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MicroRNA Analysis in Meningiomas with Different Degrees of Tissue Stiffness: A Potential Tool for Effective Preoperative Planning

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BACKGROUND AND OBJECTIVES: Meningioma, the most common primary intracranial tumor, presents challenges in surgical treatment because of varying tissue stiffness. This study explores the molecular background of meningioma stiffness, a critical factor in surgical planning and prognosis, focusing on the utility of microRNAs (miRNAs) as diagnostic biomarkers of tissue stiffness.

METHODS: Patients with meningiomas treated surgically at the University Hospital Brno were included in this study. Total RNA, isolated from tumor tissue samples, underwent quality control and small RNA sequencing to analyze miRNA expression. Differentially expressed miRNAs were identified, and their association with tumor stiffness was assessed.

RESULTS: This study identified specific miRNAs differentially expressed in meningiomas with different stiffness levels. Key miRNAs, such as miR-31-5p and miR-34b-5p, showed significant upregulation in stiffer meningiomas. These findings were validated using reverse transcription-quantitative polymerase chain reaction, revealing a potential link between miRNA expression and tumor consistency. The expression of miR-31-5p was most notably associated with the stiffness of the tumor tissue (sensitivity = 71% and specificity = 83%).

CONCLUSION: This research highlights the potential of miRNAs as biomarkers for determining meningioma tissue stiffness. Identifying specific miRNAs associated with tumor consistency could improve preoperative planning and patient prognosis. These findings pave the way for further exploration of miRNAs in the clinical assessment of meningiomas.

KEY WORDS: Meningioma, Tissue stiffness, MicroRNA

With an annual incidence of approximately 8 to 10 cases per 100 000 people, meningioma is the most common primary intracranial tumor.[1,2] Surgical treatment is the main treatment, with the aim of total resection of the tumor. The prognosis of patients with meningioma is determined by the anatomic location, the radicality of the resection, and the histological grade of the tumor according to the World Health Organization.[3,4] The radicality of the surgical procedure is mainly determined by the localization of the meningioma and its relationship to the surrounding structures.[5] An equally important factor influencing the course and the outcome of surgery is the

ABBREVIATIONS: ECM, extracellular matrix; **EMT,** epithelial-mesenchymal transition; **logFC,** log2 fold-change; **miRNAs,** microRNAs; **QC,** quality control; **T2WI,** T2-weighted imaging.

stiffness/consistency of the tumor tissue. Very rigid meningiomas often adhere to the skull base, growing around a crucial cerebral artery. Complete removal becomes challenging because of the exceptionally high risk of injuring or closing the artery. Consequently, the stiffness of the meningioma significantly affects the surgical procedure's course, the size of the residual tumor, postoperative care, and the patient's prognosis.[6-9] Despite its substantial influence on patient management, meningioma stiffness has not been thoroughly studied to date. Genetic predisposition, among other factors, plays an essential role in the etiological development of meningiomas; this study aims to identify biomarkers in tumor tissue capable of recognizing solid and thus surgically challenging cases. These biomarkers could potentially be further identified as circulating molecules in the patient's peripheral blood or cerebrospinal fluid, significantly streamlining surgical planning.

Molecules that are certainly involved in the biological behavior of meningiomas and that would undoubtedly be useful biomarkers are microRNAs (miRNAs). These short noncoding RNAs play a key role in the regulation of gene expression at the post-transcriptional level. In the world of biology and medicine, miRNAs are increasingly recognized for their potential in the diagnosis and treatment of various diseases, including cancer. In meningiomas, some miRNAs have been described to be differentially expressed in tumors compared with healthy tissue and others regulate the meningioma cell cycle, proliferation, and apoptosis.[10-14] Moreover, miR-224 was associated with a better prognosis.[15]

For the possibility of less invasive diagnosis from patient blood or cerebrospinal fluid, of interest is the work by Carneiro et al[16] who found increased plasma levels of miR-181d in patients with meningiomas, whereas Li et al[17] identified decreased levels of miR-18a in blood serum and cerebrospinal fluid in invasive meningiomas. Global profiling of miRNA levels in the cerebrospinal fluid of patients with meningioma was also performed by Kopkova et al.[18] This study revealed that miR-140-5p and miR-196b-5p showed significantly higher levels in patients with cancer compared with the control group treated for normotensive hydrocephalus. Finally, a multiphase study by Zhi et al[19] identified a panel of 6 miRNAs occurring in serum that successfully stratified patients with meningioma from a healthy control group.

METHODS

Study Design and Patient Samples

The patients with consecutive meningioma were surgically treated in 2021 and 2022 at one Department of Neurosurgery. After resection, part of the tumor tissue was treated with formalin for histopathological evaluation, and part of the tissue was immediately placed on ice and sent to the laboratory for further processing and cryopreservation at −80°C. The study was approved by the local ethics committee. A signed informed consent was obtained from each patient before the surgery. The study methodologies conformed to the standards set by the Declaration of Helsinki. Detailed clinicopathological characteristics of the patient cohorts included in the exploratory and validation phases of the study are presented in Table 1.

Assessment of the Consistency/Stiffness of Tumor Tissues

For assessing the objective stiffness of meningiomas, we used the CUSA Excel ultrasonic aspirator (CUSA) from Integra. This device, standardly used in meningioma surgery, performs simultaneous irrigation, selective fragmentation, and aspiration of tumor tissue. It has effective power control in frequencies from 23 kHz to 36 kHz and has a very simple setup and control, where the individual frequencies are expressed as values from 0% to 100% to correspond to the minimum and maximum power of the device, ie, selective fragmentation. This scale then allows us to assess at what percentage of the instrument power the selective fragmentation of the meningioma occurs, facilitating a straightforward evaluation of the meningioma's consistency or stiffness. Because meningiomas are often composed of several parts with different stiffness, we always report the highest CUSA power used during meningioma resection. That is the CUSA power value with which total extirpation of the tumor can be performed. The analyzed samples always come from tissue aspirated with this highest power. The threshold dividing meningiomas to less and more stiff is 60% of the aspirator power.

Small RNA Sequencing

Total RNA enriched for small RNA species was isolated using the mirVana miRNA Isolation Kit (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. Twenty-three total RNA samples with RNA integrity number ≥7.0 were used for constructing libraries using the QIAseq miRNA Library Kit (Qiagen). Libraries were pooled in equimolar ratio based on their molarity, calculated using an online weight-to-moles conversion calculator for nucleic acids. The library pool underwent processing in accordance with the NextSeq System Denature and Dilute Libraries Guide.[20] Single-read sequencing with a 75 bp read length was carried out using the NextSeq 500 Sequencing System and NextSeq 500/550 High Output v2 kit (75 cycles) (all Illumina).

The prealignment quality control (QC) of the sequencing data was conducted using FastQC (version 0.11.9).[21] Reads underwent quality trimming with cutadapt,[22] and reads shorter than 15 bp were excluded from the data set. The remaining reads were then mapped to the miRbase database (version 22)[23] using the miraligner tool (version 3.2).[24] Comprehensive reports, including numerical and graphical QC output, were compiled through MultiQC (version 1.7).[25] All statistical analyses were performed in the R environment (version R4.3.1).

Differential expression analysis was carried out using the DESeq2 package (version 1.41.8) from Bioconductor (version 3.18).[26] Unsupervised clustering was conducted using the complete linkage method (farthest neighbor clustering) with the Euclidean distance metric. MiRNA molecules with expression levels exceeding 1 read per million in at least 5 samples were included in the analysis and compared across the different meningioma groups. The results were visualized using heatmaps with clustergrams. All miRNAs having Benjamini-Hochberg corrected *P*-value smaller than 0.05 were considered as significantly differently expressed. The data sets presented in this study can be found in online repositories or shared by the authors on reasonable request.

Validation of Sequencing Results

Total RNA extracted from 38 samples was reverse transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit. The expression levels were analyzed by reverse transcription-quantitative polymerase chain reaction using TaqMan Universal Master Mix II and TaqMan MicroRNA Assays (all Thermo Fisher Scientific).

TABLE 1. Clinicopathological Characteristics of the Patient Cohorts Included in the Exploratory and Validation Phases of the Study

	Exploratory phase, n = 23		Validation phase, n = 38	
Parameter	**N**	**%**	**N**	**%**
Sex				
Male	5	21.7	9	23.7
Female	18	78.3	29	76.3
Age at diagnosis (y) median (25th-75th percentile)	61.2 (46.6-69.0)		61.7 (48.8-70.0)	
World Health Organization grade				
I	18	78.3	30	78.9
II	5	21.7	7	18.4
III	0	0	1	2.6
Histological subtype				
Meningothelial	9	39.1	18	47.4
Transitional	5	21.7	5	13.2
Fibrous	4	17.4	6	15.8
Metaplastic	0	0	1	2.6
Atypic	5	21.7	7	18.4
Anaplastic	0	0	1	2.6
Simpson				
I	8	34.8	11	28.9
II	3	13.0	4	10.5
III	9	39.1	16	42.1
IV	3	13.0	7	18.4
Tumor stiffness				
≦60%	13	56.5	24	63.2
>60%	10	43.5	14	36.8

Expression levels were normalized to the average expression of hsa-let-7d-5p, hsa-miR-29a-3p, and hsa-miR-93-5p. Relative expression levels were then compared between less and more stiff tumor samples using the Mann-Whitney test and analyzed by receiver operating characteristic (ROC) in R environment.

RESULTS

Next-Generation Sequencing Analysis of MicroRNAs in Meningioma Tissue Samples With Varying Stiffness Reveals Differentially Expressed Molecules

In the exploratory phase, we conducted small RNA sequencing to identify miRNA molecules with different expression profiles between 2 groups categorized by tumor tissue stiffness. The group of less stiff meningiomas included 13 samples that could be aspirated with the CUSA Excel ultrasonic aspirator at 60% power and below. The group with the stiffer tumors included 10 samples that had to be aspirated at more than 60% of the aspirator power. Of the 798 miRNAs that were taken into the analysis, 73 miRNAs were significantly differentially expressed among 2 groups (P-value<.05), with 18 miRNAs having P-values smaller than .01 (Table 2, Figure 1A).

The top 22 significantly differentially expressed miRNAs (P-value <.05, log2 fold-change [logFC] ≥ 1 or logFC ≤ −1, and baseMean >20) were able to classify stiffer meningiomas with 70% sensitivity and 85% specificity (Figure 1B). The most significantly upregulated miRNAs (adjusted P-value <.05) in more stiff tumors were miR-124-3p, miR-675-3p, and miR-675-5p,

with the last 2 arising from the same precursor miRNA. Among the most downregulated miRNAs in stiffer meningiomas were miR-130a-3p and miR-130a-5p, also arising from the same precursor miRNA.

Validation of the Expression of Selected MicroRNAs—miR-31-5p Identifies Stiff Meningiomas With High Specificity

Based on the small RNA sequencing analysis, 9 miRNAs (miR-31-5p, miR-34b-3p, miR-34b-5p, miR-34c-5, miR-124-3p, miR-144-3p, miR-483-5p, miR-675-3p, and miR-1299) were selected for the validation phase according to the following criteria—$P < .05$; baseMean >50 reads; and logFC < −1.5 or logFC >1.5 (Figure 2).

In the validation phase, the statistical analysis of single-molecule reverse transcription-quantitative polymerase chain reaction data has shown that miR-31-5p ($P = .014$; FC = 4.2) and miR-34b-5p ($P = .018$; FC = 2.1) are significantly upregulated in stiff meningiomas (Figure 3A). Although miR-34c-5p ($P = .058$; FC = 2.2) and miR-483-5p ($P = .077$; FC = 3.7) also appear to be more expressed in stiff meningiomas, this observation is not statistically conclusive. MiR-144-3p, miR-124-3p, miR-34b-3p, miR-675, and miR-1299 showed no difference in expression between the 2 compared groups.

When specifically analyzing grade I meningiomas with varying degrees of stiffness, miR-31-5p ($P = .031$; FC = 5.3), miR-34b-5p ($P = .049$; FC = 2.4), and miR-483-5p ($P = .049$; FC = 3.2) exhibited significantly higher expression in stiff meningiomas compared with their less stiff counterparts (Figure 3B). No statistically significant trend in expression levels was observed for the other miRNAs when comparing the studied groups.

ROC analyses, aimed at identifying miRNAs with optimal distinguishing ability between less (≥60%) and more (>60%) stiff meningiomas, have revealed that miR-31-5p alone shows the best ability to distinguish stiff tissue samples with area under curve = 0.741 (95% Cl [0.546-0.936] estimated by the DeLong method with 2000 stratified bootstrap replicates) (Figure 4A). The best cutoff for splitting was selected as value which maximizes product of sensitivity and specificity (sensitivity = 71.43% with 95% CI [50-92.86] and specificity = 83.33% with 95% CI [66.67-95.83]; accuracy of such cutoff is 78.95%). Notably, in grade I meningiomas alone, miR-31-5p again demonstrates superior performance (area under curve = 0.745 with 95% CI [0.528-0.962]; sensitivity = 70% with 95% CI [40-90]; specificity = 80% with 95%CI [60-95]; accuracy = 75%) (Figure 4B). The specificity and sensitivity of other individual miRNAs and various miRNA combinations have been comparatively lower.

To identify the clinicopathological parameters on which meningioma stiffness depends, the following variables were considered: the patient's age at diagnosis, the World Health Organization grade of the tumor, the patient's sex, and the level of miR-31-5p in the tumor (Table 3). The cutoff separating samples with low and high miR-31-5p expression level was established at 0.0029667 of normalized expression based on the results of ROC analysis.

The data reveal that only the expression level of miR-31-5p in tumors is associated with meningioma stiffness, with tumors exhibiting high levels of miR-31-5p being 12.5 times more likely to be stiff (Fisher exact test P-value = .0014). No statistically significant association of other clinicopathological parameters with meningioma stiffness was confirmed. By applying the multivariate logistic regression, the level of miR-31-5p in meningiomas was confirmed as an independent stratification factor (Table 4).

DISCUSSION

Determining the consistency of meningiomas is not always straightforward. Although MRI signal intensity has been used to predict the consistency of the tumor and its histopathological subtype, there is no universally accepted method to determine the consistency of meningiomas. Although T1-weighted imaging is not considered particularly useful for predicting the consistency of a tumor, T2-weighted imaging (T2WI) has shown a good correlation with the consistency of a tumor as observed during surgery and with postoperative histopathological findings.[27,28] Figure 5 shows examples of MRI findings in 2 patients with meningioma of different stiffness (Figure 5A-5C and 5E-5F) using the methodology of measuring signal changes according to Smith et al[27] in correlation with miRNA findings.

However, although numerous publications suggest that T2WI is a promising tool for predicting tumor consistency, its efficacy has not been formally confirmed. Many of these studies are limited by small participant numbers and often rely on retrospective data, with inconsistent findings across different research. The true diagnostic accuracy of T2WI remains unclear in most cases, and where it has been assessed, the sensitivity and specificity are frequently inadequate. Furthermore, variables such as differences in MRI machine capabilities, imaging protocols, and issues with the methodological approach—specifically concerning qualitative vs quantitative evaluation—can affect the reliability and generalizability of these studies. Variations in the benchmarks used to measure tumor consistency and other disparities in how data are interpreted may also compromise the robustness of the findings. Several more advanced MRI techniques such as diffusion-weighted imaging (Figure 5C and 5F), magnetic resonance elastography, and magnetic resonance spectroscopy have been used to characterize the meningioma stiffness.[27] However, some of these modalities may require special equipment or specific training for interpretation, limiting their widespread availability and use.[27,29] In any case, evaluation of various modalities of MRI imaging in relation with miRNA analysis seems to be a desirable direction for further research.

Meningiomas are associated with various molecular alterations. Therefore, it is logical to assume that the consistency of meningiomas is also associated with a specific molecular genetic profile. In 2016, Zhao et al identified 132 genes differentially expressed between soft and hard texture meningiomas; both

TABLE 2. Significantly Differentially Expressed miRNAs Between Less (≤60%) and More (>60%) Stiff Meningiomas

microRNA	baseMean	FC (log2)	*P* value	microRNA	baseMean	FC (log2)	*P* value
miR-124-3p[a]	207	4.75	4.54×10^{-7}	miR-130a-5p	59	−0.90	1.67×10^{-3}
miR-675-3p[a]	124	3.81	2.91×10^{-6}	miR-130a-3p	29 890	−0.82	2.42×10^{-3}
miR-675-5p[a]	49	4.03	7.09×10^{-6}	miR-17-5p	2395	−1.24	1.12×10^{-2}
miR-671-3p	62	0.76	3.57×10^{-4}	miR-3126-3p	5	−1.39	1.13×10^{-2}
miR-6842-3p	11	1.33	4.31×10^{-4}	miR-340-3p	108	−0.97	1.20×10^{-2}
miR-34c-3p	30	2.29	1.44×10^{-3}	miR-30b-5p	14 153	−0.63	1.21×10^{-2}
miR-2114-3p	30	1.06	1.96×10^{-3}	miR-144-3p	3372	−1.64	1.30×10^{-2}
miR-31-5p	106	1.91	2.33×10^{-3}	miR-30e-5p	70 899	−0.30	1.57×10^{-2}
miR-34c-5p	1643	1.99	4.27×10^{-3}	miR-32-3p	109	−0.72	1.58×10^{-2}
miR-181a-5p	15 833	1.13	5.77×10^{-3}	miR-1911-5p	37	−2.70	1.61×10^{-2}
miR-425-5p	2733	0.61	6.04×10^{-3}	miR-625-3p	288	−0.82	1.73×10^{-2}
miR-92b-3p	1026	0.98	6.23×10^{-3}	miR-6726-3p	5	−0.89	2.04×10^{-2}
miR-10527-5p	9	0.91	6.49×10^{-3}	miR-1299	99	−1.70	2.42×10^{-2}
miR-2277-5p	23	0.83	7.01×10^{-3}	miR-6511b-3p	23	−0.69	2.64×10^{-2}
miR-2114-5p	301	1.23	8.00×10^{-3}	miR-625-5p	214	−0.71	2.82×10^{-2}
miR-6516-5p	8	1.15	9.53×10^{-3}	miR-19a-3p	10 976	−0.89	2.87×10^{-2}
miR-449a	21	1.04	1.01×10^{-2}	let-7d-3p	1018	−0.67	2.97×10^{-2}
miR-491-5p	161	0.89	1.01×10^{-2}	miR-1298-5p	45	−2.75	3.06×10^{-2}
miR-34b-3p	57	1.75	1.06×10^{-2}	miR-374a-3p	5241	−0.51	3.35×10^{-2}
miR-1843	76	0.75	1.13×10^{-2}	miR-1264	28	−2.08	3.39×10^{-2}
miR-181b-5p	3901	0.96	1.18×10^{-2}	miR-3160-3p	9	−1.33	3.41×10^{-2}
miR-548i	5	1.39	1.28×10^{-2}	miR-548o-3p	7	−0.98	3.89×10^{-2}
miR-548ba	7	1.59	1.30×10^{-2}	miR-590-5p	553	−0.65	4.08×10^{-2}
miR-100-5p	45 583	1.02	1.30×10^{-2}	miR-30e-3p	11 014	−0.37	4.24×10^{-2}
miR-501-3p	31	0.80	1.35×10^{-2}	miR-324-5p	921	−0.46	4.36×10^{-2}
miR-483-5p	1268	1.98	1.42×10^{-2}	miR-151a-5p	3469	−0.61	4.47×10^{-2}
miR-3129-5p	9	0.73	1.42×10^{-2}	miR-548h-3p	36	−0.64	4.56×10^{-2}
miR-504-5p	28	1.44	1.51×10^{-2}	miR-186-3p	110	−0.67	4.66×10^{-2}
miR-34b-5p	456	1.67	1.62×10^{-2}				
miR-378a-3p	1994	0.67	1.79×10^{-2}				
miR-339-3p	941	0.61	1.82×10^{-2}				
miR-361-5p	9336	0.36	2.01×10^{-2}				
miR-592	24	1.50	2.12×10^{-2}				
miR-486-3p	6	1.33	2.15×10^{-2}				
miR-21-5p	185 144	0.82	2.29×10^{-2}				

TABLE 2. Continued.

microRNA	baseMean	FC (log2)	*P* value	microRNA	baseMean	FC (log2)	*P* value
miR-1179	37	0.73	2.40×10^{-2}				
miR-671-5p	1576	0.65	2.62×10^{-2}				
miR-132-3p	1966	0.72	2.69×10^{-2}				
miR-149-5p	547	0.89	3.17×10^{-2}				
miR-549a-5p	132	0.97	3.25×10^{-2}				
miR-203b-3p	5	1.62	3.58×10^{-2}				
miR-423-3p	2125	0.56	3.70×10^{-2}				
miR-628-5p	446	0.58	4.22×10^{-2}				
miR-5010-3p	9	0.82	4.44×10^{-2}				
miR-3117-3p	16	1.08	4.62×10^{-2}				

FC, fold-change; miRNAs, microRNAs.
[a]Indicates miRNAs with adjusted *P*-value smaller than .05.

alpha-2 type I collagen and osteomodulin were the most changed molecules. Gene ontology analysis of the differentially altered genes suggested that dysfunction in extracellular matrix (ECM) assembly and disassembly could contribute to the differences between soft and hard tissue textures. In addition, pathway analysis indicated that the ECM itself was the primary factor

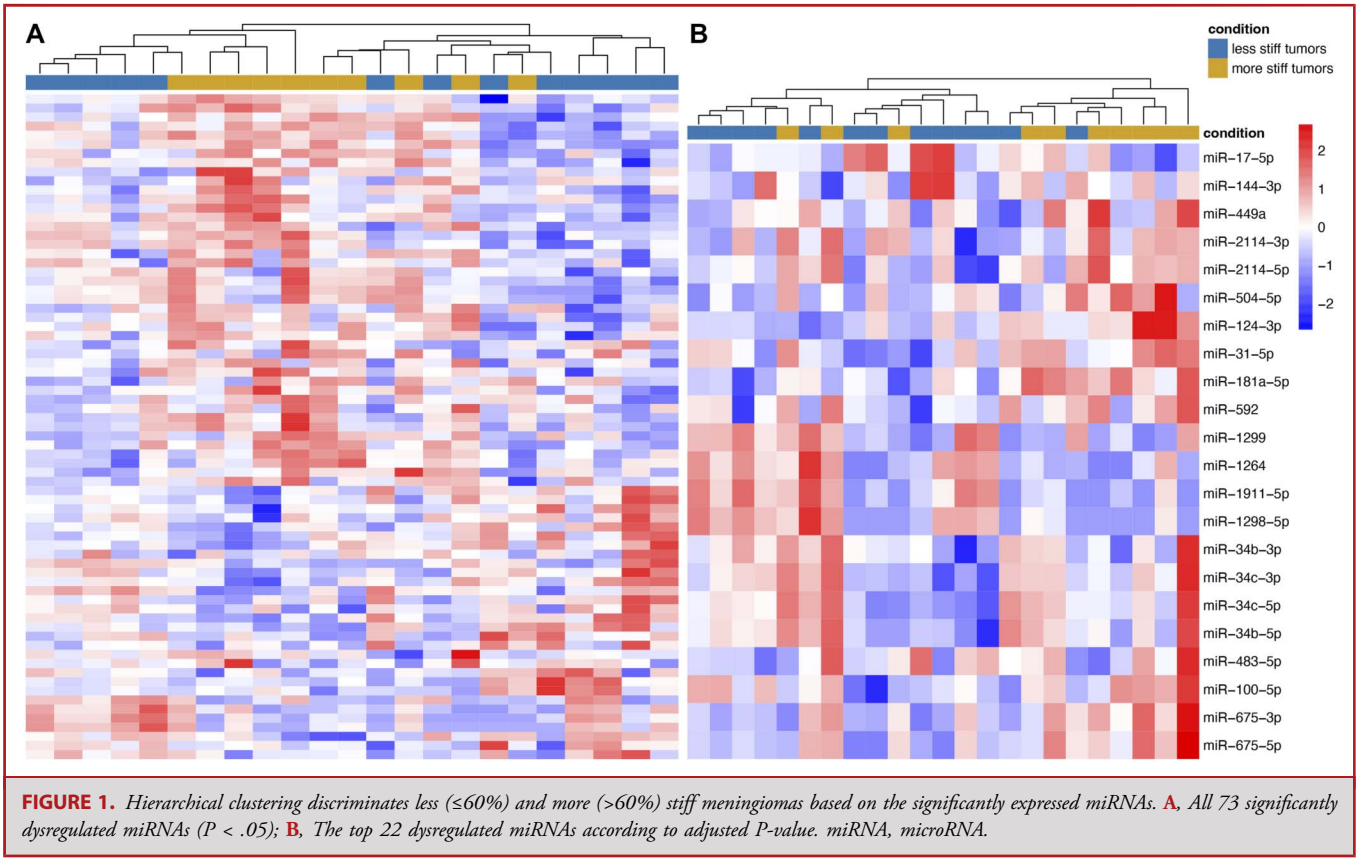

FIGURE 1. *Hierarchical clustering discriminates less (≤60%) and more (>60%) stiff meningiomas based on the significantly expressed miRNAs.* **A**, *All 73 significantly dysregulated miRNAs (P < .05);* **B**, *The top 22 dysregulated miRNAs according to adjusted P-value. miRNA, microRNA.*

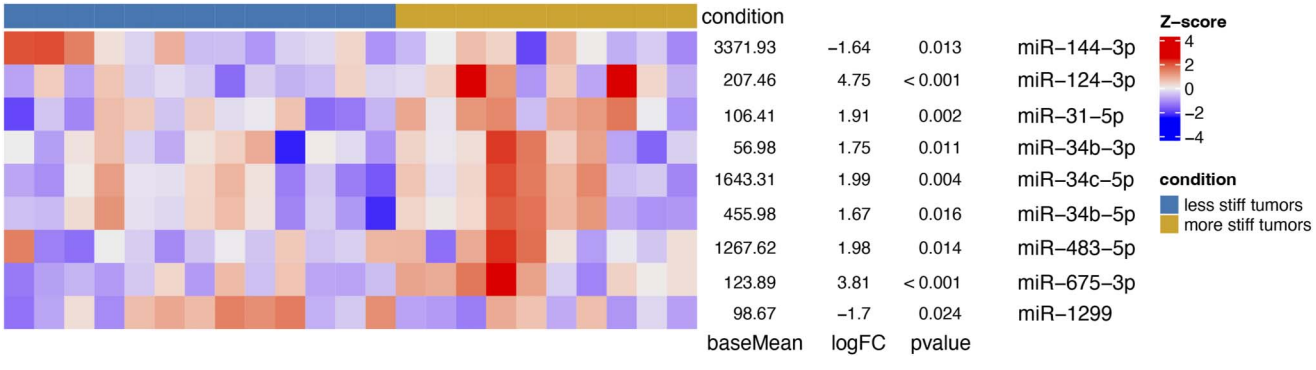

FIGURE 2. *Selected miRNAs meeting the criteria for inclusion in the validation by reverse transcription-quantitative polymerase chain reaction. miRNA microRNA.*

distinguishing the 2 subtypes.[30] However, the value of the study is diminished by the minimal number of analyzed samples, which can lead to false-positive results. Unfortunately, we did not find more research articles studying the relationship between meningiomas' texture/consistency and their molecular genetic background.

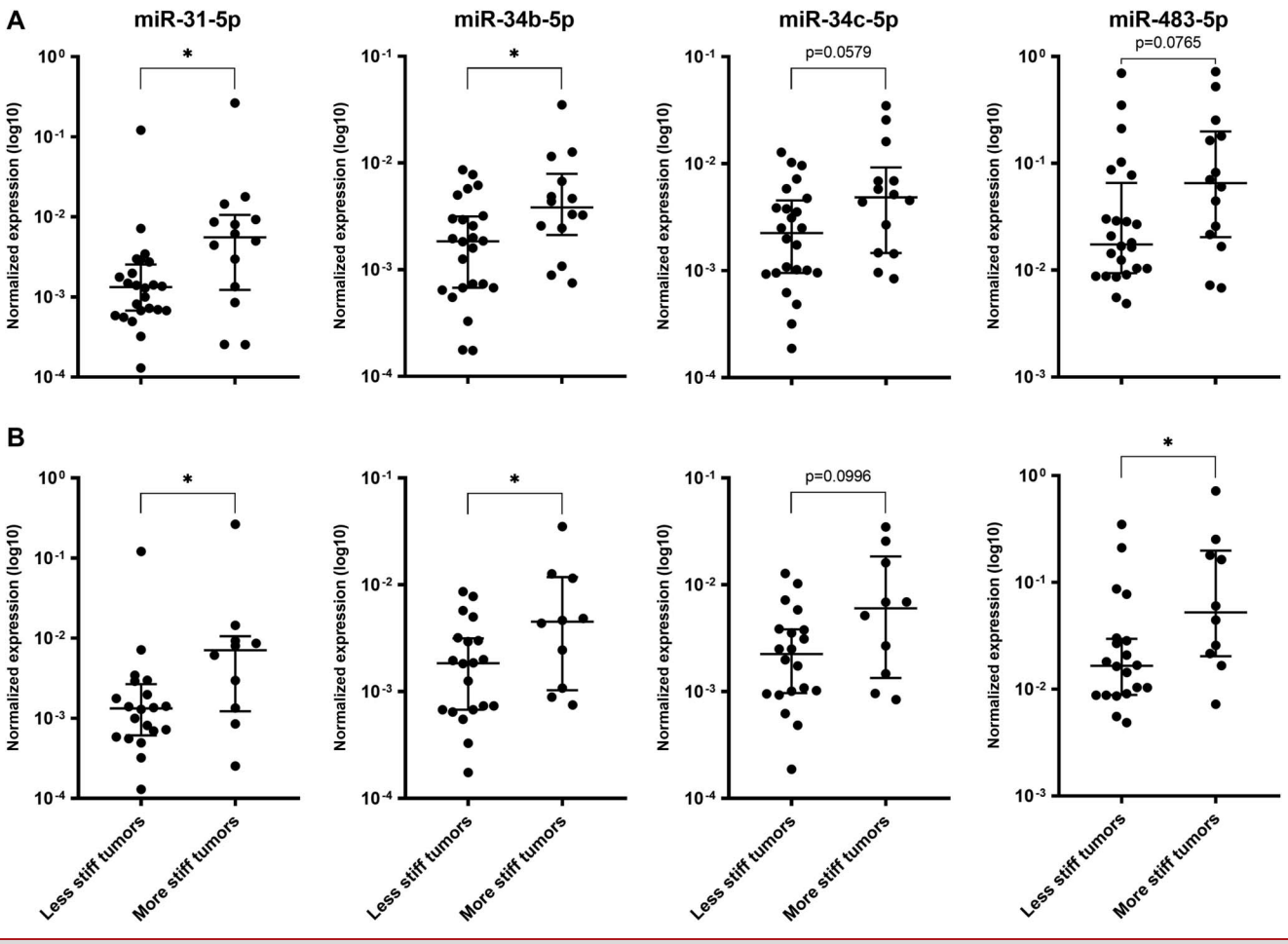

FIGURE 3. *Results of comparisons between less (≤60%) and more (>60%) stiff meningiomas based on the expression of selected miRNAs included in the validation phase.* **A**, *All meningioma samples included in the validation phase;* **B**, *World Health Organization grade I meningioma samples only. *Mann-Whitney P < .05. miRNA microRNA*

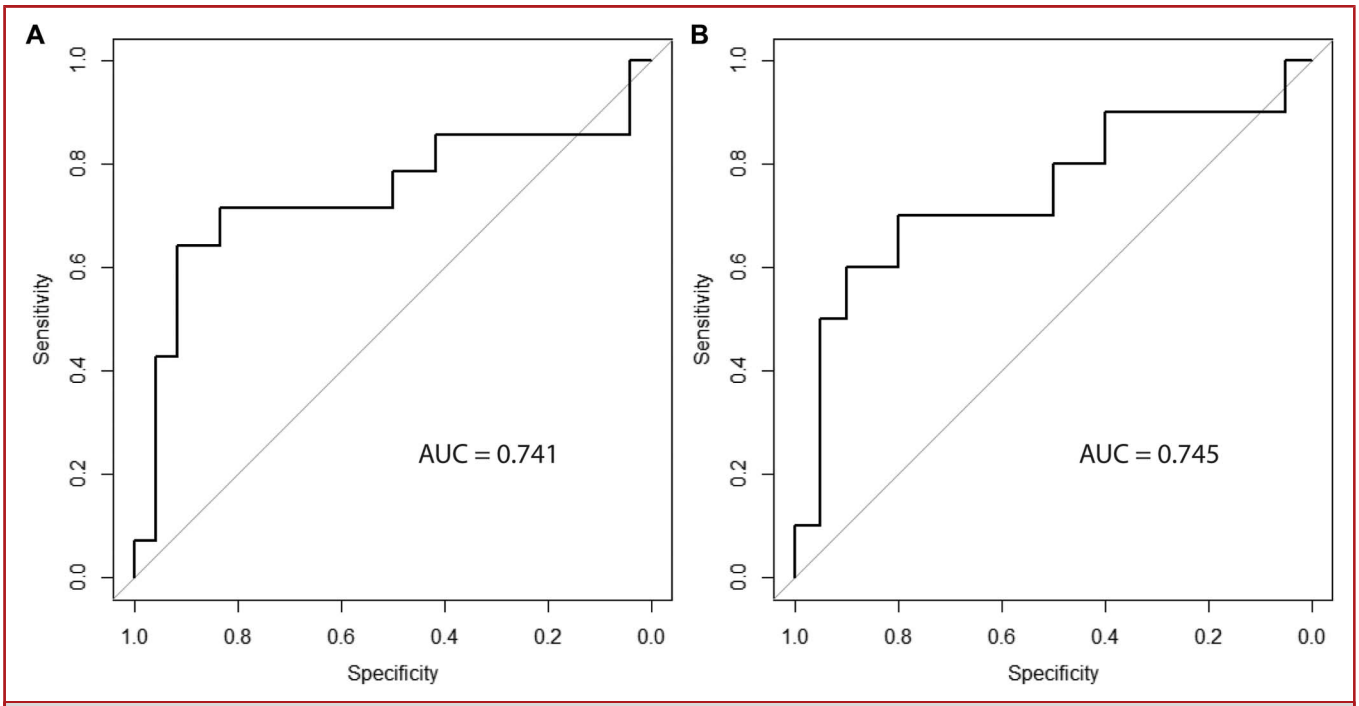

FIGURE 4. *Receiver operating characteristic curves of miR-31-5p and its distinguishing ability between less (≥60%) and more (>60%) stiff meningiomas.* **A**, *All meningioma samples included in the validation phase;* **B**, *World Health Organization grade I meningioma samples only. AUC, area under curve.*

Our study focused on miRNAs because they stand out for their high stability in formalin-fixed paraffin-embedded samples and biofluids, including peripheral blood and cerebrospinal fluid. These are all materials that are very useful for the diagnosis of meningiomas in clinical diagnostic practice.[18,31-34] In addition, the association of miRNAs with the biological characteristics of meningiomas has been described many times.[35,36] In the context of the potential use of miRNAs as predictors of tumor tissue consistency/texture, we can mention the work by Marigliano et al, who investigated correlations between advanced computer tomography (CT) imaging and miRNA expression. They found that CT texture analysis enables distinguishing normal from pathological tissues, and a higher coefficient of determination between CT-based entropy and miR-21-5p expression was evidenced in tumor vs normal tissue. This suggests the potential of miRNAs to reflect tissue characteristics.[37] No other studies describing the

TABLE 3. The Contingency Tables Showing Distributions of miR-31-5p Level, Age at Diagnosis, WHO Grade, and Patient Sex in Relation to Tumor Stiffness in Meningioma Patients

Count Total %	miR-31-5p		Age (y)		WHO grade		Sex	
	Low	High	<60	>60	I	II/III	Female	Male
Less stiff tumors (≤60%)	20	4	13	11	20	4	17	7
	52.6	10.5	34.2	29.0	52.6	10.5	44.7	18.4
More stiff tumors (>60%)	4	10	4	10	10	4	12	2
	10.5	26.3	10.5	26.3	26.3	10.5	31.6	5.3
Fisher exact test *P*-value	0.0014		Not significant		Not significant		Not significant	
Odds ratio (95% CI)	12.5 (2.57-60.7)							

WHO, World Health Organization.
Fisher exact tests were applied.

TABLE 4. Multivariate Logistic Regression of Tumor Stiffness and Clinicopathological Predictors Including miR-31-5p

Condition	OR (95% CI)	*P* value	Model accuracy
miR-31-5p, high vs low	25.24 (3.86-310.60)	.0028	86.84%
Age (y), older than vs younger than 60	7.67 (1.11-86.25)	.057	
WHO grade, I vs II/III	0.16 (0.01-1.35)	.11	
Sex, male vs female	0.04 (0.00-0.54)	.042	

OR, odds ratio; WHO, World Health Organization.

relationship between miRNAs and tissue consistency have been published to date, but these molecules are known to be important in regulating biological processes such as collagen formation, epithelial-mesenchymal transition (EMT), and fibrosis. These are interrelated processes that can affect tissue structure and stiffness. Collagen, a major component of the ECM, is overproduced

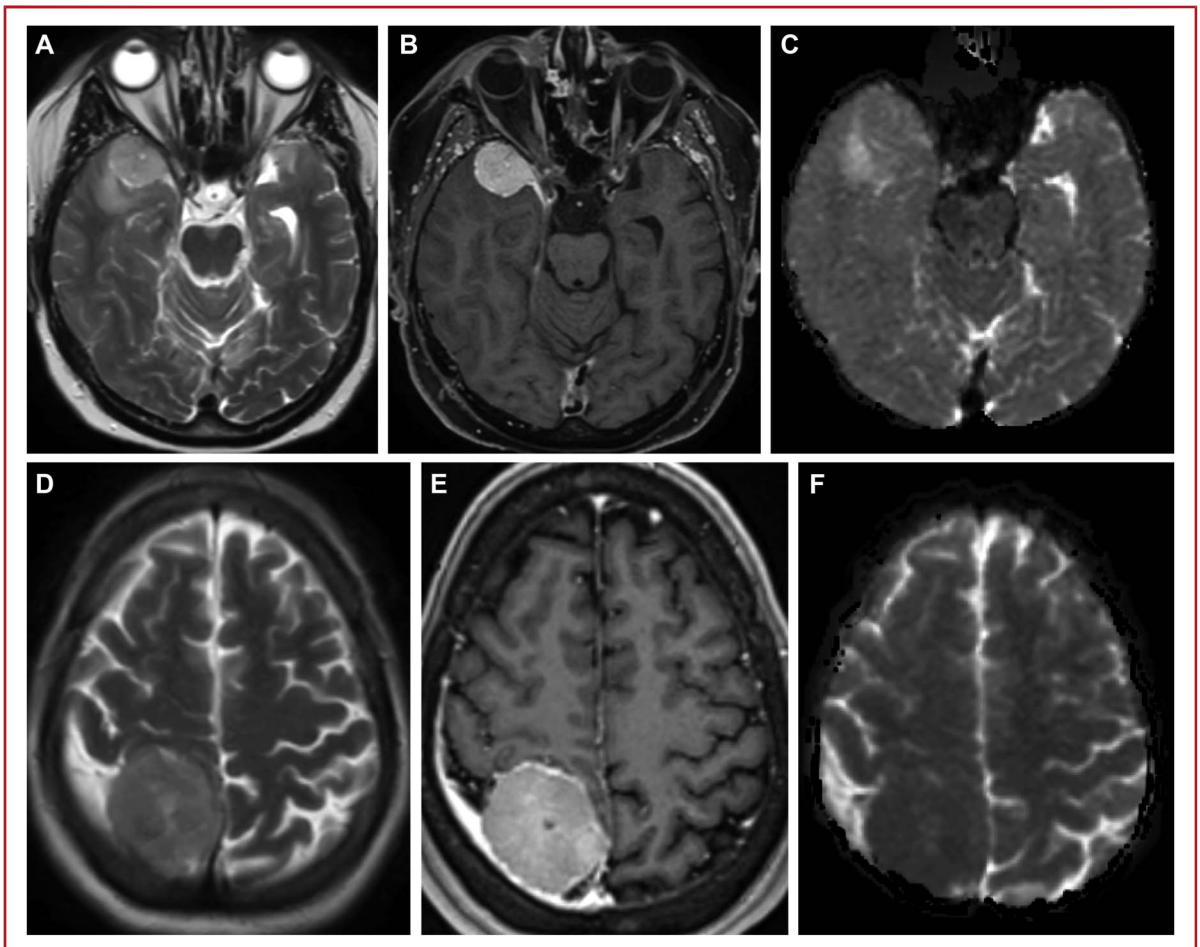

FIGURE 5. *Demonstration of MRI findings in 2 patients with meningioma of different stiffness.* **A-C**, *In the patient with a surgically verified lower stiffness (CUSA power 40%) meningothelial meningioma, higher values of T2 signal ratio of the lesion relative to the white matter of the cerebellar peduncle (2.004) were measured,* **D-F**, *whereas in the patient with a higher stiffness (CUSA power 90%) atypical meningioma, this value was markedly lower (1.126). In correlation with this finding, the measured diffusivity value was higher in the less stiff meningioma ($747*10^{-6}$ mm^2/s) compared with the more stiff meningioma ($506*10^{-6}$ mm^2/s), and the rigidity of the meningiomas was correctly classified by molecular analysis in both patients.* **A** *and* **D**, *T2-weighted image in axial plane,* **B** *and* **E**, *contrast-enhanced T1-weighted image in axial plane, and* **C** *and* **F**, *diffusivity map in axial plane calculated from multi-b-factor diffusion-weighted MRI imaging.*

during fibrosis, leading to increased tissue stiffness. EMT is a biological process where epithelial cells transform into mesenchymal cells, contributing to the production of excessive ECM, including collagen, and the development of fibrosis. This transition is associated with the loss of tissue elasticity and increased tissue thickness. These processes can also be identified in meningiomas. A typical example is fibrous meningiomas, the second most common histological subtype of meningioma found in ~50% of all cases, which is characterized by the presence of extensive fibrosis. Excessive deposition of fibrous tissue, including collagen, contributes to their characteristic histological appearance.

In our study, we confirmed miR-31-5p, miR-34b-5p, miR-34c-5p, and miR-483-5p as differentially expressed between less and more stiff meningiomas. Interestingly, miR-34b/c and miR-483 have been associated with the development of fibrosis in some studies.[38-43] Specifically, miR-34c attenuates kidney fibrosis with ureteral obstruction and is also associated with liver fibrosis.[41] Another member of the miR-34 family, miR-34b-5p, is also associated with liver fibrosis.[40] It appears that both of these molecules may be regulated by the protein kinase C-Jun N-terminal Kinase and the transcription factor Forkhead Box O, both of which help the cell cope with stress, among other things.[43] MiR-483 in turn regulates liver fibrosis in mice through binding to platelet-derived growth factor-β and tissue inhibitor of metalloproteinase 2.[39,42]

MiR-31-5p, miR-34b-5p, and miR-34c-5p are associated with EMT.[44-48] MiR-31 has been shown to enhance EMT in cervical cancer.[46] This miRNA is also highly expressed in colorectal cancer cell lines undergoing EMT. In lung cancer, miR-31 overexpression correlates with EMT markers.[48] Another study revealed that miR-34b-5p reduces collagen and elastin expression but increases matrix metalloproteinase-1, affecting processes like cell adhesion and collagen synthesis.[44] This suggests miR-34b/c's potency as an EMT suppressor.[44,45] Finally, miR-34c-5p appears to suppress EMT in non–small-cell lung cancer.[47]

So far, very little is known about the molecular background of the structural changes in meningiomas, and a great deal of effort is still needed to understand this phenomenon sufficiently. However, miRNAs will likely play a significant role in this process, and analysis of their expression patterns or levels, especially in body fluids, could be a valuable approach to predicting the stiffness of meningiomas. Together with imaging methods, they could create a powerful predictive tool that would significantly increase the efficiency of surgical planning and improve patient survival.

Limitations

The main shortcomings of this study were the limited size of the validation phase cohort and the lack of an external data set. For a deeper understanding of the whole issue leading to improved diagnosis and management of patients with meningiomas, it would be very beneficial to correlate molecular data with outcomes from advanced imaging methods in future studies. For noninvasive diagnosis, performing miRNA analysis in biofluids (peripheral blood and/or cerebrospinal fluid) will also be necessary.

CONCLUSION

This study identified specific miRNAs that are differentially expressed in meningiomas with varying stiffness, with miR-31-5p showing the best analytical performance in identifying high-stiffness tumor tissues. Thus, miRNAs seem to be promising biomarkers in this regard and, together with advanced imaging methods, could provide a comprehensive predictive tool to improve surgical planning and patient prognosis. In addition, the association of the identified miRNAs with processes such as fibrosis and EMT suggests a broader role in tumor biology that merits further investigation.

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The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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