	I, t	Convexity	–	–	–				
M26	M/58	I, t	Convexity	–	–	–				
M27	M/58	I, t	Convexity	–	–	–				
M28	M/58	I, t	Convexity	–	–	–				
M29	F/74	II, m	Spinal	+	–	+				
M30	F/39	I, t	Spinal	–	+	–				
M31	F/70	I, t	Convexity	+	–	–				
M32	F/44	II, m, rec	Convexity	–	–	–				
M33	F/15	II, m	N/A	–	–	–				
M34	M/60	I, t	Convexity	–	–	+				
M35	F/60	I, t	Spinal	–	–	–				
M36	M/55	I, f	Convexity	–	+	+	E2	Transition	CGA→TGA (57)	Nonsense (Arg→Stop)
M37	M/68	II, m	Convexity	–	+	+				
M38	M/39	II, m	Convexity	–	+	+				
M39	M/19	I, t	Convexity	+	–	+				
M40	F/40	I, t	Skull base	–	–	+				
M41	F/51	I, t	Convexity	–	–	–				
M42	M/58	I, sim	N/A	–	–	–				
M43	F/64	II, m	Parasagital	–	–	–				
M44	F/	I, t	N/A	–	–	+				
M45	F/38	I, t	Posterior fossa	–	–	+				
M46	F/38	I, t	Skull base	–	+	+				
M47	F/69	I, m	Convexity	–	–	–				
M48	F/55	I, m	Skull base	–	–	–				
M49	F/43	I, t	Convexity	–	–	–				
M50	M/70	I, m	Skull base	–	–	+				
M51	F/53	I, t	Convexity	–	–	+				
M52	M/71	II, t	N/A	+	–	–				
M53	F/63	II, N/A	Ventricular	+	–	+				
M54	F/27	II, m	Posterior fossa	–	–	–				
M55	F/63	I, t	Convexity	–	–	+				
M56	F/19	I, m	Ventricular	–	–	+	E2	Transition	CGA→TGA (57)	Nonsense (Arg→Stop)
M57	F/68	I, t	Spinal	–	–	+				
M58	F/47	I, t	Skull base	–	–	–				
M59	M/23	I, t	Tentorium	–	–	–				
M60	M/63	II, m	N/A	+	+	+				
M61	F/70	I, mic	Convexity	+	–	+				
M62	F/59	I, t	N/A	–	+	–				
M63	F/43	II, rec	Convexity	+	–	–				
M64	F/74	II, m	Convexity	+	+	+				
M65	F/43	I, t	Skull base	+	+	+				
M66	F/72	I, t	Convexity	–	+	+	E7	Transversion	GCT→GAT (211)	Missense (Ala→Asp)
M67	F/40	I, t	Convexity	–	+	–				
M68	F/37	I, t	Skull base	–	–	+				
M69	F/68	II, m	Skull base	+	–	+				
M70	F/63	I, ps	Convexity	–	–	–				
M71	F/71	I, sec	Convexity	–	–	–				
M72	F/45	I, fus	Convexity	+	–	+				
M73	F/57	I, t	Skull base	–	–	–				

Continued

Table 1 (*Continued*)

Tumor	Sex/age	Grade and classification[a]	Location	LOH 1p	LOH 14q	LOH 22q	NF2 mutations Exon	DNA sequence alt.	Nucl. change (codon)	Type of mutation
M74	F/58	II, m	Convexity	+	+	+				
M75	F/19	I, t	Skull base	+	–	+				
M76	F/77	II, N/A	Convexity	+	+	+				
M77	M/62	I, ps	Convexity	–	+	+	E11	Deletion 1-pb	ATG→AG (363)	Framshift
M78	M/19	I, N/A	Skull base	–	–	–				
M79	F/81	I, t	Skull base	+	–	–				
M80	F/40	I, ps	Spinal	–	+	+				
M81	M/69	II, sim	Convexity	+	–	+				

[a]t: transitional; m: meningotheliomatous; f; fibroblastic; fus: fusocellular; sim: simplastic; sec: secretor; mic: microcistic; rec: recurrent tumor; mult: multiple meningiomas (No NF2 patient). [b]N/A: not available

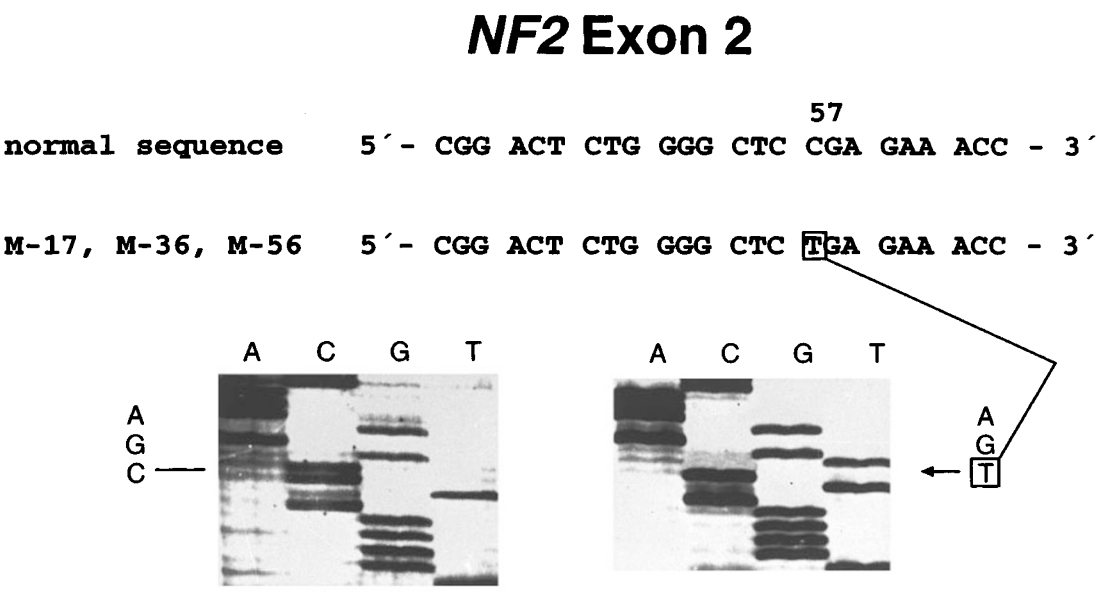

Figure 5 Representative sequencing data. Tumors M-17, M-36 and M-56 displayed an identical *NF2* gene mutation: C to T transition at codon 57 of exon 2, which changes an arginine codon (CGA) to a stop codon (TGA)

tumor presented the break point at q12 distal to *D22S32*. According to these findings, all but one sample characterized by LOH 22q displayed loss at the *NF2* region.

Screening for NF2 *mutations*

SSCP analysis revealed abnormal bands in 11 of 81 meningiomas; four mutations were localized in exon 2, two in exon 7 and five in exon 11. None of these mutations was germinal. To characterize the nucleotide sequence alterations detected by mobility shifts in SSCP analysis, we sequenced the abnormal samples after amplification by PCR; the *NF2* gene mutations detected are summarized in Table 1.

In exon 2, we identified two different mutations. The first was an A to G transition at position 189 (codon 63), with no apparent change in the protein amino acid sequence (Gly→Gly); this was detected in case M-3. The second mutation was observed in three tumors (M-17, M-36 and M-56) and was a C to T transition at nucleotide 169 of codon 57, creating a stop codon (Figure 5). The first mutation detected in exon 7 was an AT deletion at codons 206–207, causing a frameshift mutation (case M-5), whereas the second was a C to A transversion at codon 211 (nucleotide 632) that resulted in a missense Ala→Asp change (case M-66). Four different mutations were identified involving exon 11. C to T transitions were evidenced at codon 341 (nucleotide 1021) (cases M-6 and M-7), codon 337 (nucleotide 999) (tumor M-9) and codon 362 (nucleotide 1084) (tumor M-12) originating nonsense mutations consisting in Arg→Stop or Gln→Stop. The fourth mutation was a T deletion at position 1088 (codon 363), resulting in a frame shift change.

Association of anomalies

Allelic losses Thirty tumors displayed LOH at two or three chromosomal regions (Table 2). Histologically they corresponded to 11 grade I, 17 grade II and two grade III tumors. Both anaplastic grade III tumors accumulated LOH at 1p, 14q and 22q, whereas this situation was evidenced in eight grade II and one grade I tumors. The LOH1p/LOH22q association was more frequently observed (in four grade I and 7 grade II

Table 2 Association of allelic losses and tumor histology in 81 meningiomas

			Total no of cases with			*No cases with solitary anomaly*			*No cases with association*		
Grade	*No cases*	*NO LOH*	*LOH1p*	*LOH14q*	*LOH22q*	*LOH1p*	*LOH14q*	*LOH22q*	*LOH1p/22q*	*LOH14q/22q*	*LOH1p/14q/22q*
I	54	22	8	11	25	3	4	14	4	6	1
II	25	4	19	10	17	4	–	–	7	2	8
III	2	–	2	2	2	–	–	–	–	–	2
Totals	81	26	29	23	44	7	4	14	11	8	11

tumors) than the association LOH14q/LOH22q (detected in six grade I and two grade II samples). No case was found displaying LOH1p/LOH14q and a normal chromosome 22 allelic constitution.

LOH pattern and NF2 *gene mutations* All 11 tumors displaying *NF2* gene mutations showed LOH at 22q (Table 1); this was the sole allelic loss in three samples (M-6, M-12, M-56), whereas the remaining eight cases accumulated other LOH. Three tumors presented LOH at 1p, 14q and 22q; they corresponded to two grade II (M-5 and M-7) and one grade III (M-17) meningioma. LOH 14q and LOH 22q, and retention of the allelic constitution of 1p was evidenced in four grade I tumors (M-9, M-36, M-66 and M-77). Finally, one typical meningioma (M-3) displayed LOH1p and LOH22q in association. Identical *NF2* inactivant mutations at exon 2 and exon 11 were identified in three and two tumors, respectively. At codon 57 of E2, the same C to T transition was identified in two grade I and one grade III cases. The last (M-17) also displayed LOH at 1p, 14q and 22q, whereas the grade I tumors were characterized by either LOH22q (M-56) or LOH at 14q and 22q (M-36). Similar nonsense mutations due to a C to T transition at codon 341 of exon 11 were detected in cases M-6 and M-7. M-6 was a grade I meningioma which displayed LOH 22q as the sole allelic loss, whereas M-7 was a grade II tumor characterized by LOH at all three chromosomal regions analysed (1p, 14q, 22q). According to these findings, the accumulation of allelic loss at 1p and 14q, secondary to a given inactivating *NF2* gene mutation and LOH 22q, seems to be a critical step in the genesis of aggressive forms of meningioma.

Discussion

Previous cytogenetic and molecular genetic studies on meningioma suggested that a genetic model of malignant progression might explain the formation of atypical and anaplastic tumors, as a result of a mutation accumulation process. An early step in this model is the inactivation of a tumor suppressor gene on chromosome 22. At present, up to three genes of this category have been identified in this chromosome: the neurofibromatosis type 2 gene (*NF2*) (Rouleau *et al*., 1993; Trofatter *et al*., 1993), *BAM22* gene, a member of the β-adaptin family (Peyrard *et al*., 1994), and the *MN1* gene, disrupted by a balanced translocation in a meningioma cell line (Lekanne-Deprez *et al*., 1995).

In our study, we screened 979 of the 1785 nucleotides of the *NF2* cDNA corresponding to 54.8% of the *NF2* coding sequence. We found 11 cases with mutations representing about 24.5% of mutated cases if the complete coding sequence had been analysed. This frequency is similar to that found in other studies, with the sole exception of Wellenreuther *et al*. (1995), who found up to 59% of mutations in a series of 70 sporadic meningiomas. C to T transitions were identified in seven cases in our series, confirming that the CpG dinucleotides of the *NF2* gene might represent sites prone to point mutations. All 11 mutations in our series occurred at exons 2, 7 and 11 which, in agreement with De Vitis *et al*. (1996), might be considered exons of preferential involvement, although additional studies are required to verify this.

The inactivation of the *NF2* gene does not seem to play a role in the development of malignancy, as most cases displaying this anomaly as the sole alteration are generally classified as grade I meningiomas. Loss of alleles at other genome regions might therefore participate in this process. We have previously reported the association of LOH at 1p and tumor progression of meningiomas (Bello *et al*., 1994); this alteration was also frequently detected in a series of anaplastic meningiomas (Lindblom *et al*., 1994). Simon *et al*. (1995) also found an increased frequency of LOH at 1p, 14q and 10q, parallel to a higher grade of meningioma malignancy. Our results concur with these findings and suggest that up to three distinct 1p regions (1p36, 1p35-p32 and 1p22-p13) might be deleted during meningiomas tumorogenesis, indicating the presence of tumor suppressor genes in these genomic regions. No data on this subject are available, as only a small number of 1p loci had been analysed and no extensive deletion mapping was performed in meningiomas (Bello *et al*., 1994; Lindblom *et al*., 1994; Simon *et al*., 1995). Our findings agree with the data of Mertens *et al*. (1997), who proposed four 1p regions of preferential involvement in solid tumors. In fact, 1p alterations seem to be associated with a broad spectrum of human malignancies, including both solid tumors and leukemias (Schwab *et al*., 1996). A close analysis of the 1p alterations in specific tumoral subtypes will probably show the involvement of specific genes.

Simon *et al*. (1995) proposed 14q24.3-q32.33 as the critical region involved in losses in meningiomas. This fact is also shown by our data which suggest that two minimal regions of deletion would consist of 14q22-24 and 14q32, perhaps excluding the putative target of chromosome 14 deletions in colon cancer as the gene involved in atypical and anaplastic meningioma, as previously suggested by Simon *et al*. (1995). Construction of detailed partial deletion maps in meningioma, together with data from reports on other neoplasms displaying 14q losses (Takayama *et al*., 1992; Young *et al*., 1993; Fujino *et al*., 1994; Chang *et al*., 1995; Kovacs, 1993; Bandera *et al*., 1997) would support

with the hypothesis that two or more critical chromosome 14 regions may harbor tumor suppressor genes, located primarily at 14q12-q21, 14q22-q24 and 14q32 (Menon *et al*., 1997; Bandera *et al*., 1997).

Indirect evidence of the non-random role of the inactivation of loci from 1p and 14q in meningioma progression has been shown by the observations obtained in several tumors in our series which displayed identical *NF2* mutations. C to T transitions at codon 57 of exon 2 were detected in cases M-56, M-36 and M-17, and at codon 341 of exon 11 in tumors M-6 and M-7. Only those cases which, in addition to the NF2 gene inactivation, accumulated LOH at both 1p and 14q, corresponded to grade II or grade III meningiomas. Tumors M-9, M-36, M-66 and M-77 were classified as grade I, and displayed *NF2* gene inactivation, LOH at 14q and retention of alleles at 1p. The alteration at 14q probably produces grade I tumors, and LOH at 1p thus seems to be linked to the development of aggressive meningiomas, as suggested by the findings in cases M-3, M-5, M-7 and M-17. We should consider that meningiomas escaping from this progression model do exist (tumors M-37, M-38, M-65, M-75, in our series), and perhaps they follow a distinct molecular pathway, i.e. inactivation of other 14q/1p progression loci, or at 10q loci (Rempel *et al*., 1993; Simon *et al*., 1995).

Simultaneous loss at 1p and 14q is not exclusive to aggressive meningioma, as a similar association has been detected in neuroblastoma (Takayama *et al*., 1992). Cooperative inactivation of tumor progression genes located here might be a relevant event in the pathogenesis of certain tumoral subtypes. Simultaneous loss of 1p and 19q occurs in up to 75% of brain tumors with a major oligodendroglial component (Reifenberger *et al*., 1994; Bello *et al*., 1995; Kraus *et al*., 1995), thus providing evidence for the location at 1p of a tumor-related gene that frequently appears altered in association to locus anomalies from other genomic regions. Bieche *et al*. (1994) proposed the location of a tumor suppressor gene on chromosome 1p32-pter that controls the amplification of *MYC* family genes in breast tumors and, recently, the *RAD54* gene (Rasio *et al*., 1997) and *p73* (a *TP53* related gene) (Jost *et al*., 1997; Kaghad *et al*., 1997), both located at 1p, have been proposed as candidate loci for the alterations of the short arm of chromosome 1.

According to the data presented here, some atypical and malignant meningiomas may result from progression of grade I tumors and should not necessarily be considered as a separate, distinct class of meningiomas. If the existence of more than one tumor-related locus at 1p and 14q is confirmed, the involvement of different cooperative genes in these regions might suggest the existence of distinct biological forms of meningioma.

Materials and methods

Human tissue samples

Paired blood and tumor samples were obtained from 81 patients with meningiomas. All tumors were classified by histologic examination and graded according to World Health Organization (WHO) guidelines (Kleihues *et al*., 1993), as follows: 54 grade I (typical), 25 grade II (atypical), and two grade III (anaplastic) meningiomas. The tumoral cell content in all samples was estimated at approximately 75% by histologic analysis. Available information on clinico-pathological data is shown in Table 1.

DNA extraction

High molecular weight DNA was extracted from the tumor tissue and peripheral blood leukocytes by standard methods, as described previously (Rey *et al*., 1992).

RFLP analysis

DNA samples were digested to completion with restriction enzymes, electrophoresed through 0.8–1% agarose gels, transferred to nylon membranes (Zeta-Probe, Bio-Rad, Richmond, VA, USA), and hybridized to probes radiolabeled by nick-translation or random oligonucleotide priming procedures (Rigby *et al*., 1977; Feinberg and Vogelstein, 1984). A panel of DNA probes was used to detect LOH at 29 loci on chromosomes 1, 14 and 22 as follows: *D1Z2* (p1.79); *D1S76* (pCMM12.1); *D1S77* (pMCT58); *D1S7* (pMS1); *D1S57* (pYNZ2); *D1S17* (p3.18), *ACADM* (pMCAD); *F3* (pHTF8); *D1S9* (p1.04); *D1S10* (p1.08); *D1S14* (p6.02); *D1S73* (pEFD53.2); *NRAS* (pCN2); *D1S61* (pMLAJ1); *D1S66* (pHBl40); *D14S13* (pCMM101); *D14S18* (pHMZ9); *D14S16*(pTHHH37.1); *D14S17* (pEFZ18.2); *D14S20* (pMCOC12); *D14S23* (cKKA39); *D22S9* (p22/34); *IGLV* (pV3.3); *D22S20* (pFZVIA2); *D22S32* (pEFZ31); *MB* (pHM27.B2.9-MB); *PDGFB* (pPDGF-B17); *D22S80* (pFZVD11); *D22S171* (pFZIXC11).

Microsatellite analysis

LOH studies were also performed by analysis of CA repeat polymorphisms. Oligonucelotide primer pairs were obtained from GENSET SA (France) for the amplification of microsatellites from the following loci: *D1S189, D1S440, D14S66, D14S70, D14S72 and D14S77*. Each PCR was performed in a 20 μl mixture containing 100 ng of genomic DNA, 0.4 μM of each primer, 0.4 μM each of dATP, dCTP, dTTP and dGTP and 0.5 units of *Taq* polymerase in GeneAmp PCR buffer, on a PTC-100 Programmable Thermal Controller (MJ Research, Inc, Watertown, MA, USA). For all primer pairs, the mixtures were first heated to 94°C for 5 min, then subjected to 35 PCR cycles, of 94°C for 30 s, 57°C for 30 s and 72°C for 30 s, with a final 5 min extension at 72°C. To improve the results obtained with some primer pairs, the annealing temperature was varied from 52–63°C, and the extension time was increased to 1 min. The alleles were resolved on 6% polyacrylamide sequencing gels, then silver-stained as described by Bender *et al*. (1994). Scanning densitometry to determine allelic dosage was performed as described (Rey *et al*., 1992).

NF2 *gene mutation analysis*

Eight of the 17 exons of the *NF2* gene (exons 2, 5, 7, 8, 9, 11, 12 and 15, corresponding to approximately 55% of the coding region) were examined for point mutations by PCR/SSCP analysis, using the primers and PCR conditions specified by Rouleau *et al*. (1993). SSCP analysis was performed as follows: 6 μl of the radioactive PCR product were mixed with 4 μl of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). Samples were heated to 95°C for 5 min and then loaded onto a 6% non-denaturing polyacrylamide gel (with

or without 5% glycerol). A non-denaturing control was also included so that single-stranded fragments could be easily identified. The samples were electrophoresed at 10 W for 5–18 h at room temperature. Abnormal bands detected by SSCP analysis were reamplified by PCR, the DNA purified on a gel and used as a template for the sequencing reactions with the same primers used for PCR. The corresponding region of peripheral blood leucocytes derived DNA was also sequenced. Sequencing was performed using the Sequenase PCR Product Sequencing kit (USB-Amersham, UK), according to the manufacturer's indications.

Note added in proof

The following recently published papers reported interesting data on the subject: Weber R *et al*. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 14719–14724; Tse J *et al*. (1997). *Hum. Pathol.,* **28,** 779–785; Bostrom J *et al*. (1997). *Acta Neuropathol.,* **94,** 479–485 and Sulman E *et al*. (1998). *Cancer Res.,* **58,** 3226–3230.

Acknowledgements

Supported by grant SAF 95/0898, from CICYT. Ministerio de Education y Cultura. We are grateful to Paloma Nebreda for excellent technical assistance. PEL was supported by fellowship from Instituto de Cooperacion Iberoamericana (Programa MUTIS). This work was presented at the AACR special conference on Cancer of the Central Nervous System, San Diego, California, June 1997.

References

Al Saadi A, Latimer F, Madercic M and Robbins T. (1987). *Cancer Genet. Cytogenet.,* **26,** 127–141.

Bandera CA, Hiroyuki T, Behbakht K, Liu PC, LiVolsi VA, Benjamin I, Morgan MA, King SA, Rubin SC and Boyd J. (1997). *Cancer Res.,* **57,** 513–515.

Battersby RDE, Ironside JW and Maltby EL. (1986). *J. Neurol. Neurosurg. Psychiatry,* **49,** 362–368.

Bello MJ, de Campos JM, Kusak ME, Vaquero J, Sarasa JL, Pestaña A and Rey JA. (1994). *Genes Chrom. Cancer,* **9,** 296–298.

Bello MJ, Leone PE, Vaquero J, de Campos JM, Kusak ME, Sarasa JL, Pestana A and Rey JA. (1995). *Int. J. Cancer,* **64,** 207–210.

Bender B, Wiestler OD and von Deimling AA. (1994). *Biotechniques,* **16,** 204–206.

Bièche I, Champème M-H and Lidereau RA. (1994). *Cancer Res.,* **54,** 4274–4276.

Butti G, Assietti R, Casalone R and Paoletti P. (1989). *Surg. Neurol.,* **31,** 255–260.

Casalone R, Simi P, Granata P, Minelli E, Giudici A, Butti G and Solero CL. (1990). *Cancer Genet. Cytogenet.,* **45,** 237–244.

Casartelli C, Rogatto SR and Barbieri Neto J. (1989). *Cancer Genet. Cytogenet.,* **40,** 33–45.

Chang WY-H, Cairns P, Schoenberg MP, Polascik TJ and Sidransky D. (1995). *Cancer Res.,* **55,** 3246–3249.

De Vitis LR, Tedde A, Vitelli F, Ammannati F, Mennonna P, Bigozzi U, Montali E and Papi L. (1996). *Hum. Genet.,* **97,** 632–637.

Domenicucci M, Santoro A, D'Osvaldo DH, Delfini R, Cantore GP and Guidetti BJ. (1989). *Neurosurg.,* **70,** 41–44.

Fearon ER and Vogelstein B. (1990). *Cell,* **61,** 759–767.

Feinberg D and Vogelstein B. (1984). *Anal. Biochem.,* **137,** 266–267.

Fujino T, Risinger JI, Collins NK, Liu F-S, Nishii H, Takashashi H, Wetsphal EM, Barrett JC, Sasaki H, Kohler MF, Berchuck A and Boyd J. (1994). *Cancer Res.,* **54,** 4294–4298.

Harada T, Irving RM, Xuereb JH, Barton DE, Hardy DG, Moffat DA and Maher ER. (1996). *J. Neurosurg.,* **84,** 847–851.

Jost CA, Marin MC and Kaelin WG. (1997). *Nature,* **389,** 191–194.

Kaghad M, Bonnet H, Yang A, Creancier L, Biscan J-C, Valent A, Minty A, Chalon P, Lelias J-M, Dumont X, Ferrara P, McKeon F and Caput D. (1997). *Cell,* **90,** 809–819.

Kleihues P, Burger PC and Scheitauer BW. (1993). *Brain Pathol.,* **3,** 255–268.

Kovacs G. (1993). *Histopathol. (Oxf.),* **22,** 1–8.

Kraus JA, Koopmann J, Kaskel P, Maintz D, Brandner S, Schramm J, Louis DN, Wiestler OD and von Deimling A. (1995). *J. Neuropathol. Exp. Neurol.,* **54,** 91–95.

Lekanne-Deprez RH, Blanchi AB, Groen NA, Seizinger BR, Hagemeijer A, van Drunen E, Bootsma D, Koper JW, Avezaat CJJ, Kley N and Zwarthoff EC. (1994). *Am. J. Hum. Genet.,* **54,** 1022–1029.

Lekanne-Deprez RH, Riegman PH, van Drunen E, Warringa UL, Groen NA, Stefanko SZ, Koper JW, Avezaat CJJ, Mulder PGH, Zwarthoff EC and Hagemeijer A. (1995). *J. Neuropathol. Exp. Neurol.,* **54,** 224–235.

Lekanne-Deprez RH, Riegman PH, Groen NA, Warringa UL, van Biezen NA, Molijn AC, Bootsma D, de Jong PJ, Menon AG, Kley NA, Seizinger BR and Zwarthoff EC. (1995). *Oncogene,* **10,** 1521–1528.

Lindblom A, Ruttledge M, Collins VP, Nordenskjöld M and Dumanski JP. (1994). *Int. J. Cancer,* **56,** 354–357.

Lopez-Gines C, Cerda-Nicolas M, Barcia-Salorio JL and Llombart-Bosch A. (1995). *Cancer Genet. Cytogenet.,* **85,** 113–117.

Maltby EL, Ironside JW and Battersby RDE. (1988). *Cancer Genet. Cytogenet.,* **31,** 199–210.

Martuza RL and Eldridge R. (1988). *New Engl. J. Med.,* **318,** 684–687.

Memon MY. (1980). *Neurosurgery,* **7,** 262–264.

Menon AG, Rutter JL, von Sattel JP, Synder H, Murdoch C, Blumenfeld A, Martuza RL, von Deimling A, Gusella JF and Houseal TH. (1997). *Oncogene,* **14,** 611–616.

Merel P, Hoang-Xuan K, Sanson M, Moreau-Aubry A, Bijlsma EK, Lazaro C, Moisan JP, Resche F, Nishisho I, Estivill X, Delatre JY, Poisson M, Theillet C, Hulsebos T, Delattre O and Thomas G. (1995). *Genes Chrom. Cancer,* **13,** 211–216.

Mertens F, Johansson B, Höglund M and Mitelman F. (1997). *Cancer Res.,* **57,** 2765–2780.

Papi L, De Vitis LR, Vitelli F, Ammannati F, Mennonna P, Montali E and Bigozzi U. (1995). *Hum. Genet.,* **95,** 347–351.

Perry A, Jenkins RB, Dahl RJ, Moertel CA and Scheithauer BW. (1996). *Cancer,* **77,** 2567–2573.

Peyrard M, Fransson I, Xie Y-G, Huan F-Y, Ruttledge MH, Swahn S, Collins JE, Dunham I, Collins VP and Dumanski JP. (1994). *Hum. Mol. Genet.,* **3,** 1393–1399.

Poulsgard L, Ronne M and Schmidek HH. (1993). In: *Molecular Genetics of Nervous System Tumors*. Levine AJ and Schmidek HH (eds). Wiley-Liss: New York. 249–254.

Rasio D, Murakumo Y, Robbins D, Roth T, Silver A, Negrini M, Schmidt C, Burczak J, Fishel R and Croce CM. (1997). *Cancer Res.,* **57,** 2378–2383.

Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W and Collins VP. (1994). *Am. J. Pathol.,* **145,** 1175–1190.

Rempel SA, Schwechheimer K, Davis RL, Cavenee WK and Rosemblum ML. (1993). *Cancer Res.,* **53,** 2386–2392.

Rey JA, Bello MJ, de Campos JM, Kusak ME and Moreno S. (1988). *Cancer Genet. Cytogenet.,* **33,** 275–290.

Rey JA, Bello MJ, Jimenez-Lara AM, Vaquero J, Kusak ME, de Campos JM, Sarasa JL and Pestaña A. (1992). *Int. J. Cancer,* **51,** 703–706.

Rigby PW, Dieckmann M, Rhodes C and Berg P. (1977). *J. Mol. Biol.,* **113,** 237–251.

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, Demczuk S, Desmaze C, Plougastel B, Pulst SM, Lenoir G, Bijlsma E, Fashold R, Dumanski J, de Jong P, Parry D, Eldrige R, Aurias A, Delattre O and Thomas G. (1993). *Nature,* **363,** 515–521.

Ruttledge MH, Sarrazin J, Rangaratman S, Phelan CM, Twist E, Merel P, Delattre O, Thomas G, Nordenskjöld, Collins VP, Dumanski JP and Rouleau GA. (1994). *Nature Genet.,* **6,** 180–184.

Schneider BF, Shashi V, von Kap-Herr C and Golden WL. (1995). *Cancer Genet. Cytogenet.,* **85,** 101–104.

Schwab M, Praml C and Amler LC. (1996). *Genes Chrom. Cancer,* **16,** 211–229.

Simon M, von Deimling A, Larson JL, Wellenreuther R, Kaskel P, Waha A, Warnick RE, Tew JM and Menon AG. (1995). *Cancer Res.,* **55,** 4696–4701.

Takayama H, Suzuki T, Mugishima H, Fujisawa T, Ookuni M, Schwab M, Gehring M, Nakamura Y, Sugimura T, Terada M and Yokota J. (1992). *Oncogene,* **7,** 1185–1189.

Trofatter JA, MacCollin MM, Rutter JL, Murrell JR, Duyao MP, Parry DM, Eldridge R, Kley N, Menon AG, Pulaski K, Haase VH, Ambrose CM, Munroe D, Bove C, Haines JL, Martuza RL, MacDonald ME, Seizinger BR, Short MP, Buckler AJ and Gusella JF. (1993). *Cell,* **72,** 791–800.

Vagner-Capodano AM, Grisoli F, Gambarelli D, Sedan R, Pellet W and De Victor B. (1993). *Neurosurgery,* **32,** 892–900.

Wellenreuther R, Kraus JA, Lenartz D, Menon AG, Schramm J, Louis DN, Ramesh V, Gusella JF, Wiestler OD and von Deimling A. (1995). *Am. J. Pathol.,* **146,** 827–832.

Young J, Leggett B, Ward M, Thomas L, Buttenshaw R, Searle J and Chenevix-Trench G. (1993). *Oncogene,* **8,** 671–675.

Zang KD. (1982). *Cancer Genet. Cytogenet.,* **6,** 249–274.