RESEARCH ARTICLE

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MUC4 and *Caspase-3* Immunoexpression in Meningioma: A Histopathological Study Linking Mucin & Apoptotic Pathways

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Abstract

Background: The most frequent intracranial primary tumors of the central nervous system and the second most frequent tumors of the brain are meningiomas. Although most of them are benign, a subset of them is biologically aggressive, exhibiting aggressive growth behavior and brain invasion. **Materials and Methods:** Fifty-nine cases of various grades & subtypes of meningiomas were included in this retrospective study. Samples were immunohistochemically analyzed for *MUC4* & *Caspase-3* antibodies and correlated with clinico-pathologic variables. **Results:** *MUC4* was expressed in 36 (61%) cases of meningioma. Statistically, *MUC4* expression, the percentage of positivity of tumor cells, and the intensity were significantly positively correlated with the WHO grade of meningioma cases (p-value = 0.03, 0.006, and 0.002, respectively) and the meningioma histologic subtype (p-value = 0.002, 0.002, and 0.000, respectively). *Caspase-3* was expressed in 48 (81.4%) cases of meningioma. *Caspase-3* expression was statistically significantly inversely correlated with the WHO grade of the analyzed tumors (p-value= 0.005) and the meningioma histologic subtype (p-value= 0.014). There is an inverse statistically significant correlation between the intensity of *MUC4* & *Caspase-3* expression (p-value= 0.002). **Conclusion:** Our results suggest that *MUC4* is associated with higher grades of meningiomas and may have a negative impact on prognosis and recurrence rates, potentially making it a target for an agent with mucolytic effects that can help overcome chemoresistance in aggressive meningiomas. On the other hand, the expression of *Caspase-3* correlates with the grade of differentiation and certain histotypes and may be considered as an ideal target for meningioma therapeutic regimens.

Keywords: Meningioma- *Caspase-3*- *MUC4*- Immunohistochemistry

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Introduction

The most frequent intracranial primary tumors of the central nervous system (CNS) and the second most frequent tumors of the brain are meningiomas [1]. They represent more than one-third of primary CNS tumors [2], with an incidence of 9.51 per 100,000 [3], and are increasing in incidence because of better access to neuroimaging and an older population. Although meningiomas are generally thought to be benign, a subset of them is biologically aggressive, exhibiting aggressive growth behavior and brain invasion. They also frequently recur even after numerous surgical operations and are associated with resistance to therapy, which can lead to serious neurologic morbidity and even mortality [4]. The World Health Organization (WHO) histopathologic grade and extent of resection have been the main factors associated with the risk stratification for recurrence [5]. Increasing evidence suggests that tumor aggressiveness and recurrence behavior may not always be effectively predicted by tumor classification and grading [6]. Meningotheliomatous, psammomatous, transitional, fibrous, angiomatous, atypical, and anaplastic are among the several histopathologic subtypes of meningiomas [7].

Mucins are classified into two subfamilies based on their physiological and structural characteristics: secretory mucins and transmembrane mucins, which include *MUC4* [8]. Mucin-4 (*MUC4*) is a high molecular weight transmembrane glycoprotein that is expressed in different epithelia and has protective functions. It is involved in cell growth signaling. Overexpression of *MUC4* has been linked to higher tumor progression and worse prognoses in several types of carcinomas [9]. In addition to enhancing survival pathways, chemotherapy resistance, metastasis, and accelerating replication, mucin provides tumor cells a barrier that prevents drug penetration [8]. Because *MUC4* is expressed in non-neoplastic meningothelial cells, some research has found that its expression has been connected to meningothelial cell differentiation rather than aberrant genetic or epigenetic changes linked to carcinogenesis.

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Consequently, it is a helpful diagnostic marker that can be utilized to distinguish between other main non-meningothelial malignancies that have been assessed and to diagnose meningioma [10, 11].

Apoptosis -self-ordered cell death- is a basic biological event of cells that is triggered by gene control and is essential for multicellular organisms to eliminate unneeded or aberrant cells. Malignant tumor development is associated with apoptosis deficiency. Damage to DNA results in DNA fragmentation, which sets off genes necessary for apoptosis. Caspases (cysteine-aspartic proteases), a type of endo-protease, play essential functions in the cell regulatory networks that control inflammation and cell death [2, 12]. The expression of apo- and anti-apoptotic proteins is dysregulated in cancer cells, which prevents apoptosis. The cancer cell becomes immortalized because of this genetic imbalance, reflecting the abnormal cell proliferation. Therefore, caspases and other apoptotic mitochondria-dependent or non-dependent molecules are regarded as crucial targets for targeted therapy approaches that enhance the apoptosis of tumor cells [13, 14].

In meningiomas, *Caspase-3* showed low expression levels that were associated with mitotic activity, differentiation grade, and, to a lesser extent, particular histotypes [1]. Decreased caspase-3 expression is adversely influencing the response rates to chemotherapy-mediated apoptotic cell death in meningioma cells, which increases the resistance to chemotherapeutic regimens [15]. This increasing requirement to boost apoptotic rates in meningiomas has led to certain research concentrating on particular drugs as fenretinide, which is a synthetic retinoid, inducing apoptosis in tumor cell cultures in various malignancies. Remarkably, the drug induced apoptosis in all three grades of meningioma primary cells [16]. Furthermore, a commonly used anti-epileptic drug -valproic acid (VPA)- tends to initiate apoptosis by raising the levels of cleaved caspase-3 in meningioma stem cells, which also increases their radio-sensitivity [17]. Therefore, therapeutic strategies that target caspase-3 may be beneficial for meningiomas to increase apoptotic death and response rates to particular chemo-radiation regimens.

The rationale of this work was to evaluate the immunohistochemical expression of *MUC4* and *Caspase-3* in meningioma and to statistically assess their correlation with the clinico-pathological data to investigate their possible prognostic value and the possibility of targeting therapy.

Materials and Methods

After approval by the Cairo University Research Ethics Committee (REC) (code: N-87-2025), fifty-nine cases diagnosed with meningioma were collected for this retrospective cross-sectional analytical study from the Anatomic Pathology Department at Kasr Al-Ainy Hospital, Cairo University, during the period from January to December 2020.

Exclusion Criteria

Patients with poorly fixed, inadequately depicted tumors, those who had minimally represented viable tumor or cautery artifacts, cases with lost files or unavailable paraffin blocks, and incomplete data were excluded from this study.

Case Parameters

All available clinicopathological data present in the patient's request sheet had been registered, as well as other clinicopathological data, including nature of the specimen, tumor size, histopathological type, brain invasion, presence of necrosis, and WHO grade.

Histopathological Evaluation

Each paraffin block was re-cut by microtome at 4 microns thickness, then mounted on glass slides, stained by hematoxylin and eosin for re-evaluation under a light microscope by two pathologists who confirmed the diagnosis of meningioma, and the histopathologic subtype. The WHO grade was assigned to each tumor according to the criteria of the WHO classification of tumors of the central nervous system 2021 [18].

Immunohistochemical Procedure

Paraffin blocks were serially sectioned at a thickness of 4 μm, mounted on positively charged slides, and immunostained with *MUC4* (abx 173628, Abbexa, United States of America) and *Caspase-3* (31A1067, Medaysis, United States of America) monoclonal antibodies. A fully automated immunohistochemical staining protocol was applied, Dako autostainer link 48 was used, and positive controls (stomach & tonsil tissue respectively) for each antibody were applied according to the manufacturer's protocol. The primary antibodies were suppressed as negative controls in the same tumor sections.

Immunohistochemical Interpretation

If 1% of the meningioma neoplastic cells examined showed *MUC4* cytoplasmic immunostaining, the case was considered positive for *MUC4*. Each section's tumor cells that tested positive for *MUC4* were recorded, given a score between 1 and 100%, and the mean percentage was reported. If the percentage of positive neoplastic cells was greater than 50%, diffuse immunostaining was identified. The strength of immunostaining was evaluated using a four-tiered grading system: (zero or negative: no staining), (one: weak intensity; barely detectable, noticeable only with difficulty using low-power objective), (two: moderate intensity; adequately positive, moderately seen using low-power objective) and, (three: strong intensity; marked staining, grasped with ease using low-power objective) [8].

For analysis of *Caspase-3* immunohistochemical staining, an overall score was calculated by multiplying the staining intensity by the percentage of positive tumor cells. Positive results of *Caspase-3* immunohistochemical staining were determined based on brown staining of either the nucleus or cytoplasm. The percentage of positive tumor cells was rated as follows: 0, none; 1, 1±25%; 2, 26±50%; 3, 51±75%; and 4, 76±100%. Immunohistochemical staining was evaluated as follows: 0, none; 1, weak; 2, moderate; and 3, intense. When 1% of the tumor cells clearly exhibited immunohistochemistry staining, the

specimens were considered positive [19].

Statistical Analysis

All results of the present study were analyzed in the SPSS statistics software program version 26. Simple descriptive statistics were used (arithmetic mean and standard deviation) to summarize quantitative data, and frequencies were used for qualitative data. The bivariate relationship was displayed in cross-tabulations, and a comparison of proportions was performed using the chi-square test. The t-independent test was used to compare normally distributed quantitative data. All p-values are two-sided, and those ≤0.05 were used to denote statistical significance. Microscopic photos were captured using an EP50 digital camera attached to an Olympus microscope model BX 53 FN 20.

Results

Fifty-nine cases of various subtypes of meningiomas were included in this retrospective study, divided into 42 cases of grade 1 meningioma (twenty meningothelial, seventeen transitional, one angiomatous, two fibroblastic, one metaplastic, and one secretory), and 17 cases of grade 2 meningioma (fourteen atypical and three chordoid variants). The mean age of the studied cases was 46.56 years (range, 24-72); most of them (79.7%) were females. The tumors were between 2 to 16 cm in maximal diameter (mean= 6.68 cm). Recurrence was recognized in only four patients (Table 1).

Among the 59 cases of meningioma, *MUC4* was expressed in 36 (61%) cases; including 22 of 42 grade 1 (16 of 20 meningothelial, 4 of 17 transitional, and the angiomatous, and secretory ones, while none of all fibroblastic and metaplastic meningioma cases expressed *MUC4*) and 14 cases of 17 grade 2 meningioma cases (11 of 14 atypical and all chordoid variants), (Figure 1). Strong intensity was observed in 19 out of the 59 cases (32%) (13 of 42 grade 1 and 6 of 17 grade 2 meningioma cases), while 11 cases (18.6%) showed moderate staining (8 of 42 grade 1 and 3 of 17 grade 2 meningioma cases),

Table 1. Clinical Data and Tumor Characteristics of the Studied Meningioma Cases

Clinico-Pathological Features	n (%)
Age (Mean ± SD)	46.56 ± 11.299
Sex	
Male	12 (20.3)
Female	47 (79.7)
Tumor size (Mean ± SD)	6.68 ± 3.365
Histopathologic type	
Angiomatous	1 (1.7)
Secretory	1 (1.7)
Transitional	17 (28.2)
Fibroblastic	2 (3.4)
Meningothelial	20 (33.9)
Metaplastic	1 (1.7)
Atypical	14 (23.7)
Chordoid	3 (5.1)
WHO grade	
Grade 1	42 (71.2)
Grade 2	17 (28.8)
Necrosis	
Present	7 (11.9)
Absent	52 (88.1)
Microscopic Calcification	
Present	35 (59.3)
Absent	24 (40.7)
Brain Invasion	
Present	10 (16.9)
Absent	49 (83.1)
Peri-lesional Edema	
Present	23 (39)
Absent	36 (61)
Recurrence	
Present	4 (6.8)
Absent	55 (93.2)

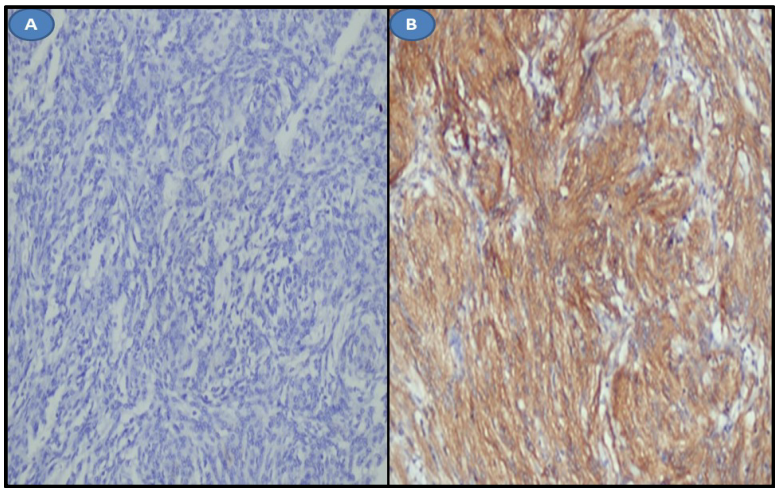

Figure 1. A) Negative immunohistochemical expression of MUC4 in a case of grade 1 meningioma (x100 original magnification). B) Positive cytoplasmic immunohistochemical expression of MUC4 in a case of grade 2 meningioma (x100 original magnification).

Table 2. Association of *MUC4* Immunostaining Intensity and Meningioma Patients' Clinicopathologic Variables and Histologic Subtypes

Clinico-Pathological Features	Negative	*MUC4* Expression				Total	P- value
		Positive					
		Weak	Moderate	Strong	P-value		
	n (%)	n (%)	n (%)	n (%)		n (%)	
Age							
<50	13 (38.2)	3 (8.8)	8 (23.5)	10 (29.4)	0.133	34 (57.6)	0.129
≥50	10 (40)	2 (8)	4 (16)	9 (36)		25 (42.4)	
Sex							
Male	6 (50)	1(8.3)	1 (8.3)	4 (33.4)	0.722	12 (20.3)	0.381
Female	17 (36.2)	4 (8.5)	10 (21.3)	16 (34)		47 (79.7)	
Tumor size							
<5cm	4 (28.6)	1 (7.1)	3 (21.4)	6 (42.9)	0. 955	14 (23.7)	0.606
≥5cm	19 (42.2)	4 (8.9)	8 (17.8)	14 (3.1)		45 (76.3)	
Histopathologic type							
Angiomatous	0 (0)	0 (0)	1(100)	0 (0)	0.000*	1 (1.7)	
Secretory	0 (0)	0 (0)	1 (100)	0 (0)		1 (1.7)	
Transitional	13 (76.5)	0 (0)	1 (5.9)	3 (17.6)		17 (28.2)	
Fibroblastic	2 (100)	0 (0)	0 (0)	0 (0)		2 (3.4)	0.002*
Meningothelial	4 (20)	0 (0)	5 (25)	11 (55)		20 (33.9)	
Metaplastic	1 (100)	0 (0)	0 (0)	0 (0)		1 (1.7)	
Atypical	3 (21.4)	2 (14.3)	3 (21.4)	6 (42.9)		14 (23.7)	
Chordoid	0 (0)	3 (100)	0 (0)	0 (0)		3 (5.1)	
WHO grade							
Grade 1	20 (47.6)	0 (0)	8 (19.1)	14 (33.3)	0.002*	42 (71.2)	0.033*
Grade 2	3 (17.6)	5 (29.4)	3 (17.6)	6 (35.4)		17 (28.8)	
Necrosis							
Present	2 (28.6)	1 (14.3)	1 (14.3)	3 (42.8)	0.848	7 (11.9)	0.547
Absent	21 (40.4)	4 (7.7)	10 (19.2)	17 (32.7)		52 (88.1)	
Microscopic Calcification							
Present	16 (45.7)	1 (2.9)	5 (14.3)	13 (37.1)	0.148	35 (59.3)	0.2
Absent	7 (29.2)	4 (16.6)	6 (25)	7 (29.2)		24 (40.7)	
Brain Invasion							
Present	3 (30)	2 (20)	2 (20)	3 (30)	0.531	10 (16.9)	0.523
Absent	20 (40.8)	3 (6.1)	9 (18.4)	17 (34.7)		49 (83.1)	
Peri-lesional Edema							
Present	11 (47.8)	2 (8.8)	5 (21.7)	5 (21.7)	0.458	23 (39)	0.266
Absent	12 (33.3)	3 (8.3)	6 (16.7)	15 (41.7)		36 (61)	
Recurrence							
Present	2 (50)	0 (0)	1 (25)	1 (25)	0.875	4 (6.8)	0.64
Absent	21 (38.2)	5 (9.1)	10 (18.2)	19 (34.5)		55 (93.2)	

and only 5 cases (8.5%) displayed weak staining (5 of 17 grade 2 meningioma cases, while none of all grade 1 meningioma cases showed weak *MUC4* staining (Table 2, Figure 2). On the contrary, no staining or staining of less than 1% of tumor cells was detected in 23 out of the 59 cases (39%). Diffuse staining (> 50% positive tumor cells) was seen in about 27% (16/59) of cases (13 of 42 grade 1 and 3 of 17 grade 2 meningioma cases), while 20 cases (33.9%) had 1-50% positive tumor cells (9 of 42 grade 1 and 11 of 17 grade 2 meningioma cases), (Table 3).

Statistically, the *MUC4* expression, the percentage of positivity of tumor cells and the intensity were significantly positively correlated with the WHO grade of meningioma cases (p-value= 0.03, 0.006 and 0.002 respectively) and the meningioma histologic subtype (p-value= 0.002, 0.002 and 0.000 respectively), whereas correlations between *MUC4* expression and other clinicopathological parameters were not evident.

Table 3. Association of *MUC4* Immunostaining Pattern (Percentage of Positive Tumor Cells) and Meningioma Patients' Clinicopathologic Variables and Histologic Subtypes

Clinico-Pathological Features	MUC4 Expression				Total	P- value
	Negative	Positive				
		< 50%	≥ 50	P-value		
	n (%)	n (%)	n (%)		n (%)	
Age						
<50	13 (38.2)	11 (32.4)	10 (29.4)	0.127	34 (57.6)	0.129
≥50	10 (40)	9 (36)	6 (24)		25 (42.4)	
Sex						
Male	6 (50)	4 (33.3)	2 (16.7)	0.584	12 (20.3)	0.381
Female	17 (36.2)	16 (34)	14 (29.8)		47 (79.7)	
Tumor size						
<5cm	4 (28.6)	5 (35.7)	5 (35.7)	0.715	14 (23.7)	0.606
≥5cm	19 (42.2)	15 (33.3)	11 (24.5)		45 (76.3)	
Histopathologic type						
Angiomatous	0 (0)	1 (100)	0 (0)	0.002*	1 (1.7)	
Secretory	0 (0)	1 (100)	0 (0)		1 (1.7)	
Transitional	13 (76.5)	1 (5.9)	3 (17.6)		17 (28.2)	
Fibroblastic	2 (100)	0 (0)	0 (0)		2 (3.4)	0.002*
Meningothelial	4 (20)	6 (30)	10 (50)		20 (33.9)	
Metaplastic	1 (100)	0 (0)	0 (0)		1 (1.7)	
Atypical	3 (21.4)	8 (57.1)	3 (21.4)		14 (23.7)	
Chordoid	0 (0)	3 (100)	0 (0)		3 (5.1)	
WHO grade						
Grade 1	20 (47.6)	9 (21.4)	13 (31)	0.006*	42 (71.2)	0.033*
Grade 2	3 (17.6)	11 (64.8)	3 (17.6)		17 (28.8)	
Necrosis						
Present	2 (28.6)	4 (57.1)	1 (14.3)	0.848	7 (11.9)	0.374
Absent	21 (40.4)	16 (30.8)	15 (28.8)		52 (88.1)	
Microscopic Calcification						
Present	16 (45.7)	9 (25.7)	10 (28.6)	0.251	35 (59.3)	0.2
Absent	7 (29.2)	11 (45.8)	6 (25)		24 (40.7)	
Brain Invasion						
Present	3 (30)	5 (50)	2 (20)	0.531	10 (16.9)	0.498
Absent	20 (40.8)	15 (30.6)	14 (28.6)		49 (83.1)	
Peri-lesional Edema						
Present	11 (47.8)	7 (30.4)	5 (21.8)	0.524	23 (39)	0.266
Absent	12 (33.3)	13 (36.1)	11 (30.6)		36 (61)	
Recurrence						
Present	2 (50)	1 (25)	1 (25)	0.887	4 (6.8)	0.64
Absent	21 (38.2)	19 (34.5)	15 (27.3)		55 (93.2)	

Caspase-3 was expressed in 48 (81.4%) cases, including 38 of 42 grade 1 (19 of 20 meningothelial, 14 of 17 transitional, and all angiomatous, secretory, fibroblastic and metaplastic meningioma cases) and 10 cases of 17 grade 2 meningioma cases (10 of 14 atypical, while none of all chordoid variant express *Caspase-3*), (Table 4, Figure 3). *Caspase-3* overall expression was found to be statistically significantly inversely correlated with the WHO grade of the analyzed tumors (p-value= 0.005) and the meningioma subtype (p-value= 0.014), whereas correlations between *Caspase-3* expression and other clinicopathological parameters were not evident.

About ninety-one percent of the studied cases that didn't express *MUC4* expressed *Caspase-3*. In addition, *Caspase-3* was expressed in about 90% and 80% of the studied cases that showed moderate and strong *MUC4* expression, respectively. Thus, there is an inverse statistically significant correlation between the intensity

Table 4. Association of *Caspase-3* Immunostaining and Meningioma Patients' Clinicopathologic Variables and Histologic Subtypes

Clinico-Pathological Features	Caspase-3 Expression			
	Negative n (%)	Positive n (%)	Total n (%)	P- value
Age				
<50	5 (14.7)	29 (85.3)	34 (57.6)	0.384
≥50	6 (24)	19 (76)	25 (42.4)	
Sex				
Male	1 (8.3)	11 (91.7)	12 (20.3)	0.304
Female	10	37	47 (79.7)	
Tumor size				
<5cm	3 (21.4)	11 (78.6)	14 (23.7)	0.796
≥5cm	8 (17.8)	37 (82.2)	45 (76.3)	
Histopathologic type				
Angiomatous	0 (0)	1 (100)	1 (1.7)	
Secretory	0 (0)	1 (100)	1 (1.7)	
Transitional	3 (17.6)	14 (82.4)	17 (28.2)	
Fibroblastic	0 (0)	2 (100)	2 (3.4)	0.014*
Meningothelial	1 (5)	19 (95)	20 (33.9)	
Metaplastic	0 (0)	1 (100)	1 (1.7)	
Atypical	4 (28.6)	10 (71.4)	14 (23.7)	
Chordoid	3 (100)	0 (0)	3 (5.1)	
WHO grade				
Grade 1	4 (9.5)	38 (90.5)	42 (71.2)	0.005*
Grade 2	7 (41.2)	10 (58.8)	17 (28.8)	
Necrosis				
Present	1 (14.3)	6 (85.7)	7 (11.9)	0.752
Absent	10 (19.2)	42 (80.8)	52 (88.1)	
Microscopic Calcification				
Present	4 (11.4)	31 (88.6)	35 (59.3)	0.086
Absent	7 (29.2)	17 (70.8)	24 (40.7)	
Brain Invasion				
Present	3 (30)	7 (70)	10 (16.9)	0.312
Absent	8 (16.3)	41 (83.7)	49 (83.1)	
Peri-lesional Edema				
Present	5 (21.7)	18 (78.3)	23 (39)	0.626
Absent	6 (16.7)	30 (83.3)	36 (61)	
Recurrence				
Present	1 (25)	3 (75)	4 (6.8)	0.735
Absent	10 (18.2)	45 (81.8)	55 (93.2)	

of *MUC4* and *Caspase-3* expression (p-value= 0.002) (Table 5).

Discussion

Several prognostic factors, such as male sex and younger age have been found to have an unfavorable impact on meningioma behavior. Higher WHO tumor grades and increased proliferation indices are additional variables linked to poor prognosis. Recurrent meningiomas have also been associated with optic nerve invasion and inadequate surgical excision. Even though the majority of meningiomas are thought to behave in a benign way, it is still crucial to investigate biomarkers and the pharmaceutical therapies that are associated with them as potential ways to improve meningioma prognosis [20-22]. The present study involved 59 meningioma cases, representing various histopathologic types and grades, and assessed the expression of *MUC4*, and *Caspase-3* using immunohistochemistry. Furthermore, the study examined the relationship between the expression of these biomarkers and available clinicopathologic variables. In

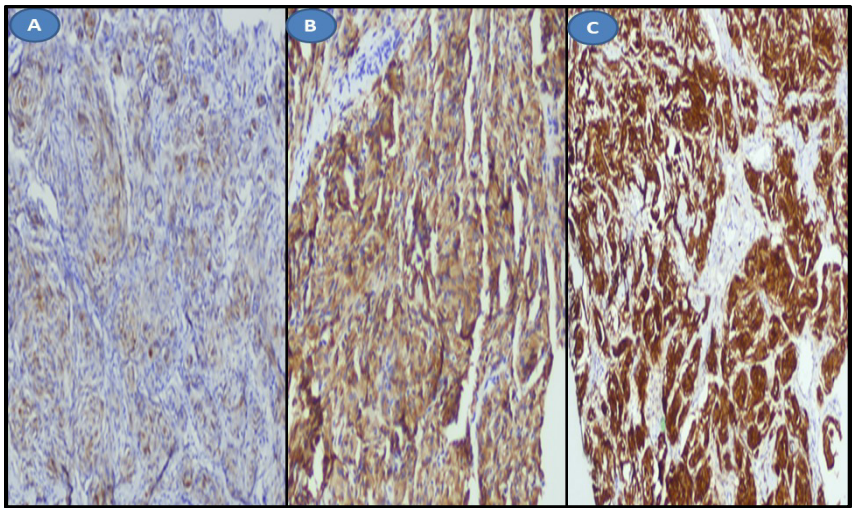

Figure 2. Cytoplasmic Immunohistochemical Expression of *MUC4* in Meningioma. (A) Weak, (B) Moderate & (C) Strong (x100 original magnification).

Table 5. Association of *MUC4* and *Caspase-3* Immunostaining in the Studied Cases

		MUC4 Expression					P- value
		Negative	Positive			Total	
		n (%)	Weak	Moderate	Strong	n (%)	
			n (%)	n (%)	n (%)		
Caspase-3 Expression	Negative n (%)	2 (18.2)	4 (36.3)	1 (9.1)	4 (36.3)	11 (12)	0.002*
	Positive n (%)	21 (43.8)	1 (2.1)	10 (20.8)	16 (33.3)	48 (88)	
	Total n (%)	23 (39)	5 (8.5)	11(18.6)	20 (33.9)	59 (100)	

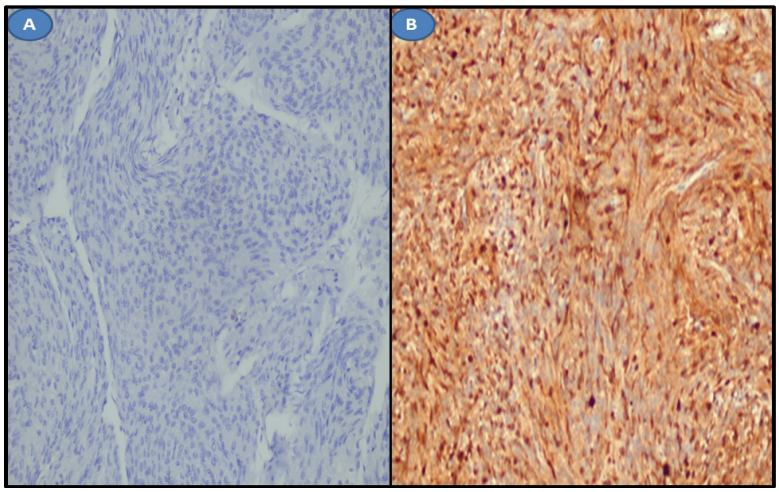

Figure 3. A) Negative immunohistochemical expression of Caspase-3 in a case of grade 2 meningioma (x100 original magnification). B) Positive cytoplasmic and nuclear immunohistochemical expression of Caspase-3 in a case of grade 1 meningioma (x100 original magnification).

this work, most of the meningioma cases (61%) expressed *MUC4*. This aligns with findings by Khalifa et al. [8]; Hasaneen et al. [10]; Matsuyama et al. [11] and Kong et al. [23] who reported that 84%, 83.3%, 92.9% and 100% of their reported meningioma cases showed *MUC4* immunohistochemical expression, respectively.

Kong et al. [23] stated that *MUC4* was expressed in all meningothelial and secretory meningioma cases (100%) . Similarly, Khalifa et al. [8] reported that the highest *MUC4* positivity (100%) was reported in meningothelial (12/12) and atypical meningioma (7/7), followed by angiomatous meningioma (75%, 3/4) while only (1/6, 16.7%) of fibroblastic meningiomas were *MUC4* positive. Nearly similar findings were reported by our study as 100 % of angiomatous (1/1), secretory (1/1) and chordoid (3/3) express *MUC4*, followed by about 80% of meningothelial (16/20) and atypical (11/14) variants expressed *MUC4*, while none of fibroblastic or metaplastic meningioma cases expressed *MUC4*. This contrasted with what was noted by Abu-Elenain et al. [9], who found lower *MUC4*

positivity in meningioma cases of meningothelial and angiomatous histologic subtypes [9]. This might be explained by the fact that there were only five cases of meningothelial meningioma in the Abu-Elenain et al. [9] study compared to the previously mentioned and our investigations.

The pattern of *MUC4* immunostaining in our study was diffuse in 27% and focal in about 34% of our studied meningioma cases. These figures were lower than that of Khalifa et al. [8] who reported that the pattern of *MUC4* immunostaining was diffuse in 44% and focal in 40% of studied meningioma cases. In the present work, among different histopathologic variants, meningothelial meningioma had the highest *MUC4* immunohistochemical mean percentage of positive tumor cells (62.5%) followed by transitional meningioma (18.8%). On the contrary, 40% of the atypical meningioma cases showed focal *MUC4* staining, while the angiomatous, and secretory subtypes showed the lowest *MUC4* immunohistochemical mean percentage of positive tumor cells (5% each). Similarly, other studies as Khalifa et al. [8] highlighted that meningothelial meningioma had the highest diffuse *MUC4* staining (69%) followed by angiomatous, transitional, and atypical meningioma; 27.4%, 27%, and 26.4% respectively. While fibroblastic meningioma showed 1% only *MUC4* positive tumor cell . In addition, Matsuyama et al. [11] reported diffuse and constant *MUC4* immunostaining in meningothelial and angiomatous meningiomas, while it was restricted to less than 5% of tumor cells in fibroblastic meningioma subtype. Also, they found that meningothelial and angiomatous tumor subtypes expressing *MUC4* most diffusely and 71% of fibrous meningioma samples were also positive for *MUC4*, although the expression was only focal or in a small number of cells.

Moreover, we found that 33.9%, 18.6% and 8.5% of the studied meningioma cases showed strong, moderate, and weak *MUC4* cytoplasmic immunostaining respectively. This is somewhat different from the figures reported by Khalifa et al. [8] who found that 16%, 40% and 28% of the enrolled meningioma cases showed strong, moderate, and weak *MUC4* cytoplasmic immunostaining respectively. In the same context, Hasaneen et al. [10] reported similar figures as among their *MUC4* positive meningioma cases, 36% showed a score 3+, 40% showed a score of 2+, and 24% cases showed a score of 1+. Consequently, a statistically significant relation was detected in the present study between the *MUC4* expression, the percentage of positivity of tumor cells and intensity and the meningioma histologic subtype (p-value= 0.002, 0.002 and 0.000 respectively). The same findings were observed by Khalifa et al. [8] who found a statistically significant correlation between *MUC4* immunostaining intensity and different meningioma histopathologic variants (p-value=0.007). On the other hand, no statistically significant relationship was noted between the *MUC4* expression and meningioma subtypes in both Abu-Elenain et al. [10] and Matsuyama et al. [11] studies .

Our study observed that *MUC4* immunoexpression was higher in WHO grade 2 meningioma cases compared to grade 1 meningioma cases, where 52.4% of WHO grade 1 were *MUC4* positive and 82.4% WHO grade 2 expressed *MUC4*. This obtained a statistically significant correlation between the *MUC4* expression and the WHO grade of meningioma cases (p-value= 0.03). This coincides with what was observed by Khalifa et al. [8] who reported that 80% of WHO grade 1 were *MUC4* positive and All WHO grade 2 and 3 expressed *MUC4* (p-value= 0.174) and concluded that *MUC4* is widely expressed and associated with higher grades of meningiomas and can adversely affect the prognosis and recurrence rate. In contrast, Abu-Elenain et al. [10] and Matsuyama et al. [11] studies stated higher *MUC4* immuno-expression in WHO grade 1 than grade 2 and grade 3 cases (p-value= 0.126 and 0.913 respectively).

The fact that the mechanism of *MUC4* expression in meningiomas is unknown may help to explain this debate. Because *MUC4* is expressed in non-neoplastic meningothelial cells with epithelioid features, some research has found that its expression has been connected to meningothelial cell differentiation rather than aberrant genetic or epigenetic changes linked to carcinogenesis. While *MUC4* was detected in all atypical and anaplastic histologic subtypes, the positive cells were limited compared to meningothelial and angiomatous subtypes, representing a possible remaining meningothelial cell nature [11].

Roukas et al. (2024) reported that all studied meningioma cases expressed *Caspase-3* [1]. Our investigation revealed nearly similar results, showing that *Caspase-3* was expressed in most of the meningioma patients we examined (81.4%). In the present study *Caspase-3* was expressed in all angiomatous, secretory, metaplastic, and fibroblastic meningioma cases (100%). In addition, 95%, 82.4% and 71.4% of meningothelial, transitional and atypical subtypes expressed *Caspase-3*, while none of the chordoid meningiomas expressed *Caspase-3*. On the other hand, Roukas et al. [15] reported that highest *Caspase-3* positivity (22%) was reported in meningothelial, followed by transitional meningioma (12%), atypical and fibroblastic (10% each), while only 4% of angiomatous meningiomas were *Caspase-3* positive [1].

Statistically, the *Caspase-3* expression was correlated significantly with the meningioma subtype (p-value= 0.014). The same findings were observed by Roukas et al. [15] who found a statistically significant correlation between *Caspase-3* immunostaining and different meningioma histopathologic variants (p-value=0.016) [1].

Regarding expression of *Caspase-3* in meningioma, our study noted that *Caspase-3* immunoexpression was higher in WHO grade 1 meningioma cases than grade 2 meningioma cases, where 90.5% of WHO grade 1 were *Caspase-3* positive and 58.8% WHO grade 2 expressed *Caspase-3*. Consequently, *Caspase-3* overall expression was found to be statistically significantly associated with the WHO grade of the analyzed tumors (p-value= 0.005). This coincides with what was noted by Roukas et al. [15] who reported that 72% of WHO grade 1 were *Caspase-3* positive, while 16% and 12% of WHO grade 2 and 3 respectively expressed *Caspase-3* (p-value= 0.002) [1].

About ninty-one percent of studied cases that didn't

express *MUC4* expressed *Caspase-3*. In addition, *Caspase-3* was expressed in about 90% and 80% of the studied cases that showed moderate and strong *MUC4* expression, respectively. Thus, there is an inverse statistically significant correlation between the intensity of *MUC4* and *Caspase-3* expression (p-value= 0.002). To our knowledge, no prior research has demonstrated this association.

Based on the above results, we concluded that *MUC4* is expressed and related to higher grades of meningiomas. It can also have a negative impact on the prognosis and recurrence rate. By targeting *MUC4*, an agent with mucolytic and proteolytic effects can help in overcoming the frequent issue of chemoresistance in aggressive meningiomas.

In contrast, the expression of *Caspase-3* correlates with the grade of differentiation and certain histotypes in the cases under investigation. It is a key player in the apoptotic process, making it a prime target for both single and combined meningioma therapeutic regimens. Patients with specific protein and gene profiles undergoing chemo-radiation regimens may benefit from increased caspase-mediated apoptotic death and improved treatment response rates if its activity is enhanced by novel therapies [24]. The lack of meningioma WHO grade 3 cases was a limitation in our study.

Author Contribution Statement

All authors contributed significantly to the study: AME participated in reading the slides, interpreting results, analyzing data, and revising the manuscript. AMA contributed to the study design, data collection, result interpretation, and data analysis, and participated in writing the manuscript. SMA contributed to the research concept, data collection, and result interpretation and participated in writing and revising the manuscript.

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Data Availability

Data are available upon request, following institutional regulations, and with official permission.

Ethical Declaration

This study protocol was approved by the Cairo University Research Ethics Committee (REC) of the Faculty of Medicine, Cairo University, and conducted following ICH (International Council for Harmonisation) GCP (Good Clinical Practice) standards, as well as relevant local and institutional regulations and guiding principles governing REC operations (code: N-87-2025,).

Conflict of Interest

The authors declare that they have no conflict of interest.

List of Abbreviations

CNS: Central nervous system.
MUC4: Mucin-4.
REC: Research Ethics Committee
VPA: Valproic acid
WHO: World Health Organization

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