miR-153-5p suppresses the proliferation, invasion and migration of malignant meningioma via the MEK/ERK signaling pathway by targeting GAB1

Weifeng Chen  
The Second Hospital of Hebei Medical University

Zhao Jiahui  
Capital Medical University

Qianlan Wu  
Handan First Hospital

Yunpeng Shi  
The Second Hospital of Hebei Medical University

Hongjing Yan  
Handan First Hospital

Wu Zhen  
Capital Medical University

Chen Lei  
Department of Neurosurgery, The Fifth Central Hospital of Tianjin

Zongmao Zhao (✉ zzm69@163.com)  
The Second Hospital of Hebei Medical University

Article

**Keywords:** MiR-153-5p, GAB1, malignant meningioma, MEK/ERK signaling pathway, proliferation, invasion, migration

**Posted Date:** September 14th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1971937/v1

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Abstract

Aims: This study aimed to determine the role and correlation of miR-153-5p and GAB1 in the proliferation, invasion and migration of malignant meningioma.

Background: Meningiomas are the most common primary intracranial tumor in adults. For meningioma, the only effective treatment and targeted therapy is excision and radiotherapy. However, some meningiomas are prone to recurring due to specific disease sites or an invasion of blood vessels and nerves, and the prognosis is poor. To discover biomarkers and develop targeted therapies, it is necessary to better understand meningioma oncogenesis.

Methods: Human meningioma tissues were collected from patients diagnosed in pathological examinations. In addition, human malignant meningioma cell lines IOMM-Lee was included. Quantitative RT-PCR was used to detect the miR-153-5p and GAB1 level in the clinical tissues and cell lines. Immunohistochemistry was used to detect GAB1 expression. The specific binding site between miR-153-5p and GAB1 was predicted using bioinformatics, and dual luciferase assay was used to confirm their specific binding relationship. In IOMM-Lee, miR-153-5p was artificially overexpressed or suppressed and target gene expression GAB1 was blocked by siRNA. Cell proliferation, invasion and migration were measured using CCK-8, Transwell and Wound-healing assays. The activity of the MEK/ERK pathway was evaluated by detecting the key factors through Western blotting.

Results: We found that miR-153-5p expression was low and GAB1 mRNA was high in meningioma tissues and IOMM-Lee cell lines. GAB1 is a direct target gene of miR-153-5p. Both miR-153-5p overexpression and GAB1 silencing inhibited malignant meningioma cells proliferation, migration and invasion, and GAB1 silencing partially blocked the effect of miR-153-5p silencing in these respects. We also confirmed that miR-153-5p might regulate MEK/ERK pathway activation by inhibiting GAB1 expression.

Conclusions: miR-153-5p suppresses the proliferation, invasion and migration of malignant meningioma via the MEK/ERK signaling pathway by targeting GAB1, defining miR-153-5p and GAB1 as a potential target for the diagnosis and treatment of malignant meningioma.

Introduction

Meningioma is a frequent type of tumor originating from the central nervous system. The World Health Organization (WHO) currently classes meningioma into three categories: WHO grade I (benign meningioma/nine subtypes), WHO grade II (atypical, clear-cell, and choroid meningioma), WHO grade III (malignant meningioma/anaplastic, papillary, and rhabdoid)\(^1\). Whereas WHO grade I meningioma has a benign clinical course with the prospect of surgical, as well as radiation treatment, WHO grade II and III, notably malignant meningioma, have a more aggressive course with a greater relapse, metastasis, along with poor rates of survival\(^2\). Malignant meningioma had a worse prognosis with ten-year overall survival (OS) rate from 14 to 34% and ten-year progression-free survival (PFS) rate of 0%\(^3\). Thus, it
is critical to explore the accounting cellular along with molecular mechanisms of malignant meningioma and identify possible targets for the development of more effective therapeutic agents.

MiRNAs are a class of endogenous non-coding short RNA molecules, which are about 18–23 nucleotides long and are remarkably conserved. MiRNAs dock to the target gene 3’untranslated region (3’UTR) to create a silencing complex, which prevents the target gene's protein translation. MiRNA molecules participate in tumor cell growth, apoptosis, infiltration, as well as metastasis. It has been revealed recently that several miRNAs, such as miRNA-200a, miRNA-145, or miRNA-224, are expressed aberrantly in meningioma. miRNA microarrays have demonstrated that abnormal miRNA expression may act as potential diagnostic and prognostic biomarkers for cancer, and many potential miRNAs were used as diagnostic biomarkers and therapeutic targets for meningiomas. MiR-153-5p is a newly discovered small-molecule coding RNA. Recently, studies have exhibited that MiR-153-5p participates in the onset and progress of various tumors, for instance, renal cell carcinoma, breast cancer, and colorectal cancer. It has also been shown that miR-153-5p over-expression enhanced the dampening influence of sh-HOTAIRM1 transfection on migration along with infiltration in glioblastoma, which illustrates miR-153-5p plays an indispensable role in the onset and progress of the malignant tumor in the central nervous system. However, the involvement of miR-153-5p in meningioma still has not been reported.

GAB1 is a member of the Grb2-associated binder (GAB) family. The GAB family has been found to regulate the signal transduction of cytokine along with growth factor receptors. Furthermore, GAB1 was shown to influence the development of colorectal cancer tumors. Sang et al reported that GAB1 regulated proliferation along with migration in intrahepatic cholangiocarcinoma. GAB1 silencing could dampen cell growth along with migration in hilar cholangiocarcinoma. Besides, GAB1 was demonstrated to predict a poor prognosis of hepatocellular carcinoma. Our previous work demonstrated that the GAB1 gene rs3805236A>G, as well as rs1397529A>C SNPs, enhanced the incidence of meningioma in the northern Chinese Han population. Additionally, rs1397529A>C may be associated with greater dural infiltration in individuals with meningioma.

According to the NCBI SNP database, the SNP rs1397529 is located in the miR-153-5p -docking site within the 3'UTR of GAB1. GAB1 SNP in the 3'UTR region could influence mRNA stability, as well as translation, which could remarkably impact gene expression through abolishing, weakening, or generating miR-153-5p docking sites, and lead to meningioma. Some studies have combined gene polymorphism with the regulatory ability of miRNAs to clarify the mechanism of their interaction in disease, but there is no report on the role of miR-153-5p and GAB1 in malignant meningioma. Hence, we demonstrated that miR-153a-5p acts as a tumor repressor via targeting GAB1 in malignant meningioma, despite being unrelated to the GAB1 SNP rs1397529.

**Methods**

**Patient Eligibility**
The flow of patient inclusion is displayed in Figure 1. All patients enrolled in this research work were from the Department of Neurosurgery, the Second Hospital of Hebei Medical University from January 2019 to May 2021. Diagnosis of meningioma was based on the 2016 revision of the World Health Organization (WHO) classification. We excluded patients with (i) a history of other tumors, and (ii) hypertension, diabetes, cerebrovascular disease, other chronic diseases, etc.

Every participant was granted written informed consent prior to surgery. Clinical data were acquired from the clinical records, including age, tumor location, gender, smoking status, intake of alcohol, tumor size, peritumor edema along with tumor stage. The tumor specimens were frozen and kept in liquid nitrogen as soon as they were excised. The Ethics Committees of Hebei Medical University's Second Hospital approved this research work. We could confirm that all methods were performed in accordance with the relevant guidelines and regulations.

**Quantitative real-time PCR analysis**

Real-time PCR was carried out as documented previously. Isolation of RNA was performed with the Trizol Kit reagent (Thermo Fisher Scientific) as described by the manufacturer. miR-153-5p expression was assessed with the MicroRNA Assay kit (Thermo Fisher Scientific), with RNU6B serving as the standardization control. Generation of cDNA was done with the M-MLV Reverse Transcriptase (Promega) to analyze GAB1 transcript expression, with GAPDH serving as the control. The quantitative PCR was performed on an ABI7300 machine. Two compound holes were set for each hole. $2^{-\Delta\Delta Ct}$ approach was adopted for relative quantification of gene expression level. The primers utilized in this work included:

- **miR-153-5p**, Forward: 5′- GGTGGCCAGTGTCATTGTTTTGT -3′; Reverse: 5′- TTGTGACTATGCAACTGGGCT -3′; U6, Forward: 5′-CTCGCTTCGGCAGCACATATACT -3′; U6, Reverse: 5′- CTGGTTGGTGTTGGTGGTCT-3′; GAB1, Forward: 5′-GAGCGGTGGTGAAGTGGTCT-3′, Reverse: 5′-CCTCGCTGTCTGCTACCAAGT-3′; GAPDH, Forward: 5′-GGAAGCTTGTCATCAATGGAAATC-3′, Reverse: 5′-TGATGACCCTTTTGGCTCCC-3′

**Immunohistochemistry**

Immunohistochemical staining of the human meningioma tissues and normal dura mater tissues was done as documented previously. Following retrieval of antigens using citric acid (pH 6.0), 1% H2O2 was employed to repress the activity of endogenous peroxidase. Thereafter, a primary monoclonal anti-GAB1 (1:200 dilution) was applied, and subsequently, secondary antibodies linked to a peroxidase-conjugated dextran polymer were applied. Diaminobenzidine was applied for visualization of the immunoreaction, and 10% hematoxylin was applied for counterstaining. In succeeding sections, the method's specificity was determined via substituting protein blocking solution for the primary antibodies. The semiquantitative integration approach was used to score immunohistochemistry data. We observed five random fields of view at high magnification (400) for every specimen. Scoring of the results was done on the basis of the following criteria: First, staining area score (SAS; ≤ 5%: 0; 5–25%: 1; 26–50%: 2;...
51–75%: 3 and > 75%: 4). Second, staining intensity (SI; light brown: 1; moderate brown: 2 and tan: 3). ICS score = SAS×SI was used to determine the Immunohistochemical (IHC) score.

**Western blotting**

We performed western blotting as documented previously. Cell lysis was done with the RIPA Lysis along with the Extraction Buffer. Lysis of the glioma cells was done with a system consisting of a protease inhibitor mixture. Prepare BCA standard according to the user manual (Thermo Scientific, US). The concentration of each sample was calculated based on standard curve analysis. Denaturation of protein samples was done using the Laemmli buffer at 95°C for five minutes before electrophoresis. The proteins were fractionated on the SDS-PAGE gel. After that, proteins were blotted onto the PVDF membrane, then inoculated with 5% (w/v) dried skimmed milk powder in TBST for one hour under gentle shaking at room temperature (RT). Afterward, they were incubated with the first antibody (GAB1, MEK1, p-ERK1/2, p-MEK1, ERK1/2, and GAPDH, Bioworld) in TBST overnight at 4°C. After being rinsed with TBST thrice (ten minutes per time), the samples were inoculated with secondary antibodies (Goat Anti-rabbit secondary antibody and goat anti-mouse secondary antibody, 1:3000 dilution, Beyotime) at room temperature away from light for 1–2 h. ECL Substrate (Clarity Max Western; Bio-Rad) was employed to visualize the generated bands, and protein bands were densitometrically assessed via the Quantity One software (Bio-Rad).

**Cell lines, cell culture, and transient transfection**

The IOMM-Lee human malignant meningioma cell line was acquired from Shanghai Baiye Biotech Co, Ltd (Shanghai, China). Lipofectamine™ 2000 was used to transiently transfect the cells (Invitrogen). IOMM-Lee cells were grown under 5% CO₂ along with 37 °C conditions in DMEM enriched with 10% FBS to confluence. After that, we digested the cells in every group, then made the solution in every group into a suspension of cells to determine the number of cells. Thereafter, we adjusted the concentration of cells to 1 × 10⁵/ml. We planted the cells 12 hours prior to treatment and their confluence was 60–70% at treatment time.

**Dual-Luciferase Assay**

The dual-luciferase enzyme assay was done with the Dual-Luciferase enzyme Reporter Assay (Promega, USA) as described by the manufacturer. In brief, we transfected the H293T cells with luciferase, renilla, and wild-type (WT) GAB1 3’UTR and mutant (MUT) GAB1 3’UTR. Cell lysis was done, then luciferase along with renilla substrate was introduced, and assessed after 24 h. pMIR-REPORT Luciferase was synthesized by Obio Technology (Shanghai, China). Luciferase enzyme reporter gene assays were done with the Dual-Luciferase Enzyme Reporter Assay System. There were four deletion mutation binding sites between mutant (MUT) GAB1 3’UTR and the wild-type miR-153-5p docking site (Figure 4A).

**CCK-8 Assays**
Cell proliferation was measured via the CCK-8 assay. IOMM-Lee cells were planted into 96-well plates (2×10^4 cells/ml; 100 µl/well). The Cell Proliferation Reagent CCK-8 (Yeasen Biotechnology (Shanghai) #40203ES76) was used to measure cell proliferation. CCK-8 reagents were introduced to the cells 24 hours, 48 hours, and 72 hours post transfection and incubated for four hours under a humidified incubator. The absorbance at 450 nm wavelength was determined with a microplate reader.

**Transwell Assays**

Twenty-four-well inserts were utilized in the transwell assays (Corning, United States). Seventy-two hours post transfection, we inoculated 2 × 10^4 cells in a serum-free medium (200 µL) enriched with 0.1% BSA. Thereafter, we introduced 600 µL of medium enriched with 10% FBS to the bottom compartment and co-inoculated for an additional 48 hours. The cells that had migrated to the insert membrane's bottom were fixed (in 4% formaldehyde), rinsed, and placed in 0.5 % triton-100. Thereafter, we counted the cells at a magnification of 200´ with a microscope.

**Wound-healing assays**

We inoculated 1×10^5 cells in 5 mL medium into 6-cm dishes. A wound line was created with a 200-L pipette tip when cells were adherent and confluent. Images of the scratches were acquired at 0,6,24, 48, and 72 h after making the scratch with an Olympus Imaging System Microscope (magnification 40×). The experiment was repeated three times, and the area was calculated using the Image J software.

**Statistical analysis**

SPSS (v23.0) and GraphPad software (v8.0) were utilized to conduct statistical analyses on the IHC and cell experimental data. McNemar's test along with one-way ANOVA was adopted to assess the IHC and cell experimental data, and subsequently by Dunnett or Bonferroni post hoc analysis. As a cut-off value for GAB1 expression, the median value was used. The Wilcoxon rank-sum test or the Kruskal-Wallis rank-sum test, as well as logistic regression, were employed to explore the correlation between clinicopathologic features and GAB1 expression. The area under the curve (AUC) and receiver operating characteristic (ROC) curves were utilized to assess the diagnostic and predictive abilities of miR-153-5p and GAB1. The Spearman correlation coefficient and statistical significance were adopted to assess the correlation analysis of gene expression. Statistical significance was defined as a p-value or FDR value < 0.05.

**Result**

**Patient Characteristics**

In total, 50 meningioma patients were recruited in this study. There were WHO grade I 30(60%), WHO grade II 15 (30%), and WHO grade III 5 (10%). There were 17 males and 33 females, aged 24-76 years, with an average age of (56.82±11.58) years.
Expression and correlation of miR-153-5p and GAB1 in normal meninge and menigioma

Since our previous study showed that GAB1 3’UTR gene polymorphism was associated with the risk of menigioma, and we predicted the direct interaction between miR-153-5p and GAB1 3’UTR on the TargetScan website (http://www.targetscan.org). In order to further explore the role of GAB1 and miR-153-5p in menigioma, we detected the expression of GAB1 and miR-153-5p in menigioma.

In contrast to normal meninge tissues, menigioma tissues expressed remarkably lower miR-153-5p and elevated GAB1 mRNA (Figure 2A1, A3). In comparison with WHO grade I, there were also lower miR-153-5p and higher GAB1 expression in WHO grade II and WHO grade III. However, there is no difference between the latter two (Figure 2A2, A4). The immunohistochemistry results showed that the expression of GAB1 was mainly found in the cellular cytoplasm (Figure 2, B1-4). Immunohistochemical staining (+++) of 28 cases (56%) and immunohistochemical staining (+++) of 7 cases (14%) of menigioma tissue group; 1 cases of normal dura mater immunohistochemical staining (+) cases (10%) and immunohistochemical staining (+++) in 0 case (0%). The number of GAB1 positive cells in the menigioma tissue group was higher than that in the normal dura mater group ($P<0.0001$) (Figure 2 C). It was exhibited that the relative expression of miR-153-5p in the tissues of menigioma patients was negatively linked with the relative expression of GAB1 mRNA by Pearson correlation ($r=-0.521$, $P<0.001$), which could be considered that both of them had a moderate degree of correlation (Figure 2D). The ROC curves were drawn (Figure 2 E-F) and the results were compared as follows: The ROC curves of miR-153-5p and GAB1 were remarkably different ($P<0.001$) in menigioma, both of them had higher accuracy for menigioma diagnosis.

Using the Mann-Whitney U test, the results showed that the dura mater invasion group had down-regulated miR-153-5p mRNA expression and up-regulated GAB1 mRNA expression compared with the dura mater non-invasion group, (all $P<0.05$, Figure 3A,B). There were also significant differences in tumor grade, (all $P<0.05$, Figure 2A2,B4). However, there was no significant correlation between the mRNA expression of both and the patient’s age, gender, and peritumoral edema ($P>0.05$). Binary logistic regression was used to assess whether both are risk factors for dural invasion and tumor grade, both miR-153-5p downregulation and GAB1 upregulation were risk factors for dural invasion and tumor grade (all $P<0.05$, Table 1).

Table 1 Correlations of miR-153-5p and GAB1 mRNA expression with clinical characteristics in menigioma patients
Stratified analysis of miR-153-5p, GAB1 mRNA, and protein expression differences according to different genotypes

Under the optimal model (recessive model), stratified analysis was performed according to the genotypes of GAB1 4 SNPs (rs3805246, rs3828512, rs3805236, and rs1397529), respectively. miR-153-5p and GAB1 mRNA expression levels were compared in different genotype subgroups. The results were not significantly different among them Figure 4.

Similarly, GAB1 protein expression level was compared in different genotype subgroups. We found there was a statistically significant in GAB1 protein expression at rs1397529 (AC vs AA). The protein expression level of GAB1 is up-regulated in meningioma patients with AC genotype compared with patients with AA genotype \( P < 0.05 \). There were no statistical differences in other sites \( P > 0.05 \). The result was shown in Table 2.

Table 2 Expression of GAB1 protein stratified analysis according to different genotypes
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<td>+</td>
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<tr>
<td></td>
<td>AA</td>
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**Transfection efficiency of miRNA and siRNA**

The transfection results indicated that FAM NC (fluorescein amidite negative control) transfected IOMM-Lee cells, and the transfection efficiency was higher after 6 hours of transfection, and the highest transfection efficiency was about 70%. The optimal transfection conditions were: FAM NC 2 μL: Lipo2000 2 μL (Figure 5A). In comparison to the NC groups, miR-153-5p contents were remarkably increased in IOMM-Lee cells inoculated with miR-153-5p mimics ($P<0.001$). By contrast, miR-153-5p expressions revealed that the miR-153-5p mimic and inhibitor were very efficient at transfection and so appropriate for further experiments (Figure 5B). Total RNA along with protein was extracted 48 hours after si-GAB1 transfection. RT-qPCR analysis revealed that si-GAB1 cell transfects had a greater than 60% reduction in GAB1 mRNA expression ($P<0.01$). This established that the siRNA was capable of successfully inhibiting GAB1 expression and could be employed in further experiments (Figure 5C).

**GAB1 was a Direct Target of miR-153-5p**

Subsequently, we further screened the downstream target of miR-153-5p through the database in TargetScan (http://www.targetscan.org), which predicted that miR-153-5p could bind with four positions of the 3’UTR of GAB1 (Figure 6A). Because previous investigations have found that the gene polymorphism of GAB1 is related to meningioma susceptibility, and the SNP rs1397529 is located in the 3’UTR, the site-directed mutation was used firstly to introduce base substitution to this miR-153-5p binding site of luciferase reporters to produce mutated plasmids using Site-Directed Gene Mutagenesis Kit (Beyotime).

There was no significant difference in luciferase activity between the GAB1 3’UTR wild-type group or mutant group and the NC group ($P>0.05$, Figure 6B). Interestingly, when all four sites predicted to target miR-153-5p binding to the GAB1 3’UTR were modified with complementary mutations, miR-153-5p was
able to regulate luciferase activity in the wild-type GAB1 3UTR group $P<0.001$ Figure 6C, and the regulatory relationship disappeared when the binding site was mutated $P<0.0001$ Figure 6C.

**miR-153-5p inhibits malignant menigioma cells proliferation, migration and invasion by targeting GAB1**

CCK-8 analysis showed that compared to the NC group, GAB1 siRNA, miR-153-5p overexpression, and GAB1 siRNA+miR-153-5p inhibitor significantly inhibited cell proliferation, while miR-153-5 silencing significantly promoted cell proliferation Figure 7A. The results of the Transwell assay showed that compared with the NC group, GAB1 siRNA, miR-153-5p overexpression, and GAB1 siRNA+miR-153-5p inhibitor significantly reduced cell invasion, while miR-153-5 silencing inhibited cell invasion (Figure 7B, 7C). According to the results of cell scratch migration assay, compared with the NC group, GAB1 siRNA, miR-153-5p overexpression, and GAB1 siRNA+miR-153-5p inhibitor significantly inhibited cell migration. However, miR-153-5 silencing significantly promoted cell migration (Figure 7D, 7E). Both miR-153-5p overexpression and GAB1 silencing could inhibit the proliferation, migration and invasion of malignant meningioma cells, and GAB1 silencing partially blocked the effect of miR-153-5p silencing on proliferation, migration and invasion of malignant meningioma cells, which suggested that miR-153-5p might inhibit malignant meningioma cell proliferation, migration, and invasion by targeting GAB1.

**miR-153-5p exerts its functions by inhibiting the GAB1-MEK/ERK axis.**

We examined the expression of the signature genes of the GAB1-MEK/ERK axis to further understand miR-153-5p's mechanism of action. Overexpression of miR-153-5p resulted in a significant decrease in GAB1 mRNA and protein expression in malignant meningioma cells (all $P < 0.05$, Figure 8A-C). Meanwhile, miR-153-5p inhibitor resulted in increased GAB1 mRNA expression, but did not reach statistical significance ($P 0.05$, Figure 8A). Instead, miR-153-5p inhibitor significantly increased GAB1 protein expression ($P < 0.05$, Figure 8B, 8C). There was an overexpression of miR-153-5p, a decrease in the expression of GAB1 protein, and a significant decrease in the levels of ERK1/2, MEK1, and p-ERK1/2 protein expressions compared to the NC group all $P < 0.01$, Figure 8B, 8C). The expression of p-MEK1 protein was also decreased. The p-MEK1 protein expression also decreased, and the result was not statistically significant. After miR-153-5p was silenced, the expression of GAB1 protein was increased, and MEK1, ERK1/2, p-MEK1, and p-ERK1/2 expressions were also up-regulated at the same time, especially the expression of ERK1/2 reached statistical significance ($P < 0.05$, Figure 8B, 8C). miR-153-5p inhibited expression of GAB1, thereby regulating activation of MEK/ERK pathways.

**Discussion**

In this study, We demonstrated that miR153-5p inhibits GAB1 expression by specifically targeting the 3'UTR of GAB1. In contrast to GAB1, miR153-5p expression in meningioma tissues is low and adversely linked with the clinical stage, which affects malignant meningioma cells proliferation, migration and invasion in vitro by directly modulating GAB1 expression. miR153-5p overexpression downregulates GAB1 expression in malignant meningioma cells, and could activate the MEK/ERK signaling pathway.
Kakkar et al found that 12 of 15 primary meningiomas (80%) were immunopositive for GAB1 protein, and 9 of 16 recurrent meningiomas (56.3%)\textsuperscript{31}. Battu et al showed that in children GAB1 protein immunopositive in meningiomas is as high as 75%, which may be involved in the invasion of meningiomas through P13K/Akt and Hedgehog signaling pathway\textsuperscript{32}. These findings may suggest that GAB1 is involved in the pathogenesis of meningiomas. In this study, we found that GAB1 protein is abnormally highly expressed in meningioma. It has been found that GAB1 gene single nucleotide polymorphisms (SNPs) are closely related to the susceptibility of meningioma and the rs1397529 polymorphism existed in the 3'UTR, which was associated with dural invasion\textsuperscript{23}. In several studies, it has been shown that polymorphisms in the 3'UTR of target genes alter miRNA regulated gene expression and the biological effects of cytokines\textsuperscript{33, 34}. The miR-153-5p and GAB1 mRNA expressions were compared after grouping all the subjects based on their genotypes and no significant differences were found. However, GAB1 protein expression at the rs1397529 (AC vs AA) site was statistically significant. The GAB1 protein in meningioma who carried the AC genotype was lower than individuals with the AA genotype. We speculate that rs1397529 risk genotype AC affects the occurrence and development of meningioma by regulating the protein expression of GAB1.

Recent studies suggest that the biological function of gene polymorphisms in the 3'UTR region is mainly to change the binding site of miRNA, so that the miRNA regulates the target gene changes, thereby participating in the formation of tumors. According to the Target Scan website, miR153-5p is bound to GAB1, but this had not been experimentally proven. A dual-luciferase reporter experiment was conducted to confirm the targeted binding relationship between miR153-5p and GAB1. In the first experiment, the site-directed mutation was used firstly to introduce base substitution to this miR-153-5p binding site rs13975290, the results did not prove that miRNA binding changes by mutating only this site. However, the binding of the two changed when we mutated all the predicted sites. The targeting relationship between the two was confirmed. Hence, the mutation effect of single nucleotide genes appeared to be relatively weak and might not affect gene expression. As an alternative, gene haplotypes might have a better effect on gene expression than single gene polymorphisms. The mutation site in linkage disequilibrium with rs1397529 might play a role in regulating gene expression in diseases. However, this speculation hasn't been verified. Our results also showed that the expression of miR153-5p in meningioma tissues has an opposite trend to that of GAB1.

Many studies have shown that miR-153-5p, as a tumor suppressor gene, contributes to the development of various malignant tumors. Overexpression of miR-153-5p has been shown to enhance the drug sensitivity of breast cancer cells to paclitaxel, inhibit the proliferation and migration of breast cancer cells, and promote apoptosis of breast cancer cells\textsuperscript{35}. In another study, miR-153 expression was lower in ovarian cancer than in adjacent tissues, which inhibited the proliferation and invasion of ovarian cancer cells and decreased their tumorigenesis potential\textsuperscript{36}. Xie et al found that miR-153-5p adsorption promoted the invasion and migration of glioblastomas, suggesting that miR-153-5p downregulation may be associated with tumor growth in glioblastomas\textsuperscript{17}. However, in another study, a different result was obtained. Li et al showed that miR-153-5p was significantly up-regulated in renal clear cell carcinoma and
promoted the generation of renal clear cell carcinoma. The exact reason for these opposing functions is unclear, but it may be related to the differential regulation of target genes.

As it is well known, miRNAs exert their biological functions mainly through the regulation of target genes. It has shown that AGO1 was a direct target of miR-153-5p and that downregulating AGO1 could partially prevent the cancer-promoting effect of miR-153-5p overexpression on renal clear cell carcinoma cells. Wang et al discovered that overexpression of miR-153-5p caused G2/M cell cycle arrest in triple-negative breast cancer cells induced by paclitaxel by downregulating the expression of the target gene CDK1. Other studies have demonstrated that miR-153-5p promoted autophagy and apoptosis of colorectal cancer cells by targeting Bcl-2 and reduced the resistance of colorectal cancer to oxaliplatin. According to Chen et al., miR-153-5p could be down-regulated in liver cancer cells in order to increase the expression of the target gene Rho GTPase-activating protein 18, which was required for tumor cells to migrate. It has also been found that MiR-153-5p targets SNAI2, which was shown to decrease the expression of IncRNA HOTAIM1 by promoting transcription and inhibiting CDH1-mediated transcription regulation, inhibiting the invasion and migration of glioblastoma cells. We have shown for the first time that miR-153-5p binds to the 3'UTR region of wild-type GAB1 and that over-expression of miR-153-5p inhibited GAB1 mRNA and protein levels. Up-regulation of GAB1 mRNA and protein, and silencing of GAB1 after co-transfection, could inhibit the effect of miR-153-5p silencing on proliferation, invasion, and migration of malignant meningioma cells. miR-153-5p inhibited malignant meningioma cell proliferation by targeting GAB1.

The functional study of GAB1 is mainly in the aspect of embryonic organ development, abnormal GAB1 expression could cause cell death and abnormal differentiation. Activation of the MEK/ERK signaling pathway promotes swelling proliferation and activation of tumor cells. Taniguchi et al showed that GAB1 promotes entering MEK/ERK-related signaling pathway promotes the growth of neuroblastoma. Kodama et al. showed that GAB1 promotes the epithelial-mesenchymal transition of hepatocytes by promoting the MEK/ERK signaling pathway. These studies suggest that GAB1 may be involved in the disease progression of meningiomas by regulating the MEK/ERK signaling pathway in meningioma. Our findings confirmed that miR-153-3p may play a role in suppressing tumorigenesis by inhibiting GAB1, thereby inhibiting ERK1/2 and MEK1.

We admit that our study has some limitations. On the one hand, malignant meningioma cells grow in an in vitro environment that is very different from that found in vivo. We would need to conduct further in vivo experiments to observe the miR-153-5p effects of targeting GAB1 on the malignant behavior of malignant meningiomas in vivo. on the other hand, on the other hand, it was not possible to verify the potential regulatory effect of risk haplotypes on transcriptional activity using dual luciferase in this study due to the relatively small sample size, and future studies will require bigger samples.

In conclusion, our research work demonstrated that miR-153-5p may target and repress GAB1, modulate the MEK/ERK signaling pathway and thereby inhibit malignant meningioma cells proliferation, migration.
and invasion. The effects of miR-153-5p on malignant meningioma cells functions may be partially due to its regulation of GAB1 expression and subsequent downstream MEK/ERK activation. Taken together, these results suggest that the newly identified miR-153-5p-GAB1-MEK/ERK axis may serve as promising therapeutic targets and prognostic biomarkers for patients with malignant meningioma.

Declarations

Funding information

This research was supported by National Key R & D Program Intergovernmental Cooperation on International Scientific and Technological Innovation of the Ministry of Science and Technology of China (2017YFE0110400); National Natural Science Foundation of China (81870984); Special Project for the Construction of Hebei Province International Science and Technology Cooperation Base (193977143D); Government funded Project on Training of outstanding Clinical Medical Personnel and Basic Research Projects of Hebei Province in the Year of 2019; Hebei Natural Science Foundation General Project—Beijing-Tianjin-Hebei Basic Research Cooperation Project (H2018206675).

Conflict of interest

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Acknowledgments

All of the authors are grateful to all the healthy volunteers and patients for their participation.

Ethical approval

The experiment was approved by the Institutional Animal Care and Use Committee of Hebei Medical University and the Experimental Ethics Committee of the Second Hospital of Hebei Medical University.

Consent to participate

Before this study, all participants gave informed written consent.

Data availability statement

The supplementary material for this article can be found online. All processed data used in this study can be obtained from the corresponding author on reasonable request.

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**Figures**
Figure 1

Workflow for inclusion and exclusion of patients
Figure 2

Expression and correlation of miR-153-5p and GAB1 in meningioma. denotes $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Figure 3
Stratified analysis of the association of miR-153-5p and GAB1 mRNA expression with clinical characteristics in meningioma patients. denotes *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figure 4

Differential expression of miR-153-5p and GAB1 mRNA stratified analysis according to different genotypes. denotes *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 
Assessment of transfection efficacy denotes *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 
Figure 6

miR-153-5p directly targeted GAB1 in IOMM-Lee cells. denotes $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. 
miR-153-5p inhibits meningioma cell proliferation, migration and invasion by targeting GAB1. denotes \( *P < 0.05, **P < 0.01, ***P < 0.001. \)
miR-153-5p exerts its functions by inhibiting the GAB1-ERK axis. \( *P < 0.05 \), \( **P < 0.01 \), \( ***P < 0.001 \).