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Identification of Meningioma Patients at High Risk of Tumor Recurrence Using MicroRNA Profiling

BACKGROUND: Meningioma growth rates are highly variable, even within benign subgroups, with some remaining stable, whereas others grow rapidly.

OBJECTIVE: To identify molecular-genetic markers for more accurate prediction of meningioma recurrence and better-targeted therapy.

METHODS: Microarrays identified microRNA (miRNA) expression in primary and recurrent meningiomas of all World Health Organization (WHO) grades. Those found to be deregulated were further validated by quantitative real-time polymerase chain reaction in a cohort of 172 patients. Statistical analysis of the resulting dataset revealed predictors of meningioma recurrence.

RESULTS: Adjusted and nonadjusted models of time to relapse identified the most significant prognosticators to be miR-15a-5p, miR-146a-5p, and miR-331-3p. The final validation phase proved the crucial significance of miR-146a-5p and miR-331-3p, and clinical factors such as type of resection (total or partial) and WHO grade in some selected models. Following stepwise selection in a multivariate model on an expanded cohort, the most predictive model was identified to be that which included lower miR-331-3p expression (hazard ratio [HR] 1.44; $P < .001$) and partial tumor resection (HR 3.90; $P < .001$). Moreover, in the subgroup of total resections, both miRNAs remained prognosticators in univariate models adjusted to the clinical factors.

CONCLUSION: The proposed models might enable more accurate prediction of time to meningioma recurrence and thus determine optimal postoperative management. Moreover, combining this model with current knowledge of molecular processes underpinning recurrence could permit the identification of distinct meningioma subtypes and enable better-targeted therapies.

KEY WORDS: Meningioma, miRNA, Prognosis, Recurrence

Among CBTRUS (Central Brain Tumor Registry of the United States) specific histology groupings, meningiomas are the most frequently reported primary intracranial tumors with the annual incidence rate of 8.33 per 100 000 population,[1] accounting for about 23.8% and 46.8% of all intracranial neoplasms in males and females, respectively.[1,2] Their recurrence cannot be predicted reliably based on histopathology itself, because even benign lesions can include tumors with distinct biological behavior and oncogenic drivers, as

demonstrated in both mesenchymal (fibroblastic) and epithelial (meningothelial) lineages of benign meningiomas.[3,4] Recurrence within 5 and 10 yr after gross total resection of World Health Organization (WHO) grade I meningiomas occurs in 12% and 19% of all cases, respectively,[5,6] whereas 5- and 10-yr recurrence rates of subtotally resected benign lesions range from 37% to 60%, and 55% to 100%, respectively.[6] This suggests that there might be key regulators with a significant effect on meningioma biology irrespective of their histopathological degree, the identification of which would allow a more accurate prediction of their behavior. The expression of miRNAs was recently proposed as just such a predictor. These noncoding small RNAs can act as oncogenes or tumor suppressors in various tumors.[4,7] A recent study[4] identified sets of miRNAs that were also deregulated in benign and high-grade meningiomas. Although a few miRNA signatures have been suggested to predict meningioma recurrence, no mutual miRNA sequences have been reported so far.[7-9] The present study was therefore conducted to shed light on these inconsistencies and extend our knowledge of the relationship between miRNA expression and meningioma recurrence.

METHODS

Patients' Description

The study was approved by the Institutional Research Ethics Committee. Of 302 patients who underwent meningioma surgery between 1990 and 2012, only 172 from whom sufficient tissue for miRNA analysis and comprehensive clinical data were available were selected. All patients signed their informed consent. Imaging/clinical follow-up were performed at 3 and 12 mo after surgery, and approximately every 24 to 72 mo thereafter if no recurrence/regrowth was detected. When meningioma recurrence/regrowth was found, additional follow-ups were scheduled. Recurrence, after total or gross total (Simpson grades I, II, and III) and incomplete (Simpson grade >III) resection, has been defined as the reappearance of any new lesion in which meningioma tissue had previously been removed, or when any remnants of tumor after primary surgery were noticed on the follow-up imaging to have grown. If significant tumor growth was noted at follow-up or meningioma became symptomatic, patients were reoperated on or underwent radiation therapy when indicated. Because of overall follow-up of the whole cohort, the 8-yr limit was used as an adequate cutoff time for recurrence for simple 2-sample analyses. This cutoff time is for descriptive purposes only and has no influence on the main results of the study.

miRNA Expression Analysis

Briefly, in the screening phase, formalin-fixed paraffin-embedded (FFPE) tumor samples were obtained from 38 patients for microarray analysis following RNA purification. Of these, 11 had primary samples of meningiomas with evidence of radiographic recurrence up to 8 yr after surgery (MRR+); 15 had meningiomas without evidence of radiographic recurrence within the same time period (MRR−); and 12 had meningiomas without radiological recurrence within the follow-up of <8 yr. Moreover, paired-matched primary and recurrent samples from 10 MRR+ patients were also analyzed. In the training phase, 59 previously unanalyzed samples were assembled for quantitative polymerase chain reaction (qPCR) analyses (20 MRR+ [primary samples], 25 MRR−, and 14 meningiomas without radiological recurrence within the follow-up of <8 yr). Finally, the validation phase included all 172 patients, including cohorts from the screening and training phases, the total comprising 37 with MRR+ (primary samples), 59 with MRR−, and 76 with meningiomas without radiological recurrence within the follow-up of <8 yr (Table 1 and Figure 1). For detailed information about RNA purification (QIAGEN, Hilden, Germany), miRNA array, and TaqMan assay (Applied Biosystems, Foster City, California), see **Text**, **Supplemental Digital Content 1**.

Statistical Methods

Statistical analyses were performed with R (R Foundation for Statistical Computing, Vienna, Austria) and Bioconductor. Wilcoxon exact one-sample tests and Cox regression analysis were applied to the microarray data to identify differentially expressed miRNAs and miRNAs with a significant time-to-relapse (TTR) effect. Univariate adjusted and nonadjusted Cox regression models were selected for qPCR data following the building of a full multivariate model and consequent stepwise selection method, which provided factors for the final multivariate model. Each prognostic factor was characterized by HR (hazard ratio) and P value. For more information, see **Text**, **Supplemental Digital Content 1**.

RESULTS

Screening Phase

The microarray analysis revealed 49 miRNAs with their expression strongly dependent on TTR in meningiomas at various risks of recurrence (Figure 2A), according to the Cox regression model of TTR. Only mature miRNA with higher abundance (intensity of fluorescence >5) were analyzed in all microarray experiments. Decreasing gene expression as risk of recurrence increases is typical for most miRNAs. Only 12 miRNAs increased expression following recurrence; among them were members of the miR-320 family showing the highest HR values. Seven highly abundant and biologically relevant miRNAs were subsequently selected for further validation by qPCR. That set included hsa-miR-15a-5p, hsa-miR-19b-3p, hsa-miR-30e-5p, hsa-miR-107, hsa-miR-146a-5p, hsa-miR-320c, and hsa-miR-331-3p; their characteristics are shown in **Figure**, **Supplemental Digital Content 2**. Additionally, 4 miRNAs that exhibited stable expression and did not correlate with recurrence status or other clinical characteristics were selected for qPCR normalization (let-7b-5p, miR-324-5p, miR-181b-5p, and miR-1281) (**Figure**, **Supplemental Digital Content 3**).

Paired Analysis

A total of 41 mature miRNAs exhibited differential expression in paired-matched primary and recurrent MRR+ samples (Figure 2B). Most of these were expressed less strongly in the recurrent samples, with only about 13 of them expressing more strongly in recurrent samples. Interestingly, the sample pairs formed 2 main clusters according to differentially expressed miRNAs, with some miRNAs changing their level of expression in opposite ways. One of these clusters contained only patients
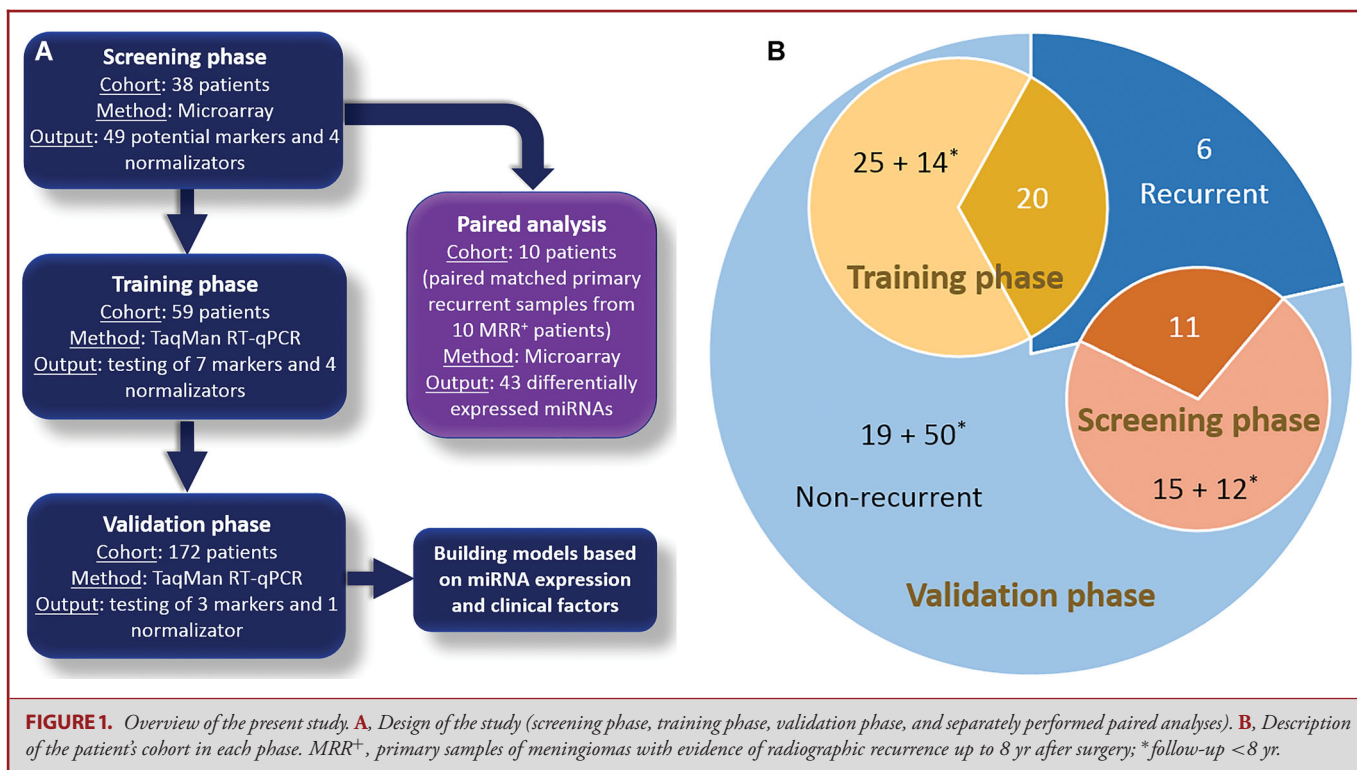
TABLE 1. The Clinical-Pathological Characteristics of Meningioma Patients Included in the Data Set at All Phases of the Study

Cohort		Screening phase 38				Training phase 59				Validation phase 172			
	n	MRR⁻	MRR⁺	P value	Totalᵃ	MRR⁻	MRR⁺	P value	Totalᵃ	MRR⁻	MRR⁺	P value	Totalᵃ
Female/male	n (%)	13/2 (86.7/13.3)	7/4 (63.6/36.4)	.348	28/10 (73.7/26.3)	21/4 (84.0/16.0)	10/10 (50.0/50.0)	.034	40/19 (67.8/32.2)	47/12 (79.7/20.3)	19/18 (51.4/48.6)	.007	115/57 (66.9-33.1)
Age (yr)	Median (IQR)	54 (43-61)	53 (45-61)	.812	54 (43-64)	51 (43-61)	48 (41-61)	.912	50 (43-60)	56 (45-62)	52 (42-64)	.596	56 (45-65)
WHO grade I/II/III	n (%)	9/2/3 (64.3/14.3/21.4)	3/5/3 (27.3/45.5/27.3)	.128	14/12/11 (37.8/32.4/29.7)	24/1/0 (96.0/4.0/0.0)	16/4/0 (80.0/20.0/0.0)	.155	51/8/0 (86.4/13.6/0.0)	51/3/4 (87.9/5.2/6.9)	21/11/4 (58.3/30.6/11.1)	.001	125/31/14 (73.5/18.2/8.2)
Convexity/skull base meningioma	n (%)	6/9 (40.0/60.0)	7/4 (63.6/36.4)	.427	17/21 (44.7/55.3)	8/17 (32.0/68.0)	8/12 (40.0/60.0)	.807	23/36 (39.0/61.0)	20/39 (33.9/66.1)	18/19 (48.6/51.4)	.221	72/100 (41.9/58.1)
Simpson grade I-III/>III	n (%)	12/3 (80.0/20.0)	8/3 (72.7/27.3)	1	30/7 (81.1/18.9)	21/2 (91.3/8.7)	9/11 (45.0/55.0)	.003	40/17 (70.2/29.8)	47/8 (85.5/14.5)	20/17 (54.1/45.9)	.002	127/39 (76.5/23.5)
Add-on radiotherapy (yes/no)	n (%)	4/11 (26.7/73.3)	6/5 (54.5/45.5)	.228	13/25 (34.2/65.8)	3/22 (12.0/88.0)	12/8 (60.0-40.0)	.002	15/44 (25.4/74.6)	10/49 (16.9/83.1)	22/15 (59.5/40.5)	<.001	36/136 (20.9/79.1)
Follow-up (mo)	Median (IQR)	149.3 (113.7-198.1)	99.8 (87.6-123.4)	.005	100.9 (66.8-144.7)	161.2 (124.4-202.2)	138.3 (102.3-191.8)	.233	124.4 (85.5-181.5)	146.2 (124.5-202.6)	120.0 (87.3-173.4)	.004	95.7 (65.8-145.7)
Time to relapseᵇ	%	–	–	–	64.5 ± 8.8	–	–	–	61.3 ± 6.9	–	–	–	72.5 ± 4.0

IQR, interquartile range (first quartile through third quartile); MRR⁺, patients with meningiomas with evidence of radiographic recurrence up to 8 yr after surgery; MRR⁻, patients with meningiomas without evidence of radiographic recurrence up to 8 yr after surgery; WHO, World Health Organization.

ᵃIncluding patients with follow-up <8 yr without recurrence.

ᵇKaplan-Meier estimate of survival at the time of 8 yr after surgery: 8-yr survival ± standard error.

FIGURE 1. *Overview of the present study.* **A**, *Design of the study (screening phase, training phase, validation phase, and separately performed paired analyses).* **B**, *Description of the patient's cohort in each phase.* MRR$^+$, *primary samples of meningiomas with evidence of radiographic recurrence up to 8 yr after surgery;* *follow-up <8 yr.*

with convexity meningiomas and who were generally older and presented with higher histopathological WHO grades at diagnosis. Only 2 miRNAs, miR-193b-3p and miR-27a-3p, exhibited deregulation between paired-matched primary and recurrent samples of MRR$^+$ and also showed dependence on TTR within the screening phase. These were not chosen for further validation of relapse prediction. Interestingly, miR-30c-5p was deregulated in recurrent samples of MRR$^+$, whereas the expression of the closely related miR-30e-5p, miR-30b-5p, and miR-30a-5p was found to be dependent on TTR in the screening phase, which indicates that miR-30 is significantly involved in meningioma pathogenesis; it was therefore also selected for further validation.

Training Phase

The expression of candidate miRNAs was normalized against miR-181, which exhibited the most stable basal expression over all samples within the qPCR data (**Figure, Supplemental Digital Content 4**). A univariate analysis confirmed the differences in some of the miRNA's expression in primary samples of MRR$^+$ observed in microarray experiments. The following miRNAs exhibited significant dependence on TTR within the samples using adjusted or nonadjusted models in their expression: hsa-miR-15a-5p, hsa-miR-146a-5p, and hsa-miR-331-3p (Table 2). Nonadjusted models used only certain miRNAs as prognostic factors, whereas the adjusted model also included clinical factors such as age, sex, WHO grade, tumor location, and type of

resection. All 3 miRNAs were selected for final validation on the extended cohort.

Validation Phase

The 3 miRNAs selected in the training phase were tested in 172 patients, including cohorts from the screening and training phases. The final model was developed using the complete set of ΔCt values for the cohort that had a realistically unbalanced ratio of MRR$^+$ and MRR$^-$ patients. Adjusting clinical factors were included in the Cox regression model for the final analysis of TTR in patients. Univariate analyses once again confirmed the miR-331-3p as the most promising prognostic factor. In the analysis of each marker separately, miR-331 gave the highest HR and the lowest *P* value among other miRNA-based univariate models. This analysis was also performed on the total resection subgroup in order to exclude the influence of such a strong prognostic factor that probably has no molecular background. Notably, this analysis produced similar results. Moreover, miR-15a was not a statistically significant candidate in the present models. Investigating the influence of clinical adjusting factors, the type of resection had the strongest prognostic value in all models. However, WHO grade in the subgroup of patients with a total resection was the strongest prognostic factor within the miR-146a- and miR-15a-based models. The results from the univariate Cox regression models are summarized in Table 3. The multi-variate model was built in order to test whether miRNA represents real additional value in meningioma recurrence prognostication. The stepwise selection method with fixed clinical adjusting factors
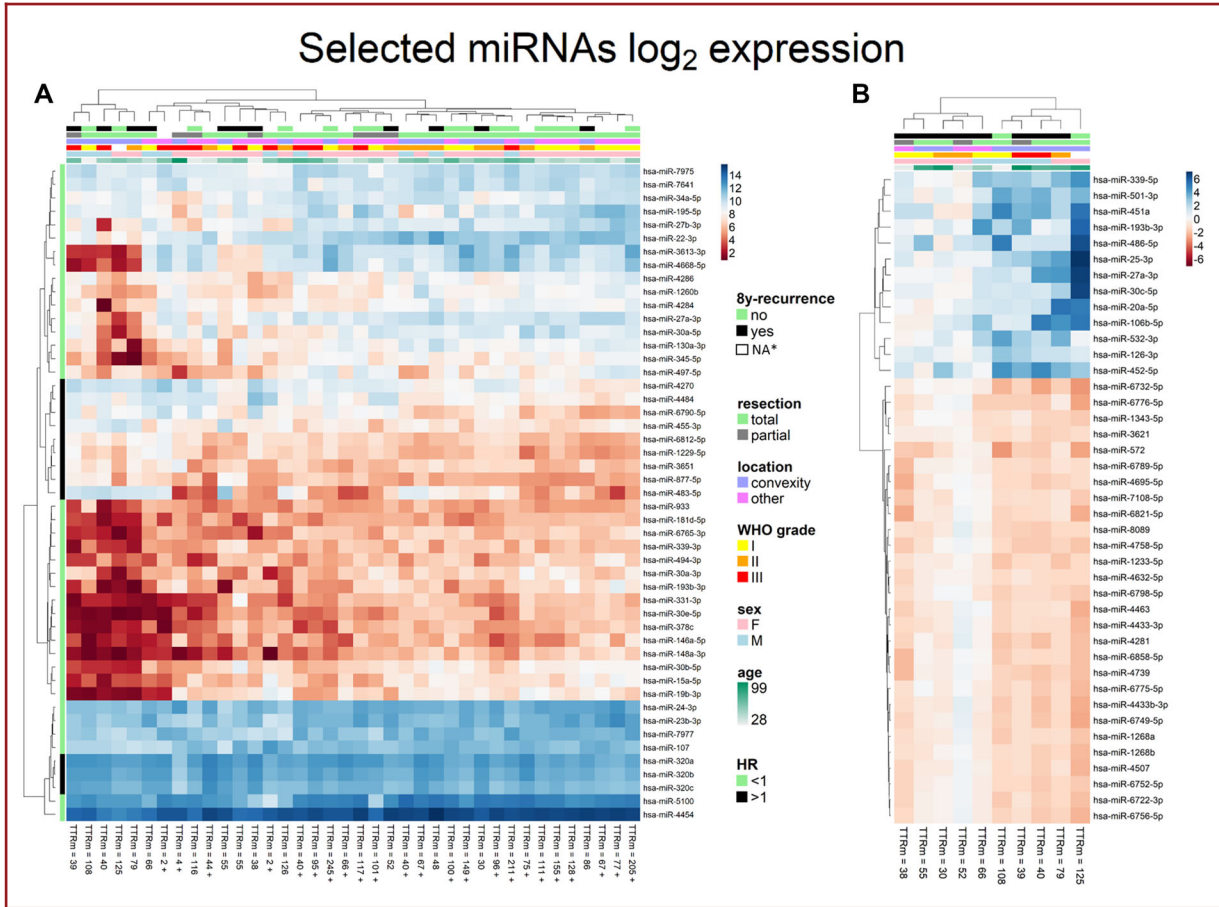
FIGURE 2. *Microarray data.* **A**, *Unsupervised hierarchical clustering of 49 differentially expressed miRNAs in MRR$^+$ and MRR$^-$ based on logarithmic values (log$_2$) of miRNA's expression compared to recurrence in 8 yr of follow-up, sex, histopathological grade, type of resection, tumor location, and age at diagnosis. Hazard ratio (HR) is also marked for each miRNA.* **B**, *Differentially expressed miRNAs between primary and recurrent samples of MRR$^+$ from paired analyses. TTRm, time to relapse in months; + censored if there is no event during follow-up period; * no evidence of radiographic recurrence, but follow-up <8 yr.*

was used to select the most important prognostic factors in the multivariate model. The only significant ($P < .001$) candidate identified by the model was miR-331-3p, the extent of resection being the only other significant clinical factor, other factors having only supportive functions. The final model is shown in Figure 3. Among all tested models, WHO grades and miR-146a-5p also appeared to be predictors. The question remains, whether balanced cohorts in terms of Simpson grades in the validation phase would lead to similar results, or would alternatively confirm the role of the extent of tumor resection as a predictor of recurrence.

DISCUSSION

miRNA Profile and Meningioma Recurrence

A link between aberrant miRNA expression and meningioma recurrence has so far been identified in only 3 studies.[7-9]

However, none of the miRNAs overlaps with the set of 49 deregulated miRNAs identified during the screening phase of the present study. This discrepancy might stem from differences in sample size or cohort homogeneity.[8] For example, in contrast to the present study, the other cited studies[7-9] included Asian cohorts comprising various numbers of patients ranging from 103 to 230 and a mixture of recurrent and nonrecurrent tumors (see **Table**, **Supplemental Digital Content 5**). Other differences between the approaches exist in the methodology, study design, and sample type. For example, only one study performed miRNA-profiling analysis incorporating all 3 phases,[7] whereas the screening phase was omitted in the other 2 studies[8,9]; moreover, one of them selected only one candidate miRNA for analysis without utilizing screening and training dataset.[9] These factors combined might have led to the eventual selection of different miRNAs. Regarding tissue samples, one of the cited studies prospectively analyzed circulating miRNAs from

TABLE 2. Univariate Adjusted and Nonadjusted Cox Regression Models of Time to Relapse From Training Phase for Each Measured miRNA

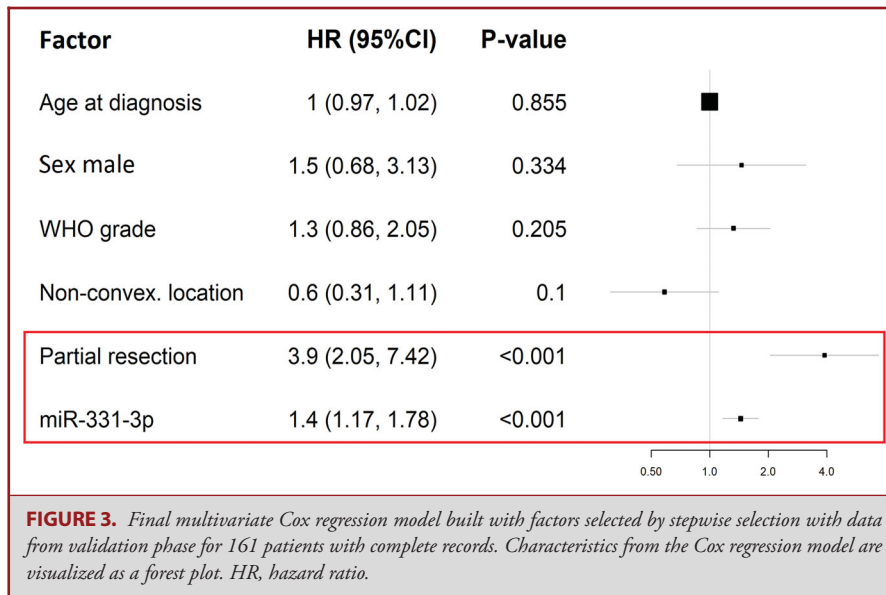|	Nonadjusted model					Adjusted model				
Factor	HR	1/HR	95% CI range		P value	HR	1/HR	95% CI range		P value
miR-107	1.25	0.80	0.86	1.80	.240	0.94	1.06	0.63	1.41	.761
miR-331-3p	1.76	0.57	1.14	2.70	.010	1.57	0.64	0.93	2.65	.090
miR-15a-5p	1.15	0.87	0.97	1.35	.103	1.25	0.80	1.01	1.55	.038
miR-19b-3p	1.27	0.79	0.90	1.78	.175	1.25	0.80	0.88	1.77	.214
miR-30e-5p	1.11	0.90	0.89	1.38	.357	1.21	0.83	0.90	1.64	.209
miR-320c	1.23	0.81	0.79	1.92	.364	0.89	1.12	0.49	1.62	.710
miR-146a-5p	1.55	0.65	1.13	2.13	.007	1.42	0.70	1.00	2.02	.053

Significant miRNAs in at least one model are marked in bold.

TABLE 3. Univariate Adjusted Cox Regression Models of Time to Relapse From Validation Phase for Each Measured miRNA

	All patients						Patients after total resection				
Factor	HR	1/HR	95% CI range		P value	Factor	HR	1/HR	95% CI range		P value
miR-146a-5p based model											
Age at diagnosis	0.99	1.01	0.97	1.01	.436	Age at diagnosis	1.01	0.99	0.98	1.04	.709
Sex: male	1.68	0.59	0.84	3.37	.142	Sex: male	1.15	0.87	0.47	2.82	.759
WHO grade	1.63	0.61	1.07	2.50	.024	**WHO grade**	1.86	0.54	1.06	3.24	.029
Nonconvex. location	0.63	1.60	0.33	1.18	.148	Nonconvex. location	0.54	1.85	0.23	1.25	.150
Partial resection	3.16	0.32	1.67	6.00	4.24E-04	–	–	–	–	–	–
miR-146a-5p	1.34	0.74	1.10	1.63	.003	**miR-146a-5p**	1.37	0.73	1.07	1.76	.014
miR-15a-5p based model											
Age at diagnosis	0.99	1.01	0.97	1.01	.254	Age at diagnosis	0.99	1.01	0.97	1.02	.656
Sex: male	2.77	0.36	1.45	5.27	.002	Sex: male	1.69	0.59	0.72	3.95	.228
WHO grade	1.37	0.73	0.91	2.08	.132	**WHO grade**	1.85	0.54	1.07	3.18	.027
Nonconvex. location	0.69	1.46	0.37	1.28	.236	Nonconvex. location	0.65	1.55	0.28	1.50	.310
Partial resection	3.67	0.27	1.92	7.02	8.52E-05	–	–	–	–	–	–
miR-15a-5p	0.94	1.06	0.84	1.05	.283	miR-15a-5p	0.96	1.04	0.82	1.11	.573
miR-331-3p based model											
Age at diagnosis	1.00	1.00	0.97	1.02	.827	Age at diagnosis	1.01	0.99	0.98	1.04	.443
Sex: male	1.43	0.70	0.67	3.03	.354	Sex: male	1.12	0.89	0.44	2.87	.811
WHO grade	1.33	0.75	0.86	2.05	.200	WHO grade	1.53	0.65	0.86	2.73	.146
Nonconvex. location	0.58	1.72	0.31	1.10	.095	Nonconvex. location	0.46	2.17	0.19	1.11	.085
Partial resection	3.87	0.26	2.03	7.38	4.11E-05	–	–	–	–	–	–
miR-331-3p	1.45	0.69	1.17	1.79	.001	**miR-331-3p**	1.43	0.70	1.10	1.87	.007

Significant factors are marked in bold.

patients' serum,[7] whereas the others[8,9] used tumor tissue either as snap-frozen or FFPE samples, and collected data retrospectively. Moreover, although quantitative reverse transcription PCR was used in the training and validation phases of all 3 cited studies,[7,8] we used microarrays in our screening phase, which is a more advanced technique, as it targets all miRNAs. However, the differences between our results and those of other studies might also stem from the fact that other gene regulation mechanisms play a more robust role in meningioma recurrence.[10] The previous studies also reported that the upregulation of miR-190a[8] and miR-409-3p[7] and downregulation of miR-29c-3p[8]

and miR-219-5p[8] were associated with increased recurrence rates, whereas the effect of miR-224 expression was inconsistent in the different studies. Its downregulation was associated with increased recurrence,[7] whereas Wang et al[9] found the opposite, which they attributed to the activation of the ERG2-BAK-induced apoptosis pathway. Based on the high biological relevance and differential expression profile resulting from the Cox regression model and hierarchical clustering analysis, 7 miRNAs were selected for qPCR validation on an independent cohort, namely miR-15a-5p, miR-19b-3p, miR-30e-5p, miR-107, miR-146a-5p, miR-320c, and miR-331-3p. Following normalization against stably expressed

Factor	HR (95%CI)	P-value
Age at diagnosis	1 (0.97, 1.02)	0.855
Sex male	1.5 (0.68, 3.13)	0.334
WHO grade	1.3 (0.86, 2.05)	0.205
Non-convex. location	0.6 (0.31, 1.11)	0.1
Partial resection	3.9 (2.05, 7.42)	<0.001
miR-331-3p	1.4 (1.17, 1.78)	<0.001

FIGURE 3. *Final multivariate Cox regression model built with factors selected by stepwise selection with data from validation phase for 161 patients with complete records. Characteristics from the Cox regression model are visualized as a forest plot. HR, hazard ratio.*

miR-181, adjusted and nonadjusted models were used to test for effects on the TTR with estimated P values $< .1$. Among the miRNAs, the most significant positive prognostic factors were selected as being miR-15a-5p ($P = .038$), miR-146a-5p ($P = .053$), and miR-331-3p ($P = .09$). Subsequent testing of the 3 miRNAs on an expanded cohort using stepwise selection in the multivariate model identified the most effective predictive model to be the miR-331-3p-based model. The model which incorporated clinical factors identified those patients with a high miR-331-3p expression (HR 1.44; $P < .001$) and total/gross total meningioma resection (HR 3.90; $P < .001$) as cases with a significant influence on TTR. Other clinical factors played only a supportive role.

Having compared various models, the second most effective model was identified to be the one that included miR-146a-5p. In addition to the miRNA, the WHO grades and the extent of tumor resection were also good prognosticators. Factors identified as indicating a low propensity to recur were totally/gross totally removed (HR 3.16; $P < .001$) benign meningiomas (HR 1.63; $P = .024$) with upregulated miR-146a-5p expression (HR 1.34; $P = .003$). In a total/gross total resection subgroup analysis, both miRNAs remained significant predictive factors. Additionally, in the model with miR-146a-5p, the WHO grading system still functioned as a prognosticator.

miR-15a

miR-15 has been reported to suppress tumors in colon[11] and prostate cancer.[12] Following miR-15 transfection of tumor cells, the apoptosis rate increased significantly, probably because of the inhibition of nuclear factor kappa B (NF-κB) that promotes the transcription of antiapoptotic factors, such as Bcl-2 and Bcl-XL.[11] Additionally, miR-15b has been shown to reduce the invasion of glioma cells and angiogenesis by downregulation of neuropilin-2 (NRP-2) that interacts with vascular endothelial growth factor (VEGF), or through deactivation of the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway signaling pathway.[13] NRP-VEGF interactions have been shown to promote developmental angiogenesis and metastases.[14] Thus, further investigations are warranted in order to verify which of these mechanisms, if any, is applied in meningioma biology. MEK-ERK as a key signaling pathway[15] and a highly expressed VEGF/VEGF receptor[16] have already been found in meningiomas.

miR-146a-5p

miR-146a is known to suppress gastric cancer cell invasion and metastasis,[17] and knocking out its expression in C57BL/6 mice leads to the development of myeloid sarcomas and lymphomas.[18] A study on gliomas[19] found that a combined treatment with gamma-linolenic acid (GLA) and irradiation led to miR-146a overexpression. Because surgical resection supplemented with irradiation is a standard therapy for recurrent meningiomas,[6] it would be desirable to determine whether this combined therapy has a similar beneficial effect on miR-146a-5p expression in meningiomas. The importance of miR-146 in meningioma recurrence is suggested by the existence of a negative feedback loop between signal transducer and activator of transcription 3 (*STAT3*) and NF-κB involving miR-146b,[20] and the fact that *STAT3* expression was significantly higher in recurrent WHO grade I and/or grade II meningiomas than in nonrecurrent ones.[21] *STAT3* targets miR-146b, which reduces IL-6 production by downregulating NF-κB. This is the final step in the negative feedback loop because IL-6 activates *STAT3*.[20] Upon

miR-146b downregulation, its uninhibited target gene NF-*κ*B induces increased IL-6 production followed by *STAT3* activation. Notably, Johnson et al[21] reported that *STAT3* activation was markedly stronger in WHO grade II meningiomas than those of lower grade. High *STAT3* activation was also identified in 2 out of 3 recurrent WHO grade I meningiomas and in none out of 3 nonrecurrent lesions. Additionally, a strong STAT3 phosphorylation/activation signal was observed in 2 out of 4 recurrent WHO grade II meningiomas and one out of 3 nonrecurrent tumors.[21] Although the suppression of IL-6 by miR-146a leading to reduced *STAT3* expression has not yet been demonstrated in meningiomas,[22] these findings highlight the potential therapeutic value of miR-146a in these tumors. Combining GLA with irradiation might be an effective treatment strategy for overexpression of the miRNA, deactivation of the STAT3 pathway, and, thus, reducing the likelihood of meningioma recurrence.

miR-331-3p

The pronounced downregulation of miR-331-3p in glioblastomas suggests its role as tumor suppressor, possibly by upregulating *NRP-2* expression[23] or influencing targets such as receptor tyrosine-protein kinase erbB-2 (*HER2*),[24] deoxyhypusine hydroxylase,[25] phosphatase and tensin homolog/protein kinase B (PTEN/AKT), astrocyte-elevated gene-1 (*AEG-1*),[26] transcription factor *E2F1*,[27] *HER2/PI3K/AKT*,[28] or kallikrein-related peptidase 4.[29] However, which proteins are targets of miR-331-3p in meningiomas remains unclear. Phosphoinositide-3-kinase (PI3K)/AKT, as a key regulator of cell survival in cancers, has already been associated with meningiomas.[30] Moreover, AEG-1-depleted meningioma cells undergo apoptosis via phospho-AKT and Bcl-2 suppression.[31] It is therefore rational to suspect that a negative impact of miR-331-3p deregulation on meningioma might be carried out through these signaling pathways.

miRNA Profile in Paired Analyses

Recent findings regarding the variability of gene mutations being dependent on meningioma localization,[32] along with the observations that some genetic factors found in these tumors are important embryonic stem cell regulators,[33] suggest that meningiomas may derive from early progenitors/cancer stem cells, whereas their histogenetic origin may be site specific. Of note, based on a differential expression of 41 miRNAs in the paired analyses, 2 clusters were created, one of them predominantly comprising convexity meningiomas with 13 miRNAs highly expressed in their recurrent samples. Because miRNAs are involved in the regulation of embryonic stem cell development and signaling,[34] these findings further support the view that biological properties of meningiomas may be derived from site-specific progenitor/cancer stem cells regulated by respective miRNAs. Moreover, as WHO grade II and III meningiomas are significantly more frequent in the younger patients,[35] it seems that a specific expression profile of the 13 miRNAs can recognize recurrent high-grade meningiomas in older subjects as a biologically distinct tumor subgroup. Basically, this may indicate 2 distinct biological mechanisms required for tumor recurrence, with the mechanism observed in older subjects requiring just the upregulation of the miRNAs. However, because of a small cohort in the paired analyses, all this remains speculative and requires further validation.

Limitations of the Study

The main shortcomings of the present study were its retrospectivity, the limited size of the validation phase cohort, and the lack of an external dataset. As the dataset was unbalanced in terms of Simpson grades, the question remains whether such balancing of the cohort would lead to results similar to those of an unbalanced one. Moreover, miRNA expression profiles in the MRR$^+$ group might be prejudiced by the fact that only some symptomatic patients with meningioma recurrence underwent reoperation, whereas others were irradiated, and those without clinical manifestation and significant tumor growth were left untreated.

CONCLUSION

Our findings indicate that the miRNA-based model can serve as a novel predictor of meningioma recurrence and can thus help in determining an optimal postoperative surveillance regime to identify patients who may benefit from early retreatment. Moreover, combining the model with molecular mechanisms governing meningioma recurrence, such as miRNA targets and associated signaling pathways, might help to identify clinically distinct meningiomas and better target their treatment. Finally, because the literature indicates that no mutual miRNA predictors have yet been identified, a prospective randomized multicenter controlled trial is justified in order to resolve this ongoing discrepancy.

Disclosures

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