RESEARCH ARTICLE

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Downregulation of lncRNA-MALAT1, Altered Immunohistochemical Expression of Cyclin D1 Protein and E-Cadherin Protein in Correlation to Meningioma Grades

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Abstract

Objectives: Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is known to be upregulated in the tumors with ability to metastasize. In contrast, long non-coding Ribonuclei acid (lncRNA) MALAT1 was reported by some studies to be downregulated in meningioma cells. E-cadherin and Cyclin D1 are prognostic indicators and possible attractive targets for the treatment of recurring and aggressive meningioma. This study aimed to evaluate the expression level of lncRNA MALAT1, as well as Cyclin D1 and E-cadherin immunohistochemical expression in meningiomas (Grade 1 and 2) and their association with the clinicopathological parameters of the studied cases. **Materials and Methods:** Quantitative determination of relative expression levels of lncRNA-MALAT1 in 64 cases of meningioma to 5 controls of normal dura mater was performed, in addition to evaluation of E-cadherin and Cyclin D1 immunohistochemical expression. The results were tested for association with the clinicopathological parameters. **Results:** lncRNA-MALAT1 expression were downregulated in 49/64 of the cases of meningioma (76%) in comparison to control. There were significant association of expression of lncRNA-MALAT1 with grade, brain invasion and increased mitosis (p=0.007, 0.04, 0.006 respectively). There were also significant associations of strong E-cadherin and negative Cyclin D1 proteins expression with grade 1 (p = 0.02, 0.004), low mitosis (p=0.03, and 0.04) and brain invasion (p=0.04, 0.03) respectively. Additionally, a significant weak negative correlation between E-cadherin and Cyclin D1 expression was fond, yet no significant correlation between lncRNA MALAT1 expression and either of E-cadherin or Cyclin D1 expression could be achieved. **Conclusion:** lncRNA MALAT1 is downregulated in meningiomas and associated with increased aggressiveness. Overexpressed cyclin D1 and decreased E-cadherin expression are also associated with high grade meningioma

Keywords: lncRNA MALAT1- Cyclin D1- E-cadherin- Meningioma- Grade

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Introduction

Meningiomas are the most frequent among primary central nervous system (CNS) tumors, accounting for 37.6%. Meningioma incidence increases with age, with a strong increase after 65 years [1]. Meningiomas of the CNS WHO grade 1 are non- slowly growing and can be cured by total surgical resection. Unfortunately, about 20% of meningiomas have a more aggressive clinical course, with local recurrences, brain invasion and advancement to higher grade, providing a therapeutic obstacle [2].

Although meningioma subtyping and grading are mainly based on histologic features, the 2021 WHO classification suggests using molecular biomarkers to help in meningioma grading. The recently introduced molecular features into the 2021 WHO classification help to enhance risk assessment and unveil new therapeutic options [3]. Molecular studies performed on even slowly growing benign meningiomas could declare tumorigenesis and help to detect targets for prevention of progression to malignancy [4].

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an important member of the long non-coding Ribonuclei acid (lncRNA) family, was first detected in non-small cell lung cancer and was described to be upregulated in more aggressive metastasizing tumors [5]. MALAT-1 affects tumorigenesis through different mechanisms, including the MALAT-1/miR-

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183/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) and Wnt/β-catenin signalling pathways [6].

The tumorigenic role of MALAT-1 centers on cell proliferation and limitation of apoptosis. These actions occur through modification of different microRNAs by epigenetic and genetic regulation of genes such as Von Hippel–Lindau (VHL), Cyclin D1 (CCND1), and E-cadherin [7]. More recent work added that MALAT-1 impacts a number of immune cells involved in the defense against cancer [8]. Xu et al. [9] concluded that MALAT1 can promote immune evasion of tumor cells by interacting with significant transcription factors and signalling pathways, hence affecting chemoresistance, immunotherapy responses.

Despite the established pro-oncogenic function of MALAT-1 in various cancers, few more recent studies reported MALAT-1 to have a tumor suppressor-like function. Such findings need to be thoroughly studied and established as they contradict a substantial amount of evidence confirming MALAT-1's pro-oncogenic role [5].

The role of MALAT-1 in brain tumors is questionable. While some studied showed MALAT-1 to be downregulated in gliomas, others reported it as an oncogene promoting proliferation and inhibiting apoptosis in glioma cells by depressing Rap1b.112 Fibroblast Growth Factor 2 (FGF2) through MALAT-1–stimulated angiogenesis under hypoxic conditions [6].

Regarding meningiomas, not much is understood about the function of MALAT1 apart from its role in regulation of MicroRNA 145 which affects invasiveness of aggressive meningioma [10]. lncRNA MALAT1 was shown by some to be downregulated in meningioma cells and its overexpression was associated with reduced meningioma aggressiveness [11]. Cyclin D1, a significant oncogene that has been demonstrated to be overexpressed in a number of cancers; such overexpression also correlates with the degree of malignancy. In meningioma, Cyclin D1 was reported to predict increased recurrence rate, thus, representing an important prognostic marker and a possible target for the treatment of meningiomas [12].

E-cadherin is an important regulator of cell-to-cell adhesion and thus it can mediate tumor cell motility, invasion and additionally tumor cell proliferation. It has been discovered that a lower expression of E-cadherin is linked to a higher risk of invasion and metastatic potential. in various tumors including meningioma [13, 14].

To guide meningioma targeted therapy in the future, the majority of meningioma molecular biomarkers still need to be studied in clinical trials [15]. Our goal was to find out how much the lncRNA MALAT1, Cyclin D1, and E-cadherin proteins were expressed in Grade 1 and 2 meningiomas, and whether or not these expressions were related to the clinicopathological characteristics of the studied cases

Materials and Methods

This is a retrospective observational cross-sectional study. A total of 64 cases of CNS Grade 1 and 2 meningioma and 5 controls of normal dura were obtained from the archives of the pathology department at kasr Al-ainy hospital as stored, formalin fixed, paraffin embedded blocks.

Exclusion criteria included history of chemo- or radiotherapy or any other treatment before the operation. Cases with extensive necrosis, crushing or cautery artifact were also excluded. G*POWER software 3.1.9.7 was used to calculate the sample size. After reviewing the literature for relevant studies investigating similar markers, over expression of MALAT1 in cancerous tissue compared to normal counterpart was 4.7:1 [16], over expression of Cyclin D1 was reported in 60% of GII Meningiomas [12], and E-cadherin staining intensity decreased in atypical meningiomas [13]. Confidence of 95% and power of 80%, an effect size of 0.8; the minimum required sample size was 64 cases [17].

Before starting, our work was approved by the Ethics committee of Faculty of Medicine, Cairo University, Egypt (approval no. N-414-2023, Date: 11-11-2023). For patients' privacy, their names were replaced by numbers. Only such numbers were used on the slides, in the data sheet and during statistical analysis.

Data Collection

The collected clinical data for each case including age, sex, site, radiological size and presence of peri-tumoral edema, as well as history of recurrence were obtained from the pathology request forms.

Histopathological examination

Hematoxylin-eosin (H&E) stained sections were prepared for histological examination, including confirmation of the diagnosis, WHO grading, detection of WHO histopathological variants, the presence of necrosis, mitotic count and brain invasion for each case. The cases were categorized in each parameter into present or absent. Mitosis was categorized into low or high based on a cutoff of 4 Figures / 10 high power fields (HPFs); the figure adopted by the WHO for grading of meningiomas into grade 1 and 2 [3].

Real-Time Polymerase Chain Reaction (RT-PCR)

The collected blocks were sectioned at 10 μm thickness. Each sample included 3 sections and was preserved at -80◦C for RNA extraction then reverse transcribed into complementary DNA (cDNA) then quantitative determination of lncRNA MALAT1 relative expression levels using (qRT-PCR).

RNA Extraction

The guidelines provided by the maker were followed utilising the miRNeasy mini kit for extracting total RNA from serum (Qiagen, Valencia, CA, USA). The NanoDrop®-1000 with the use of a spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), the purity of the RNA samples was assessed.

cDNA reverse transcription

Reverse transcription (RT) of extracted RNA was performed using RT2 first strand kit from Qiagen in Germany (the miScript II RT kit (Qiagen, Valencia, CA,

USA) to produce cDNA following the manufacturer's guidelines.

Quantitative real-time PCR for lncRNA MALAT-1 detection was performed using Quantitect SYBR green PCR master mix and MALAT-1 primer assay [Human MALAT-1: (LPH18065A, Thermofisher)] in a 25 µl per well reaction volume. Additionally, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Qiagen, Germany, Cat. No. 330701, GeneGlobe ID- QT00079247) was used as an internal control for lncRNA-MALAT1.

The method of ΔCt was employed to assess the expression levels. Using equation $2^{-\Delta\Delta Ct}$, the fold change in MALAT-1 expression levels was determined [18].

Immunohistochemistry

Two sections on positively charged slides were obtained from each block and immunostained using a Ventana Benchmark Ultra machine automated staining system. The used primary antibodies were anti-Cyclin D1 monoclonal rabbit antibody (Dako-USA-clone EP12 Catalog #IR083- USA) and anti-E-cadherin monoclonal rabbit antibody (Ventana clone EP 700Y, Tucson, AZ, USA). Positive controls prepared from normal breast tissue for E- cadherin and from mantle cell lymphoma for cyclin D1 were used. Negative controls were also performed by using PBS instead of primary antibody.

Evaluation of immunohistochemical results

E-cadherin expression was detected as brownish membranous/ cytoplasmic staining in tumor cells. The staining was scored by three independent pathologists. Four staining categories were identified (negative, +, ++, +++) based on the percentage of stained tumor cells and the staining intensity (negative, weak, moderate, and strong).These categories included "negative" (no detectable staining), "+" (weak staining in ≤70% of tumor cells or moderate staining in ≤30% of tumor cells), "++" (weak staining in > 70% of tumor cells, or moderate staining in 31–70% of tumor cells, or strong staining in ≤30% of tumor cells), and "+++" (moderate staining in > 70% of tumor cells or strong staining in > 30% of tumor cells) [19].

Cyclin D1 expression was detected as brownish staining in the tumor cells nuclei. Four staining categories were identified (negative, +, ++, +++). These categories included negative (no detectable staining), + (staining from 1-25% of the tumor cells), ++ (staining from 26-50% of the tumor cells), +++ (staining from 51- 75% of the tumor cells) and ++++ (staining from 76-100% of the tumor cells) [14].

Statistical analysis

IBM (statistical package for the social sciences (SPSS) software package version 20 was used to analyse the data. (Armonk, NY: IBM Corp). The statistical analysis included:

- Descriptive statistics of clinico-pathological parameters of the studied cases (Frequency and percentages for categorical variables, Mean ± Standard Deviation for numerical continuous parametrical variables).

- The expression pattern of lncRNA MALAT 1 by RT-PCR (Fold change) (Mean ± SD), Cyclin D1 & E-cadherin proteins by immunohistochemistry (expression score) (frequency, %).

- Investigating the association of lncRNA MALAT 1 expression, Cyclin D1 & E-cadherin with clinicopathological parameters (Chi square, using Mann Whitney U and Kruskal–Wallis test)

- Correlating the expression of lncRNA MALAT 1 by RT-PCR with Cyclin D1 & E-cadherin proteins by immunohistochemistry using Spearman correlation tests.

- The diagnostic accuracy of MALAT1 in differentiating grade 1 and 2 meningiomas was evaluated by analyzing the receiver-operating-characteristic (ROC) and calculating the area under the curve (AUC). An AUC of >0.5 was considered significant discriminator. Detection of the cutoff point with the highest sensitivity and specificity was calculated from the coordinate points of the ROC curve.

- The statistical significance was determined at <0.05 for all values.

Results

The clinicopathological characteristic of the studied cases

Regarding the clinicopathological features of studied 64 cases of meningioma. The mean age at diagnosis was 64.3±12.04. 50 cases (78.1%) were females. The mean radiologic tumor size was 6.6±3.2 cm. 46 cases (71.9%) were WHO grade 1 and 18 cases (28.1%) were WHO grade 2. Brain invasion, hemorrhage, necrosis, radiologic peritumoral edema and calcification were detected in (17.2%, 15.6%, 10.9%, 39.1% and 60.9%) respectively. The mitotic count was considered high in 18.7%. The most prevalent histopathological variants in the study were meningothelial followed by transitional (57.8% and 28.1%) respectively (Figure 1 A-G). Only 4 cases (6.2%) were recurrent cases Table 1.

The most prevalent E-cadherin expression scores in the studied cases were negative and weak (50%, and 25%) respectively, however moderate and strong expressions were detected in 7.8%, and 17.2% respectively (Figure 2: A-F). Similarly, the most prevalent Cyclin D1 expression scores in the studied cases were negative and weak expressions (40.6% and 23.4% respectively) however moderate, and strong expressions were present in 20.3% and 15.7% respectively. In our study no cases with score ++++ Cyclin D1 were detected (Figure 3: A-D). The mean lncRNA MALAT1 expression detected in our cases was 66±1.30, Table 1.

lncRNA MALAT1 expression and its association with clinicopathological parameters of cases

lncRNA MALAT1 expression was downregulated in 49/64 of meningioma cases (76%) in comparison to 5 controls (dura matter) using independent T test for comparing means in parametric data (p=0.04). The mean of lncRNA MALAT1 in meningioma was 0.66±1.3-fold change relative to the normal dura (control) as illustrated in Table 2

As regard the association of lncRNA MALAT1 expression with the clinicopathological features of the

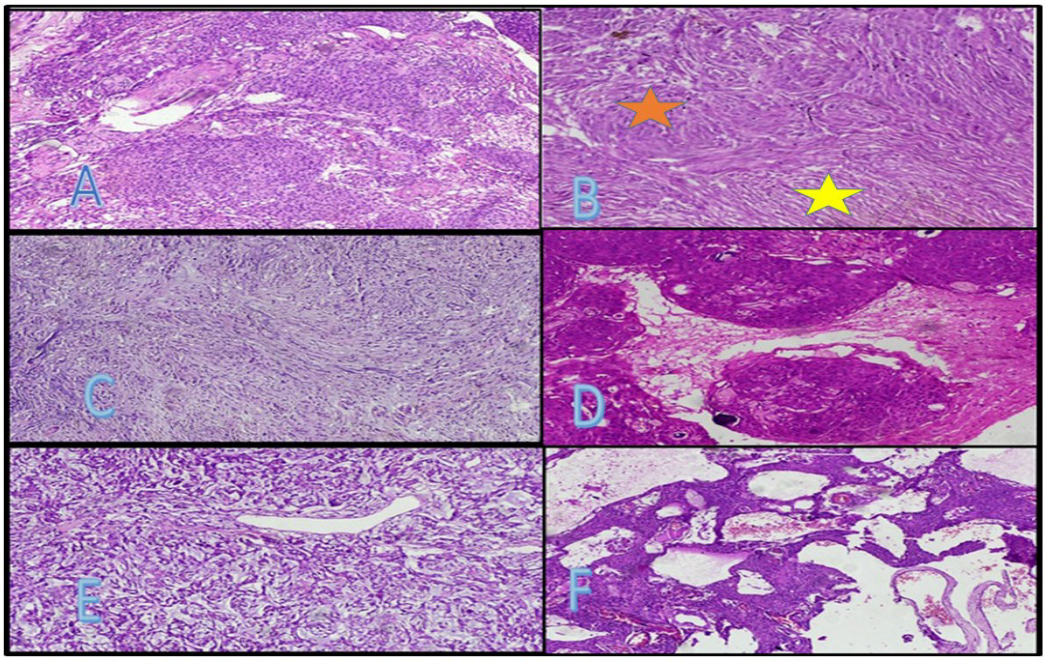

Figure 1. H&E Stained Sections of Meningioma Cases; Meningothelial (A x200), Transitional (B x200) showing meningothelial component (red star) and fibroblastic component (yellow star), fibroblastic (C x200), Meningothelial with brain invasion (D x200), Chordoid (Ex 200), and Angiomatous (F x200).

studied cases using Mann Whitney U Mann Whitney U and Kruskal–Wallis tests as illustrated in Table 3, there were significant association of expression of lncRNA MALAT1 with grade, brain invasion and mitosis (p=0.007, 0.04, 0.006). Downregulation was more evident with grade 2 cases, the presence of brain invasion and high mitotic count. However, lncRNA MALAT1 expression didn't show any significant association with other clinicopathological parameters.

ROC curve of lncRNA MALAT1 in relative expression levels for meningioma diagnosis

The ROC curve of lncRNA MALAT1 expression levels was plotted between sensitivity (y-axis) and 1–specificity (x-axis) (Figure 4).

Regarding, ROC curve analysis of lncRNA MALAT1 relative expression levels; lncRNA MALAT1 with an area under the curve (AUC) of 0.857 (95% confidence interval (CI) = 0.751-0.963, p= 0.009). The sensitivity and

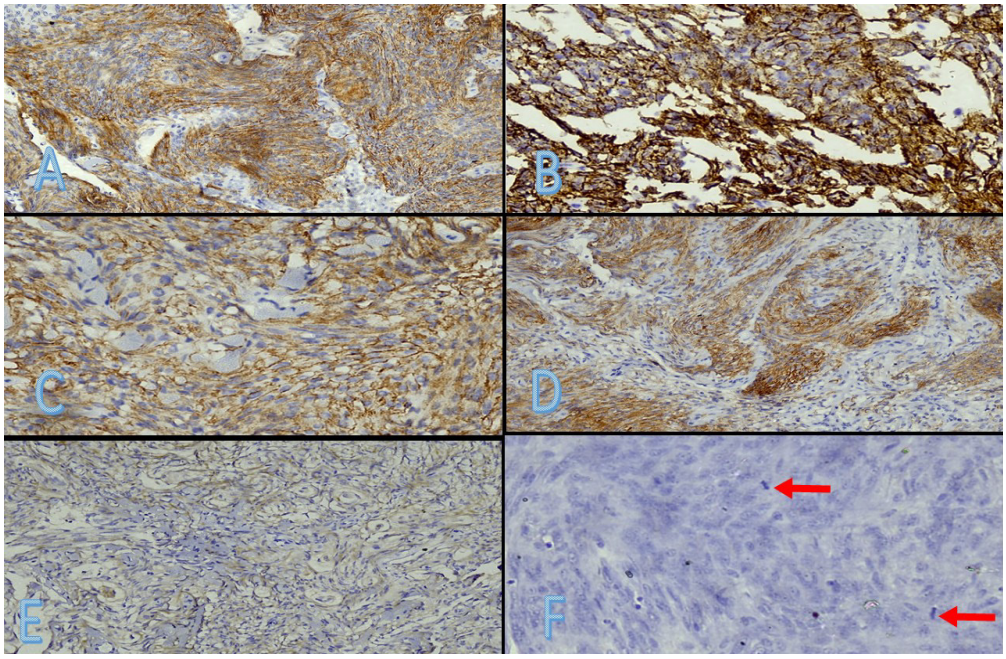

Figure 2. Immunohistochemical Staining Pattern of E-cadherin in Meningioma. Diffuse strong staining pattern in meningothelial GI more intense in whorls (A* 200), Strong membranous staining (B, C*400), moderate staining pattern (D *200). Weak focal membranous staining pattern (Ex 200), and negative staining (Fx200) red arrows indicate mitosis.

Table 1. Clinicopathological Characteristics of the Studied Cases

Number (%) 64(100)		
Age (y)	Mean±SD	64.3±12.04
Sex	F	50 (78.1)
	M	14 (21.9)
WHO Grade	G1	46 (71.9)
	G2	18 (28.1)
Radiologic tumor size	Mean±SD	6.6±3.2
Brain invasion	Absent	53 (82.8)
	Present	11 (17.2)
Hemorrhage	Absent	54 (84.4)
	Present	10 (15.6)
Necrosis	Absent	57 (89.6)
	Present	7 (10.9)
Recurrence	Absent	60 (95.8)
	Present	4 (6.2)
Histopathological type	Meningothelial	37 (57.8)
	Transitional	18 (28.1)
	Others	9 (14.1)
Mitosis	Low	52 (81.3)
	High	12 (18.7)
Radiologic Peri-tumoral oedema	Absent	39 (60.9)
	Present	25 (39.1)
Calcification	Absent	25 (39.1)
	Present	39 (60.9)
E-cadherin	Negative	32 (50)
	+ (weak)	16 (25)
	++ (moderate)	5 (7.8)
	+++ (Strong)	11 (17.2)
Cyclin D1	Negative	26 (40.6)
	+ (weak)	15 (23.4)
	++ (moderate)	13 (20.3)
	+++ (Strong)	10 (15.7)
lncRNA MALAT1	Mean±SD	0.66±1.3

Table 2. lncRNA MALAT1 Expression (M±SD in the Studied Cases of Meningioma versus Control

lncRNA MALAT1	Mean ± Std. Deviation	95% Confidence Interval		Sig. (2-tailed)
		upper	lower	
control	2.6±2.9	3.3	1.4	0
case	0.17±0.2	4.8	0.002	0.04

Independent sample T test , two sided P.value<0.05

Table 3. Difference in the mean± standard Deviation of Expression of lncRNA MALAT1 in Different Groups of theClinicopathological Parameters of the Studied Cases

			Mean±SD
Sex	F	50	0.37±0.42
	M	14	0.47±0.51
WHO Grade	GI	46	0.46±0.49
	GII	18	0.21±.21
Brain invasion	No	53	0.42±0.47
	Yes	11	0. 24±0.23
Necrosis	No	60	0.38±0.45
	Yes	4	0.42±0.39
Histopathological type	Mengiothelial	37	0.41±0.47
	Transitional	18	0.40±0.42
	Others	9	0.33±0.42
Mitosis	No	52	0.43±0.46
	Yes	12	0.19±0.2
Peritumoral oedema	No	39	0.31±0.33
	Yes	25	0.51±0.56
Calcification	No	25	0.27±0.29
	Yes	39	0.46±0.5

Mann Whitney U tests, Kruskal-Wallis test two sided P.value<0.05

specificity of lncRNA MALAT1 were calculated using a cutoff of 0.8765 for lncRNA MALAT1 relative expression in cases of meningioma and revealed 85% sensitivity and 100% specificity at this point (Table 4).

Association of E-cadherin protein expression with the clinicopathological parameters of the studied cases

E-cadherin protein expression in the studied cases was negative, weak, moderate and strong in 50%, 25%, 7.8% and 17.2% respectively Figure 2 (A-F).

The association of E-cadherin protein expression with the clinicopathological characteristics of the studied cases using Chi-square test is illustrated in Table 5. We reported a significant association of strong E-cadherin protein expression with grade 1; all grade 2 cases showed negative to weak expressions (p = 0.02), low mitosis (p = 0.03) and negative brain invasion (p = 0.03). All the 4 recorded recurrent cases showed negative or weak expression of E-cadherin (p=0.001). There were no significant associations of E-cadherin with the rest of studied parameters (Table 5).

Association of Cyclin D1 protein expression with the clinicopathological parameters of studied cases

Cyclin D1 expression in the studied cases was negative, +1, +2 and +3 in 40.6%, 23.4%, 20.3% and 15.7% respectively, there was no +4 in the studied cases Figure 3 (A-D).

The association of Cyclin D1 protein expression with the clinicopathological characteristics of the studied cases using Chi-square test is illustrated in Table 6. There were significant association of increased Cyclin D1

Table 4. ROC Curve Analysis of LNC-RNA-MALAT1

Area Under the Curve	P value	95% Confidence Interval				
		Lower Bound	Upper Bound	Cut off	Sensitivity %	Specificity %
0.857	0.009	0.751	0.963	0.8765	85%	100%

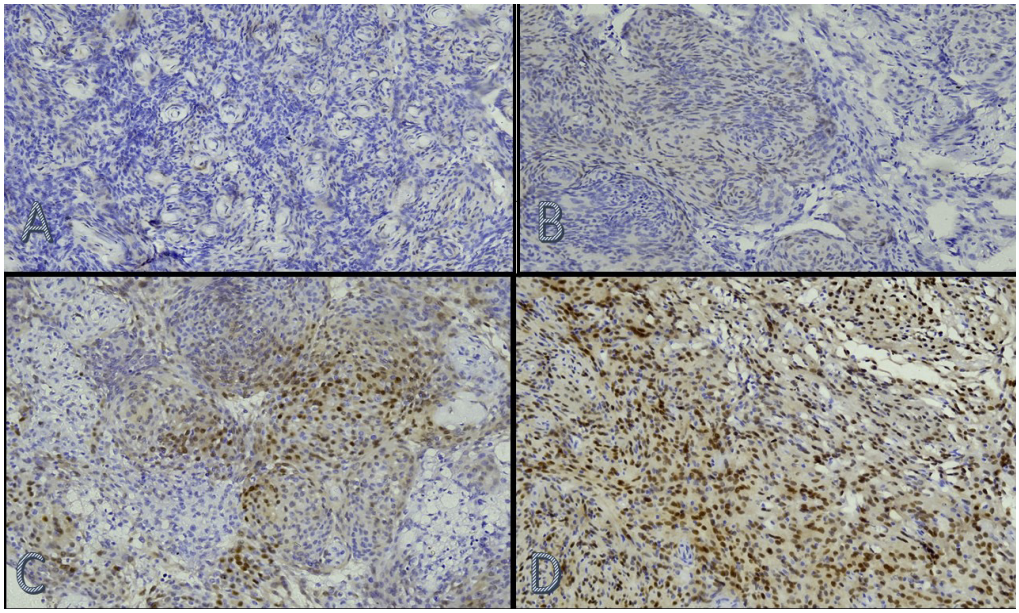

Figure 3. Immunohistochemical Staining Patterns of Cyclin D1 in Meningioma Cases. Negative staining (A x200). Focal weak positive nuclear staining (B x200). Moderate nuclear staining (C x200) and Strong diffuse nuclear staining (D x200)

expression with high grade, larger size, the presence of brain invasion, and high mitosis (p = 0.004, 0.005, 0.03, 0.04) respectively. There were no significant associations of Cyclin D1 protein expression with the rest of the studied parameters

Correlation between lncRNA MALAT1, E-cadherin, and Cyclin D1 protein expressions in the studied cases

By testing the link between the immunohistochemical expression patterns of E-cadherin, and Cyclin D1 and gene expression levels of lncRNA MALT1 using the Spearman Bivariate correlation test (Non-parametric Bivariate

Table 5. Association of E-cadherin Protein Expression Scores and Clinicopathological Parameters of Meningioma

			Expression of E-cadherin				
			Negative	+ (weak)	++ (Moderate)	+++ (strong)	P
Age (y)	Mean±SD		46.3±10.2	46±11.5	51±16.7	40.7±15	0.4
Sex	F	50	25	13	5	7	0.5
	M	14	7	3	0	4	
WHO Grade	G1	46	19	11	5	11	0.02*
	G2	18	13	5	0	0	
T size	Mean±SD		6.9±3.5	6.2±3.4	6.5±2.6	5.8±2.6	0.8
Brain invasion	Absent	53	24	13	5	11	0.04*
	Present	11	8	3	0	0	
Necrosis	Absent	57	28	13	5	11	0.08
	Present	7	4	3	0	0	
Histopathological type	Meningothelial	37	19	9	5	4	0.4
	Transitional	18	9	5	0	4	
	Others	9	4	2	0	3	
Mitosis	Low	52	26	10	5	11	0.03*
	High	12	6	6	0	0	
Peritumoral oedema	Absent	39	20	11	2	6	0.4
	Present	25	12	5	3	5	
Calcification	Absent	25	12	8	2	3	0.8
	Present	39	20	8	3	8	
Recurrence	Absent	60	29	15	5	11	0.001*
	Present	4	3	1	0	0	

Chi square, Kruskal-Wallis ANOVA, two sided P.value<0.05

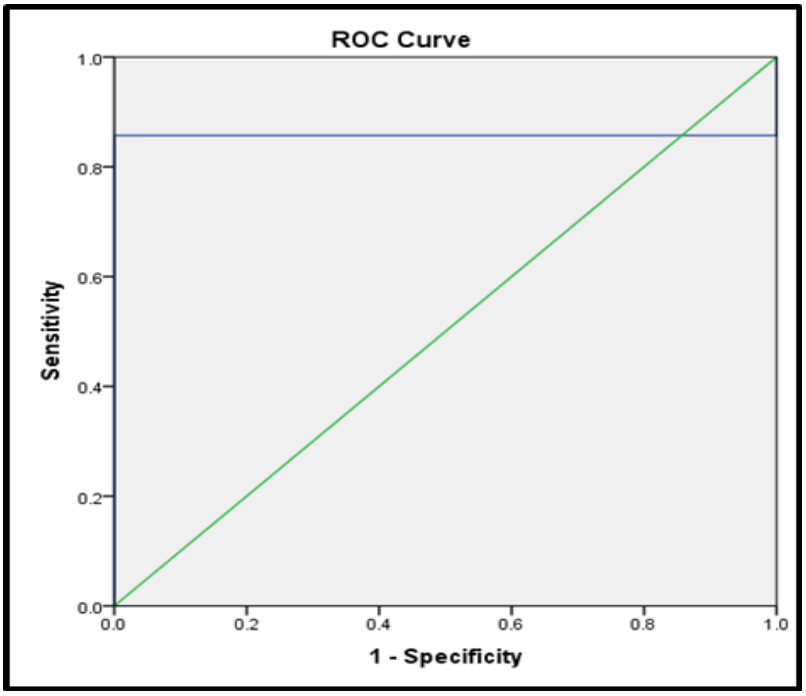

Figure 4. The ROC Curve of lncRNA MALAT1 Relative Expression Levels in Meningioma Cases

correlation), a significant weak negative correlation between E-cadherin and Cyclin D1 was observed (Correlation Coefficient: -0.322 Sig:0.009). However, there was no significant, either positive or negative, correlation between lncRNA MALAT1 expression and each of E- cadherin or Cyclin D1 expression (Table 7).

Discussion

The prognosis of meningioma depends on multiple variables including tumor site, grade, and patient's age. The overall prognosis of meningioma is favorable especially for low grade cases [20]. Recurrence in meningiomas is a

Table 6. Association of Cyclin D1 Protein Expressions Status and Clinicopathological Parameters

			Expression of Cyclin D1				
			Negative	+ (weak)	++ (Moderate)	+++ (strong)	P
Age (y)	Mean±SD		47.2±11	45.2±12.5	45.1±8	47±15.8	0.9
Sex	F	50	21	11	12	6	0.4
	M	14	5	3	2	4	
WHO Grade	G1	46	23	12	8	3	0.004*
	G2	18	3	3	5	7	
T size	Mean±SD		5.7±2	5.9±2.8	7±3.7	9.5±4.2	0.005*
Brain invasion	Absent	53	25	13	9	6	0.03*
	Present	11	1	2	4	4	
Necrosis	Absent	57	24	13	13	7	0.1
	Present	7	2	2	0	3	
Histopathological type	Meningothelial	37	14	6	10	7	0.6
	Transitional	18	8	7	1	2	
	others	9	4	2	2	1	
Mitosis	Low	52	25	12	9	6	0.04*
	High	12	1	3	4	4	
Peritumoral odema	Absent	39	16	13	2	8	0.1
	Present	25	7	8	6	4	
Calcification	Absent	25	11	6	4	4	0.5
	Present	39	12	15	4	8	
Recurrence	Absent	60	26	12	13	9	0.05
	Present	4	0	3	0	1	

Chi square, Kruskal-Wallis ANOVA, two sided P.value<0.05

Table 7. Correlation between LNC-RNA-MALAT& E Cadherin and Cyclin D1 Expressions in Meningiomas

Correlations test					
			lncRNAmalat1	Ecadherin	cyclinD1
Spearman's rho	lncRNAmalat1	Correlation Coefficient	1	0.107	0.036
		Sig. (2-tailed)	.	0.406	0.779
		N	64	64	64
	Ecadherin	Correlation Coefficient	0.107	1	-.322**
		Sig. (2-tailed)	0.406	.	0.009
		N	64	64	64
	cyclinD1	Correlation Coefficient	0.036	-.322**	1
		Sig. (2-tailed)	0.779	0.009	.
		N	64	64	64

challenging issue due to limitations of complete surgical resection aiming to preserve the surrounding normal brain tissue in addition to the unsatisfactory response to adjuvant radiotherapy, chemotherapy and hormonal therapy [21].

Many studies explored the field of lncRNA role in initiation and propagation of meningiomas aiming to use the expression patterns of lncRNA as prognostic markers in meningioma by studying their relation to histopathological grades and other known parameters affecting prognosis [11].

Metastasis-associated lung adenocarcinoma transcript (MALAT1) is a lncRNA that has been shown to be involved in various cancers, where it plays a role in regulating gene expression, tumor cell proliferation, invasion, and metastasis. However, the specific role of MALAT1 in meningiomas is not as well characterized as in some other tumors [5, 10]. As research in this area continues to evolve, new findings may provide a deeper understanding of how MALAT1 functions in meningiomas and its potential implications for treatment [10].

MALAT-1 has been also reported to regulate tumor associated immunity; a study performed by Adewunmi et al., 2023 on triple negative breast cancer showed that lncRNA Malat1 downregulation reduces the tumour microenvironment's immunosuppressive characteristics, boosting CD8+ T-cell infiltration while lowering immunosuppressive tumor-associated macrophages (TAM) and myeloid-derived suppressor cells (MDSC). Additionally, they demonstrated that in a preclinical model, combining immune checkpoint inhibition or chemotherapy with MALAT1 targeted therapy may enhance therapeutic results [22]

Our work aimed to study the differential expression of lncRNA MALAT1 in meningiomas in comparison to normal dura and also in different grades of meningiomas (grade 1 and 2). Understanding those expression levels could help to clarify its potential role in meningioma biology. E-cadherin and Cyclin D1 are potential prognostic markers in cancer generally and in meningioma particularly [14, 23]. Their expression levels in meningioma may help risk stratification. Studying E-cadherin and Cyclin D1 expression and correlating such expression with lncRNA MALAT1 expression levels can help to detect its tumorigenic and prognostic role in meningioma.

Our studied cases were predominantly of grade 1 (71.9%), affecting female patients with a mean age at presentation of 64.3±12.04. Despite not being an epidemiological study, this work captures the majority of low grade meningiomas (70–90%) that have been described in the literature, as well as old age and female predominance [24].

In our study, lncRNA MALAT1 expression was studied in 64 cases of meningioma compared to 5 control (dura matter) using independent T test for comparing means in parametric data (p=0.04). The mean of lncRNA MALAT1 in meningioma was 0.66±1.3-fold change relative to the normal dura as illustrated in Table 2. The sensitivity and specificity of lncRNA MALAT1 were calculated using a cut-off value of 0.8765 for lncRNA MALAT1 relative expression in cases of meningioma and revealed 85% sensitivity and 100% specificity at this point (Figure 4 & Table 4), concluding a downregulation of lncRNA MALAT1 in cases of meningioma. This finding is consistent with Eraky 2023 [11].

In concordance with our results, MALAT1 levels were found to be downregulated in endometrial carcinoma [6]. The anti-tumorigenic role of lncRNA MALAT1 is explained by inhibiting microRNA-145, which is an oncogenic microRNA [6, 11]. As far as we are aware, no comparable studies evaluated MALAT1 expression in relation to different grades of meningioma in the literature. Interestingly, our study revealed that downregulation of lncRNA MALAT1 in meningioma is significantly associated with grade 2 cases, brain invasion and high mitosis (p=0.007, 0.04, 0.006) respectively (Table 3). These finding agreed with other reports showing that lncRNA MALAT1 overexpression can lead to reduced tumor invasiveness & its downregulation can increase tumor cell migration and help progression of other various tumors [6-7, 10- 11, 25].

In contrast, Duan et al., 2023 suggested that MALAT1 in glioblastomas acts as a competing endogenous RNA (ceRNA) for miR-199a which spromotes tumor growth and spread. Moreover, MALAT1 can promote angiogenesis and epithelial-to-mesenchymal transition (EMT) in colorectal cancer by sponging miR-126-5p. It can also activate P65 and β-catenin facilitating head and neck squamous cell carcinoma (HNSCC) progression [26].

Arun et al., 2020 also suggested that MALAT1 is upregulated in different cancers such as lung, breast,

prostate and colon compared to corresponding normal tissues and confirmed the association of MALAT1 overexpression with shorter metastasis-free survival and worse prognosis [5]. E-cadherin is a cell-to-cell adhesion molecule with crucial role in epithelial cell behaviour, tissue integrity, as well as suppression of tumor development and progression [27]. Suppressed E-cadherin expression or function plays an important role in tumor progression, as E-cadherin downregulation in epithelial tumors decreases cellular adhesion leading to increased motility, tumor invasion and metastasis [28].

E-cadherin protein expression was lost in 13/18 of our GII cases with none of them showing intermediate or strong expression (Table 5 and Figure 2 (a-f)). Such finding reflects significant association between E-cadherin positivity and meningioma grades. Such finding agreed with others [23, 27] and can be explained by the loss of contact inhibition leading to enhanced tumor cell proliferation and invasiveness [29].

In contrast, others reported overexpression of E-cadherin in high-grade tumors as high-grade oral squamous cell carcinoma and clear cell renal cell carcinoma [30]. They suggested that E-cadherin overexpression in such aggressive tumors reflects abnormal differentiation of tumor cells and does not function any biological role by itself [30].

In our study, there was a significant correlation between E-cadherin expression and high mitosis (p=0.03) Table 5 and Figure 2-f). Our twelve cases with high mitosis (>4 / 10 HPFs) showed 50% negative and 50% weak expression of E-cadherin. This agreed with several studies concluding that E-cadherin decreased expression is significantly higher in cases with increased proliferation and lymph node metastasis [14, 31- 32].

Our study also detected a significant correlation between loss of E- cadherin expression and brain invasive meningioma cases (P=0.04) (Table 5). Decreased expression of one of the important cell surface regulators (E-cadherin) leads to disruption of cellular and increased tumor cell motility [27]. This agreed with Kim et al., 2023 who reported decreased E-cadherin expression to be associated with brain invasive meningioma having high recurrence rate [33].

The current study does not identify any significant difference in E-cadherin expression among the histopathological variants of meningioma (p=0.4) (Table 5). This was compatible with the results of Shimada et al. [34]. Although, those results are against the fact that tissue morphogenesis depends upon cell adhesion molecules that help forming the characteristic microscopic features of meningiomas, such as whorls in meningothelial variant, our finding can be explained by the uneven distribution of the histopathological variants in our study including only one case for each of secretory, angiomatous and metaplastic variants, 2 cases of fibrous variant and the majority of cases being meningothelial or transitional. One interesting finding is that we and Shimada et al., 2005 noticed more intense E-cadherin staining in the whorles.

Despite lack of survival data, 3 of our 4 known recurrent cases showed negative E-cadherin expression and the remaining one showed weak positivity reflecting a statistically significant possible association of E-cadherin loss with recurrence in meningiomas (p=0.001) (Table 5). Similar finding was reported by Wallesch et al. [35]

Currently, there is abundant data that disorders of cyclins, cyclin-dependent kinases (CDK) or their inhibitory proteins play an important role in different cancers. The most frequent of such disorders are related to cyclin D1, which can promote gene transcription and tumor growth, inhibit apoptosis and mediate chemoresistance [36]

In our study, cyclin D1 expression showed significant correlation with high histologic grade, larger tumor size and high mitosis (p=0.004, 0.005 and 0.04) respectively (Table 6 and Figure 3 (a-d)); findings that all suggest cyclin D1 expression as a poor prognostic factor. Our results agreed with Cheng et al. [12]. Absence of 4+ cyclin D1 score in our study could be explained by absence of grade 3 (anaplastic meningioma).

Our study also revealed significant association of cyclin D1 expression and brain invasion (p=0.03) (Table 6), a result also agreed upon by Cheng et al., 2015. This can be explained by the interaction of cyclin D1 with matrix metalloproteinase-9 (MMP-9), increasing its expression. Increased MMP-9 expression is implicated in the degradation of the extracellular matrix and increased invasiveness of the neoplastic cells [12].

In a trial to identify the nature of the relation between the patterns of E-cadherin and Cyclin D1 expression and the gene expression levels of lncRNA MALT1 using the Spearman Bivariate correlation test (Non parametric Bivariate correlation), we detected a significant weak negative correlation between E-cadherin and Cyclin D1 (Correlation Coefficient: -0.322 Sig:0.009) (Table 7). Such negative correlation is explained by the role of each protein in tumor initiation, invasion and propagation. High grade meningiomas are highly proliferative (strong expression of Cyclin D1 and invasive (lost E-cadherin expression) [12, 24]. However, we couldn't achieve positive or negative significant correlation between lncRNA MALAT1 expression and each of E-cadherin and Cyclin D1 positivity. This may be possibly explained by the relatively small number of studied cases in our work, thus encouraging further studies on wider scale of cases to identify the exact definite role of lncRNA MALAT1 in meningioma pathogenesis. To our knowledge, no comparable studies in the literature have studied such associations.

Important limitation for our study is being a small retrospective cohort study. Such type of study is more susceptible to bias. Information on exposure and confounding variables may be unavailable, inadequate, or difficult to obtain as we depend upon the recorded data in the pathology reports and request forms with no available survival data. This made a difficulty in survival analysis especially multivariate regression models.

In conclusion, despite the small number of our cases and lack of survival data, we can conclude that lncRNA MALAT1 downregulation occurs in meningiomas and is associated with more aggressive tumor characteristics. Although, overexpressed cyclin D1 and decreased

E-cadherin expression are associated with aggressive meningioma features, our study couldn't document any significant association between such proteins and lncRNA MALAT1. Nevertheless, our results suggest lncRNA MALAT1 as a prognostic marker and possible target for meningioma therapy.

Author Contribution Statement

Conceptualization: NAS, EE, MK, RT. Data curation: PES. Formal analysis: NAS. Funding acquisition: all author. Investigation: NAS. Methodology: NAS, EM, MK. Project administration: PES. Resources: EE, MK. Software: NAS. Supervision: NAS, PES. Validation: NAS, PES. Visualization: EE, MK, NAS. Writing – original draft: EE, MK, RT. Writing – review & editing: NAS, PES. Approval of final manuscript: all authors.

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Approval

The research was approved by the Ethics committee of Faculty of Medicine, Cairo University, Egypt (approval no. N-414-2023, Date: 11-11-2023)

Conflict of Interest

The authors declare no conflict of interest.

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