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Novel evaluation of the expression patterns CD44 and MMP9 proteins in intracranial meningiomas and their relationship to the overall survival

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Abstract

Background: Meningiomas are common primary brain neoplasms. CD44 is a cell surface glycoprotein receptor that is involved in matrix-mediated cell signaling and cell–matrix adhesion. Matrix metalloproteinase-9 (MMP-9) plays important role in angiogenesis and tumor invasion. The expression of CD44 protein membranous and cytoplasmic (CD44M and CD44C) has been reported in several tumors (such as lobular carcinoma, renal cell carcinoma, sinonasal melanoma, and lymphoma) except CNS tumors.

Methods: This study addressed the expression of CD44M and CD44C and MMP9 proteins in intracranial meningiomas and their relationship to overall survival. The expression patterns of CD44M&C and MMP-9 proteins were examined in 32 cases of benign meningiomas, 12 cases of atypical meningiomas, and 6 cases of anaplastic meningiomas using immunohistochemical staining methods.

Results: There was more evidence of CD44M expression in atypical and anaplastic meningioma ($p = < 0.001$). Interestingly, Spearman correlation analyses revealed significant positive correlation between CD44M and MMP9 protein ($r = 0.572$, $p = < 0.001$) in spite of the negative correlation between MMP9 and CD44 score ($r = -0.035$ $p = 0.405$). There was a significant association between Ki67 protein expression and the grade of meningiomas ($p < 0.001$) and gender ($p = 0.026$). There was a significant correlation between overall survival (OS) and age, gender, tumor grade, and Ki-67.

Conclusions: Extensive CD44M expression in high-grade meningioma may reflect a tendency toward more invasive power of meningioma cells into surrounding structures (dura, bone, and brain).CD44M/MMP-9 axis presented by this study is open for future investigations.

Keywords: CD44, MMP9, Ki67, Meningiomas

Background

Meningioma is one of the most common primary brain neoplasms, accounting for nearly 30% of all central nervous system tumors [1, 2]. Meningioma is thought to originate from the arachnoid cap cells of the leptomeninges. Meningioma is graded from I to III according to World Health Organization (WHO) 2016 classification of central nervous system tumors.

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Recently, brain invasion is considered a criterion for the diagnosis of atypical meningioma [1, 2]. Mostly, grade I meningioma is characterized by benign behavior in contrast to grade II/III meningiomas that have a poor prognosis [3, 4].

CD44 is a cell surface glycoprotein receptor that is ligand to hyaluronan; an extracellular matrix molecule, being implicated in matrix-mediated cell signaling and in cell–matrix adhesion. It is expressed in almost all human cells. It is important for regulating cell adhesion, migration, angiogenesis, proliferation, and inflammation [5]. CD44 is assumed to be related to tumor invasion and metastatic ability. It was studied on several tumor types, including meningiomas [6]. The expression of CD44 protein (membranous and cytoplasmic expression) has been reported in several tumors such as infiltrating lobular carcinoma [7], renal cell carcinoma [8], sinonasal melanoma [9], and lymphoma [10].

Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade extracellular matrix components and take part in normal physiological processes of tissue remodeling. MMP-9 has been assigned an important role during angiogenesis and tumor invasion [11]. MMP expression in meningiomas has been studied in relation to tumor invasiveness, malignancy, or recurrence [12, 13].

A major issue relates to the recurrence of totally resected (Simpson's grade I) meningiomas and to the identification of its predicting factors. Indeed, despite total macroscopic resection of meningiomas, relapses do occur; however, the reasons behind this phenomenon are still not fully clarified. It has been suggested that the recurrence of totally resected (Simpson's grade 1) meningiomas may be related to the presence of microscopic clusters of neoplastic cells left in the dura mater or in the arachnoid membrane [14, 15].

The expression of CD44 in meningioma patients with intracranial meningiomas is still controversial. Few studies demonstrated marked expression in high-grade meningiomas [16, 17], in contrast to another which concluded that the expression is more extensive in benign meningioma than atypical meningioma [18, 19]. Concerning MMP-9, authors have reported on parallel increase in MMP-9 expression in high-grade meningioma compared to benign meningioma [20].

To date, there are no data detailing the pattern of expression of CD44 (CD44 M&C) in meningiomas and its relationship to MMP-9. To the best of our knowledge, the present report is the first to investigate the correlation between CD44 M&C expression pattern and MMP-9 in intracranial meningiomas and their relationship to OS.

Materials and methods

Patients and tumor samples

This retrospective study was carried out at the Pathology Department, Faculty of Medicine- Al-Azhar University (Assuit branch, Assuit, Egypt) in the period between April 2015 and June 2020. The patients were followed up until May 2020. The specimens included paraffin-embedded tumor blocks representing 32 cases of benign meningiomas, 12 cases of atypical meningiomas, and 6 cases of anaplastic meningiomas. The Hematoxylin and Eosin-stained slides were initially and independently reviewed by two neuropathologists without prior knowledge of the clinical data. The histopathological diagnoses were rendered according to the revised WHO 2016 classification criteria of CNS tumors using standard histological criteria [2]. The demographic and clinical data were obtained retrospectively from chart review files of the Neurosurgery Departments, Assiut University and South Valley University. Patient confidentiality was strictly maintained throughout this study. The patients have given written consent at the date of surgical operation for the use of their tissue within these studies. The study was reviewed and approved by the Local Ethics Committee, Assiut University.

Immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) slides from the meningiomas tissue blocks were retrieved from the pathology lab and included for the IHC study. The FFPE blocks were cut into 3–4-μm thickness and then put on positively charged glass slides. Sections were de-paraffinized and rehydrated, followed by antigen retrieval, which was done with Tris–EDTA in a water bath at 90 °C for 45 min. The primary Mouse Anti-Human CD44 Std. Clone SPM521, Dilution 1:100) (CATALOG #: E17361, Spring bioscience, Pleasanton, California, USA).

Ki-67 primary monoclonal mouse antibody (clone MIB-1) (Code Number: M7240, Dilution 1:50 Dako) and mouse monoclonal antibody against human MMP9 (CloneVIIC2; Dilution 1:100, Lab Vision Corp., Freemont, California, USA) were applied. Each incubated for one hour at room temperature. A universal staining kit "Ultra Vision Detection System Anti-Polyvalent, HRP/DAB (ready-to-use)" (catalog #TP-015-HD, LAB VISION Corp., Fremont, CA, USA) was applied following the manufacturer's instructions. The sections were then counterstained with Mayer's Hematoxylin. The immunostaining was performed using appropriate positive and negative controls. Replacement of the primary antibody with a blocking buffer was used to generate negative control slides.

Evaluation of CD44, MMP9, and Ki67 expression:

A general assessment was made of the expression pattern, taking into account the location, particularly for CD44 (cytoplasm and/or cell membrane) and the amount of positivity detected.

Staining scores for MMP-9 and CD44 were established semi-quantitatively as described by Okada et al. [20] Specifically, the immunohistochemical score for each antibody was calculated by optical analysis using the sum of the percentage of positive cells (0: none; 1: 1–30%; 2: 31–60%; 3: 61–100%) and staining intensity graded from 1 to 3 (1: weak; 2: moderate; and 3:strong). The scores were determined by concordance among the scores of two independent reviewers unaware of the clinicopathological data. From a statistical point of view, immunohistochemical scores of 4 or greater were considered to represent high expression, and those of 3 or lower were to indicate low expression. Evaluation of Ki-67 was performed under a high-power objective (magnification 400 ×), and distinct nuclear staining was recorded as positive.

The areas with the highest number of positive tumor nuclei (hot spots) were selected and an average of 1000 tumor cells from the areas of maximum labeling were counted for analysis [21]. A Ki-67 value of 4% was used as a cutoff point to determine low and high Ki-67 expression, as suggested by Perry et al. [22].

Statistical analysis

The collected data were verified, coded by the researcher, and analyzed using the Statistical Package for Social Sciences (IBM-SPSS/PC/VER 21). Descriptive statistics: mean, standard deviation, median, range, frequencies, and percentage were calculated. Test of significances: Chi- square and Fisher Exact tests were used to compare the difference in the distribution of frequencies among different groups. Student t test and Mann–Whitney U test were calculated to test the mean differences in continuous variables between groups (parametric and non-parametric). The Kaplan–Meier method was used to analyze survival, and the log-rank test was used to estimate differences in survival. Multivariate Cox Hazard regression analysis was calculated to investigate the significant factors influencing OS (Hazard Ratio -HR-, 95% confidence interval -95% CI-). Correlation analysis was used to test the association between variables (Spearman's rank correlation). A significant p value was considered when it was less than 0.05.

Results

Clinicopathological characteristics

The current study included 50 patients (9 male and 41 female patients) with intracranial meningioma of various grades; 32 benign (GI), 12 atypical (GII), and 6 anaplastic (GIII). In the group of benign meningiomas; 18 were meningothelial, 8 fibroblastic, 2 psammomatous, 2 microcystic, and 2 angiomatous.

The patients' age ranged from 17 – 67 years with a mean age of 50.22 ± 12.56. For statistical purposes, the studied cases were divided into two groups; 32 benign (GI) meningiomas, 18 atypical, and anaplastic meningiomas (GII/GIII).

Complete excision, including dura and bone (Simpson grade 1), had been performed in 10/50 meningiomas (20%). Complete excision plus apparently reliable coagulation of dural attachments (Simpson grade 2) had been gained in 36/50 cases (72%). Complete excision of the solid tumor, but with insufficient dural coagulation or bone excision (Simpson grade 3), had been performed in 2/50 cases (4%). Tumor biopsy (Simpson grade 4) had been gained in 2/50 (4%).

Four patients had died of their tumors during the follow-up period. The median OS of these patients was 4.9 years (range 3.95–4.93) using Kaplan–Meier estimates. The clinical characteristics of the patients are listed in Table 1.

Table 1 Clinical characteristics of patients with intracranial meningiomas:

Histological type	Number (%)	Age (mean ± SD)	Sex (No/%)		Simpson grade				OS	
			Male	Female	I	II	III	IV	Alive	Dead
Benign (GI)	32	54.19 ± 9.55	4 (12.5)	28 (87.5)	8 (25.0)	22 (8.8)	–	2 (6.3)	32	0
Meningothelial	18 (36.0)	55.67 ± 9	2 (22.2)	16 (39.0)	6 (60.0)	12 (33.3)	–	0.0		
Fibroblastic	8 (16.0)	53.25 ± 10.38	2 (22.2)	6 (14.6)	2 (20.0)	4 (11.1)	–	2 (100.0)		
Microcystic	2 (4.0)	45 ± 0	0 (0.0)	2 (4.9)	0.0	2 (5.6)	–	0.0		
Psammomatous	2 (4.0)	65 ± 0	0 (0.0)	2 (4.9)	0.0	2 (5.6)	–	0.0		
Angiomatous	2 (4.0)	43 ± 0	0 (0.0)	2 (4.9)	0.0	2 (5.6)	–	0.0		
Atypical (GII)	12	38.5 ± 15.17	2 (16.7)	10 (83.3)	2 (20.0)	8 (22.2)	2 (100.0)	0.0	10	2
Anaplastic (GIII)	6 (12%)	52.5 ± 6.02	3 (50.0)	3 (50.0)	0 (0.0)	6 (16.7)	0 (0.0)	0.0	4	2

Association between immunohistochemical markers and clinicopathological parameters

All cases of meningiomas express the CD44 (cytoplasmic and/or membranous), cytoplasmic MMP-9, and nuclear Ki-67.

CD44 expression

The mean CD44 score was 3.38 ± 0.87 for benign (GI) and 4.67 ± 0.8 for atypical and anaplastic meningiomas (GII/III). There was a significant association between CD44 scores and different grades (I vs II/III; Fig. 1A–C) of meningiomas ($p = < 0.001$) and Simpson grade ($p = 0.026$). Table 2.

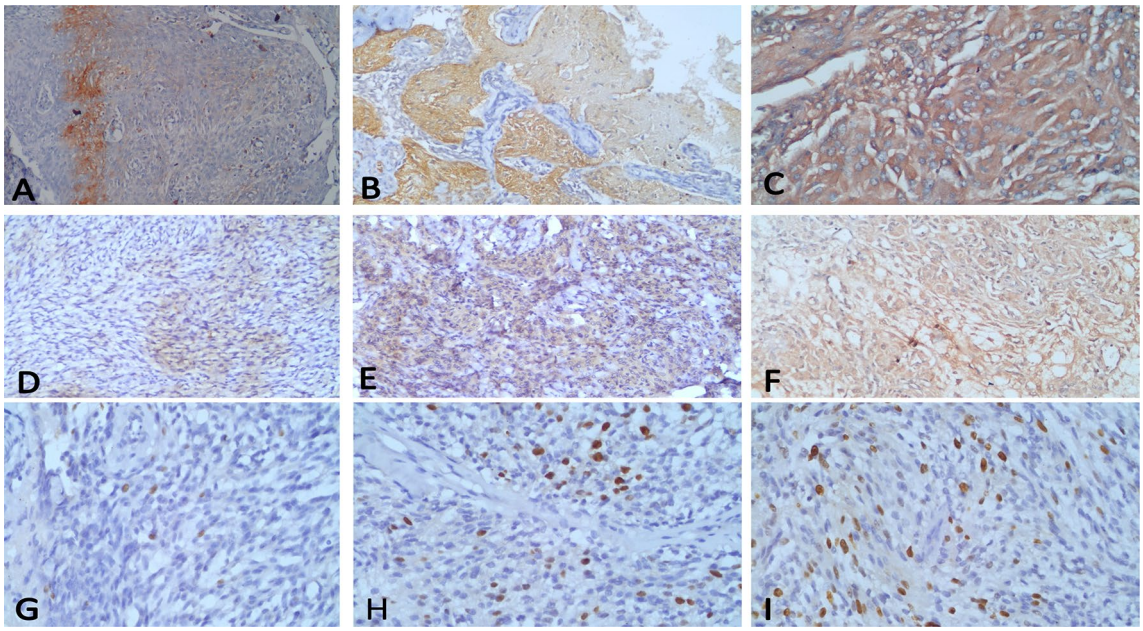

Fig. 1 Immunohistochemistry finding for CD44, MMP9 and Ki67 in meningiomas. CD44 expression **A**–**C** demonstrated low score in GI meningioma (**A**) and high score in brain invasive meningioma (**B**) and anaplastic meningioma GIII (**C**) (original magnification A and Bx200 Cx400). MMP9 expression in meningiomas **D**–**F** showed the highest score in anaplastic meningioma (**F**), (original magnification A and Bx200 Cx400). Ki67 expression in meningiomas (**G**–**I**) demonstrated high Ki-67 LI in both atypical and anaplastic meningioma (original magnification × 400)

Table 2 Summary of clinicopathological data of meningioma patients

Parameter	No.	CD44			MMP-9			Ki67%		
		Mean ± SD	Median (IQR)	*p* value	Mean ± SD	Median (IQR)	*p* value	Mean ± SD	Median (IQR)	*p* value
Age group										
• < 50 years	24	3.83 ± 1.1	4 (2)	= 0.935	4.50 ± 1.6	5 (3)	= 0.261	8.45 ± 7.9	6 (5)	= 0.115
• ≥ 50 years	26	3.85 ± 1.1	4 (1)		4.04 ± 1.6	4 (2)		5.51 ± 4.8	4 (4)	
Sex										
• Female	41	3.80 ± 1.1	4 (2)	= 0.356	4.20 ± 1.5	5 (3)	= 0.402	6.55 ± 6.2	5 (5)	= 0.026*
• Male	9	4.00 ± 0.7	4 (1)		4.56 ± 1.9	5 (2)		8.60 ± 6.1	13 (11)	
Grade										
• G I	32	3.38 ± 0.9	3 (1)	< 0.001**	3.81 ± 1.5	4 (2)	< 0.001**	3.28 ± 2.2	3 (3)	< 0.001**
• G II–III	18	4.67 ± 0.8	4 (1)		5.06 ± 1.4	5.5 (1)		13.89 ± 6.8	14 (10)	
Simpson grade										
• I	10	3.40 ± 1.4	3 (1)	= 0.026*	3.80 ± 1.9	3.5 (2)	= 0.527	7.80 ± 6.7	3 (3)	= 0.845
• II	36	3.89 ± 1.0	4 (1)		4.42 ± 1.5	5 (3)		6.58 ± 5.4	6 (5.5)	
• III–IV	4	4.50 ± 0.6	4.5 (1)		4.00 ± 1.2	4 (2)		7.80 ± 5.2	8 (7)	

* significant *p* value < 0.05, ** significant *p* value < 0.01

The expression pattern of CD44 (Cytoplasmic and/or membranous) was evaluated in all studied meningiomas. Within those with benign (GI) meningiomas; 20/32 (62.5%) express the membranous pattern; however, 10/12 (83.3%) and 6/6 of atypical and anaplastic express the membranous pattern, respectively (Fig. 2A, B). The differences in the expression pattern between different grades of meningiomas were statistically significant ($p = < 0.001$). The details of the expression pattern of CD44 (Fig. 2C, D) are listed in Table 3.

MMP-9 expression

The mean MMP-9 score of benign (GI) meningiomas was 3.81 ± 1.5, while atypical and anaplastic meningiomas (GII/III) were 5.06 ± 1.4. There was a significant association between MMP-9 scores and pathological grades of meningiomas ($p < 0.001$; Fig. 1D–F). There was no significant correlation between MMP-9 and other clinicopathological variables (Table 2).

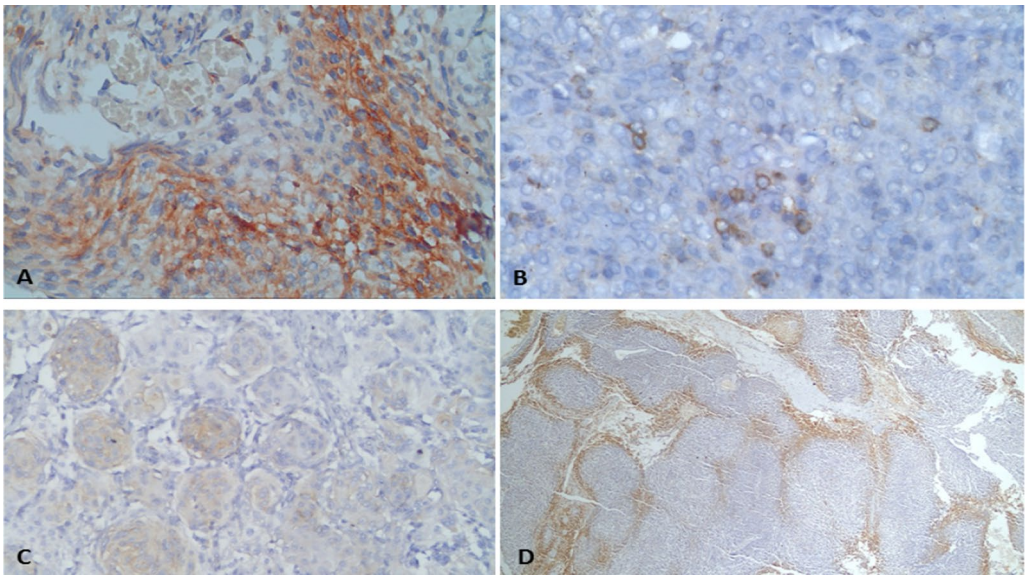

Fig. 2 CD44 expression pattern: **A** CD44 cytoplasmic expression in meningothelial meningioma (original magnification × 400).(**B**) CD44 membranous expression (center of pic) in anaplastic meningioma (original magnification × 600). **C** CD44 expression in the whorls of meningothelial meningioma (original magnification × 200). **D** CD44 expression with peripheral arrangement in atypical meningioma (original magnification × 40)

Table 3 The CD44 protein expression patterns in intracranial meningiomas

	Types						P value
	Benign (n = 32)		Atypical (n = 12)		Anaplastic (n = 6)		
CD44C*							
−	0	0.0	0	0.0	3	50.0	< 0.001**
+	16	50.0	2	16.7	0	0.0	
++	4	12.5	2	16.7	3	50.0	
+++	12	37.5	8	66.7	0	0.0	
CD44M*							
−	12	37.5	2	16.7	0	0.0	< 0.001**
+	14	43.8	8	66.7	0	0.0	
++	6	18.8	2	16.7	3	50.0	
+++	0	0.0	0	0.0	3	50.0	

CD44C*, cytoplasmic expression; CD44M*, Membranous expression

Ki-67 expression

The mean Ki-67 score within benign meningiomas (GI) was 3.28 ± 2.2, while that of atypical (Fig. 3) and anaplastic meningiomas (GII/III) were 12.08% ± 8%. Ki-67 expression showed a significant association with meningiomas grade ($p < 0.001$; Fig. 1G–I) and gender ($p = 0.026$) (Table 2).

Correlation between the immunohistochemical markers:

Significant positive correlation was noted between Ki-67 and CD44 score, Ki-67 and MMP-9, CD44 membranous expression and MMP-9 ($r = 0.263$, $p = 0.032$), ($r = 0.256$ $p = 0.037$), ($r = 0.572$, $p = < 0.001$), respectively. Interestingly, however, there was negative correlation between MMP9 and CD44 score ($r = -0.035$, $p = 0.405$), there was positive correlation between MMP9 and CD44 membranous expression ($r = 0.242$, $p = 0.045$). The details of Spearman's correlation between immunohistochemical markers are listed in Table 4.

Table 4 The correlation among the expression values of Ki67, CD44, and MMP9 protein expression in intracranial meningiomas

Parameter	Ki67 rho* (*p* value)	MMP9	CD44 score
Total (n = 50)			
CD44 Score	0.263 (= 0.032)	− 0.035 (= 0.405)	1
CD44-Cytoplasmic	0.036 (= 0.402)	− 0.257 (= 0.036)	1
CD44-Membranous	0.572 (< 0.001)	0.242 (= 0.045)	1
MMP9	0.256 (= 0.037)	1	− 0.035 (= 0.405)

* Spearman's ranked correlation coefficient

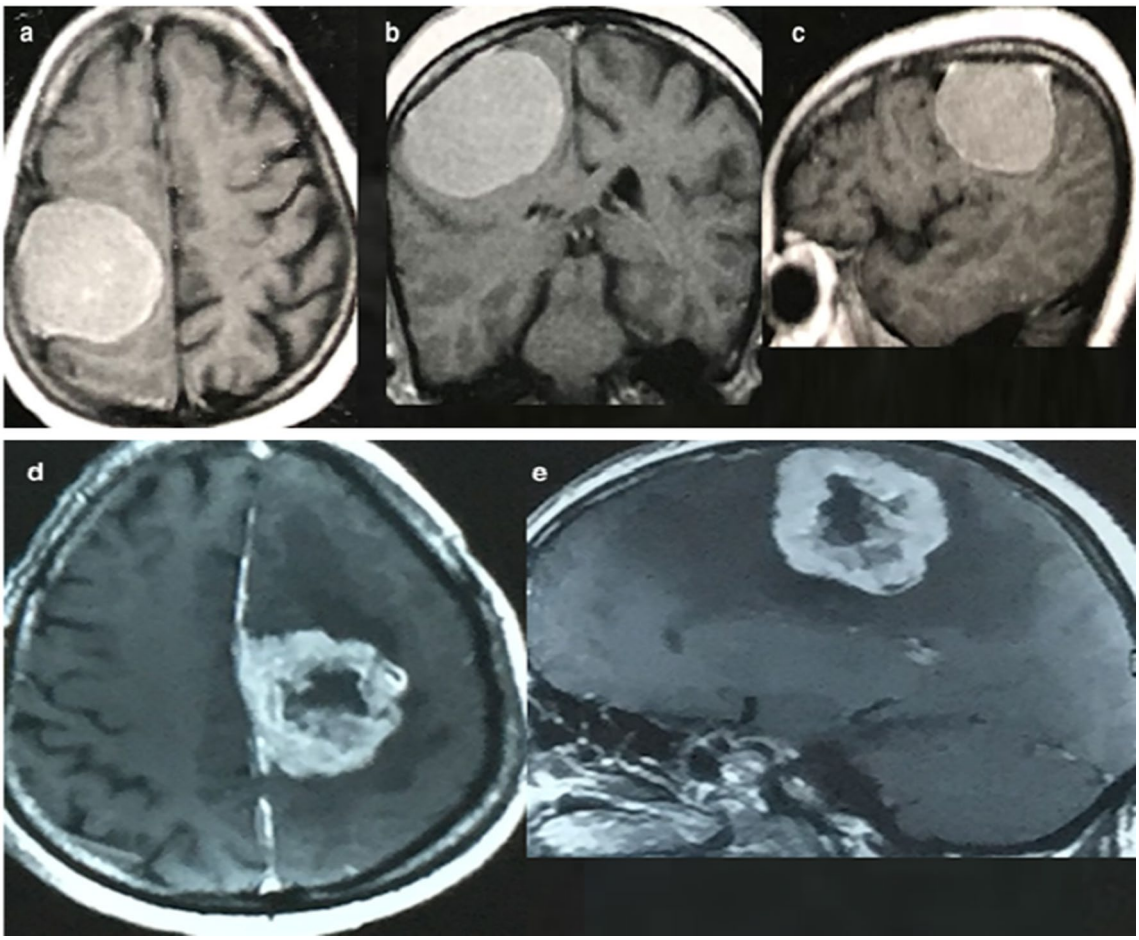

Fig. 3 MRI examples of different types of meningioma: **a** axial, **b** coronal and **c** Sagittal post-contrast T1 weighted images, show large right convexity, well defined, homogenously enhanced meningioma. Histopathology revealed transitional meningioma (WHO grade I). **d** Axial and **e** Sagittal post-contrast T1WI: a left well defined lobulated parasagittal extra axial dural based mass (meningioma). It shows avid enhancement with area of central degeneration and associated with moderate vasogenic edema and mass effect evident by effacement of the adjacent sulci and mild midline shift. Histopathology revealed atypical meningioma (WHO grade II)

Survival analyses

Overall survivals were analyzed by the expression of each immunohistochemical marker, age, gender, tumor grade, and Simpson grade (II-IV). On univariate analysis, a significant correlation was found between OS and age group (≥ 50 years), gender (male), and Grade II/III ($p = 0.041$, $p = 0.012$, $p = 0.014$) respectively.

The expression of immunohistochemical markers (CD44 and MMP9) did not correlate with OS; however, Ki-67 showed a statistically significant correlation with OS (OS: HR, 1.136 95% confidence interval (CI) 1.025–1.259, $p = 0.015$).

On multivariate analysis, still age, GII/III and Ki-67 significantly correlated with OS ($p = 0.046$, $p = 0.047$, $p = 0.048$), respectively. Additionally, CD44 score and CD44 cytoplasmic were associated with poor OS (OS: HR, 1.881 95% confidence interval (CI) 1.121–4.123, $p = 0.044$) (OS: HR, 2.401 95% confidence interval (CI), 1.095–5.446, $p = 0.046$) respectively. The details of univariate correlation and multivariate analysis are listed in Table 5.

According to the Kaplan–Meier curve, there was a significant correlation between median OS and age (≥ 50 years; $p = 0.031$), histological grade (GII/III; $p = 0.005$), and nearly significant for Ki-67 (≥ 4 cut-off point; $p = 0.068$). Interestingly, CD44 cytoplasmic revealed poor OS ($p = 0.048$); however, no association was found between median survival and CD44 membranous (Fig. 4).

Discussion

To the best of the authors' knowledge, the expression patterns of CD44, MMP-9 proteins, and Ki-67 proliferation marker in meningiomas are poorly understood. Moreover, the correlations among these expression patterns and the clinicopathological features of meningiomas have not been fully addressed by the previous studies. The current study examined the correlation between CD44 M&C, MMP-9, and Ki-67 protein expression and the clinicopathological variables (histopathological grades of meningiomas, age, gender, and Simpson grade) and the overall survival of the patients.

In agreement with other investigations, this study revealed overexpression of CD44 protein in atypical and anaplastic meningiomas as compared to their benign counterparts [16, 17]. In the current study, most cases showed both CD44 cytoplasmic and membranous protein expression patterns; however, the membranous expression was more evident in atypical and anaplastic meningiomas [23]. These findings concur with the previous studies that reported diffuse strong membranous staining in atypical and high-grade meningiomas (II/III) meningiomas [24].

Interestingly, some studies reported a decline in CD44 expression in high-grade meningiomas [18, 19]. Freitag et al. [25] reported a significantly high expression of CD44 mRNA in low-grade tumors than in high-grade meningiomas. This discrepancy between CD44 results in the literature may be reasoned to several possibilities. Importantly, the heterogeneity of CD44 proteins in meningiomas is secondary to its post-translational modifications and differential splicing [26]. Also, the variability in the scoring methodologies among the different studies explains this discrepancy.

This current study revealed on multivariate analysis and Kaplan Meier that the CD44 cytoplasmic expression was a poor prognostic factor. In contrast, Kamamoto et al. [17] reported the lack of correlation between CD44 and overall survival but found a correlation with PFS.

Table 5 Cox Proportional Hazard Regression analysis for overall survival in patients with intracranial meningiomas

Variable	Univariate		Multivariate	
	HR (95% CI) *	*p* value	HR (95% CI)	*p* value
Age group (≥ 50 years)	1.784 (1.024–2.927)	= 0.041*	1.143 (1.011–3.154)	= 0.046*
Sex (male)	4.713 (1.664–8.479)	= 0.012*		
Grade (GII-III)	5.725 (1.422–9.046)	= 0.014*	3.197 (1.055–6.201)	= 0.047*
Ki67%	1.136 (1.025–1.259)	= 0.015*	1.095 (1.025–1.259)	= 0.048*
CD44 score	1.765 (0.723–4.306)	= 0.212	1.881 (1.121–4.123)	= 0.044*
CD44 (Cytoplasmic)	1.993 (0.591–6.728)	= 0.266	2.401 (1.095–5.446)	= 0.046*
CD44 (Membranous)	1.758 (0.612–5.051)	= 0.294	1.212 (0.702–7.811)	= 0.425
MMP9	1.471 (0.661–3.272)	= 0.344		
Simpson Grade II-IV	1.209 (0.488–5.085)	= 0.495		

HR hazard ratio, *CI* confidence interval

* significant *p* value < 0.05

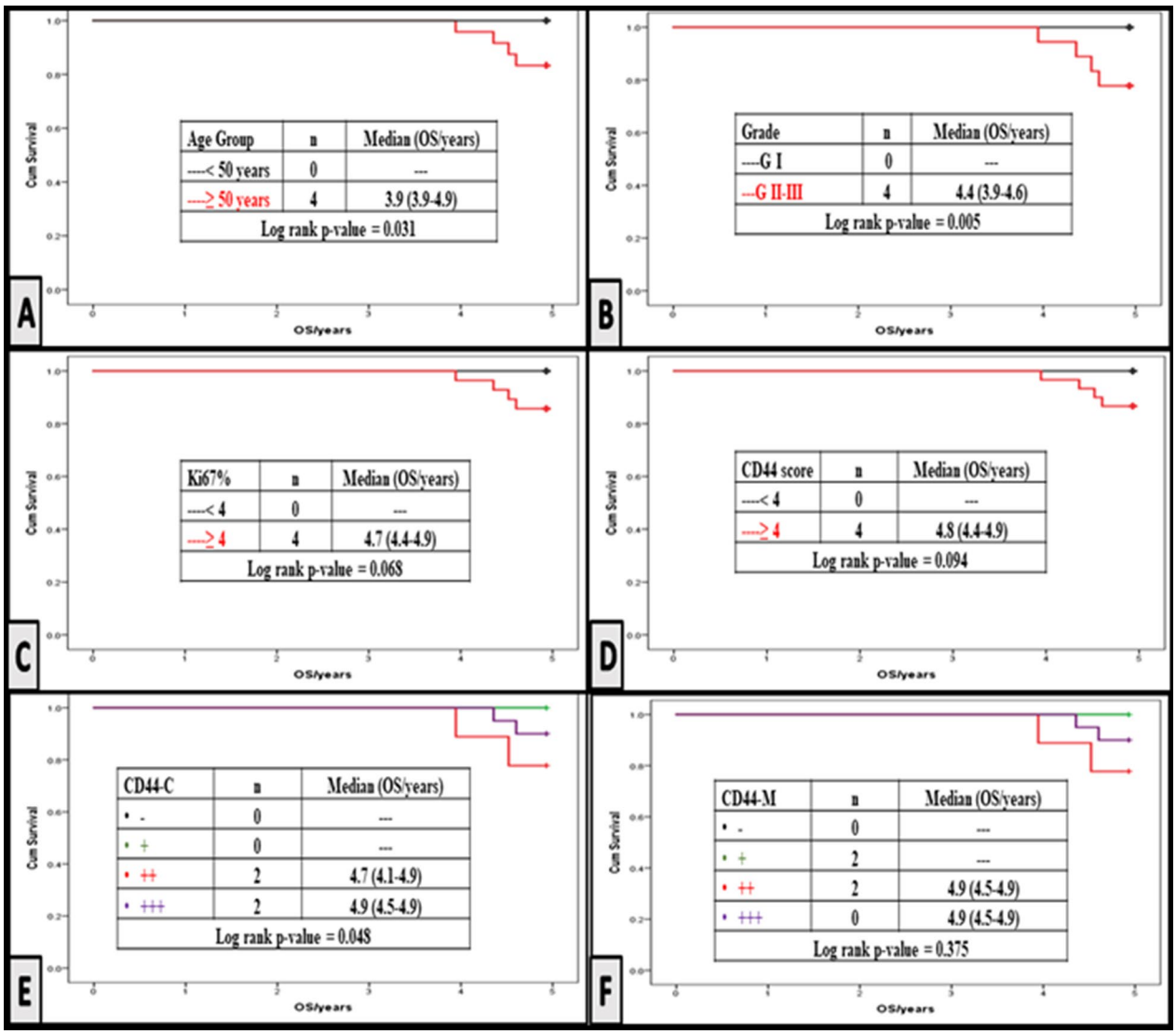

Fig. 4 Kaplan–Meier survival curves for the effect each factor on overall survival: **A** Age, **B** Grade, **C** Ki67%, **D** CD44 Score, **E** CD44-C and **F** CD44-M. *OS* overall survival

This discrepancy may be reasoned to the use of different staining methodologies or study groups.

CD44 is a transmembrane protein, so the delocalization of CD44 to the cytoplasm may cause alterations in the cell–cell and cell-extracellular matrix interactions. Accordingly, cytoplasmic staining in this series may reflect the production of aberrant CD44 proteins by the malignant cells [9]. Taken as a whole, this current study and the previous investigations indicate some differences in biological function and timing of expression of the membranous and cytoplasmic of CD44, according to the degree of invasion, i.e., stage-dependent manner).

Interestingly, the grading of meningiomas depends mainly on the invasion of adjacent structures (brain). Taken together, extensive CD44 membranous expression in high-grade meningioma may reflect a tendency toward more invasive power of neoplastic cells into surrounding structures (dura, bone, and brain). These findings strengthen the putative role of membranous CD44 protein in the cellular progression of meningiomas.

The pathogenetic roles of CD44 in the development of atypical and high-grade meningiomas may be reasoned to increased motility of the malignant cells and alterations in the cell adhesion [17, 27]. Several experimental leads support these notions. For instance, the loss of

the membranous CD44 protein expression in sinonasal melanomas is associated with increased motility of the melanoma cells resulting from the loss of cellular adhesion [28]. Additionally, the cell adhesion by CD44 is predominantly mediated through the extracellular domain, while co-localization of the cytoplasmic domain of CD44 with cytoskeletal proteins, particularly actin, stabilizes the interactions with the substrate. Moreover, the overexpression of membranous CD44 protein in the invasive lobular carcinoma was significantly more often observed in lymph node-negative patients than in patients with lymph node metastases [7].

The overexpression of MMP-9 protein in the atypical and high-grade meningiomas in the current study is in agreement with the previous investigations [29]. MMP-9 protein belongs to a family of gelatinases that degrades the extracellular matrix and the basal lamina components such as collagen IV, fibronectin, and laminin [30]. Interestingly, some studies [29] showed a significant correlation between high MMP-9 expression and high histological grade in tumors. Also, an association between the MMP-9 mRNA level and the degree of brain invasion has been reported [31]. When the impact of MMP-9 on survival was examined, we did not find any correlation between them. This finding is reasoned to the paucity of the absence of recurrence in the patients included in the study. To the best of our knowledge, only Barresi et al. [12] and Okada et al. [20] reported high MMP-9 expression as a prognostic independent variable on multivariate analysis.

To the best of the authors' knowledge, the correlation between CD44 and MMP-9 proteins in meningiomas has not been addressed by the previous studies. Here, this study reports a positive correlation between MMP-9 protein and membranous CD44 protein expression.

Alternatively, a negative correlation with CD44 protein expression (the expression score and the cytoplasmic staining pattern) was detected. Our results are in accordance with findings in other tumors such as malignant melanoma [32], prostatic carcinoma [33], and mammary carcinoma [34]. Gupta et al. [35] reported that cell surface interaction of CD44 and MMP-9 proteins increases the migration and the invasion of PC3 cells. In contrast, Lee et al. [8] demonstrated a correlation between CD44 and MMP-9 proteins in clear RCC, who did not find this correlation in non-clear renal cell carcinoma. The discrepancy among the different studies concerning the relationship between CD44 and MMP-9 protein expression may be explained in the light of findings reported by Desai et al. [36]. Desai and his colleagues indicated a decrease in the surface expression of CD44 in the prostate cancer cell (PC3) knock-down of MMP-9 and a decrease in the activation of MMP-9 in cells null for CD44 [36].

In addition, downregulation of MMP-9 protein expression in PC3 cells switches CD44 isoform expression from CD44s to CD44v6 which is more glycosylated [35].

Taken together, the correlation of MMP-9 and CD44 protein expression depends mainly on the pattern of membranous expression more than on the cellular level of CD44 protein, whether CD44 is linked with MMP-9 proteins at the surface of the cell in intracranial meningiomas. CD44M/MMP-9 axis in atypical and anaplastic meningiomas may be at the basis of the higher invasive potential toward the brain parenchyma and bone of these neoplasias [32, 35, 36].

The results of the current study are in line with the previous investigations that demonstrated the correlation between meningioma grade and the Ki-67 labeling indices [37]. Moreover, Ki-67 labeling indices were the most important independent prognostic parameters in high-grade meningiomas [38]. In agreement with other studies, this study revealed a significant positive correlation among the indices of the proliferative marker Ki-67 and CD44 [16, 19] and MMP-9 proteins expression values [39] protein expression. In contrast, Barresi et al. [12] and Okada et al. [20] did not find any correlations between Ki-67 and MMP-9 protein expression. This discrepancy probably reflects the small sample size of the study groups (only three cases of atypical meningioma) and the use of different cutoff points for scoring the Ki-67 labeling index [12, 20].

Conclusions

Our work demonstrated statistically significant high expression of CD44, MMP-9, and Ki-67 scores in high-grade meningiomas. Our findings illustrated, for the first time, a positive correlation between CD44M and MMP-9 protein expression in meningiomas. It also revealed that the CD44 cytoplasmic is a poor prognostic factor ($p < 0.001$). Accordingly, CD44M/MMP-9 protein axis is a promising biomarker for meningiomas prognosis prediction and therapeutic implications. Expression of CD44 membranous and cytoplasmic require quantitative and qualitative assessment and their relationship to MMP-9, OS, and PFS in large-scale studies to further validate our results in the future.

Abbreviations

MMP: Matrix metalloproteinase; WHO: World Health Organization; MRI: Magnetic resonance imaging; FFPE: Formalin-fixed paraffin-embedded; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval.

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Availability of data and materials
The data and materials of this manuscript are available for sharing.

Declarations

Ethics approval and consent to participate
Patient confidentiality was strictly maintained throughout this study. The patients have given written consent at the date of surgical operation for the use of their tissue within these studies. The study was reviewed and approved by the Local Ethics Committee of Assiut University.

Consent for publication
Written consent has been obtained from every included patient regarding publishing their details and images.

Competing interests
There is no conflict of interest in this manuscript.

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References

1. Unteroberdörster M, Michel A, Darkwah Oppong M, Jabbarli R, Hindy NE, Wrede KH, Sure U, Pierscianek D. The 2016 edition of the WHO classification of primary brain tumors: applicable to assess individual risk of recurrence in atypical meningioma? A single-center experience. J Neurol Surg A Cent Eur Neurosurg. 2021. https://doi.org/10.1055/s-0040-1720987.
2. Louis D, Ohgaki H, Wiestler O, Cavenee WK. WHO classification of tumours of the central nervous system, Revised. 4th ed. Lyon: IARC Press; 2016.
3. Durand A, Labrousse F, Jouvet A, et al. WHO grade II and III meningiomas: a study of prognostic factors. J Neurooncol. 2009;95:367–75.
4. Zhao P, Hu M, Zhao M, Ren X, Jiang Z. Prognostic factors for patients with atypical or malignant meningiomas treated at a single center. Neurosurg Rev. 2015;38:101–7.
5. Naruse M, Shibasaki K, Yokoyama S, Kurachi M, Ishizaki Y. Dynamic changes of CD44 expression from progenitors to subpopulations of astrocytes and neurons in developing cerebellum. PLoS ONE. 2013;8(1):e53109.
6. Rooprai HK, Liyanage K, King A, Davies D, Martin K, Pilkington GJ. CD44 expression in human meningiomas: an immunocytochemical, immunohistochemically and flow cytometric analysis. Int J Oncol. 1999;14(5):855–60. https://doi.org/10.3892/ijo.14.5.855.
7. Berner HS, Nesland JM. Expression of CD44 isoforms in infiltrating lobular carcinoma of the breast. Breast Cancer Res Treat. 2001;65(1):23–9. https://doi.org/10.1023/a:1006417412046.
8. Lee YM, Kim JM, Lee HJ, Seong IO, Kim KH. Immunohistochemical expression of CD44, matrix metalloproteinase2 and matrix metalloproteinase9 in renal cell carcinomas. Urol Oncol. 2019;37(10):742–8. https://doi.org/10.1016/j.urolonc.2019.04.017.
9. Regauer S, Ott A, Berghold A, Beham A. CD44 expression in sinonasal melanomas: is loss of isoform expression associated with advanced tumour stage? J Pathol. 1999;187(2):184–90. https://doi.org/10.1002/(SICI)1096-9896(199901)187:2%3c184::AID-PATH216%3e3.0.CO;2-2.
10. Lemma SA, Kuusisto M, Haapasaari KM, Sormunen R, Lehtinen T, Klaavuniemi T, Eray M, Jantunen E, Soini Y, Vasala K, Böhm J, Salokorpi N, Koivunen P, Karihtala P, Vuoristo J, Turpeenniemi-Hujanen T, Kuittinen O. Integrin alpha 10, CD44, PTEN, cadherin-11 and lactoferrin expressions are potential biomarkers for selecting patients in need of central nervous system prophylaxis in diffuse large B-cell lymphoma. Carcinogenesis. 2017;38(8):812–20. https://doi.org/10.1093/carcin/bgx061.
11. Iwado E, Ichikawa T, Kosaka H, Otsuka S, Kambara H, Tamiya T, Kondo S, Date I. Role of VEGF and matrix metalloproteinase-9 in peritumoral brain edema associated with supratentorial benign meningiomas. Neuropathology. 2012;32(6):638–46. https://doi.org/10.1111/j.1440-1789.2012.01312.x.
12. Barresi V, Vitarelli E, Tuccari G, Barresi G. MMP-9 expression in meningiomas: a prognostic marker for recurrence risk? J Neurooncol. 2011;102(2):189–96. https://doi.org/10.1007/s11060-010-0312-8.
13. Beltran E, Matiasek K, De Risio L, de Stefani A, Feliu-Pascual AL, Matiasek LA. Expression of MMP-2 and MMP-9 in benign canine rostrotentorial meningiomas is not correlated to the extent of peritumoral edema. Vet Pathol. 2013;50(6):1091–8. https://doi.org/10.1177/0300985813481610.
14. Kinjo T, al-Mefty O, Kanaan I. Grade zero removal of supratentorial convexity meningiomas. Neurosurgery. 1993; 33(3):394–9; discussion 399. https://doi.org/10.1227/00006123-199309000-00007.
15. Kamitani H, Masuzawa H, Kanazawa I, Kubo T. Recurrence of convexity meningiomas: tumor cells in the arachnoid membrane. Surg Neurol. 2001;56(4):228–35. https://doi.org/10.1016/s0090-3019(01)00582-1.
16. Mostafa RR, Khairy RA. CD44 expression in meningioma and its correlation with proliferation indices. J Clin Diagn Res. 2017;11(8):EC12–5. https://doi.org/10.7860/JCDR/2017/28438.10379.
17. Kamamoto D, Saga I, Ohara K, Yoshida K, Sasaki H. Association between CD133, CD44, and nestin expression and prognostic factors in high-grade meningioma. World Neurosurg. 2018;S1878–8750(18):32890. https://doi.org/10.1016/j.wneu.2018.12.067.
18. Figarella-Branger D, Roche PH, Daniel L, Dufour H, Bianco N, Pellissier JF. Cell-adhesion molecules in human meningiomas: correlation with clinical and morphological data. Neuropathol Appl Neurobiol. 1997;23(2):113–22.
19. Arsene D, Comănescu M, Ardeleanu C. Adhesion cell molecules as potential markers of aggressiveness in meningiomas. Rom J Morphol Embryol. 2014;55(2 Suppl):585–9.
20. Okada M, Miyake K, Matsumoto Y, Kawai N, Kunishio K, Nagao S. Matrix metalloproteinase-2 and matrix metalloproteinase-9 expressions correlate with the recurrence of intracranial meningiomas. J Neurooncol. 2004;66(1–2):29–37. https://doi.org/10.1023/b:neon.0000013474.01161.58.
21. Reid MD, Bagci P, Ohike N, Saka B, Erbarut Seven İ, Dursun N, Balci S, Gucer H, Jang KT, Tajiri T, Basturk O. Calculation of the Ki67 index in pancreatic neuroendocrine tumors: a comparative analysis of four counting methodologies. Mod Pathol. 2015;28(5):686–94.
22. Perry A, Stafford SL, Scheithauer BW, Suman VJ, Lohse CM. The prognostic significance of MIB-1, p53, and DNA flow cytometry in completely resected primary meningiomas. Cancer. 1998;82(11):2262–9.
23. Gassoum A, Arbab MA, Aldeaf SAH, Elhassan LA, Bashier BM, Saad MSA, et al. CD44 expression in sudanese meningioma patients. Int J Recent Sci Res. 2016;7(6):11900–4.
24. Lewy-Trenda I, Omulecka A, Janczukowicz J, Papierz W. CD44 expression in human meningiomas: an immunohistochemical analysis. Pol J Pathol. 2004;55(1):33–7.
25. Freitag D, McLean AL, Simon M, Koch A, Grube S, Walter J, Kalff R, Ewald C. NANOG overexpression and its correlation with stem cell and

differentiation markers in meningiomas of different WHO grades. Mol Carcinog. 2017;56(8):1953–64. https://doi.org/10.1002/mc.22653.
26. Cichy J, Pure E. The liberation of CD44. J Cell Biol. 2003;161:839–43. https://doi.org/10.1083/jcb.200302098.
27. Harwood CA, Green MA, Cook MG. CD44 expression in melanocytic lesions: a marker of malignant progression? Br J Dermatol. 1996;135(6):876–82. https://doi.org/10.1046/j.1365-2133.1996.d01-1089.x.
28. Lacy BE, Underhill CB. The hyaluronate receptor is associated with actin filaments. J Cell Biol. 1987;105(3):1395–404. https://doi.org/10.1083/jcb.105.3.1395.
29. Reszec J, Hermanowicz A, Rutkowski R, Turek G, Mariak Z, Chyczewski L. Expression of MMP-9 and VEGF in meningiomas and their correlation with peritumoral brain edema. Biomed Res Int. 2015;2015: 646853. https://doi.org/10.1155/2015/646853.
30. von Randow AJ, Schindler S, Tews DS. Expression of extracellular matrix-degrading proteins in classic, atypical, and anaplastic meningiomas. Pathol Res Pract. 2006;202(5):365–72. https://doi.org/10.1016/j.prp.2006.01.012.
31. Nordqvist AC, Smurawa H, Mathiesen T. Expression of matrix metalloproteinases 2 and 9 in meningiomas associated with different degrees of brain invasiveness and edema. J Neurosurg. 2001;95(5):839–44. https://doi.org/10.3171/jns.2001.95.5.0839.
32. Yu Q, Stamenkovic I. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes Dev. 1999;13(1):35–48. https://doi.org/10.1101/gad.13.1.35.
33. Desai B, Rogers MJ, Chellaiah MA. Mechanisms of osteopontin and CD44 as metastatic principles in prostate cancer cells. Mol Cancer. 2007;6:18.
34. Joseph C, Alsaleem M, Orah N, Narasimha PL, Miligy IM, Kurozumi S, Ellis IO, Mongan NP, Green AR, Rakha EA. Elevated MMP9 expression in breast cancer is a predictor of shorter patient survival. Breast Cancer Res Treat. 2020;182(2):267–82. https://doi.org/10.1007/s10549-020-05670-x.
35. Gupta A, Cao W, Sadashivaiah K, Chen W, Schneider A, Chellaiah MA. Promising noninvasive cellular phenotype in prostate cancer cells knockdown of matrix metalloproteinase 9. Sci World J. 2013;2013:493689. https://doi.org/10.1155/2013/493689.
36. Desai B, Ma T, Zhu J, Chellaiah MA. Characterization of the expression of variant and standard CD44 in prostate cancer cells: identification of the possible molecular mechanism of CD44/MMP9 complex formation on the cell surface. J Cell Biochem. 2009;108(1):272–84. https://doi.org/10.1002/jcb.22248.
37. Dictor M, Ehinger M, Mertens F, Akervall J, Wennerberg J. Abnormal cell cycle regulation in malignancy. Am J Clin Pathol. 1999;112(1 Suppl 1):S40-52.
38. Liu N, Song SY, Jiang JB, Wang TJ, Yan CX. The prognostic role of Ki-67/MIB-1 in meningioma: a systematic review with meta-analysis. Medicine (Baltimore). 2020;99(9):e18644. https://doi.org/10.1097/MD.0000000000018644.
39. Panagopoulos AT, Lancellotti CL, Veiga JC, de Aguiar PH, Colquhoun A. Expression of cell adhesion proteins and proteins related to angiogenesis and fatty acid metabolism in benign, atypical, and anaplastic meningiomas. J Neurooncol. 2008;89(1):73–87. https://doi.org/10.1007/s11060-008-9588-3.

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