Posttranscriptional deregulation of signaling pathways in meningioma subtypes by differential expression of miRNAs

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Background. Micro (mi)RNAs are key regulators of gene expression and offer themselves as biomarkers for cancer development and progression. Meningioma is one of the most frequent primary intracranial tumors. As of yet, there are limited data on the role of miRNAs in meningioma of different histological subtypes and the affected signaling pathways.

Methods. In this study, we compared expression of 1205 miRNAs in different meningioma grades and histological subtypes using microarrays and independently validated deregulation of selected miRNAs with quantitative real-time PCR. Clinical utility of a subset of miRNAs as biomarkers for World Health Organization (WHO) grade II meningioma based on quantitative real-time data was tested. Potential targets of deregulated miRNAs were discovered with an in silico analysis.

Results. We identified 13 miRNAs deregulated between different subtypes of benign meningiomas, and 52 miRNAs deregulated in anaplastic meningioma compared with benign meningiomas. Known and putative target genes of deregulated miRNAs include genes involved in epithelial-to-mesenchymal transition for benign meningiomas, and Wnt, transforming growth factor–β, and vascular endothelial growth factor signaling for higher-grade meningiomas. Furthermore, a 4-miRNA signature (miR-222, -34a*, -136, and -497) shows promise as a biomarker differentiating WHO grade II from grade I meningiomas with an area under the curve of 0.75.

Conclusions. Our data provide novel insights into the contribution of miRNAs to the phenotypic spectrum in benign meningiomas. By deregulating translation of genes belonging to signaling pathways known to be important for meningioma genesis and progression, miRNAs provide a second in line amplification of growth promoting cellular signals. MiRNAs as biomarkers for diagnosis of aggressive meningiomas might prove useful and should be explored further in a prospective manner.

Keywords: histological subtypes, meningioma, miRNA, qRT-PCR.
2, a gene located on 22q, is more frequent in fibroblastic than in meningothelial meningioma, suggesting different drivers for tumorigenesis in the mesenchymal (fibroblastic) and epithelial (meningothelial) lineages of benign meningioma. Deregulated signaling pathways associated with meningioma include Wnt, hedgehog, transforming growth factor–β (TGFβ), mitogen-activated protein kinase (MAPK)/phosphatidylinositol-3 kinase (PI3K), and vascular endothelial growth factor (VEGF) pathways, and a role for EMT in meningioma genesis has been recently proposed.

Despite their undisputed importance in various cancer types, only a few miRNAs have been associated with meningiomas, including miR-200a, -145, -190, -29c-3p, and -219-5p. MiR-200a was found downregulated in benign meningioma, and its overexpression inhibited meningioma cell growth in vitro and in vivo. Targets of miR-200a include β-catenin, a major component of the Wnt signaling pathway frequently involved in meningioma genesis, and nonmuscle heavy chain IIb, a protein involved in cell migration and division. Another study provided evidence that a combination of high expression of miR-190 and low expression of miR-29c-3p and miR-219-5p significantly correlates with increased recurrence rates in meningiomas. The only study on differential miRNA expression between low-grade and high-grade meningiomas focused on 5 known cancer-related miRNAs, showing that miR-145 is significantly downregulated in WHO grades II and III meningiomas. When reexpressed in higher-grade meningioma cell lines in vitro, miR-145 expression led to decreased proliferation and migratory potential and an increased sensitivity for apoptosis, suggesting an important antimigratory and antiproliferative role for miR-145 in higher-grade meningiomas.

In the present study we aim at deepening the knowledge of miRNA deregulation in meningiomas of different histological subtypes and grades and exploring the clinical usability of miRNAs for prediction of higher-grade meningioma. Additionally, we try to link deregulated miRNAs to signaling pathways associated with meningioma initiation and progression using their validated and potential targets derived from an in silico analysis.

Materials and Methods

Patient Samples

For array analysis, we retrospectively selected 55 patients with meningioma of different histological subtypes and WHO grades. As a validation set for quantitative real-time (qRT-PCR, we collected samples from 125 consecutive patients who received surgery for meningioma in our local neurosurgery department between January 2012 and October 2013 (see Supplementary Table S1 for details). Samples were obtained at the time of surgery with patients’ informed consent, subsequently frozen in liquid nitrogen, and stored at −70°C. All tumors have been reclassified according to the current WHO grading. After confirmation of representativeness and histological subtypes of the kryoslides by a neuropathologist, adjacent slides were used for RNA isolation. Total RNA including miRNA was isolated with the miRNeasy Kit (Qiagen) according to manufacturer’s instructions. Structural integrity of RNA was validated on a Bioanalyzer (Agilent Technologies). In the validation set, 30 tumors were excluded from analysis (13 were already part of the array set, 10 tumor slides were not representative, and 7 tumors did not yield sufficient RNA concentration or quality), leaving a total of 95 samples available for qRT analysis.

MiRNA Expression Profile

Expression of 1205 human miRNAs was determined using Agilent human miRNA microarray (miRBase release 16.0) according to manufacturer’s protocol. In short, 100 ng total RNA including miRNAs was dephosphorylated and subsequently labeled through addition of a cyanine dye (Cy3-pCp). Labeled RNA is hybridized to the microarray containing 40 replicates for each of the 1205 features for exactly 20 h. After washing and drying, the array was scanned in an Agilent microarray scanner, and expression values were calculated using the Agilent Feature Extraction Tool.

Data Processing and Statistical Classification

Raw data were normalized with quantile normalization. Differentially expressed miRNAs (at least 2-fold) among meningothelial, fibroblastic, and transitional meningioma and among WHO grades I, II, and III meningioma were identified using a pairwise 2-sided t-test (P < .05) (for the full list, see Supplementary Table S2). P-values were adjusted for multiple testing by using the Benjamini–Hochberg procedure with false discovery rate (FDR) correction (http://www.jstor.org/stable/2346101). We also performed pairwise classification of the different meningioma subsets in a total of 12 different classification scenarios using support vector machines (SVMs) with linear kernel (see Supplementary Tables S3 and S4) as implemented in the R e1071 package. SVMs were evaluated by applying a standard 5-fold cross-validation. To account for variations in the random partitioning into sample subsets, we repeated the cross-validation runs 20 times. In addition we carried out the same procedure using randomly permuted class labels, such that 20 so-called permutation tests were applied for each subset size to test for potential overtraining.

Validation of Differentially Expressed MiRNAs

We performed a 2-step validation of the array results using qRT-PCR. Initially, we profiled 12 deregulated miRNAs in the array samples to validate the array findings. We used the miScript PCR System (Qiagen) for cDNA synthesis and RT-PCR according to the manufacturer’s recommendations. In short, 200–500 ng total RNA including miRNA was used for first strand synthesis using the miScript II RT Kit in a total volume of 20 μL. The generated cDNA served as input in the subsequent RT-PCR using either 1 μL of a 1:50 (500 ng input) dilution or 1 μL of a 1:5 (200 ng input) dilution per PCR (depending on the primer assay used) with the miScript SYBR Green PCR Kit and miScript Primer Assays on a StepOnePlus Real-Time PCR System (Applied Biosystems). The short RNA RNA66B was used as endogenous control. All reactions were performed in duplicates, and mean cycle threshold (CT) values of the duplicates were used to calculate the ΔCT for each miRNA compared with the endogenous control in each sample. Mean ΔCT was calculated for each group, and significant expression
differences between the groups were determined using a 2-tailed t-test \( (P < .05) \). To generalize our findings, we performed profiling of 6 miRNAs (miR-34a*, -136, -195, -376c, and -497) that proved deregulation in the array set in an independent validation set of 95 meningioma samples accordingly (200 ng RNA input per RT reaction, 1 μL of 1:5 diluted RT reaction as input per PCR). Raw ΔCT values for all samples and miRNAs are given in Supplementary Table S5. Clinical utility of these miRNAs for differentiation of WHO grade I versus grade II meningioma was assessed with a receiver operator characteristics analysis and SVM (radial kernel)-based classification analysis using qRT data of the validation and array sample sets as the training and test set, respectively. Permutation tests (10,000-fold) have been conducted to exclude possible overtraining of the model.

In silico Analysis for Identification of Putative Novel Targets

After validation of downregulation of miR-34a*, -136, -195, -376c, and -497 in higher-grade meningioma, we performed an in silico analysis in order to identify novel putative target genes potentially regulated by these miRs. Target gene prediction was done using miRDB and miRWalk.\(^{34,15}\) As downregulation of a potentially regulating miRNA should lead to overexpression of the target gene/protein, we searched for an overlap of the predicted targets with (i) genes overexpressed in higher-grade or metabolically aggressive low-grade compared with benign low-grade meningiomas\(^{16}\) and (ii) proteins overexpressed in higher-grade compared with low-grade meningiomas in a recent proteomic study.\(^{17}\)

Results

In order to identify differentially expressed miRNAs in meningioma subtypes, we performed miRNA expression profiling of 1205 miRNAs in 55 meningioma samples, including meningothelial, fibroblastic, transitional, atypical, and anaplastic meningioma. We computed pairwise median expression differences between each of the aforementioned groups and identified significantly deregulated miRNAs, defined as miRNAs with an at least 2-fold median expression difference and an FDR-adjusted \( P \)-value \( < .05 \). Based on these criteria, a total of 57 miRNAs have been found to be differentially expressed in meningiomas of different subtypes. Hierarchical clustering to these 57 miRNAs revealed 2 clusters, one containing 6 atypical, 10 anaplastic, and only 3 WHO grade I meningiomas (2 meningothelial and 1 transitional), and the second cluster consisting of the remaining 30 benign, 4 atypical, and 2 anaplastic meningiomas (Fig. 1). We also performed pairwise classification of samples based on their expression profiles using SVMs. For example, for differentiation of meningothelial from fibroblastic meningiomas, we reached an overall accuracy of 89.3% using a 5-miRNA profile (more detailed information on classification scenarios and significantly deregulated miRNAs is in Supplementary Tables S2–S4).

Differentially Expressed MiRNAs in WHO Grade I Meningioma Subtypes

Focusing on only the common type of meningiomas, a total of 13 miRNAs were more than 2-fold differentially expressed among different histological subtypes. In detail, 7 miRNAs were downregulated in meningothelial meningiomas with miR-222, -195, and -497 downregulated in meningothelial compared with both fibroblastic or transitional meningioma, whereas differential expression of miR-223, -224*, 199a-3p, and -5p was only significant compared with fibroblastic meningioma. Six miRNAs were significantly upregulated in meningothelial meningioma, with miR-181a being significant for both meningothelial versus fibroblastic and versus transitional meningioma and 5 (miR-181b, -204, -218, -451, and -873) significantly deregulated in only meningothelial versus fibroblastic meningioma. There were no significantly deregulated miRNAs between transitional and fibroblastic meningiomas (Fig. 2A). Interestingly, 3 of the 6 upregulated miRNAs were located on chromosome 9.

Differentially Expressed MiRNAs in WHO Grades II and III Meningiomas

In order to obtain a comprehensive picture of miRNA deregulation during meningioma progression, we compared the median expression of miRNAs in atypical and anaplastic meningiomas with the median expression in grade I meningiomas (all histological subtypes combined and each subtype separately). In atypical meningioma, we found downregulation of miR-30a*, -146b-5p, and -873 compared with meningothelial meningioma and downregulation of miR-29c, -199-5p, and -3p and upregulation of miR-181a compared with fibroblastic meningioma. There were no miRNAs significantly deregulated in transitional meningioma alone or all common-type meningiomas combined compared with grade II meningioma. Additionally, we found overexpression of miR-21 in grade III compared with grade II meningioma.

In grade III meningioma, we identified a total of 51 deregulated miRNAs compared with either WHO grade I subtype alone or combined (Fig. 2B). In detail, totals of 7, 24, and 30 miRNAs were significantly deregulated in grade III meningioma compared with meningothelial, fibroblastic, and transitional meningiomas, respectively. The 51 significantly deregulated miRNAs are distributed among 11 chromosomes with a significant enrichment on chromosomes 14 (12 miRNAs) and 9 (6 miRNAs) as revealed by GeneTrail analysis of miRNA precursor genes \( (P < .05) \).\(^{18}\) The miRNAs on 14q are located within 2 clusters: 3 miRNAs are located at 14q32.2, about 10–20 kb down-stream of the gene MEG3, and 9 miRNAs are located at 14q32.31, an intergenic miRNA cluster spanning about 42 kb with 42 known miRNAs. Five of the 6 deregulated miRNAs on chromosome 9 are located on 9q, with 2 (ie, miR-23b and miR-27b) lying ~150 bp apart from each other on 9q22.32.

Quantitative Real-Time PCR Validation

In order to validate our findings, we chose a 2-step validation approach using qRT–PCR. We selected 12 miRNAs for confirmation of deregulated expression in the same 55 samples used for array analysis (see Supplementary Table S6). We were able to confirm a 3- to 5-fold downregulation of miR-222, -195, -497, and -199a-3p in meningothelial meningioma compared with transitional and fibroblastic meningiomas, and of miR-199a-3p in meningothelial compared with fibroblastic

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meningioma (Supplementary Fig. S1A). Furthermore, for 9 of 12 miRNAs we found decreasing expression with increasing tumor grade, but due to high interindividual variation among the samples of the same tumor subtype, this trend was only significant for 6 miRNAs (miR-195, -218, -497, -376c, -136, and -34a*) in WHO grade III versus grade I meningiomas (Supplementary Fig. S1B). Additionally, miR-34a* showed significant differential expression between grades II and III and miR-101 between

Fig. 1. Hierarchical clustering of the array samples based on expression of the 57 miRNAs with significant expression differences among WHO grades or histological subtypes. Cluster A contains 6 grade II (orange), 10 grade III (red), and only 3 grade I meningiomas, and cluster B consists of the remaining 30 grade I, 4 grade II, and 2 grade III meningiomas. Meningothelial, transitional, fibroblastic, grade II, and grade III meningiomas are indicated with blue, green, yellow, orange, and red bars, respectively.
grades I and II meningiomas. The strongest downregulation was detected for miR-376c and -136, with \( \sim 15 \) -fold decrease from fibroblastic to grade III meningioma.

For independent confirmation of results, we performed additional qRT-PCRs for 6 miRNAs in 95 consecutively collected meningioma samples (ie, validation set, Fig. 3). We could confirm significant downregulation of miR-195, -497, -376c, and -34a* in grade III compared with grade I meningioma. Additionally, miR-497 and -376c were significantly downregulated in meningothelial compared with fibroblastic meningioma (see Supplementary Table S6).

**Receiver Operator Characteristics Analysis**

To assess clinical utility of miRNA expression as a biomarker, we generated SVM-based prediction models for every possible combination of miR-136, -195, -222, -497, -376c, and -34a* to classify WHO grade I from grade II meningioma. The best
results for a single miRNA model were achieved for miR-136 and -34a*, with areas under the curve (AUCs) of 0.769 and 0.718 in the training set and 0.741 and 0.659 in the test set, respectively (Supplementary Table S9, Fig. 4). The best model based on the combination of miR-222, -497, -34a*, and -136 achieved a specificity, sensitivity, and AUC of 0.97, 0.57, and 0.82 in the training set and 0.91, 0.60, and 0.75 in the test set, respectively.

Validated Target Genes of Meningioma Deregulated MiRNAs

To complete the picture of deregulated miRNA expression and its potential influence on gene expression in meningiomas, we conducted a PubMed search for each of the miRNAs successfully validated in qRT-PCR analysis and searched for experimentally validated target genes. The inclusion criterion for experimental validation was direct interaction between miRNA and the 3′ untranslated region of the target gene, as evidenced by a luciferase assay system (Table 1, references included in Supplementary material). In total, we found evidence for 38 and 19 validated gene targets for miR-195 and -497, respectively, with 14 targets common to both miRNAs (reflecting the common seed sequence of the miR-15 family). Considerably fewer validated targets were found for miR-376c (6 targets), -136, and -34a* (4 targets each). For several of the validated targets, overexpression in meningioma, especially in higher-grade meningioma, has been previously demonstrated. Pathways affected by deregulation include cell cycle (cyclin D1–D3 [CCND1–3], Wee1), apoptosis (B-cell lymphoma 2 [Bcl-2], X-linked inhibitor of apoptosis protein [XIAP]), TGFβ (TGFα, TGFβ receptor 1 [TGFBR1], activin A receptor type 1C [ACVRL1]), and MAPK signaling (growth factor receptor-bound protein 2 [GRB2], v-raf-1 murine leukemia viral oncogene homolog 1 [RAF1]).

Putative Novel Target Genes of Deregulated MiRNAs in Meningiomas Associated With Progression or Recurrence

Searching for novel putative target genes of deregulated miRNAs in meningiomas, we performed an in silico analysis where we compared the predicted target genes of miR-34a*, -136, -195, -376c, and -497 with genes or proteins overexpressed in higher-grade or recurrent meningiomas from 2 recent publications. Combining the results of these publications, 122 genes and 111 proteins were overexpressed in higher-grade or recurrent meningiomas, with a total of 31 and 23 genes being putative novel targets of miR-34a*, -136, -195, -376c, or -497 and 3 (CCND2, CCNE1, and IGF1R) being already validated targets of one of these miRNAs (Table 2). Putative novel targets include proteins involved in metabolic processes (arachidonate 15-lipoxygenase type B [ALOX15B], uridine diphosphate–glucose pyrophosphorylase 2 [UGP2], aldehyde dehydrogenase [ALDH]6A1, ALDH7A1, and fructosamine-3-kinase-related protein [FN3KRP]), cytoskeletal proteins (spectrin beta non-erythrocytic [SPTBN]1 and 2, desmoglein 2 [DSG2], tropomyosin 2 [TPM2], and septin [SEPT]2 and 11), and proteins involved in known deregulated signaling pathways in meningiomas (erythrocyte membrane protein band 4.1 like 5 [EPB41L5], signal peptide CUB domain epidermal growth factor like [SCUBE3], phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2β [PIK3C2B], and Crk-like protein [CRKL]).

Discussion

Here we provide, to the best of our knowledge, the first report of deregulated miRNA expression between the histological subtypes of benign meningioma. Our microarray data suggested 7 miRNAs significantly downregulated (miR-199a-3p/5p, -222, -223, -224*, -195, and -497) and 5 miRNAs significantly upregulated (miR-181a/b, -204, -218, -451, and -873) in...
Fig. 4. Receiver operator characteristics (ROC) analysis. ROC curves for SVM-based prediction models for differentiating WHO grade I from grade II meningiomas using expression of miR-34a* and -136, separately, and the 497/34a*/136/222 combined signature in training (left panels) and test set (right panels). AUC value for each analysis is given.
malignant meningothelial compared with fibroblastic meningioma. Interestingly, several of these miRNAs play roles in regulation of EMT, a process that increases cell migratory potential, especially during metastasis. In detail, miR-204-19 has been shown to repress EMT, while miR-222 is overexpressed in aggressive breast cancer showing a more mesenchymal phenotype.20 The deregulation of miRNAs involved in EMT in meningioma supports the possible role of EMT in meningioma development and progression, as has been recently suggested.8 Quantitative RT-PCR confirmed downregulation of miR-199a-3p, -136, -376c, -195, and -497 and detected additional downregulation of miR-34a* and -377 (previously not seen in array analysis) in meningothelial meningioma. In an independent sample set, downregulation of miR-497 as well as miR-376c was further confirmed.

Searching for miRNAs associated with meningioma progression, we detected 2 clusters of miRNAs on chromosome 14q32.2 (DLK1-DIO3 cluster) and 14q32.31 that harbor a total of 11 miRNAs significantly downregulated in WHO grade III meningioma compared with grade I meningioma (predominantly transitional subtype). Loss of chromosome 14q is a frequent and well-known event associated with a higher grade and more aggressive behavior in meningioma.21 However, the relevant putative tumor suppressor genes involved in meningioma progression are still unknown. The DLK1-DIO3 cluster is important during embryonal development and tumorigenesis, expressing the genes DLK1, RTL1, and DIO3 (paternal allele), and MEG3 and MEG8 (maternal allele). Interestingly, MEG3 is downregulated due to promoter hypermethylation in meningothelial meningioma compared with fibroblastic meningioma. Interestingly, several of these miRNAs play roles in regulation of EMT, a process that increases cell migratory potential, especially during metastasis. In detail, miR-204-19 has been shown to repress EMT, while miR-222 is overexpressed in aggressive breast cancer showing a more mesenchymal phenotype.20 The deregulation of miRNAs involved in EMT in meningioma supports the possible role of EMT in meningioma development and progression, as has been recently suggested.8 Quantitative RT-PCR confirmed downregulation of miR-199a-3p, -136, -195, and -497 and detected additional downregulation of miR-34a* and -377 (previously not seen in array analysis) in meningothelial meningioma. In an independent sample set, downregulation of miR-497 as well as miR-376c was further confirmed.

Table 1. Experimentally validated target genes of meningioma-deregulated miRNAs

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target Gene</th>
<th>Known Deregulation in Meningioma</th>
</tr>
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<tbody>
<tr>
<td>miR-136</td>
<td>PTEN, BCL2, MTDH, PPP2R2A</td>
<td>BCL2, S1, BCL2S2, MTDHS2, BCL2S4</td>
</tr>
<tr>
<td>miR-34a*</td>
<td>XIAP, TNF, SP4, JUN</td>
<td>JUN, IGFl, S8, 16, TGfl, S17</td>
</tr>
<tr>
<td>miR-376c</td>
<td>ACVR1, ALKS, TGFa, GRB2</td>
<td>MIIR, S2, 4, 5</td>
</tr>
</tbody>
</table>

Table 2. Overexpressed genes potentially regulated by deregulated miRNAs in higher-grade meningiomas

<table>
<thead>
<tr>
<th>Gene Symbols</th>
<th>Serna et al16</th>
<th>Sharma et al17</th>
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<tbody>
<tr>
<td>Total no. of deregulated genes/proteins</td>
<td>222</td>
<td>294</td>
</tr>
<tr>
<td>Total no. of genes/proteins overexpressed</td>
<td>146</td>
<td>111</td>
</tr>
<tr>
<td>Overlap with predicted targets of miR-34a*, -136, -195, -376c, and -497</td>
<td>31</td>
<td>23</td>
</tr>
</tbody>
</table>

References S1–S76 included in Supplementary material.

Experimentally validated targets are indicated in bold.
high-grade meningiomas.22 Additionally, 7 of the miRNAs in this cluster regulate expression of transcription factors, including TWIST1, that are linked to EMT in cancer.23 Upregulation of the 14q32.2 cluster has been shown for hepatocellular carcinoma,24 defining a novel, stem cell–like subtype of liver cancer. Downregulation of miRNAs in the 14q32.31 cluster has recently been shown for renal, breast, and ovarian cancer; glioblastoma;25 gastrointestinal stromal tumors;26; and neuroblastoma.27 Additionally, we found downregulation of miR-34 in WHO grade III meningioma, whose precursor is located on 1p36, a region deleted in up to 90% of grade III meningioma and strongly associated with meningioma progression.28

In our study, we confirmed downregulation of miR-34a, -195, -136, and -376c in higher-grade meningiomas with qRT-PCR. Experimentally validated and putative novel target genes of these miRNAs, which show overexpression in high-grade or aggressive meningiomas, can be linked to several pathways implicated in meningioma genesis and progression, such as the Wnt/β-catenin pathway and the TGFβ, MAPK/PI3K, and VEGF pathways (see Table 1 and Supplementary Table S8 for references). In detail, several components of the Wnt/β-catenin pathways are validated targets of the aforementioned miRNAs, including CCND1, the jun proto-oncogene (JUN), VEGF, and Bcl2-like protein 2 (BCL2L2), or overexpressed putative targets such as EBP41L5. In general, cytoplasmic β-catenin is either (i) bound to the adenomatous polyposis coli (APC) complex, marked with ubiquitin, and subsequently degraded or (ii) bound with E-cadherin as a linker between the plasma membrane and actin skeleton regulating cell–cell contacts at the adherens junction. In meningioma, there are multiple reports of decreased E-cadherin expression at the plasma membrane and correlated with increasing tumor grade or invasive growth.10,29,30 There are also reports of downregulation of membrane-bound and upregulation of cytoplasmic or nuclear β-catenin in higher-grade meningiomas.8,31 In the nucleus, β-catenin binds to T-cell factor–lymphoid enhancer factor transcription factor, inducing transcription of its target genes, including CCND1, JUN, VEGF, and BCL2L2.32–35 Overexpression of VEGF and CCND1 in meningiomas has been shown previously. During mouse gastrulation, overexpression of EBP41L5, a member of the band 4.1 protein family, has been shown to destabilize membrane-bound E-cadherin and thereby promote EMT.96 Therefore, we propose that downregulation of miRNAs that specifically regulate translation of Wnt-responsive genes provides a second in line of amplification of the already active Wnt signal in meningioma, resulting in promotion of proliferation and EMT.

Aside from Wnt signaling, TGFβ signaling is also altered in meningioma and plays a dual role in cell proliferation and EMT. Depending on ligand and cellular context, the TGFβ signal has been known to either suppress growth of epithelial cells and low-grade cancers, including benign meningiomas,97 or promote proliferation of mesenchymal cells and metastasis in high-grade cancers.38 TGFβ signaling may be influenced by loss of translational repression of ACVR1C, TGFBR1, and ACVR2A as validated targets of miR-376c and -195 or by overexpressed SPTBN1 or SCUBE3 as putative targets of downregulated miR-376c, thereby enhancing TGFβ signals in higher-grade meningiomas. In detail, SPTBN1 has been shown to bind Smad3 upon activation of TGFβ signaling and facilitate the transport of the Smad protein complex into the nucleus.39 Of note, SPTBN1 has been identified as the molecular binding partner of neurofibromatosis 2 and DAL-1, two proteins associated with meningioma initiation.40,41 Additionally, activation of TGFβ signaling by SCUBE3 promotes EMT and invasiveness in lung cancer cells.42

Several components of MAPK/PI3K signaling are targets of deregulated miRNAs in meningioma, including GRB2, RAF1, MAPK8, TGFBR1, and conserved helix-loop-helix ubiquitous kinase (CHUK; validated), or PIK3C2B (predicted). Of note, PIK3C2B binds to GRB2 in cytoplasm and is translocated to epidermal growth factor receptor upon activation.43 MAPK and DAPK1 phosphatidylinositol-3-phosphate signaling contribute to proliferation and regulation of apoptosis in malignant meningiomas.44 Angiogenesis in meningioma is promoted by differential overexpression of VEGF, a validated target gene of miR-195, in different WHO grade I meningiomas (10-fold overexpressed in meningothelial compared with fibroblastic meningioma) and even stronger expression in higher-grade meningiomas.45,46

Recently, alterations in metabolic pathways have been implicated in high-grade meningiomas and in the subgroup of clinically aggressive low-grade meningiomas, featuring differential levels of several metabolites, indicating increased membrane turnover, proliferation, energy demand, and hypoxic conditions in the tumor.16,47 Several validated and putative target genes of downregulated miRNAs overexpressed in higher-grade meningioma are elements of metabolic pathways and might reflect the metabolic aggressiveness of these meningiomas, including fatty acid synthase (FASN), adenylate kinase 3 (AK3), and UGP2. In detail, increased membrane turnover and proliferation include changes in fatty acid metabolism and degradation. Overexpression of FASN, a validated gene target of miR-195, has been recently shown for higher-grade and especially recurrent WHO grade I meningiomas.48,49 Increased energy demand might be reflected by upregulation of AK3, a mitochondrial enzyme involved in generation of ATP, which is a putative target of miR-376c. Hypoxic conditions regulate expression of many enzymes in glycolgen metabolism, including UGP2,50 a putative target of miR-376c, which has recently been shown to be overexpressed in atypical meningioma.51

Differential of clinically aggressive from benign grade I meningioma remains a great challenge, and even histological diagnosis of grade II meningioma can prove difficult in some cases. Therefore, biomarkers that distinguish between benign grade I meningioma and more aggressive grade I or grade II meningiomas would be of substantial clinical benefit. Unfortunately, the patient cohort in our study did not include recurrent grade I meningioma cases, so further research is needed to identify miRNAs relevant for such cases. But here we tried to explore the usefulness of miRNAs as biomarkers for grade II meningiomas. Using the qRT data, we were able to build an SVM prediction model based on 4 miRNAs (miR-222, -34a*, -136, and -497) that differentiated grade II from grade I meningiomas with an AUC of 0.75 and a specificity of 0.91, but a low sensitivity of 0.60. Although the predictive power of this model is too low for clinical implementation, it nevertheless provides a sound starting point for further analysis. As we included only 6 miRNAs as possible miRNA candidates for our model, further
miRNAs should be tested in order to improve sensitivity of our model.

Taken together, we were able to identify deregulated miRNAs in WHO grade I meningiomas that play a role in EMT and support the previous evidence of different drivers for tumorogenesis of fibroblastic and meningothelial meningiomas. Furthermore, we identified heavy downregulation of miRNAs clustered on 14q32 in WHO grade III meningioma that regulate translation of genes belonging to signaling pathways known to be important for meningioma genesis and progression, providing a second in line amplification of growth promoting cellular signals. We also provided first evidence for a 4-miRNA signature for diagnosis of grade II meningioma, although a prospective study should be conducted to confirm clinical usefulness of this signature.

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Conflict of interest statement. The authors disclose no potential conflicts of interest.

References


