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Clinicopathological variables, immunophenotype, chromosome 1p36 loss and tumour recurrence of 247 meningiomas grade I and II

Juan Ruiz, Armando Martínez, Susana Hernández, Horacio Zimman, Milagros Ferrer, Cristina Fernández, Mamen Sáez, Jose A. López-Asenjo and Julian Sanz-Ortega

Department of Pathology and Neurology, Hospital Clínico San Carlos, Madrid, Spain

Summary. The WHO grading scheme distinguishes benign (grade I), atypical (grade II) and anaplastic (grade III) meningiomas. Both atypical and anaplastic meningiomas exhibited an overall increased rate of recurrence, but between 15-20% benign meningiomas will also exhibit an unfavourable clinical course with recurrence before 10 years despite aggressive surgery. We investigated 247 cases of meningiomas grade I and II. The immunohistochemical expression of 30 different molecular biomarkers of cell adhesion molecules, cell-cycle and apoptosis regulators and checkpoints was analyzed. We also determined apoptosis by in-situ hybridization (APOPDETEK™) and loss of chromosome 1p36 by FISH. The study revealed a statistically significant co-variation ($p<0.05$) between meningiomas grade II associated with several clinicopathological features (Simpson grade of clinical resection, necrosis, nuclear atypia, macronucleoli, transition to small cell, sheet-like growth, high cellularity), increased expression of several biomarkers of tumour proliferation (Cyclin A, Cyclin E, MIB-1 or MDM2), proteases (Cathepsin D) or cell-adhesion (CD44) and lower expression of progesterone receptors than meningiomas grade I. The presence of Psammoma bodies or the location at convexity were protective prognostic factors for tumour recurrence while high cellularity and early age of onset (<57 year-old) were indicators of increased recurrence risk. The expression of COX-2, γ-catenin, Topoisomerase IIa, VEGF and MIB-1 was significantly higher in the cohort of recurrent meningiomas. Meningiomas with chromosome 1p36 loss showed a higher recurrence rate (33.3%) than meningiomas with normal chromosome 1p36 (18%). Increased COX-2 expression in recurrent meningioma may also suggest a putative role of COX-2 inhibitors as a chemopreventive treatment for recurrence.

Key words: Meningioma, Recurrence, Immunohistochemical, COX-2, 1p deletion

Offprint requests to: Dr. Julian-Sanz-Ortega, Department of Pathology and Neurology, Hospital Clínico San Carlos, C/ Prof. Martin Lagos s/n, 28040 Madrid, Spain. e-mail: jsanzo.hcsc@salud.madrid.org

Introduction

Meningiomas account for approximately 20-30% of all primary central nervous system tumours (Lusis and Gutman, 2004). They are usually considered to be benign. Nevertheless, between 15-50% of meningiomas will exhibit an unfavourable clinical course with recurrence before 10 years, despite aggressive surgery. In 2000 the WHO published a revised grading scheme for meningiomas to differentiate grade I (benign), grade II (atypical) and grade III (anaplastic o malignant) (Louis et al., 2000, 2007; Burger et al., 2002). Both atypical and anaplastic meningiomas exhibited an overall increased rate of recurrence, even after gross total resection (Simpson, 1957). While local 5-year recurrence rates are 5% for benign meningiomas, they are about 40% for totally resected atypical meningiomas. However, the 10-year regrowth rate for histologically benign meningiomas rise up to 15-25%. The histopathological based grading system has proved useful in predicting prognosis and in defining treatment regimens for meningiomas. However, there remains considerable variability in clinical outcomes within each grade, especially among atypical meningiomas. Existing criteria do not adequately predict the likelihood of tumour recurrence (Whittle et al., 2004).

Numerous efforts have been made to evaluate

different immunohistochemical assays in meningioma prognosis, analyzing the proteins involved in the regulation of cell cycle and apoptosis checkpoints, adhesion molecules or other molecular pathways (Louis et al., 2000; Amatya et al., 2001; Sandberg et al., 2001; Burger et al., 2002; Roessler et al., 2002; Milenkovic et al., 2008; Panagopoulos et al., 2008). The evaluation of Ki-67/MIB-1 labelling index, p53, p16 protein accumulation or progesterone receptors, among others, have been proposed as prognostic indicators but their association with tumour grade or clinical outcome is still controversial (Bruna et al., 2007; Vankalakunti et al., 2007; Claus et al., 2008; Terzi et al., 2008). The purpose of this study was to identify molecular biomarkers of recurrence unique to grade I and II of meningiomas and to unravel underlying molecular mechanisms driving meningioma tumorigenesis. The information obtained could potentially be translated into more effective and less toxic targeted therapies.

Materials and methods

Selection of cases

Our study was planned as a retrospective observational study, cohort study. Two hundred and fifty six consecutive intracranial meningioma cases (from 1980-2005) were obtained from the Hospital Clinico San Carlos files, Madrid, Spain. Histopathological specimens were separately evaluated by two pathologists (J.R and A.M.) according to the WHO criteria. After the new histopathological review of all available histological slides and reports, 4 cases proved to be haemangiopericytomas and 3 sarcomas.

Clinical parameters included in our study were: sex, age, surgery and death date, tumour recurrence recorded as either present or absent, primary tumour site (two categories: cerebral convexity and other location), Simpson grade of surgical resection, single or multiple meningioma, adjuvant radiotherapy, tumour size and clinical follow-up (recurrence-free survival and overall survival). Date of tumour relapse or last contact was evaluated as the end of the study. The time of relapse was determined as the date at which tumour progression or recurrence was confirmed by CT or MRT.

The histopathological parameters were: histopathological subtype, WHO grading system (grade I, II and III) and the presence or absence of haemorrhage, Psammoma bodies, necrosis, nuclear atypia, macronucleoli, cellular pleomorphism, transition to small cell, sheet-like growth, high cellularity or brain invasion. Increased mitotic activity was recorded when 4 or more mitosis per 10 high-power fields were detected.

We analyzed several proteins that have an important role in the generation and progression of tumour cells: proteins associated with cell cycle, apoptosis, proliferation biomarkers, angiogenesis or tumour invasion (Table 1).

Tissue microarray construction

Tissue microarrays (Kononen et al., 1998; Simon et al., 2004) were constructed from formalin-fixed, paraffin-embedded tissue blocks using a manual tissue arrayer (Beecham Instrument, Hackensack, USA). An H&E stained section from each donor block was examined and the areas of interest were identified and marked. We constructed 4 TMAs that contained two 1mm cores representative of each case. The final arrays represented 247 meningiomas, 8 normal meninges and 4 tonsils.

Immunohistochemical staining of tumours

All immunohistochemical sections were stained with Dako Autostainer (Glostrup, Denmark) utilizing standard immunohistochemical techniques. Serial sections of 4 µm were deparaffinized and rehydrated. Table 1 summarizes the antibodies (n=30), dilutions and antigen retrieval methods that we used. After peroxidase blocking, sections were incubated with primary antibody for 30 minutes at room temperature, washed, then incubated with secondary antibody. Finally, sections were incubated with DAB (3,3'-diaminobenzidine substrate) for 5 minutes, counterstained with hematoxylin, dehydrated and coverslipped.

The immunostaining for each antigen was evaluated independently by two pathologists (J.R. and J.S.) using a blind method. The observers had no information about the specimens examined. Standard criteria were used to classify the immunohistochemical findings as positive or negative for each marker (Table 1), using an Olympus BX40 microscope (100x magnification). Figure 1 shows examples of MIB-1, gamma catenin or Cox-2 immunostainings.

FISH analysis of chromosome 1p36 deletions

Test and reference probe mixtures for chromosomes 1 (1p36/1q25) were purchased from Vysis (Des Plaines, IL). The 1p36 probe was 400 kb in length and was pre-labelled with an orange fluorophore. The 1q25 reference probe was 620 kb in length, pre-labelled with a green fluorophore.

Three-micron sections of TMA were deparaffinized in xylene, rehydrated through an ethanol series and then the slides were transferred to dH2O and washed twice. The slides were incubated at 37°C in a humidified chamber for 20 min. and washed in dH_2O, then dehydrated through an ethanol series. After air drying at room temperature, the slides were placed on a 37°C warming tray and 2 µL of the probe mix was applied. The section and probe mix were then co-denatured at 95°C for 20 min, and finally placed in a 37°C humidified chamber overnight. Post hybridisation, the slides were washed for 2 min in 0.4M SSC/0.3% NP-40 at 37°C. They were then transferred to 2M SSC/0.1% NP-40 at

room temperature for 30 s to 1 min. The slides were allowed to air dry in the dark before 6 µL of 4'-6-diamidino-2-phenylindole (DAPI) counterstain was applied. They were then cover slipped, wrapped in aluminium foil and stored in a -20°C freezer until they were scored.

Sections were examined under a multiple filter Nikon50i fluorescence microscope. Under x1000 oil immersion, two investigators each assessed 20 nuclei at 10 different sites for a total of 200 nuclei. Nuclei were scored for reference to test probe signal ratio. Reference to test probe signal ratio of 2:2, in more than 50% of cells was taken to indicate no deletion. Reference to test probe signal ratio of 2:1 was regarded as indicating

Table 1. Antibodies, dilutions and antigen retrieval.

Antibody	Clone		Dilut. (1/x)	Antigen-Retrival condition	% of cells Category (+ or -)	Intensity	Loc.	References13-30
ApopDETEK™	-	Enzo Life Sciences	-	-	≥ 75%=(+) <75%=(-)	NA	N	Ng et al., 1998
Bcl-2	124	DAKO	100	AR1	≥ 15%=(+) <15%=(-)	NA	N	Maiuri et al., 2007
E-Cadherin	NCH-28	DAKO	100	AR1	>0%=(+) 0%=(-)	NA	C, M	Schwechheimer et al., 1998; Loussouarn et al., 2006
Caspase 3a	C92-605	BD Bio-sciences	20	AR1	>0%=(+) 0%=(-)	NA	C	---
ß-Catenin	1	DAKO	200	AR1	>0%=(+) 0%=(-)	NA	C, M	Shimada et al., 2005; Utsuki et al., 2005
γ-Catenin	15	Transduction Lab	750	AR1	>0%=(+) 0%=(-)	NA	C, M	Shimada et al., 2005
Catepsin D	DB2000	DAKO	50	AR1	1=≥ 20% 2=<20%	1=++/+++ 2=-/+	C	Castilla et al., 2003
CD44	DF1485	Novocastra	50	AR1	>0%=(+) <=%=(-)	NA	C, M	Suzuki et al., 1996
Cyclin A	6D6	Novocastra	50	AR1	≥ 3%=(+) <3%=(-)	NA	N	Nakabayashi et al., 2003
Cyclin D1	DCS-6	DAKO	50	AR	≥ 5%=(+) <5%=(-)	NA	N	---
Cyclin E	13A3	Novocastra	10	AR1	≥ 5%=(+) <5%=(-)	NA	N	---
COX-2	RB-9072-R7	Lab Vision	RTU	AR1	>10%=(+) <10%=(-)	NA	C, M	Lin et al., 2003
EGFR	2-18C9	PharmDxTM	10	AR2	1=≥ 50% 2=<50%	1=++/+++ 2=-/+	M	Anderson et al., 2004
EMA	E29	DAKO	300	AR1	1=≥ 50% 2=<50%	1=++/+++ 2=-/+	C, M	---
Her2	Herceptest	DAKO	1	AR1	1=≥ 10% 2=<10%	1=+/++/+++ 2=-	M	Loussouarn et al., 2006
MIB-1	MIB-1	Master Diagnostica	100	AR1	≥ 4%=(+) <4%=(-)	NA	N	Amatya et al., 2004
MDM2	5B10C	Novolink	50	AR1	≥ 5%=(+) <5%=(-)	NA	N	Amatya et al., 2004
MMP9	RB-9234-R7	Lab vision	25	AR1	0=0% 1=<30% 2=<60% 3=<100%	0=- 1=+ 2=++ 3=+++	C	Okada et al., 2004
p16	E6H4	DAKO	RTU	AR1	≥ 10%=(+) <10%=(-)	NA	N	Korshunov et al., 2003
p21	SX118	DAKO	25	AR1	≥ 10%=(+) <10%=(-)	NA	N	Kamei et al., 2000
p27	57	Transduction Lab	1000	AR1	>0%=(+) 0%=(-)	NA	N	Korshunov et al., 2003
p53	D07	DAKO	40	AR1	≥ 10%=(+) <10%=(-)	NA	N	Karamitopoulo et al., 1999
ß-PDGF	2B3	Cell Signalling	100	AR1	NA	0=- 1=+ 2=++ 3=+++	C	---
PTEN	18H6	Novocastra	50	AR1	≥ 50%=(+) <50%=(-)	NA	N	---
Progesterona receptor	OM1D	DAKO	20	AR1	≥ 33%=(+) <33%=(-)	NA	N	Loussouarn et al., 2006
Survivin	RB-1629-P	Neomarkers	2000	AR1	1=≥ 50% 2=<50%	1=++/+++ 2=-/+	C	Sasaki et al., 2002
ß–TGF	RB-9246-R7	Neomarkers	RTU	AR4	>0%=(+) 0%=(-)	NA	C, M	Johnson et al., 2004
TIMP-2	3A4	Santa Cruz	500	AR1	>0%=(+) 0%=(-)	NA	C, M	Paek et al., 2002
Topo II	3F6	Novocastra	20	AR1	≥ 4%=(+) <4%=(-)	NA	N	Roessler et al., 2002; Tanaka et al., 1999
VEGF	AR360-5R	BD Biosciences	RTU	AR4	1=≥ 75% 2=<75%	1=++/+++ 2=-/+	C, M	Maiuri et al., 2007; Paek et al., 2002

CB: citric buffer. BM: Baño maría. PK: Proteinase K AR1: CB 10mM pH 6.5 15´ Steam AR2: Peroxide Blk 5´ + PK 5´, AR3: CB pH 6.5 + PK 10´ 37º, AR4: CB pH 9 45´ BM 95º. Intensity: + = weak, ++ = moderate, +++ = strong. Categories: The sum of percentage and the intensity. N: Nucleus, C: Cytoplasm, M: Membrane, Loc.: location, NA: Not assessable.

deletion (Fig. 1). Two or more copies of the sequence being probed with a disproportionate number of reference probe signals (e.g. 3:2 or 4:3) were considered to indicate imbalance. Imbalance ratios may indicate deletion, but, equally, may be due to artefact or nuclear truncation. Such cells were scored but not counted as either deleted or normal.

Statistical Analysis

Statistical analysis was performed using SPSS software, version 15.0 (SPSS, Inc. Chicago, IL). The distribution of each molecular biomarker expression on several tumour features as WHO grade, invasion of CNS, and recurrence was compared either by the chi square χ^2 test for categorical variables or using Fisher exact test.

Results

A summary of the clinical data distribution for the meningiomas included in our series (n=247) has been provided in Table 2a. Meningiomas were more predominant in female (73.7%, n=182) patients, had a mean age of 57 years-old (range: 45-67), had a mean size of 3.2 cm (range 2.38-5 cm) and lateral convexity (30.4%) was the most common location. Complete surgical resection of meningiomas (Simpson grade 1, 2 and 3) was determined in 138 cases, partial removal (Grade 4) in 24 cases and no grade 5 cases were included in the study. Insufficient data for the Simpson grading scale was observed in 85 cases. The mean follow-up period in our series was 7.70 years (range 1.60-13.25). Tumour recurrence was determined in 49 out of 247 meningiomas (19.8%).

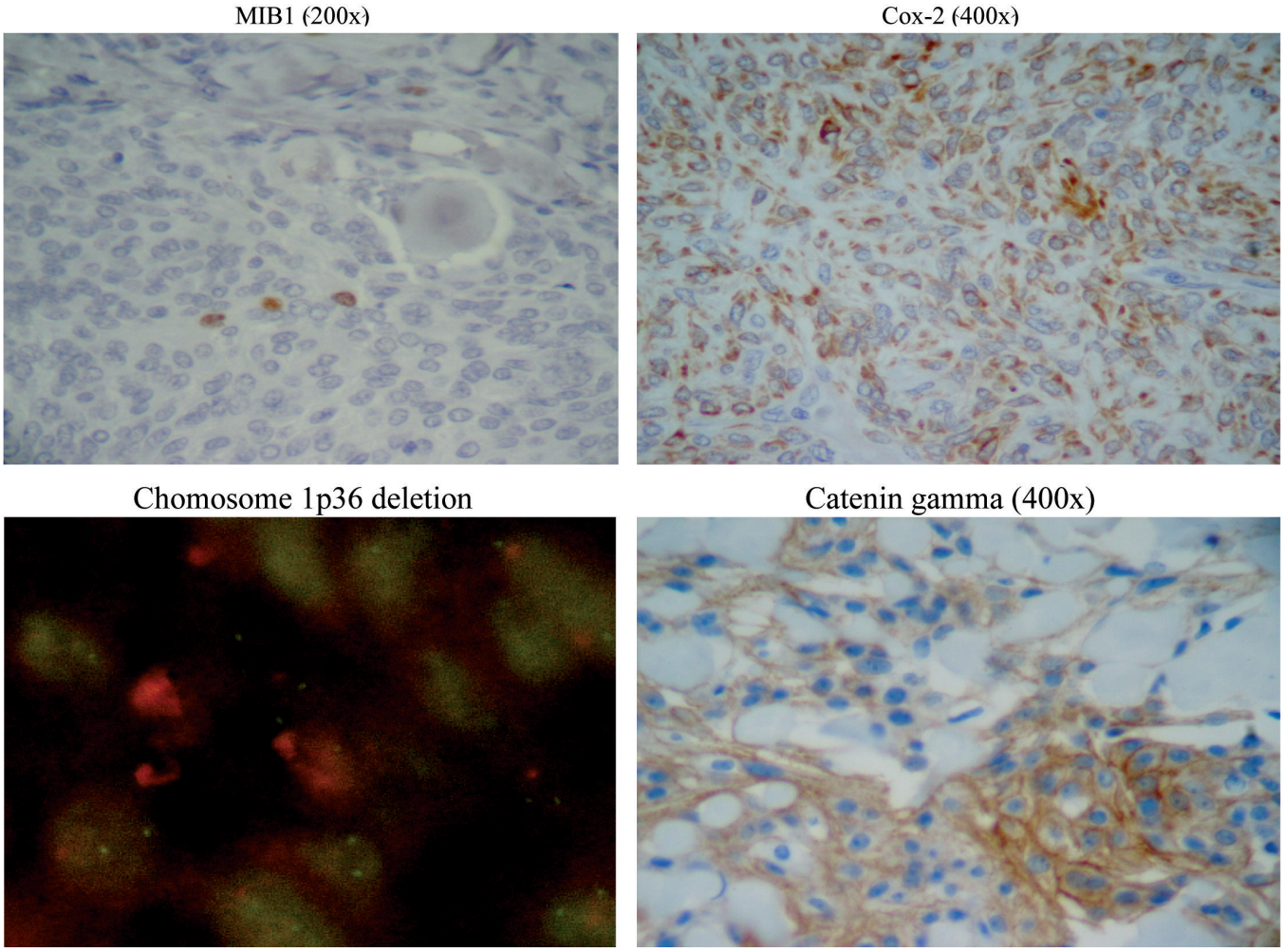

Fig. 1. Immunostaining for MIB1, Cox-2 and γ Catenin at different magnifications. Example of a case showing a deletion of chromosome 1p36 by FISH: Each nuclei shows two green dots representing two alleles hybridizing with the chromosome 1q control probe and one red dot representing the 1p35 chromosomal region with only one allele.

Table 2a. Clinical Variables (n=247).

Variable	Evaluation	Frequency (%)
Sex	Male	65 (26.3%)
	Female	182 (73.7%)
Age (years)*		57 (45-67); (n =205)
Follow-up (years) *		7.70 (1.60-13.25)
Survival	Alive	156 (63.2%)
	Dead	53 (21.4%)
	Unknown	38 (15.4%)
Recurrence	Yes	49 (19.8%)
	No	198 (80.2%)
Location	Lateral Convexity	75 (30.4%)
	Parasagittal Median	25 (10.1%)
	Anterior base	37 (15.0%)
	Posterior base	8 (3.2%)
	Posterior fossa	22 (8.9%)
	Spinal region	22 (8.9%)
	Others (cutaneus)	1 (0.4%)
	Skull	57 (23.1%)
Simpson grade of clinical resection	Grade 1	94 (38.15)
	Grade 2	35 (14.2%)
	Grade 3	9 (3.6%)
	Grade 4	24 (9.7%)
	Grade 5	0 (0%)
	Not known	85 (34.4%)
Adjuvant Treatment	Yes	2 (0.8%)
	No	245 (99.2%)
Multiplex meningioma	Yes	2 (0.8%)
	No	245 (99.2%)
Longest diameter (cm.) *		3.25 (2.38-5)

Table 2b. Histopathological Data (n=247).

Variable	Evaluation	Frequency (%)
Grade, WHO	I	208 (84.2%)
	II	39 (15.8%)
	III	0 (0%)
Hipercellularity	Yes	49 (19.8%)
	No	198 (80.2%)
Necrosis	Yes, light	29 (11.7%)
	No	207 (83.8%)
	Yes, extensive	11 (4.5%)
Lobular pattern loss ("sheeting")	Yes	51 (20.6%)
	No	196 (79.4%)
Nuclear atypia with macronucleoli	Yes	65 (26.3%)
	No	182 (73.7%)
Transition to small cell.	Yes	8 (3.2%)
	No	239 (96.8%)
Brain invasion	Yes	10 (4%)
	No	237 (96%)
Psammoma bodies	Yes	134 (54.3%)
	No	113 (45.7%)
Nuclear atypia	Yes	204 (82.6%)
	No	42 (17.4%)
Haemorrhage	Yes	131 (53%)
	No	116 (47%)
	Others	178 (72.1%)
Mitotic activity	4 or more mitosis per 10 HPF	227 (93.8%)
	Less than 4 mitosis per 10 HPF	15 (6.2%)

*: Data was expressed as mean and range.

Table 2b shows all the histopathological findings in our series. Following the WHO criteria 208 cases were considered to be grade I and 39 cases were considered to be grade II meningiomas. No grade III (anaplastic) meningiomas were included in our study.

The histopathological subtypes of meningiomas were distributed in our series as follows: Transitional (48.2%), Fibrous (15.0%), Atypical (12.1%), Psammomatous (7.3%), Meningotheliomatous (6.9%), Angiomatous (3.2%), Microcystic (2.4%), Secretory (1.6%), Metaplastic (1.6%), Clear cell (0.8%), Chordoid (0.8%), Lymphoplasmacyte-rich (0%), Rhabdoid (0%), Papillary (0%) and Anaplastic (0%).

Variables associated with grade II, brain invasion or recurrence

The association of clinicopathological variables with grade, brain invasion or recurrence was analyzed (Table 3a). The study revealed a statistically significant co-variation ($p<0.05$) between WHO grade II meningiomas associated with Simpson grade of clinical resection, necrosis, nuclear atypia, macronucleoli, transition to small cell, sheet-like growth, high cellularity, mitosis and brain invasion. Brain invasion was associated with grade, increased cellularity, nuclear atypia, macronucleoli and mitosis. Tumour recurrence was associated with age at diagnosis, high cellularity, location and presence of pPsammoma bodies.

The analysis of molecular biomarkers (Table 3b) revealed a statistically significant co-variation between grade II meningiomas associated with increased protein expression of cathepsin D, CD44, Cyclin A, Cyclin E, MIB-1 and MDM2. Tumour recurrence was associated with overexpression of γ-catenin, COX-2, VEGF, Topoisomerase II alpha and MIB-1. No prognostic molecular biomarkers were associated with brain invasion.

Discussion

In the present study, the overall recurrence rate we found was 17.5% for grade I and 27% for grade II meningiomas, although these differences were not statistically significant (p=0.17) (Table 3a). The WHO grading system is useful to predict tumour recurrence, especially for meningioma grade III (Louis et al., 2007). However, there remains considerable variability in the reported clinical outcomes within grades I (benign) and II (atypical), especially among atypical meningiomas. We, like other authors (Whittle et al., 2004), believe that the existing criteria do not adequately predict the likelihood of tumour recurrence of meningiomas grade I and II and that there is a need for new predictive markers. Table 3a shows other clinicopathological variables that may be useful prognostic factors, such as the presence of Psammoma bodies or the location at convexity as protective prognostic factors for tumour recurrence or, the high cellularity and early age of onset

(<57 year-old) as indicators of increased recurrence risk. Psammoma bodies may indicate a slower tumour growth and better differentiation. The high cellularity is a morphological feature frequently found in meningiomas grade II. Considering separately the histopathological features (high cellularity, nuclear atypia, haemorrhage,

Table 3a. Statistical association (Chi-square test) between clinicopathological variables **(Table 3a)** or molecular biomarkers **(Table 3b)** with WHO grade, brain invasion or tumour recurrence at any time of follow-up in grade I and II meningiomas (n=247).

Marker	Value	WHO grade		Brain invasion		Recurrence	
		1	2	yes	No	Yes	No
Gender	M	50 (76.9)	15 (23.1)	5 (7.7)	60 (92.3)	13 (20.0)	52 (80.0)
	F	158 (86.8)	24 (13.2)	5 (2.7)	177 (97.3)	36 (19.8)	146 (80.2)
	p	0.061		0.083		0.970	
Age at diagnosis	57+	101 (85.6)	17 (14.4)	5 (4.2)	113 (95.8)	19 (16.1)	99 (83.9)
	<57	70 (84.3)	13 (15.7)	4 (4.8)	79 (95.2)	25 (30.1)	58 (69.9)
	P	0.806		0.844		0.018	
Simpson, grade	1	80 (87.0)	12 (13.0)	4 (4,3)	88 (95.7)	19 (20.7)	73 (79.3)
	2-5	49 (74.2)	17 (25.8)	3 (4.5)	63 (95.5)	20 (30.3)	46 (69.7)
	p	0.042		0.953		0.165	
WHO grade	1			0 (0)	206 (100)	36 (17.5)	170 (82.5)
	2			9 (24.3)	28 (74.7)	10 (27.0)	27 (73.0)
	p			<0.001		0.172	
Location	convex	78 (78.8)	21 (21.2)	3 (3.0)	96 (97.0)	15 (15.2)	84 (84.8)
	other	77 (84.6)	14 (15.4)	6 (6.6)	85 (93.4)	31 (34.1)	60 (65.9)
	p	0.301		0.248		0.002	
Hipercellularity	Yes	23 (46.9)	26 (53.1)	5 (10.2)	44 (89.9)	16 (32.7)	33 (67.3)
	No	185 (93.4)	13 (6.6)	5 (2.5)	193 (97.5)	33 (16.7)	165 (8.3)
	p	<0.001		0.015		0.012	
Necrosis	Yes	13 (44.8)	16 (55.2)	4 (13.8)	25 (86.2)	6 (20.7)	23 (79.3)
	No	195 (89.4)	23 (10.6)	6 (2.8)	212 (97.2)	43 (19.7)	175 (80.3)
	p	<0.001		0.005		0.903	
Loss of lobular pattern	Yes	24 (47.1)	27 (52.9)	5 (9.8)	46 (90.2)	13 (25.5)	38 (74.5)
	No	184 (93.9)	12 (6.1)	5 (2.6)	191 (9.,4)	36 (18.4)	160 (81.6)
	p	<0.001		0.019		0.256	
Pleomorphism	Yes	4 (44.4)	5 (55.6)	0 (0)	9 (100)	2 (22.2)	7 (77.8)
	No	204 (85.7)	34 (14.3)	10 (4.2)	228 (95.8)	47 (19.8)	191 (80.2)
	p	0.001		0.530		0.855	
Macronucleoli	Yes	39 (60.0)	26 (40.0)	4 (6.2)	61 (93.8)	17 (26.2)	48 (73.8)
	No	169 (92.9)	39 (15.8)	6 (3.3)	176 (96.7)	32 (17.6)	150 (82.4)
	p	<0.001		0.316		0.137	
Transition to small cell	Yes	3 (37.5)	5 (62.5)	1 (12.5)	7 (87.5)	1 (12.5)	7 (87.5)
	No	205 (85.8)	34 (14.2)	9 (3.8)	230 (96.2)	48 (20.1)	191 (79.9)
	p	<0.001		0.218		0.597	
Brain invasion	Yes	0 (0)	10 (100)			2 (20.0)	8 (80.0)
	No	205 (88.0)	28 (12.0)			44 (18.9)	189 (81.1)
	p	<0.001				0.930	
Psammoma	Yes	116 (86.6)	18 (13.4)	6 (4.5)	128 (95.5)	14 (10.4)	120 (89.6)
	No	92 (81.4)	21 (18.6)	4 (3.5)	106 (96.5)	35 (31.0)	78 (69.0)
	p	0.269		0.709		<0.001	
Nuclear atypia	Yes	179 (87.7)	25 (12.3)	5 (2.5)	199 (97.5)	40 (19.6)	164 (80.4)
	No	28 (66.7)	14 (33.3)	4 (9.5)	38 (90.5)	9 (21.4)	33 (78.6)
	p	0.001		0.026		0.788	
Hemorrhage	Yes	101 (77.7)	29 (22.3)	8 (6.2)	122 (93.8)	33 (25.4)	97 (74.6)
	No	106 (91.4)	10 (8.6)	2 (1.7)	114 (98.3)	16 (13.8)	100 (86.2)
	p	0,012		0.208		0.066	
PMN infiltration	Yes	52 (75.4)	17 (24.6)	6 (8,7)	63 (91,3)	15 (21.7)	54 (78.3)
	No	156 (88.1)	21 (11.9)	4 (2,3)	173 (97,7)	34 (19.2)	143 (80.8)
	p	0,013		0.022		0.655	
Mitosis	<4	205 (90.3)	22 (9.7)	6 (2.6)	221 (97.4)	46 (20.3)	181 (79.7)
	4+	0 (0)	15 (100)	2 (13.3)	13 (86.7)	3 (20.0)	12 (80.0)
	p	<0.001		0.025		0.980	

Table 3b.

Marker	Value	WHO grade		Brain invasion		Recurrence	
		1	2	Yes	No	yes	No
ApopDETEK	positive	6 (100%)	0 (0%)	0 (0%)	6 (100%)	1 (16.7%)	5 (83.3%)
	negative	35 (87.5%)	2 (12.5%)	1 (2.5%)	39 (97.5%)	4 (10.0%)	36 (90.0%)
	p	0,359		0,695		0,625	
Bcl-2	positive	35 (89.7%)	4 (10.3%)	0 (0%)	39 (100%)	9 (23.1%)	30 (76.9%)
	negative	156 (83.4%)	31 (16.6%)	7 (3.8%)	180 (96.2%)	37 (19.8%)	150(80.2%)
	p	0.321		0.220		0.642	
E-Cadherin	positive	42 (77.8%)	12 (22.2%)	2 (3.7%)	52 (93.3%)	13 (24.1%)	41 (75.9%)
	negative	166 (86.5%)	26 (13.5%)	7 (3.6%)	185 (96.4%)	36 (18,.8%)	156(82.2%)
	p	0.119		0.984		0.387	
Caspase 3a	positive	179 (83.3%)	36 (16.7%)	9 (4.2%)	206 (95.8%)	46 (21.4%)	169(78.6%)
	negative	25 (92.6%)	2 (7.4%)	0 (0%)	27 (100%)	3 (11.1%)	24 (88.9%)
	p	0.209		0.279		0.210	
, Catenin	positive	30 (78.9%)	8 (21.1%)	1 (2.6%)	37 (97.4%)	9 (23.7%)	29 (76.3%)
	negative	173 (85.2%)	30 (14.8%)	8 (3.9%)	195 (96.1%)	40 (19.7%)	163(80.3%)
	p	0.330		0.696		0.576	
Á Catenin	positive	86 (82.7%)	18 (17.3%)	2 (1.9%)	102 (98.1%)	28 (26.9%)	76 (73.1%)
	negative	114 (85.7%)	19 (14.3%)	6 (4.5%)	127 (95.5%)	19 (14.3%)	114(85.7%)
	p	0.525		0.274		0.015	
Cathepsin D	positive	63 (77.8%)	18 (22.2%)	4 (4.9%)	77 (95.1%)	19 (23.5%)	62 (76.5%)
	negative	129 (87.8%)	18 (12.2%)	5 (3.4%)	142 (96.6%)	28 (19.0%)	119(81.0%)
	p	0.048		0.568		0.431	
CD44	positive	9 (64.3%)	5 (35.7%)	1 (7.1%)	13 (92.9%)	2 (14.3%)	12 (85.7%)
	negative	198 (85.3%)	34 (14.7%)	9 (3.9%)	223 (96.1%)	37 (20.3%)	185(79.7%)
	p	0.036		0.548		0.587	
Cyclin A	positive	7 (46.7%)	8 (53.3%)	1 (6.7%)	14 (93.3%)	5 (33.3%)	10 (76.7%)
	negative	196 (87.5%)	28 (12.5%)	8 (3.6%)	216 (96.4%)	42 (18.8%)	182(81.2%)
	p	< 0.001		0.542		0.169	
Cyclin D1	positive	133 (85.3%)	23 (14.7%)	3 (1.9%)	153 (98.1%)	27 (17.3%)	169(82.7%)
	negative	68 (84%)	13 (16%)	5 (6.2%)	76 (93.8%)	20 (24.7%)	61 (75.3%)
	p	0.791		0.086		0.176	
Cyclin E	positive	3 (42.9%)	4 (57.1%)	0 (0%)	7 (100%)	0 (0%)	7 (100%)
	negative	202 (86%)	33 (14%)	8 (3.4%)	227 (96.6%)	48 (20.4%)	187(79.6%)
	p	0.002		0.620		0.182	
COX-2	positive	17 (85%)	3 (15%)	0 (0%)	20 (100%)	8 (40.0%)	12 (60.0%)
	negative	191 (84.1%)	36 (15.9%)	10 (4.4%)	217 (95.6%)	41 (18.1%)	186(81.9%)
	p	0.920		0.338		0.018	
EGFR	positive	101 (81.5%)	23 (18.5%)	2 (1.6%)	122 (98.4%)	22 (17.7%)	102(82.3%)
	negative	103 (88.8%)	13 (11.2%)	6 (5.2%)	110 (94.8%)	27 (23.3%)	89 (76.7%)
	p	0.111		0.125		0.288	
EMA	positive	197 (84.9%)	35 (15.1%)	10 84.3%)	222 (95.7%)	48 (20.7%)	184(79.3%)
	negative	9 (69.2%)	4 (30.8%)	0 (0%)	13 (100%)	1 (7.7%)	12 (92.3%)
	p	0.133		0.445		0.254	
HER2	positive	105 (86.1%)	17 (13.9%)	5 (4.1%)	117 (95.9%)	26 (21.3%)	96 (78.7%)
	negative	103 (82.4%)	22 (17.6%)	5 (4.0%)	120 (96.0%)	23 (18.4%)	102(81.6%)
	p	0.430		0.969		0.566	
MIB-1	positive	6 (54.5%)	5 (45.5%)	1 (9.1%)	10 (90.9%)	6 (54.5%)	5 (55.5%)
	negative	196 (86%)	32 (14%)	8 (3.5%)	220 (96.5%)	42 (18.4%)	86 (81.6%)
	p	0.005		0.342		0.003	
MDM2	positive	16 (64%)	9 (36%)	2 (8.0%)	23 (92.0%)	6 (20.4%)	19 (79.6%)
	negative	183 (87.6%)	26 (12.4%)	6 (2.9%)	203 (97.1%)	40 (19.1%)	169(80.9%)
	p	0.002		0.182		0.563	
MMP9	positive	116 (84.7%)	21 (15.3%)	7 (5.1%)	130 (94.9%)	25 (18.2%)	112(81.8%)
	negative	90 (84.9%)	16 15.1%)	2 (1.9%)	104 (98.1%)	24 (22.6%)	82 (77.4%)
	p	0.960		0.187		0.397	
p16	positive	5 (45.5%)	6 (54.5%)	2 (18.2%)	9 (81.8%)	3 (27.3%)	8 (72.7%)
	negative	201 (86.3%)	32 (14.7%)	7 (3%)	226 (97.0%)	46 (19.7%)	187(80.3%)
	p	< 0.001		0.091		0.542	
p21	positive	54 (73%)	20 (27%)	5 (6.8%)	69 (93.2%)	17 (23.0%)	57 (77.0%)
	negative	144 (88.9%)	18 (11.1%)	3 (1.9%)	159 (98.1%)	32 (19.8%)	130(80.2%)
	p	0.002		0.053		0.572	
p27	positive	99 (83.9%)	19 (16.1%)	3 (2.5%)	115 (97.5%)	23 (19.5%)	95 (80.5%)
	negative	102 (87.2%)	15 (12.8%)	3 (3.4%)	113 (966%)	25 (21.4%)	92 (78.6%)
	p	0.475		0.693		0.721	
p53	positive	6 (85.7%)	1 (14.3%)	0 (0%)	7 (100%)	0 (0%)	7 (100%)
	negative	202 (84.2%)	38 (15.8%)	10 (4.2%)	230 (95.8%)	49 (20.4%)	191(79.6%)
	p	0.912		0.581		0.182	
PDGF	positive	76 (84.4%)	14 (15.6%)	2 (2.2%)	88 (97.8%)	21 (23.3%)	69 (76.7%)
	negative	125 (84.5%)	23 (15.5%)	7 (4.7%)	141 (95.3%)	27 (18.2%)	121(81.8%)
	p	0.998		0.325		0.343	
PTEN	positive	194 (85.8%)	32 (14.2%)	6 (2.7%)	220 (97.3%)	44 (19.5%)	182(80.5%)
	negative	4 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)	4 (100%)
	p	0.417		0.741		0.326	
RP	positive	190 (86%)	31 (14%)	7 (3.2%)	214 (96.8%)	47 (21.3%)	174(78.7%)
	negative	15 (68.2%)	7 (31.8%)	2 (9.1%)	20 (90.9%)	2 (9.1%)	20 (90.9%)
	p	0.028		0.161		0.175	
Survivin	positive	130 (83.3%)	26 (16.7%)	6 (3.8%)	150 (96.2%)	37 (23.7%)	119(76.3%)
	negative	75 (86.2%)	12 (13.8%)	3 (3.4%)	84 (96.6%)	12 (13.8%)	75 (86.2%)
	p	0.554		0.875		0.065	
ß TGF	positive	26 (92.9%)	2 (7.1%)	0 (0%)	28 (100%)	3 (10.7%)	25 (89.3%)
	negative	169 (82.8%)	35 (17.2%)	8 (3.9%)	196 (96.1%)	43 (21.1%)	161(79.9%)
	p	0.175		0.286		0.197	
TIMP2	positive	163 (84.5%)	30 (15.5%)	7 (3.6%)	186 (96.4%)	42 (21.8%)	151(78.2%)
	negative	40 (85.1%)	7 (14.9%)	1 (2.1%)	46 (97.9%)	7 (14.9%)	40 (85.1%)
	p	0.912		0.608		0.295	
Topoisomerase II	positive	4 (50%)	4 (50%)	0 (0%)	8 (100%)	7 (87.5%)	1 (12.5%)
	negative	198 (85.7%)	33 (14.3%)	8 (3.5%)	223 (96.5%)	42 (18.2%)	189(81.8%)
	p	0.006		0.592		< 0.001	
VEGF	positive	12 (75%)	4 (25%)	1 (6.3%)	15 (93.8%)	8 (50.0%)	8 (50.0%)
	negative	193 (85.4%)	33 (14.6%)	8 (3.5%)	218 (96.5%)	41 (18.1%)	185(81.9%)
	p	0.264		0.580		0.002	
Chromosome 1p36	normal	137(84.5%)	25(15.5%)	6(3.8%)	156(96.2%)	29 (18%)	133(82%)
	1p loss	20 (74%)	7(21%)	2(7.5%)	25(92.5%)	9(33.3%)	18(66.6%)
		0.176		0.320		0.073	

Criteria for evaluation of immunohistochemical findings is summarized in Table 1.

necrosis or mitosis) that are frequently found in meningiomas grade II, only high cellularity is a significant prognostic factor for meningioma recurrence (Table 3a) .

Numerous previous efforts have focused on the evaluation of meningioma growth fraction as a prognostic marker (Kunishio et al., 2000; Korshunov et al., 2002, 2003; Roessler et al., 2002; Bruna et al., 2007; Terzi et al., 2008; Uzüm and Ataoglu, 2008). We found that MIB-1 overexpression was associated with overall recurrence (Table 3b). We believe this marker may be clinically informative and useful. Another molecular biomarker that indicates increased proliferative activity is Topoisomerase IIα .

The cyclo-oxygenase-2 (COX-2) is an enzyme catalysing fatty acid oxidation causally linked to the progress from normal cell growth through hyperplasia on to neoplasia and cancer (Lin et al., 2003; Buccoliero et al., 2007) and also protects cells from apoptosis induced by various cellular stresses. COX-2 inhibits detachment-induced apoptosis (anoikis) in cancer from activation of the PI-3K/Akt pathway (Choi et al., 2005). The cases in our series that showed COX-2 overexpression (n=20) showed a statistically significant increase of VEGF (p=0.006), PDGF (p=0.05), TGFb (p=0.05) and HER2. (Fig. 2). Therefore, COX-2 over-expression may indicate a subgroup of meningiomas with increased "stress" and growth stimulation and a higher recurrence rate, independent of the WHO grading system. Chemoprevention based on COX-2 inhibitors has already been achieved in a wide range of cancer or as adjuvant therapy of established disease (Ragel et al., 2007). The increased COX-2 expression in recurrent meningioma may also suggest a putative role for COX-2 inhibitors to prevent recurrence (Ragel et al., 2003, 2007).

Losses of chromosome 1p36 are frequently found in meningeal neoplasms and have been previously reported to be increased in the pathogenesis of atypical and anaplastic tumours (Boström et al., 2001; López-Ginés et al., 2004; Maillo et al., 2007; Nakane et al., 2007; Guan et al., 2008; Pfisterer et al., 2008). We have not found significant differences for 1p losses between benign and atypical meningiomas. Tumour recurrence was observed in 33.3% of the meningiomas showing chromosome 1p36 loss, and 18% in the meningiomas with no chromosomal deletion, although these differences were not statistically significant (p=0.17).

The study revealed statistically significant differences ($p<0.05$) between meningiomas grade I and II. Meningiomas grade II were found to be associated with several clinicopathological features: Simpson grade of clinical resection, high cellularity, necrosis and nuclear atypia, macronucleoli, transition to small cell, sheet-like growth, haemorrhage, polymorphonuclear neutrophils infiltration and mitosis counting (>4/10HPF). No significant differences were found between meningioma grade and gender, age at diagnosis, tumour location or the presence of Psammoma bodies. All the clinicopathological findings in our series are virtually identical to those reported previously (Perry et al., 1999, 2001). Meningiomas grade II showed overexpression of several biomarkers that indicate increased growth fraction: Cyclin A, Cyclin E, MIB-1 (Ki67), MDM2 or Topoisomerase II alpha. The use of mitoses counting or immunostainings such as MIB-1 to detect increased growth fraction is controversial. In our series, both can be used indistinctly in the differential diagnosis between meningiomas grade I and II, but MIB-1 has a stronger association with tumour recurrence, as we will discuss later. Meningiomas grade II also overexpressed other biomarkers that seem to be instrumental in the mechanism of tumour invasion: CD44 and Cathepsin D. The strong expression of the cell-adhesion molecule CD44 observed in atypical meningiomas has already been reported by others (Suzuki et al., 1996; Lewy-Trenda et al., 2004). Protease Cathepsin D positive immunostaining was detected in 50% of the atypical meningiomas in our series, a novel finding consistent with the more aggressive behaviour of meningiomas grade II. Meningioma grade II also showed lower expression of progesterone receptors than meningiomas grade I.

In conclusion, the presence of a high cellularity in meningeal neoplasms, MIB-1 or Topoisomerase IIa, gamma catenin, and cyclooxigenase 2 overexpression are additional useful prognostic factors to predict recurrence for benign and atypical meningiomas. The present study also provides a clinicopathological and immunohistochemical characterization of meningiomas grade I and II. The loss of chromosome 1p36 is a frequent event in meningiomas that needs further evaluation. COX-2 positive immunostaining is associated with increased presence of growth factors, increased cellular stress and suggests a putative role for COX-2 inhibitors in this subgroup of meningiomas.

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References

Amatya V.J., Takeshima Y., Sugiyama K., Kurisu K., Nishisaka T., Fukuhara T. and Inai K. (2001). Immunohistochemical study of Ki-67 (MIB-1), p53 protein, p21WAF1, and p27KIP1 expression in benign, atypical, and anaplastic meningiomas. Hum. Pathol. 32,970-975.

Boström J., Meyer-Puttlitz B., Wolter M., Blaschke B., Weber R.G., Lichter P., Ichimura K., Collins V.P. and Reifenberger G. (2001). Alterations of the tumor suppressor genes CDKN2A (p16(INK4a)), p14(ARF), CDKN2B (p15(INK4b)), and CDKN2C (p18(INK4c)) in atypical and anaplastic meningiomas. Am. J. Pathol. 159, 661-669.

Bruna J., Brell M., Ferrer I., Gimenez-Bonafe P. and Tortosa A. (2007). Ki-67 proliferative index predicts clinical outcome in patients with atypical or anaplastic meningioma. Neuropathology 27, 114-120.

Buccoliero A.M., Castiglione F., Degl'Innocenti D.R., Arganini L., Taddei A., Ammannati F., Mennonna P. and Taddei G.L. (2007). Cyclooxigenase-2 (COX-2) overexpression in meningiomas: real

time PCR and immunohistochemistry. Appl. Immunohistochem. Mol. Morphol. 15, 187-192.

Burger P.C., Scheithauer B.W. and Vogel F.S. (2002). Intracranial meninges. In: Surgical pathology of the nervous system and its coverings. 4th ed. Churchill Livingstone. Elsevier Science. USA pp 49-71.

Choi E.M., Kwak S.J., Kim Y.M., Ha K.S., Kim J.I., Lee S.W. and Han J.A. (2005). COX-2 inhibits anoikis by activation of the PI-3K/Akt pathway in human bladder cancer cells. Exp. Mol. Med. 30, 199-203.

Claus E.B., Park P.J., Carroll R., Chan J. and Black P.M. (2008). Specific genes expressed in association with progesterone receptors in meningioma. Cancer Res. 68, 314-322.

Guan Y., Hata N., Kuga D., Yoshimoto K., Mizoguchi M., Shono T., Suzuki S.O., Tahira T., Kukita Y., Higasa K., Yokohama N., Nagata S., Iñaki T., Sasaki T. and Hayashi K. (2008). Narrowing of the regions of allelic losses of chromosome 1p36 in meningioma tissues by an improved SSCP analisis. Int. J. Cancer 122,1820-1826.

Kononen J., Bubendorf L., Kallioniemi A., Bärlund M., Schraml P., Leighton S., Torhorst J., Mihatsch M.J., Sauter G. and Kallioniemi O.P. (1998). Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat. Med. 4, 844-847.

Korshunov A., Shishkina L. and Golanov A. (2002). DNA topoisomerase II-alpha and cyclin A immunoexpression in meningiomas and its prognostic significance: an analysis of 263 cases. Arch. Pathol. Lab. Med. 26, 1079-1086.

Korshunov A., Shishkina L. and Golanov A. (2003). Immuno-histochemical analysis of p16INK4a, p14ARF, p18INK4c, p21CIP1, p27KIP1 and p73 expression in 271 menigiomas correlation with tumor grade and clinical outcome. Int. J. Cancer 104, 728-734.

Kunishio K., Morisaki K., Matsumoto Y. and Nagao S. (2000). DNA topoisomerase II alpha protein and mRNA expression in intracranial meningiomas. Brain Tumor Pathol. 17, 105-110.

Lewy-Trenda I., Omulecka A., Janczukowicz J. and Papierz W. (2004). CD44 expression in human meningiomas: an immunohistochemical analysis. Pol. J. Pathol. 55, 33-37.

Lin C.C., Kenyon L., Hyslop T., Hammond E., Andrews D.W. and Curran W.J. Jr (2003). Cyclooxygenase-2 (COX-2) expression in human meningioma as a function of tumor grade. Am. J. Clin. Oncol. 26, S98-102.

López-Ginés C., Cerda-Nicolas M., Gil-Benso R., Callaghan R., Collado M., Roldan P. and Llombart-Bosch A. (2004). Association of loss of 1p and alterations of chromosome 14 in meningioma progression. Cancer Genet. Cytogenet. 148, 123-128.

Louis D.N., Scheithauer B.W., Budka H., von Deimling A. and Kepes J.J. (2000). Meningiomas. In: World Health Organization classification of tumours. Pathology and genetics: tumours of central nervous system. Kleihues P. and Cavanee W. (eds). Lyon. pp 175-184.

Louis D.N., Ohgaki H., Wiestler O.D., Cavenee W.K., Burger P.C., Jouvet A., Scheithauer B.W. and Kleihues P. (2007). The 2007 WHO Classification of Tumours of the Central Nervous System. Acta Neuropathol. 114, 97-109.

Lusis E.A. and Gutmann D.H. (2004) Meningioma: an update. Curr. Opin. Neurol. 17, 687-692.

Maillo A., Orfao A., Espinosa A.S., Sayagues J.M., Merino M., Sousa P., Lara M. and Tabernero M.D. (2007). Early recurrences in histologically benign/grade I meningiomas are associated with large tumors and coexistence of monosomy 14 and del (1p36) in the ancestral tumor cell clone. Neuro. Oncol. 9, 438-446.

Milenkovic S., Marinkovic T., Jovanovic M.B., Djuricic S., Berisavac I.I. and Berisavac I. (2008). Cyclin D1 Immunoreactivity in menongimas. Cell. Mol. Neurobiol. 28, 907-913.

Nakane Y., Natsume A., Wakabayashi T., Oi S., Ito M., Inao S., Saito K. and Yoshida J. (2007). Malignant transformation-related genes in meningiomas: allelic loss on 1p36 and methylation status of p73 and RASSF1A. Neurosurgery 107, 398-404.

Panagopoulos A.T., Lancellotti C.L., Veiga J.C., de Aguiar P.H. and Colquhoum A. (2008). Expression of cell adhesion proteins and proteins related to angiogenesis and fatty acid metabolism in benign, atypical, and anaplastic meningiomas. J. Neurooncol. 89, 73-87.

Perry A., Scheithauer B.W., Stafford S.L., Lohse C.M. and Wollan P.C. (1999). ‘‘Malignancy’’ in meningiomas: a clinicopathologic study of 116 patients. Cancer 85, 2046–2056.

Perry A., Chicoine M.R., Filiput E., Miller J.P. and Cross D.T. (2001). Clinicopathologic assessment and grading of embolized meningiomas: a correlative study of 64 patients. Cancer 92, 701-711.

Pfisterer W.K., Coons S.W., Abouñ-Enein F., Hendricks W.P., Scheck A.C. and Preul M.C. (2008). Implicating chromosomal aberrations with meningioma growth and recurrence: results from FISH and MIB-I analysis of grades I and II meningioma tissue. J. Neurooncol. 87, 43-50.

Ragel B.T. and Jensen R.L. (2003). New approaches for the treatment of refractory meningiomas. Cancer Control. 10, 148-158.

Ragel B.T., Jensen R.L. and Couldwell W.T. (2007). Inflamatory response and meningioma tumorigenesis and the effect of cyclooxigenase-2 inhinitors. Neurosurg. Focus 23, E7.

Roessler A., Gatterbauer B. and Kitz K. (2002). Topoisomerase II alpha as a reliable proliferation marker in meningiomas. Neurol. Res. 24, 241-243.

Sandberg D.I., Edgar M.A., Resch L., Rutka J.T., Becker L.E. and Souweidane M.M. (2001). MIB-1 staining index of pediatric meningiomas. Neurosurgery 48, 590-597.

Simon R., Mirlacher M. and Sauter G. (2004). Tissue microarrays. Biotechniques 36, 98-105.

Simpson D. (1957). The recurrence of intracranial meningiomas after surgical treatment. J. Neurol. Neurosurg. Psychiatry 20,22-39.

Suzuki S.O., Iñaki T., Kitamoto T., Mizoguchi M., Fukui M. and Tateishi J. (1996). Differential expression of CD44 variants among meningioma subtypes. J. Clin. Pathol.: Mol. Pathol. 49, 140-146.

Terzi A., Saglam E.A., Barak A. and Soylemezoglu F. (2008). The significance of immunohistochemical expression of Ki-67, p53, p21 and p16 in meningiomas tissue arrays. Pathol. Res. Pract. 204, 305-314.

Vankalakunit M., Vasishta R.K., Das Radotra S. and Khosla V.K. (2007). MIB-1 immunolabelling: a valuable marker in prediction of benign recurring meningiomas. Neuropathology 27, 407-412.

Whittle I.R., Smith C., Navoo P. and Collie D. (2004) Meningiomas. The Lancet 363, 1535-1544.

Uzüm N. and Ataoglu G.A. (2008) Histopathological parameters with ki-67 and bcl-2 in th prognosis of meningiomas according to WHO 2000 classification. Tumori 94, 389-397.

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