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ORIGINAL PAPER

Caveolin-1 in meningiomas: expression and clinico-pathological correlations

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Abstract Caveolin-1 (Cav-1) protein has been documented in several neoplasms with a controversial role in cell proliferation, tumour development and progression. The aim of the present study was to investigate the Cav-1 immunohistochemical expression in human meningiomas. Sixty-two cases, classified as 11 meningothelial (17%), 12 transitional (19%), 5 fibrous (8%), 3 microcystic (5%), 3 secretory (5%), 1 clear cell (2%), 1 chordoid (2%) and 26 (42%) atypical meningiomas, were selected from our pathological files. Clinico-pathological data, including Ki-67 values and survival data were also available. Ten leptomeningeal samples were utilized as normal tissue control. For each case, a polyclonal antibody against Cav-1 was applied and an intensity distribution (ID) score was determined. The Cav-1 immunoexpression was found in 95% of meningiomas with a variable ID score, while only minimal, not uniform, reactivity was noted in non-neoplastic meninges. Of note, higher Cav-1 ID score was significantly correlated with tumour site, Simpson's grade, histological type, higher histologic grade, Ki-67 labelling index $\geq 4\%$ and clinical course. Kaplan–Meier curves demonstrated a significantly worse survival in patients with higher Cav-1 ID score, Ki-67 $\geq 4\%$ and 2–3 Simpson grade. Multivariate analysis indicated that only Ki-67 was an independent prognostic factor. Increased immunoexpression of the Cav-1 seems to be associated with the biological aggressiveness of meningiomas, reflecting a worse prognosis.

Keywords Caveolin-1 · Meningiomas · Ki-67 · Prognosis

Introduction

Caveolin-1 (Cav-1) protein is the major component of caveolae, specialized, flask-shaped, plasmalemmal compartments in which vesicular transport processes [1, 5, 32] and signal transduction mechanisms [2, 4, 26, 30, 32] take place. The Cav-1 is most strongly expressed in endothelial cells, adipocytes, fibroblasts and smooth muscle cells [31], but its presence has also been documented in other sites, including mammary gland [22], lung alveolar epithelia [25], renal tubules [4], astrocytes [6], dorsal root ganglion [12] and Schwann cells [23].

The Cav-1 seems to play an important role in cell proliferation and tumour development since it has been shown that its tyrosine-14 phosphorylation results in growth stimulation [22]. Nonetheless, it may also exert a tumour suppressor activity by inhibiting several

Dedicated to Professor Dazio Batolo on the occasion of his retirement.

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Table 1 Clinicopathological characteristics and Cav-1 immuno-expression data of 62 analysed meningiomas

Case	Sex	Age	Site	Histotype	Grade	Ki-67(%)	SI	ASP	ID-SCORE	Simpson	Status	FU (months)	Recurrences (DFI)
1	M	31	Basal	Atypical	II	8.0	2	4	8	1	NED	94	No
2	M	38	Parasagittal	Atypical	II	10.0	2	3	6	3	DOD	80	Yes [16]
3	F	57	Parasagittal	Atypical	II	5.0	2	4	8	2	NED	112	No
4	M	74	Basal	Meningothelial	I	3.0	2	3	6	3	DID	42	_
5	F	67	Basal	Transitional	I	0.5	2	3	6	1	DOID	5	_
6	M	60	Basal	Meningothelial	I	1.0	1	4	4	2	NED	112	No
7	F	76	Basal	Transitional	I	0.5	1	0	0	1	NA	_	_
8	F	59	Basal	Meningothelial	I	4.0	3	3	9	1	NA	_	_
9	M	75	Basal	Secretory	I	0.5	3	2	6	2	DOID	2	_
10	F	71	Convexity	Atypical	II	6.0	3	3	9	1	NED	91	No
11	F	49	Convexity	Meningothelial	I	1.0	1	2	2	1	NED	117	No
12	F	77	Basal	Secretory	I	0.5	1	0	0	2	DID	12	_
13	M	67	Convexity	Atypical	II	10.0	1	2	2	1	NED	120	No
14	F	76	Convexity	Microcystic	I	1.0	1	1	1	2	DID	76	_
15	M	54	Basal	Meningothelial	I	1.0	1	4	4	1	NED	114	No
16	F	63	Parasagittal	Atypical	II	1.0	2	2	4	1	NA	_	_
17	F	64	Parasagittal	Atypical	II	5.0	2	3	6	2	NA	_	_
18	F	73	Parasagittal	Atypical	II	5.0	2	4	8	3	NED	114	No
19	F	78	Basal	Transitional	I	1.0	1	3	3	3	NA	_	_
20	M	65	Basal	Meningothelial	I	4.0	2	4	8	1	DOID	0	_
21	M	64	Convexity	Atypical	II	5.0	2	2	4	1	AWD	94	No
22	M	56	Parasagittal	Atypical	II	5.0	2	3	6	1	DOD	80	Yes [16]
23	F	66	Parasagittal	Atypical	II	10.0	2	4	8	2	DOD	59	Yes [40]
24	M	66	Parasagittal	Atypical	II	6.0	3	4	12	1	NED	108	No
25	F	54	Convexity	Transitional	I	0.5	2	3	6	1	NED	100	No
26	M	80	Parasagittal	Transitional	I	1.0	2	3	6	1	NED	119	No
27	M	63	Convexity	Transitional	I	1.0	1	1	1	1	NED	118	No
28	M	75	Convexity	Meningothelial	I	2.0	3	2	6	1	DID	50	No
29	M	63	Convexity	Meningothelial	I	1.0	2	2	4	1	NED	115	No
30	F	70	Basal	Transitional	I	1.0	1	4	4	2	NED	119	No
31	F	59	Basal	Fibrous	I	1.0	1	3	3	1	NED	106	No
32	F	57	Parasagittal	Transitional	I	1.0	1	3	3	1	NED	101	No
33	F	21	Convexity	Clear cell	II	0.5	1	1	1	1	NED	98	No
34	F	70	Convexity	Atypical	II	10.0	2	4	8	1	AWD	101	Yes [41]
35	F	79	Basal	Meningothelial	I	0.5	1	0	0	1	NED	119	No
36	M	73	Parasagittal	Atypical	II	30.0	2	4	8	2	DOD	38	Yes [38]
37	F	47	Basal	Secretory	I	1.0	2	2	4	3	NED	96	No
38	F	48	Parasagittal	Transitional	I	1.0	1	3	3	1	NA	_	–
39	F	72	Basal	Atypical	II	10.0	2	3	6	2	NA	_	–
40	M	53	Parasagittal	Microcystic	I	4.0	2	3	6	1	NED	110	No
41	M	61	Basal	Atypical	II	5.0	2	4	8	3	NA	–	–
42	F	70	Parasagittal	Atypical	II	4.0	2	3	6	2	NA	–	–
43	F	65	Convexity	Fibrous	I	1.0	1	2	2	1	NA	–	–
44	F	60	Convexity	Atypical	II	10.0	2	3	6	1	DOID	7	_
45	F	70	Basal	Fibrous	I	1.0	1	2	2	1	NED	96	No
46	F	64	Parasagittal	Fibrous	I	3.0	1	2	2	1	NED	108	No
47	F	77	Convexity	Transitional	I	1.0	2	2	4	1	NED	106	No
48	M	40	Parasagittal	Atypical	II	3.0	3	3	9	1	NED	106	No
49	F	84	Parasagittal	Atypical	II	30.0	2	2	4	2	DOID	9	–
50	F	66	Convexity	Transitional	I	1.0	2	2	4	1	NED	117	No
51	F	75	Convexity	Atypical	II	5.0	2	1	2	1	NED	106	No
52	F	61	Basal	Meningothelial	I	2.0	2	4	8	3	NA	_	–
53	F	74	Convexity	Atypical	II	15.0	2	4	8	1	NED	91	No
54	F	53	Parasagittal	Atypical	II	8.0	2	3	6	3	AWD	91	Yes [38]
55	M	71	Basal	Transitional	I	0.5	2	3	6	2	DOID	7	–
56	M	77	Parasagittal	Atypical	II	10.0	3	4	12	2	DOD	41	Yes [24]
57	M	75	Parasagittal	Atypical	II	20.0	2	3	6	3	DOD	14	Yes [14]
58	M	65	Convexity	Meningothelial	I	1.0	2	2	4	1	NED	111	No

Table 1 continued

Case	Sex	Age	Site	Histotype	Grade	Ki-67(%)	SI	ASP	ID-SCORE	Simpson	Status	FU (months)	Recurrences (DFI)
59	M	75	Convexity	Chordoid	II	5.0	1	1	1	1	DOID	1	–
60	F	59	Convexity	Fibrous	I	2.0	2	2	4	1	NED	103	No
61	F	51	Parasagittal	Atypical	II	5.0	2	3	6	1	AWD	96	Yes [31]
62	M	43	Convexity	Microcystic	I	1.0	2	3	6	1	NED	95	No

SI cav-1 staining intensity, *ASP* cav-1 area of staining positivity, *FU* follow up, *DFI* disease free interval, *NED* no evidence of disease, *AWD* alive with disease, *DOD* dead of disease, *DOID* dead of intercurrent disease, *DID* dead of independent disease

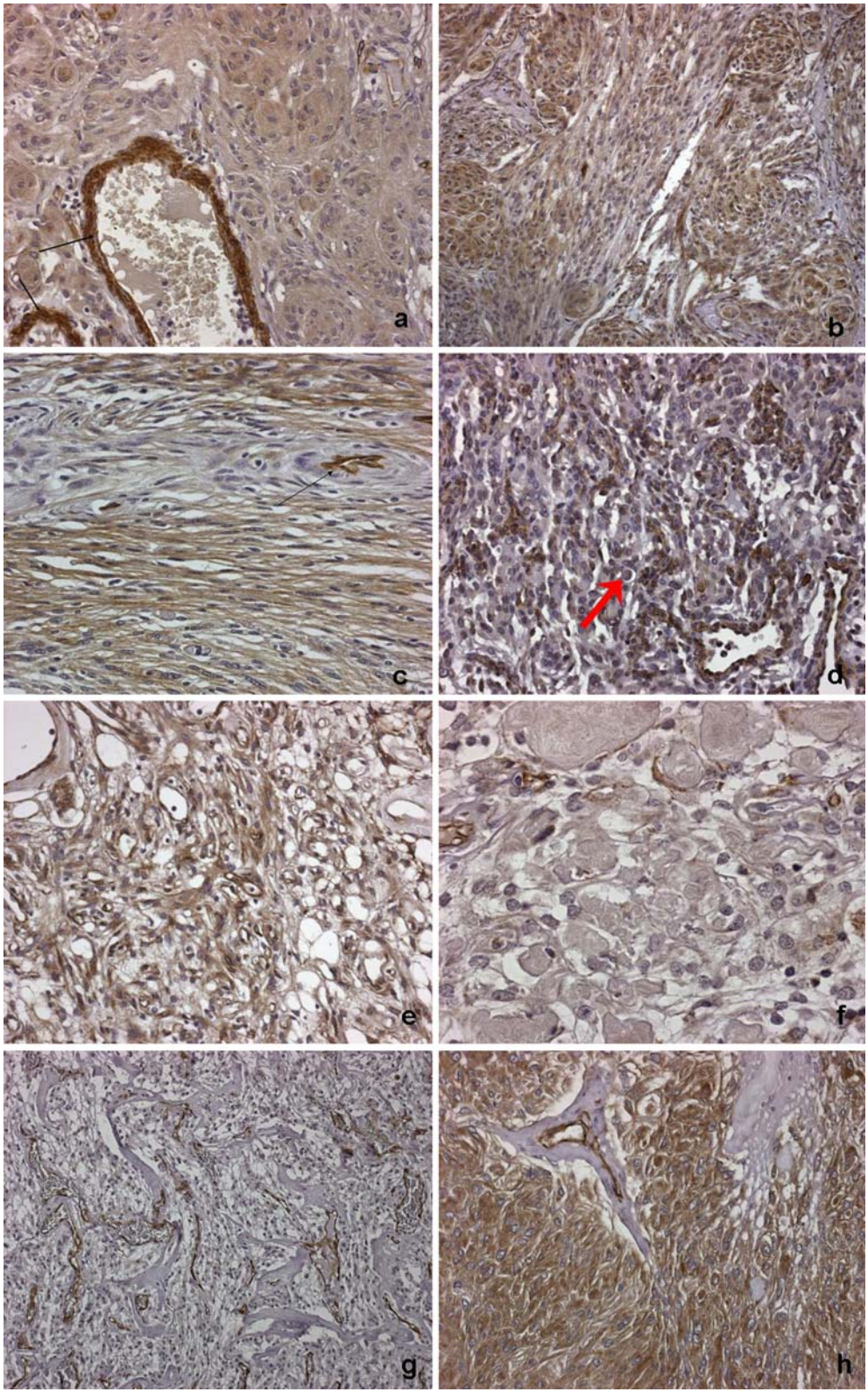
proto-oncogene signalling products [29]. Indeed, down-regulation of the Cav-1 expression has been demonstrated in a variety of neoplastic cell lines, including human breast and colon carcinoma cells [3, 27], while a subsequent re-expression of the Cav-1 in these cells clearly reduced the frequency of tumour formation [3, 22]. On the other hand, elevated Cav-1 expression levels have been reported in some malignancies, such as prostate adenocarcinoma, squamous cell as well as pleomorphic carcinomas of the lung, oesophageal squamous carcinoma and renal cell carcinoma [14, 15, 17, 18, 24, 40]. Moreover, positive correlations between Cav-1 overexpression and disease progression or appearance of metastases have been found in human prostate carcinoma [39, 40], pleomorphic lung carcinoma [24] and renal cell carcinoma [14, 15]. In the central nervous system, the Cav-1 expression has been documented in astrocytoma cell lines [7, 33] but its role in these neoplasms has not been fully ascertained. The aim of the present study was to investigate the Cav-1 immunohistochemical expression in human meningiomas, comparing different morphologic subtypes to normal leptomeningeal tissue. To the best of our knowledge, the Cav-1 expression in these tissues has not been previously reported.

Materials and methods

Sixty-two cases of surgically resected meningiomas obtained from 37 (60%) female and 25 (40%) male patients (age range 21–84 years; mean age 63.5 years), collected between 1996 and 1998, were taken from the files of the Unit of Pathology, M. Bufalini Hospital, Cesena, Italy. More precisely, 26 cases, diagnosed as atypical meningiomas, were randomly selected. Subsequently a comparable number of cases comprising both meningothelial and transitional histotypes were considered. Cases of meningiomas displaying a more unusual histotype and occurring in the same years were also recruited. All cases were histologically re-evaluated according to the WHO Classification [21]. Finally, the study cohort comprised: 11 meningothelial (17%), 12 transitional (19%), 5 fibrous (8%), 3 microcystic (5%), 3 secretory (5%), 1 clear cell (2%), 1 chordoid (2%) and 26 (42%) atypical meningiomas. Tumour sites were divided into three categories: convexity (34%), parasagittal (34%) and basal (32%). For each case, Simpson's grade of surgical resection [34] as well as immunohistochemical assessment of growth fraction determined by Ki-67 labelling index (LI) was also available. On the basis of Simpson's grade, two main groups were considered: the first one (63%) representing grade 1 tumours (complete excision, including dura and bone), the second group (22 + 15% cases) comprising both grade 2 (complete excision plus apparently reliable coagulation of dural attachments) and grade 3 (complete excision of the solid tumour, but insufficient dural coagulation or bone excision) meningiomas. Follow-up data, including patients survival and recurrences, were available in 82% cases. Recurrence was defined as detection of recurrent tumour by neuroradiological investigations in those patients with a previous complete surgical excision.

Ten samples of human leptomeninges, obtained at autopsy from patients without any brain disease, were utilized as normal tissue controls.

All meningeal specimens, fixed in 10% neutral formalin for 24 h at room temperature, were embedded in paraffin at 55°C and cut into 4 μm thick sections for subsequent immunohistochemical study. Briefly, the intrinsic endogenous peroxidase activity was blocked with 0.1% H_2O_2 in methanol for 20 min; and then, normal sheep serum was applied for 30 min to prevent unspecific adherence of serum proteins. Sections were successively incubated at 4°C overnight with the polyclonal rabbit antibody against Cav-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; working dilution 1:500); a sheep anti-rabbit immunoglobulin antiserum (Behring Institute; w.d. 1:25) was applied and the bound primary antibody was visualized by avidin–biotin–peroxidase detection using the Vectastain Rabbit/Mouse Elite Kit, according to the manufacturer's instructions. To reveal the immunostaining, the



sections were incubated in darkness [36] for 10 min with 3-3′ diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO, USA), in quantities of 100 mg in 200 ml 0.03% hydrogen peroxide in phosphate-buffered saline (PBS). Nuclear counterstaining was performed by Mayer's haemalum. Specificity of

◀**Fig. 1** **a** A meningothelial meningioma displaying a grade 1 intensity positive immunoreaction for Cav-1. Endothelial cells of haematic vessels are strongly positive for Cav-1 (*arrows*) (Cav-1 stain; original magnification ×200). **b** Cav-1 immunostaining is evident in transitional meningioma, combining whorled meningothelial cells and elongated "fibroblastic" elements (Cav-1 stain; original magnification, ×100). **c** A fibrous meningioma showing a grade 2 intensity positive immunostaining for Cav-1. The *arrow* indicates the strong positivity for Cav-1 in endothelial cells (Cav-1 stain; original magnification, ×200). **d** Secretory meningioma with characteristic eosinophilic inclusions (*red arrow*). Neoplastic cells were virtually negative for Cav-1, whereas a strong reaction was evident in endothelial cells (*black arrow*) (Cav-1 stain; original magnification, ×200). **e** A microcystic meningioma exhibiting a strong immunoreaction in endothelial cells and a grade 2 intensity staining for Cav-1 in the neoplastic component (Cav-1 stain; original magnification, ×200). **f** Clear cell meningioma displayed a grade 1 intensity immunoreaction only in scattered neoplastic cells, whereas endothelial cells appeared to be strongly stained (Cav-1 stain; original magnification, ×400). **g** Chordoid meningioma was virtually negative for Cav-1 stain. A strong reaction was evidenced in endothelial cells. (Cav-1 stain; original magnification, ×100). **h** A grade 3 intensity immunostaining was evident in neoplastic cells and endothelial cells of an atypical meningioma. A necrosis focus is also evident at the right corner of the figure (Cav-1 stain; original magnification, ×200)

the Cav-1 binding was assessed by three kinds of controls: (1) omitting the primary antiserum, (2) replacing it with normal rabbit serum and (3) previously absorbing it with its homologous antigen. Specimens of adipose tissue as well as endothelium and smooth muscle of the vessels present within the examined tissue represented the positive controls.

Immunostained sections were examined by light microscopy using a 20× and 40× objective lens and a 10× eyepiece. Two pathologists using a double-headed microscope performed the assessment of immunostained sections on a consensus basis. The Cav-1 expression was based on the presence of cytoplasmic and/or membranous staining. Immunostaining intensity was graded as (0) negative, (1) weak, (2) moderate, (3) strong; the stained area recorded as percentage of positive cells was rated as follows: 0 (≤ 10%), 1 (11–25%), 2 (26–50%), 3 (51–75%), 4 (> 75%), according to the procedure described by Joo et al. [15]. A Cav-1 intensity-distribution (ID) score for each case was generated by multiplying the values of the two variables. Cases displaying an ID score 0 were considered as negative.

In sections obtained from the same tissue blocks, Ki-67 antigen was unmasked by antigen retrieval procedures (10 mM, pH 6.0 sodium citrate buffer heated in a microwave oven for 3 cycles × 5 min); and then, Ki-67 antiserum (clone MIB-1, DAKO, Glostrup, Denmark; w.d. 1:50) was applied for 30 min at room temperature. The Ki-67 LI was calculated as mean percentage by counting the stained nuclei of tumour cells for 1,000 cells in three representative neoplastic fields; all degrees of nuclear staining intensity were taken in consideration. A Ki-67 value of 4% was utilized as a cut-off point to determine low and high Ki-67 expression, as suggested by Perry et al. [28].

For statistical analyses, cases were subdivided on the basis of the ID score into two groups: (1) 0–4: low ID score; (2) 6–12: high ID score. Chi-square test was used

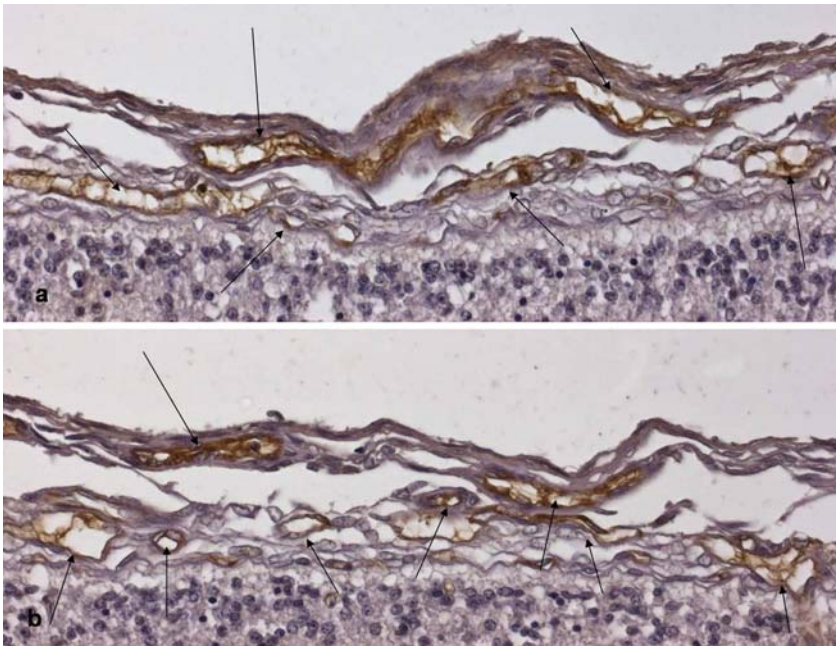


Fig. 2 Normal human leptomeninges displaying only a slight reactivity for Cav-1. Endothelial cells in subarachnoidal vessels were strongly labelled by Cav-1 antibody (*arrows*) (Cav-1 stain; original magnification ×200)

Table 2 Statistical correlation between Cav-1 ID score and clinico-pathological parameters analysed throughout Chi-square test

Variable	Cav-1 ID score		Chi-square	*P*
	0–4	6–12		
Gender				
Male	8	17		
Female	21	16	3.673	NS
Age				
≤ 65 years	16	16		
> 65 years	13	17	0.276	NS
Site				
Convexity	14	7		
Basal	10	10		
Parasagittal	5	16	7.870	0.01955
Simpson's grade				
Grade 1	22	17		
Grade 2–3	7	16	3.921	0.04769
Histotype				
Meningothelial	6	5		
Transitional	8	4		
Unusual	10	3		
Atypical	5	21	14.843	0.00196
Grade				
I	22	12		
II	7	21	9.724	0.00182
Ki-67 LI				
< 4%	24	10		
≥ 4%	5	23	17.149	0.00003
Clinical corse				
Alive	22	17		
Dead from meningiomas	2	11	5.818	0.01586
Recurrences				
Not	17	10		
Yes	0	3	4.358	0.03681

to analyse the correlation between Cav-1 ID score and clinico-pathological characteristics. Overall survival and recurrence-free interval were assessed by the Kaplan–Meier method, with the date of primary surgery as the entry data. The end point was length of survival to death for meningioma or intercurrent diseases strictly related with it, such as status epilepticus, diabetes insipidus with electrolytic imbalance, metachronous meningiomas. Patients who died of other causes were censored. The Mantel–Cox log-rank test was applied to assess the strength of association between survival time or recurrence-free interval and each of the parameters (age and gender of the patient, site, Simpson's grade, histotype, histologic grade, Cav-1 ID score and Ki-67 LI of the tumour) as a single variable. Successively, a multivariate analysis (Cox regression model) was utilised to determine the independent effect of each variable on survival.

A probability (*P*) value less than 0.05 was considered statistically significant. Data were analysed using the SPSS package version 6.1.3 (SPSS Inc., Chicago, IL, USA).

Table 3 Univariate and multivariate survival analyses in 52 patients with meningioma

Univariate analysis				
Parameter		X^2	*df*	*P* value
Sex		4.04	1	0.0443
Age		2.88	1	0.0899
Site		4.52	2	0.1041
Simpson's grade		6.49	1	0.0108
Histotype		2.07	3	0.5578
Histological grade		1.42	1	0.2342
Ki-67		6.02	1	0.0141
Cav-1 ID score		6.45	1	0.0111
Multivariate analysis				
Variable	β	SE	Exp (β)	*P* value
Ki-67	0.0973	0.0290	1.1022	0.0008

Results

The clinico-pathological characteristics of the analysed meningiomas and the corresponding Cav-1 immunohistochemical data are shown in Table 1. Variable Cav-1 immunolabeling was found in 95% of meningiomas. The observed variability related to both intensity of staining and quantity of stained cells. More specifically, 43/62 meningiomas exhibited a moderate to strong immunostaining and 38/62 showed an evident reaction in more than 50% of cells. Immunoreactivity was observed along the cell membrane and the cytoplasm, demonstrated by the presence of stained brown granular immunoreaction products (Fig. 1). In contrast, in all ten cases of non-neoplastic meninges, the Cav-1 stained cells were lower than 10% with a grade 1 of staining intensity (Fig. 2).

Significantly higher Cav-1 ID scores (≥ 6) were observed in meningiomas with a parasagittal location in comparison to those located at convexity or at the base of the skull (Table 2). Taking into consideration the two groups of meningioma with different Simpson's grade, a significant difference was achieved (Table 2). Given the small number of meningiomas displaying a fibrous, microcystic, secretory, clear cell and chordoid histotype, these tumours were all lumped together in a unique group, in the attempt to obtain quantitatively comparable categories for the statistical analysis. Therefore, when the Cav-1 immunoexpression was analysed in relation to the different histological subtypes of meningiomas, four histopathologic groups were considered: meningothelial, transitional, atypical and unusual variants (fibrous, microcystic, secretory, clear cell, chordoid). Atypical meningiomas displayed a significantly higher Cav-1 ID

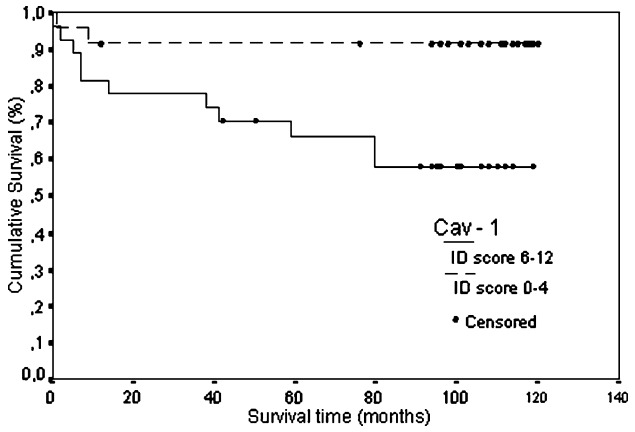


Fig. 3 Kaplan–Meier meningioma-specific survival curve according to Cav-1 ID score. The survival of patients with higher Cav-1 ID score was significantly worse than that of patients with a lower ID score ($P = 0.01$)

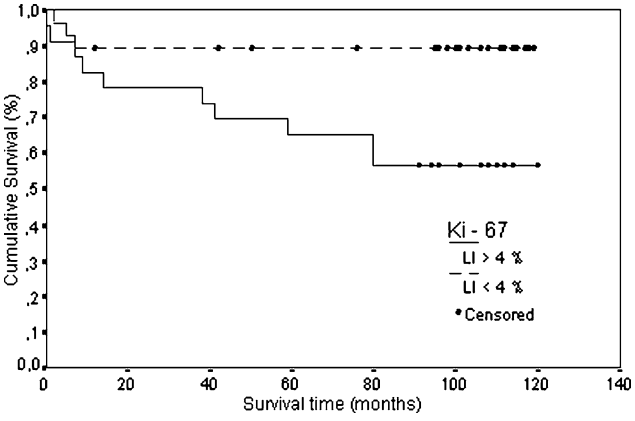


Fig. 4 Kaplan–Meier meningioma-specific survival curve according to Ki-67 LI. The survival of patients with higher Ki-67 LI was significantly worse than that of patients with a lower LI ($P = 0.01$)

score (Table 2). When cases were stratified by grade, significant differences in the Cav-1 ID score were found between WHO grade I and grade II tumours, these latter ones displaying a higher Cav-1 positivity (Table 2). No statistically significant differences in the Cav-1 immunostaining were observed with respect to gender and age of patients (Table 2). A variously represented Ki-67 nuclear reactivity was found in meningiomas, with a rate of stained cells ranging from 0.5 to 30% (mean value 4.72%). Significantly higher Cav-1 ID score was encountered in cases displaying Ki-67 LI levels equal or greater than 4% (Table 2). Regarding the clinical course, significantly higher Cav-1 ID score was found in patients who died of neoplastic disease, and in those who developed disease recurrences (Table 2).

Table 4 Univariate analysis for recurrence-free survival time in 30 patients with a predetermined grade 1 Simpson meningiomas

Parameter	X^2	*df*	*P* value
Sex	0.10	1	0.7474
Age	0.80	1	0.7763
Site	4.01	2	0.1345
Histotype	5.72	3	0.1260
Histological grade	4.93	1	0.0265
Ki-67	5.72	1	0.0168
Cav-1 ID score	4.26	1	0.0390

Univariate analysis identified sex, Simpson's grade of surgical resection, Ki-67 LI and Cav-1 ID score as significant prognostic factors for meningioma-specific survival (Table 3); in particular, the survival of patients with higher Cav-1 ID score (≥ 6) was significantly ($P = 0.01$) worse than that of patients with lower Cav-1 ID score (< 6). The survival curves of patients with low and high Cav-1 ID score as well as Ki-67 LI are illustrated in Figs. 3 and 4, respectively. Moreover, multivariate analysis indicated that only Ki-67 LI was an independent prognostic factor (Table 3).

The prognostic value of aforementioned parameters was confirmed by both univariate and multivariate analyses ($P < 0.05$), when the survival analysis was conducted by considering patients dead of intercurrent disease as censored.

Among the 30 patients with an available follow up that showed a predetermined Simpson's grade 1 resection, only three had developed recurrences. Follow up ranged from 16 to 120 months. The mean recurrence-free time was 96 months. All recurrent cases displayed a Cav-1 ID score ≥ 6. Univariate analysis showed that a Cav-1 ID score ≥ 6, a Ki-67 LI $\geq 4\%$ and a high histologic grade were significant prognostic factors for recurrence (Table 4).

Discussion

Since the first report of Cav-1 involvement in tumorigenesis [12], numerous studies on the expression of this protein in different types of human neoplasms and neoplastic cell lines have been performed [3, 17, 18, 22, 37–40]. The Cav-1 down-regulation has been found in certain tumours [3, 22, 37, 38], while its over-expression has been documented in others [8, 14, 15, 17, 18, 40, 41], suggesting thus the behaviour of Cav-1 is tissue-dependent. Indeed, by immunohistochemistry, a significant loss of the Cav-1 expression has been demonstrated in both ovarian adenocarcinomas [37] and malignant soft tissue sarcomas [38], in line with a

tumour suppressor action of Cav-1 in different kinds of malignancies. By contrast, in oesophageal squamous cell carcinomas, as well as in clear cell renal carcinoma, the Cav-1 immunoreactivity has been correlated with tumour spread and metastases and found to be inversely related to overall survival rate [15, 17]; additionally, the Cav-1 over-expression correlates with the Gleason score and lymph node involvement in prostatic carcinomas [40]. Consequently, these latter studies have suggested a potential role for the Cav-1 as histo-prognostic marker, able to identify an increased risk of metastases and final poor outcome. Interestingly, in lung neoplastic tissue, the staining intensity and the immunopositivity for Cav-1 were different in adenocarcinomas (AD) in comparison to squamous cell carcinomas (SCC), with loss or weakness in the former and over-expression in the latter [18]. Therefore, it can be hypothesized that the function of Cav-1 differs according to tissue type, as demonstrated by its distinct immunoexpression in AD and SCC. These diverse effects can be explained by differing activation states of different domains of the Cav-1 and altered interactions with binding partners [10].

In the present study, we analysed the Cav-1 immunoexpression in human meningiomas and corresponding normal meninges in order to evaluate the distribution pattern of this protein and its possible prognostic utilization. In all cases of normal leptomeninges, only a slight, occasional, Cav-1 immunoreactivity was noted, whereas positive immunostaining was detected in 59/62 meningiomas; in particular, 43/62 meningiomas exhibited a moderate to strong immunostaining and 38/62 showed a reaction in more than 50% of cells. These data strongly suggest an association between the overexpression of Cav-1 in leptomeninges and the acquisition of their neoplastic phenotype. Moreover, when all cases were stratified for grade or histotype, significant differences in the Cav-1 immunoexpression were achieved, being parasagittal site, grade II and atypical histological subtype the parameters characterized by higher Cav-1 appearance. Within grade II tumours, both the clear cell and the chordoid analysed meningiomas represented an exception, showing a low Cav-1 ID score; nevertheless, the extreme paucity of these variants did not allow us to obtain statistical results or specific histopathological correlations. No statistically significant differences in the Cav-1 immunostaining were found with respect to gender and age of patients. In the aim to investigate the relevance of Cav-1 immunostaining in relation to growth fraction, we also analysed our immunohistochemical data in comparison to Ki-67 LI, earlier performed in the same series of meningiomas. Since according to the guidelines proposed by Perry et al. [28], levels of Ki-67 equal or greater than 4% have been associated with an increased rate of recurrence, we utilized this percentage as the cut-off value to identify tumours characterized by a negative prognostic parameter; interestingly, in cases displaying Ki-67 levels equal or greater than 4%, a significantly higher Cav-1 ID score ($P < 0.001$) was encountered. The correlation between Cav-1 immuno-expression and Ki-67 LI might be related to a suitable role of this protein in the cellular mitotic processes, as documented by its changes during the cell cycle, with a maximal expression in the G2/M phase in human mammary epithelial cells [22]. Nevertheless, if the activity of Cav-1 in relation to cell-cycle progression may be also realized by interacting with the MAP kinase cascade [11] or by regulating the cytokinetic process require further investigations.

In our study, we also examined the recurrence-free interval in the group of Simpson's grade 1; among the 30 considered cases, 3 had developed recurrences. The reason why meningiomas undergoing complete surgical resection may recur is still an open question, although some authors have suggested that microscopic clusters of neoplastic cells left in the dura mater or in the arachnoid membrane could be responsible for recurrence, hypothesizing that this event depends upon the biological activity of meningioma tumour cells [16, 20]. However, by univariate analysis, we found that recurrent tumours of our series displayed a significantly higher Cav-1 ID score (≥ 6) and Ki-67 LI ($\geq 4\%$), this latter finding being in accordance with results extensively reported by other authors [9, 13, 19, 35]. Hence, we speculate that surgically not-removed microscopic neoplastic foci, displaying a higher Cav-1 immunohistochemical expression and a higher growth fraction, similar to the principal tumour, may possess an intrinsic higher capability to proliferate and, thus, to give rise to a recurrent meningioma. Unfortunately, due to the paucity of recurrent cases in our cohort, we were not able to perform a multivariate analysis to assess whether the Cav-1 ID score is an independent factor for meningioma-specific recurrence rate. On the other hand, our study also suggests that the Cav-1 expression is correlated with survival in human meningiomas. In fact, a significantly higher Cav-1 ID score appeared significantly correlated to a worse survival of patients, even if it was not an independent prognostic factor for meningioma-specific survival, with the Ki-67 LI representing the only independent variable. Therefore, the prognostic significance of Cav-1 expression in these neoplasms appears to be comparable to that observed in clear cell renal carcinoma and squamous

cell oesophageal carcinoma [15, 17], both suggesting a role for the Cav-1 as a negative albeit not independent prognostic factor for patient-specific tumour survival.

In conclusion, in our view, increased Cav-1 expression may be an additional indicator of meningioma biological aggressiveness, reflected by its association with atypical subtype, high histologic grade and growth fraction, and resulting as a predictor of worse clinical outcome and recurrence.

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