MiR-30b-5p inhibits proliferation and promotes apoptosis of medulloblastoma cells via targeting MYB proto-oncogene like 2 (MYBL2)

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ABSTRACT

Medulloblastoma (MB) is the most common malignant brain tumors among children. MiR-30b-5p is a potential tumor suppressor in a variety of human cancers. However, its expression and function in MB remain poorly understood. This study aimed to investigate the expression, role and regulatory mechanism of miR-30b-5p in MB. The expression of miR-30b-5p in MB tissues and cell lines was detected by real-time PCR. The effects of miR-30b-5p on cell proliferation and apoptosis were monitored by CCK-8 (Cell Counting Kit-8) assay, colony formation assay and flow cytometry, respectively. Bioinformatics database TargetScan predicted the target genes of miR-30b-5p. The interaction between miR-30b-5p and MYB proto-oncogene Like 2 (MYBL2) was determined by luciferase reporter gene assay. We demonstrated that the expression of miR-30b-5p was significantly downregulated in MB. Upregulated miR-30b-5p could inhibit the proliferation and induce apoptosis of MB. Moreover, overexpressed miR-30b-5p could increase the expression of BAX but decrease that of Bcl-2. Downregulated miR-30b-5p exerted the opposite effect. MYBL2 was proved to be the target gene of miR-30b-5p and was negatively regulated by miR-30b-5p. These results indicate that miR-30b-5p inhibits the progression of MB via targeting the expression of MYBL2.

Significance of this study

What is already known about this subject?

- Medulloblastoma (MB) is a malignant childhood brain tumor.
- MicroRNAs (miRNAs) are well known to regulate the expression of genes involved in many biological processes.
- miRNAs are well known to regulate the expression of genes involved in many biological processes, such as proliferation, apoptosis, metastasis, tumorigenesis and progression.
- As a tumor suppressor, miR-30b-5p participates in the regulation of human malignant tumor progression.

What are the new findings?

- MiR-30b-5p exerted an antitumor role in MB.
- Upregulated miR-30b-5p could inhibit the proliferation and induce apoptosis of MBs.
- Moreover, overexpressed miR-30b-5p can increase the expression of BAX but decrease that of Bcl-2.
- MYBL2 (MYB proto-oncogene Like 2) was proven to be the target gene of miR-30b-5p and was negatively regulated by miR-30b-5p.

How might these results change the focus of research or clinical practice?

- The results may provide new implications for novel therapeutic approaches for MB.

INTRODUCTION

Medulloblastoma (MB) is a malignant brain tumor widely seen in childhood.[1 2] Although encouraging progress in diagnosis and cancer therapy has been achieved in the past decade, the overall survival rate still remains unfavorable, and most patients succumb to progressive disease.[3 4] Hence, an in-depth investigation into the underlying mechanism of MB is highly desirable to provide a novel therapeutic regimen and improve the prognosis of patients.

MicroRNAs (miRNAs) are a class of RNA molecules containing approximately 18–25 nucleotides, specifically binding to the 3′-untranslated region (3′-UTR) of target genes,[5] and it is widely accepted that they regulate the expression of genes involved in many biological processes, such as proliferation, apoptosis, metastasis, tumorigenesis and progression.[6–10] The abnormal expression of miRNA is closely related to the tumorigenesis and progression of MB.[11 12] It was reported that the loss of miR-34a accelerates the formation of MB.[13] MiR-31 impedes the proliferation of MB cells by inhibiting DNA replication.[14] MiR-30 family members, including miR-30a-5p and miR-30e-5p, are downregulated in MB, suggesting that they play an antitumor role in MB.[15] However, little is known concerning the role and regulatory mechanism of miR-30b-5p in the progression of MB.

MYB transcription factor family consists of three members: MYB (c-Myb), MYBL1 (A-Myb) and MYBL2 (B-Myb).[16] MYBL2 (MYB

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Table 1 Primer sequences of genes

Gene	Primer sequence
miR-30b-5p	F: 5′-GGGCTGTAAACATCCTACAC-3′
	R: 5′-GGGCTGTAAACATCCTACA-3′
U6	F: 5′-CTCGCTTCGGCAGCACA-3′
	R: 5′-AACGCTTCACGAATTTGCGT-3′

F, forward primer; R, reversed primer.

proto-oncogene Like 2) is a vital regulatory factor involved in the physiological processes of cells, such as cell cycle process, cell survival and cell differentiation.[16] The expression level of MYBL2 is upregulated in a variety of tumors, which drives the tumorigenesis and progression of cancers, such as liver cancer and breast cancer.[17 18] A line of evidence has shown that the high expression of MYBL2 in neuroblastoma can serve as an indicator of poor prognosis, which is associated with the increased risk of death and worse overall survival.[19] Bioinformatics prediction suggests that there are potential binding sites between miR-30b-5p and the 3′UTR of MYBL2. The purpose of this study was to investigate the expression and function of miR-30b-5p in MB and the interaction between miR-30b-5p and MYBL2.

MATERIALS AND METHODS

Subjects

Thirty-five cases of MB and adjacent non-tumor tissues were collected from patients presented to Children's Hospital affiliated to Zhejiang University. All tissues were immediately frozen in liquid nitrogen after surgical resection and stored until further use. Written informed consent was signed by guardians of the participants.

Cell lines and cell culture

Human MB cell lines (D425 cells, D341 cells, D283 cells and DAOY cells) were purchased from the American Type Culture Collection (Manassas, Virginia, USA). All cell lines were cultured in Dulbecco Modified Eagle's medium (Hyclone, Logan, Utah, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, Grand Island, New York, USA), 100 U/mL penicillin and 100 µg/mL streptomycin, and incubated at 37°C in 5% CO_2. The culture medium was replaced at an interval of 3 to 4 days. 0.25% trypsin (Americesco, Framingham, Massachusetts, USA) was used for subculture.

Cell transfection

MiR-30b-5p mimics, mimic negative control (miR-con), miR-30b-5p inhibitor and scramble control (anti-miR) were purchased from RiboBio (Guangzhou, China). DAOY and D425 cells were inoculated into six-well plates at a density of 1×10^5 cells/mL and incubated at 37°C with 5% CO_2 for 24 hours. DAOY and D425 cells were transfected with miR-30b-5p mimics, miR-con, miR-30b-5p inhibitors and anti-miR-con, respectively, using Lipofectamine 3000 (Invitrogen; Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the supplier's instructions. The transfection efficiency was detected by real-time PCR (RT-PCR).

RT-PCR analysis

Total RNA from MB tissues and cells was extracted by TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA of 1 µg was reversely transcribed using the SuperScript First-Strand cDNA System (Invitrogen). Then quantitative RT-PCR (qRT-PCR) analysis was performed. The relative gene expression of miR-30b-5p was calculated using the $2^{-\Delta\Delta CT}$ method. The sequences of primers were shown in table 1.

Cell Counting Kit-8 (CCK-8) assay

DAOY and D425 cells were harvested in the logarithmic phase and trypsinized with 0.25% trypsin. Afterward, cells were cultured in 96-well plates for 24 hours. Enhanced CCK-8 solution of 10 µL (Biossci Biotechnology, Wuhan,

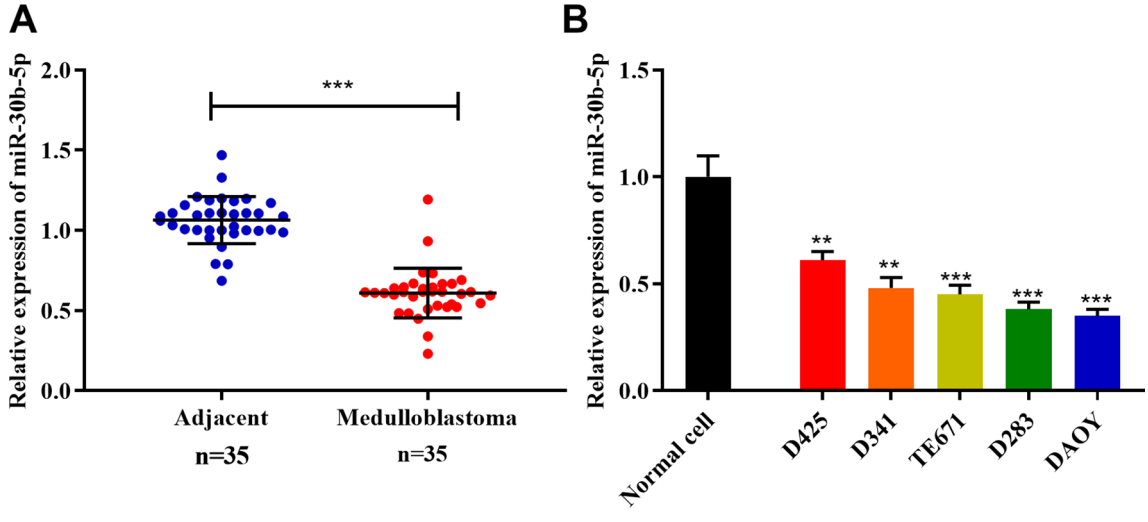

Figure 1 The expression of miR-30b-5p in medulloblastoma (MB) tissues and cell lines was significantly low. (A) The expression of miR-30b-5p in MB cells and adjacent cells of 35 patients was detected by quantitative real-time PCR. The results showed that the expression of miR-30b-5p was significantly lower in cancer tissues. (B) The expressions of miR-30b-5p in five kinds of MB cells (D425 cells, D341 cells, TE671 cells, D283 cells and DAOY cells) was significantly lower than in normal cells. **p<0.01 and ***p<0.001.

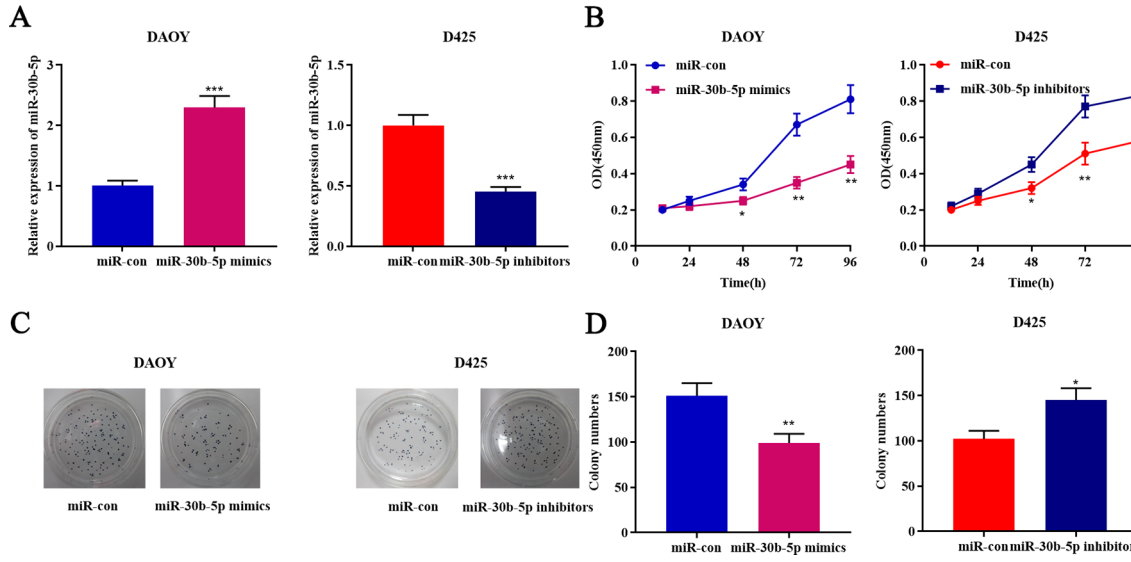

Figure 2 MiR-30b-5p played a vital role in the proliferation of medulloblastoma (MB). (A) The expression of miR-30b-5p in DAOY cells transfected with miR-30b-5p mimics and D425 cells transfected with miR-30b-5p inhibitors were detected by quantitative real-time PCR. The results showed that the cell models of overexpressed miR-30b-5p and lowly expressed miR-30b-5p were successfully constructed. (B) Cell Counting Kit-8 (CCK-8) assay showed that the proliferation ability of DAOY with overexpressed miR-30b-5p was significantly decreased, whereas D425 cells with low miR-30b-5p expression exhibited slow proliferating rate. (C and D) Colony formation assay indicated that the number of colony formation of DAOY cells with overexpressed miR-30b-5p decreased significantly, while the number of colony formation of D425 cells with inhibited miR-30b-5p increased significantly. *p<0.05, **p<0.01 and ***p<0.001. OD, optical density.

China) was added to continue the culture. One hour later, the absorbance value (optical density value) of each well was measured at a wavelength of 450 nm on the microplate reader.

Colony formation assay

DAOY cells and D425 cells were inoculated into six-well plates, with 1×10^3 cells in each well. After 2 weeks of culture, the culture medium was abandoned and the plate was rinsed carefully with phosphate-buffered saline (Biossci Biotechnology) twice. Cells were then fixed with 4% paraformaldehyde for 10 min. Crystal violet solution of 1 mL was added to each well to stain the colonies, and then the number of colony formation was recorded.

Apoptosis assay

Apoptosis was analyzed using a fluorescein isothiocyanate (FITC) Annexin V cell apoptosis detection kit (Ruibo, Guangzhou, China) following the manufacturer's instructions. Briefly, cells were collected, washed and stained with FITC-annexin V and propidium iodide, and then cell apoptosis was detected by flow cytometry (Becton Dickinson, Mountain View, California, USA).

Western blot analysis

The cells were lysed with Radioimmunoprecipitation buffer (Beyotime Biotechnology, Shanghai, China). After high-speed centrifugation, the supernatant was collected and heated in a water bath at 100°C for 10 min to denature the protein. The concentration of the protein was determined by BCA protein assay kit (Beyotime Institute of Biotechnology, Haimen, China). Then sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed and after that the protein was transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, Massachusetts, USA). After the PVDF membrane was washed with Tris buffered saline Tween solution, MYBL2 antibody (catalog no: ab12296, 1:1000; Abcam, Shanghai, China), Bcl-2 antibody (catalog no: ab32124, Abcam, 1:1000), BAX (catalog no: ab53154, 1:1000; Abcam) and β-actin antibody (catalog no: ab20272, 1:1000; Abcam) were added to incubate the PVDF membrane overnight at 4°C. The membrane was then washed and incubated with the secondary antibody (Hubei Biossci Biotechnology) for 1 hour at room temperature. After washing, the immunoreaction was performed using the hypersensitive enhanced chemiluminescence detection system (Hubei Biossci Biotechnology).

Bioinformatics analysis and dual luciferase reporter gene assay

The potential target gene of miR-30b-5p was predicted by TargetScan 7.0 software. Wild-type (WT) or mutant (MUT) MYBL2 was subcloned into pGL3 Basic vector (Promega, Madison, Wisconsin, USA) and then transfected into HEK293T cells. The cells were then inoculated in 24-well plates at a density of 5.0×10^3 cells/well. MiR-30b-5p or miR-con was cotransfected with WT MYBL2 or MUT type MYBL2 report vector into HEK293T cells. Luciferase activity was measured at 48 hours after transfection. Each group of experiments was repeated three times.

Statistic analysis

SPSS 17.0 (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Measurement data were presented as

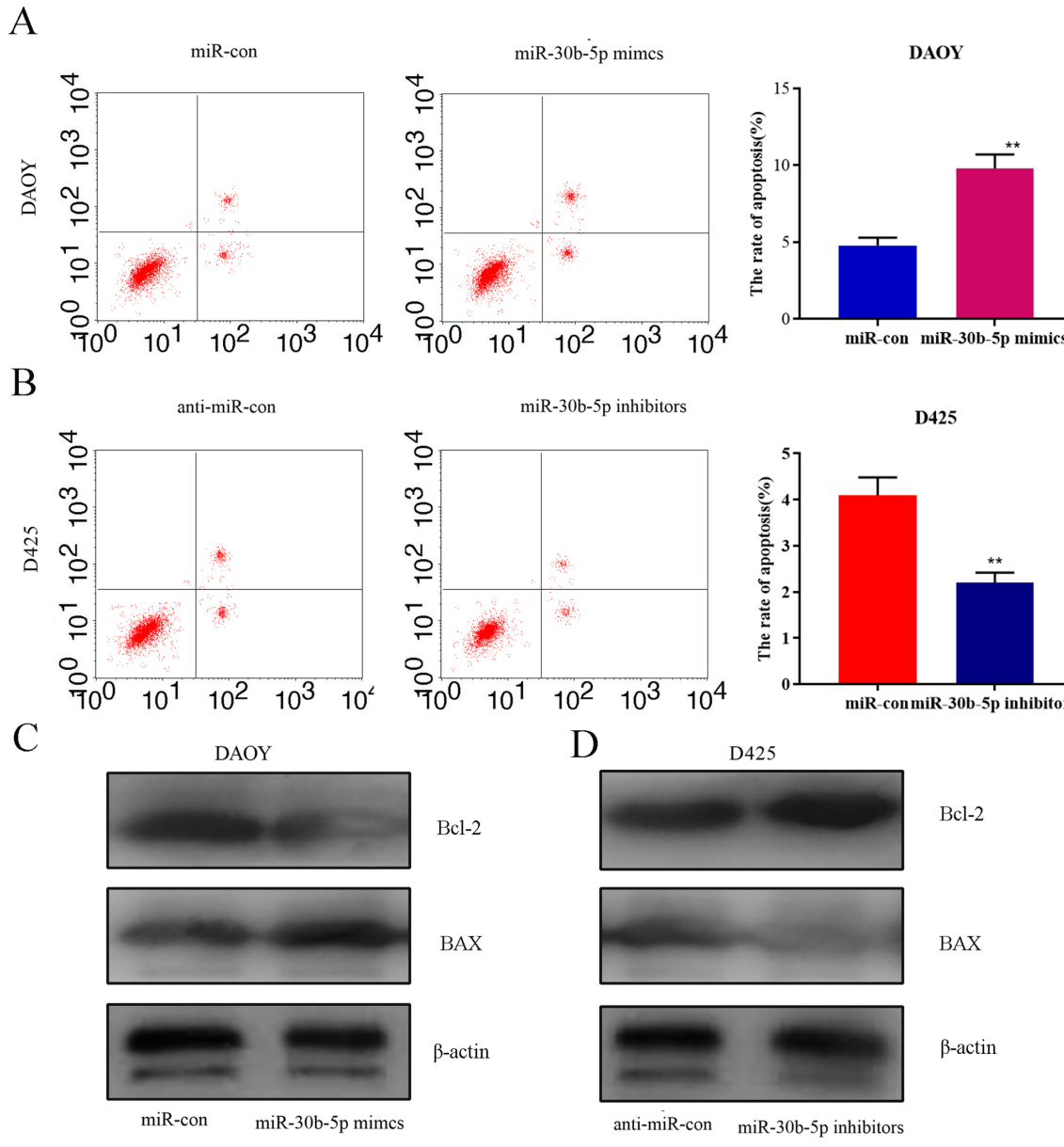

Figure 3 MiR-30b-5p was significantly correlated with apoptosis in medulloblastoma (MB). (A and B) Flow cytometry showed that the proportion of DAOY apoptosis with overexpressed miR-30b-5p was significantly increased, while the downregulation of miR-30b-5p expression significantly decreased the proportion of apoptosis in D435 cells. (C and D) Western blot demonstrated that the expression of Bcl-2 was significantly downregulated and the expression level of BAX was upregulated in DAOY cells with upregulated miR-30b-5p. However, the expression of Bcl-2 was significantly upregulated and the expression of BAX was significantly downregulated in D425 cells transfected with miR-30b-5p inhibitors. **p<0.01.

mean±SD. t-test was carried out to analyze the difference of measurement data. $p<0.05$ indicated statistical significance.

RESULTS

MiR-30b-5p displayed low expression in MB tissues and cell lines

To compare the expression of miR-30b-5p in MB tissues and adjacent tissues, RT-PCR was performed, and the results of which showed that the expression of miR-30b-5p in MB tissues was significantly lower than that in normal tissues (figure 1A). Compared with the normal cells, the expression level of miR-30b-5p was significantly decreased in five kinds of MB cell lines (figure 1B). These results suggested that miR-30b-5p exerted an antitumor role in MB.

MiR-30b-5p impeded MB proliferation in vitro

To determine the role of miR-30b-5p in MB, we selected DAOY cells and D425 cells for further analysis. MiR-con and miR-30b-5p mimics were transfected into DAOY cells, and anti-miR-con and miR-30b-5p inhibitors were transfected into D425 cells, respectively. The transfection efficiency was detected by qRT-PCR. The results showed that miR-30b-5p was highly expressed in the miR-30b-5p mimic group and lowly in the miR-30b-5p inhibitors group

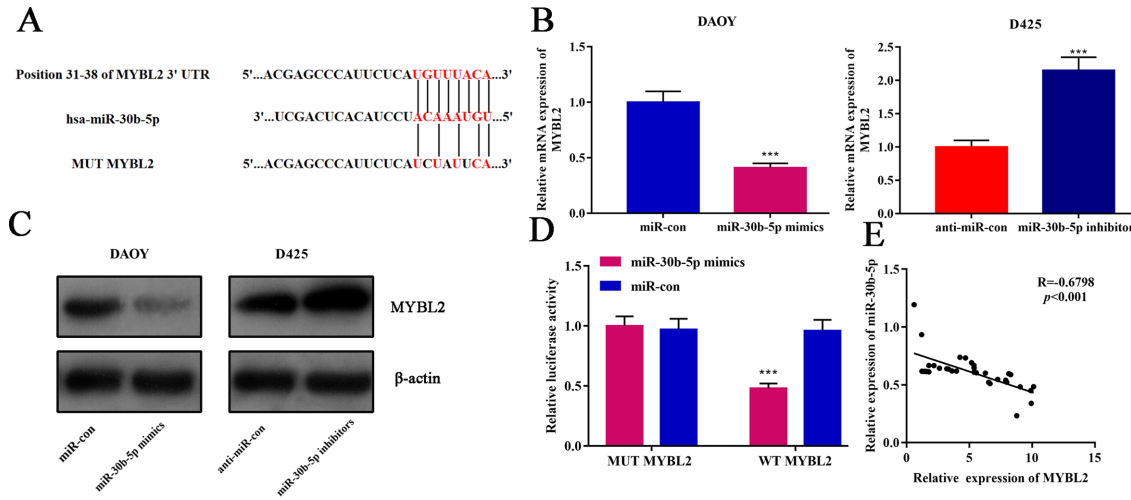

Figure 4 MiR-30b-5p directly targeted MYBL2 in medulloblastoma (MB). (A) TargetScan predicted MYBL2 was one of the candidate target genes for miR-30b-5p, and their binding site was shown. (B, C) Forty-eight hours after transfection, the expressions of MYBL2 mRNA and MYBL2 protein in DAOY cells and D425 cells were detected by quantitative real-time PCR and western blot, and it was found that miR-30b-5p inhibited the expression of MYBL2. (C) Wild-type (WT) or mutant-type (MUT) MYBL2 luciferase reporter gene plasmid and miR-30b-5p mimics or miR-con were transfected into HEK293T cells. MiR-30b-5p mimics transfection that could significantly reduce the luciferase activity of WT type, but had no significant effect on the luciferase activity of MUT. ***p<0.001.

compared with the control groups (figure 2A). In addition, CCK-8 assay indicated that the proliferation of DAOY cells with overexpressed miR-30b-5p was repressed markedly compared with that in the control group. On the contrary, downregulated miR-30b-5p would boost the proliferation of D425 cells (figure 2B). Colony formation assay showed that the number of colonies formed by DAOY cells with upregulated miR-30b-5p was reduced significantly. Conversely, inhibition of miR-30b-5p expression increased the number of colony formation of D425 cells. These results suggested that miR-30b-5p played an inhibitory role in regulating the proliferation of MB cells.

MiR-30b-5p regulated MB apoptosis

The results of flow cytometry analysis suggested that, as expected, the proportion of apoptosis was significantly increased in DAOY cells with overexpressed miR-30b-5p compared with that in control group (figure 3A). However, the apoptosis rate was significantly lower in D425 cells after miR-30b-5p was inhibited (figure 4B). Western blot demonstrated that the expression of Bcl-2 was significantly decreased and the expression of BAX was increased after miR-30b-5p transfection. However, the transfection of miR-30b-5p inhibitors caused the opposite effects.

MiR-30b-5p directly targeted MYBL2 in MB

TargetScan indicated that MYBL2 was one of the candidate targets of miR-30b-5p and their binding site was shown in figure 4A. In addition, overexpressed miR-30b-5p resulted in a significant decrease of MYBL2 in DAOY cells at mRNA level, while inhibited miR-30b-5p exerted an opposite effect (figure 4B). Western blot demonstrated that MYBL2 expression was markedly suppressed in DAOY transfected with miR-30b-5p mimics, and D425 transfected with miR-30b-5p inhibitors exhibited a markedly increase in the expression of MYBL2 (figure 4C). After the WT or MUT type MYBL2 luciferase reporter gene plasmid and miR-30b-5p mimics or miR-con were transfected into HEK293T cells, it was found that the luciferase activity of WT reporter was significantly decreased by miR-30b-5p mimics transfection, but there was no significant effect on MUT reporter (figure 4D). The results of the correlation between the expression level of miR-30b-5p and MYBL2 showed that miR-30b-5p could negatively regulate the expression of MYBL2 in MB samples (figure 4E). These results indicated that miR-30b-5p could negatively modulate the expression of MYBL2 via binding to 3′UTR of MYBL2.

DISCUSSION

MiRNA is implicated in the biological process of MB that functions as an oncogene or suppressor gene.[20–25] It is reported that many members from the miR-30 family are lowly expressed in MB, which is related to the acceleration of the formation and growth of MB.[15] This study confirmed that miR-30b-5p exhibited a low expression in MB, thus playing an antitumor role. From the point of view of mechanism, miR-30b-5p could inhibit the proliferation and promote the apoptosis of MB by negatively regulating the expression of MYBL2.

Accumulating studies show that miRNA is a key regulator of tumorigenesis and metastasis.[26–28] The in-depth exploration of the relationship between miRNA and the biological behaviors of MB can provide a novel target for early diagnosis, monitoring progress and prognosis assessment.[29] It is well documented that a majority of miR-30 family members are involved in the progression of malignant tumors, such as gallbladder cancer, gastric cancer and ovarian cancer.[30–32] From the point of view of mechanism, miR-30b directly targets the expression of homeobox A1 to impede the proliferation, migration and invasion of esophageal cancer cells.[33] MiR-30b specifically binds to Snail 3′UTR to restrain epithelial–mesenchymal transition

of pancreatic cancer stem cells.[34] In gastric cancer, miR-30b represses the migration and invasion of tumor cells by targeting the expression of EIF5A2.[35] These studies validate the inhibitory role of miR-30b in a variety of tumors.

MiR-30b-5p is one of the members of miR-30 family and is confirmed as a tumor suppressor in multiple cancers, such as liver cancer and gastric cancer.[36 37] Further studies validate that miR-30b-5p can inhibit the proliferation, metastasis and epithelial–interstitial transformation of renal clear cell carcinoma by targeting G-protein subunit α-13.[38] In this study, we found that the expressions of miR-30b-5p in MB tissues and cell lines were downregulated. CCK-8 assay and colony formation assay suggested that the proliferation of MB cells with overexpressed miR-30b-5p was significantly arrested. However, the transfection of miR-30b-5p inhibitors showed the opposite effect. Subsequently, we detected the apoptosis of MB cells, and as expected, the results showed that the proportion of apoptosis increased observably after miR-30b-5p mimics transfection, while miR-30b-5p inhibitor exerted the opposite effect. Western blot indicated that upregulated miR-30b-5p inhibited the expression of Bcl-2 and promoted the expression of BAX. Hence, we concluded that miR-30b-5p was a tumor suppressor in MB.

MYBL2 is abnormally upregulated in a variety of tumors including neurological tumors.[16–18] In terms of mechanism, the interaction of MYBL2 and IGFBP-5 promoter increases its transcriptional activity, thereby accelerating the proliferation of neuroblastocytes.[39] Besides, MYBL2 enhances cell survival rate by activating antiapoptotic genes such as ApoJ/clusterin and BCL2.[40] MiR-30 regulates B-myb expression to affect cell senescence.[41] In addition, the proliferation of glioma cells can be modulated by miR-30e-5p/MYBL2 axis.[42] Based on this, it is noteworthy whether miR-30b-5p can regulate the expression of MYBL2. In the present study, bioinformatics database predicted that there was at least one potential binding site between miR-30b-5p and the 3′UTR of MYBL2. MiR-30b-5p mimics significantly decreased not only the expression of MYBL2 both at mRNA and protein level but also the luciferase activity of WT MYBL2 reporter, but had no significant effect on MUT type MYBL2. Subsequently, we found that there was a negative correlation between miR-30b-5p and MYBL2 expression in MB samples. Therefore, we concluded that miR-30b-5p directly targeted 3′UTR of MYBL2 and negatively regulated its expression level in MB.

In summary, our findings present novel experimental data that can identify miR-30b-5p as a tumor-suppressive miRNA in MB. MiR-30b-5p restrains the proliferation and facilitates the apoptosis of MB cells by pairing with 3′UTR of MYBL2. Our results reveal the key roles miR-30b-5p/MYBL2 axis plays in MB, which provides a potential molecular target for the therapy of MB. In the following studies, in vivo studies are required to further validate our demonstrations.

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Competing interests None declared.

Patient consent for publication Not required.

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Data availability statement Data are available on reasonable request. The data used to support the findings of this study are available from the corresponding author on request.

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REFERENCES

1 Menyhárt O, Giangaspero F, Győrffy B. Molecular markers and potential therapeutic targets in non-WNT/non-SHH (group 3 and group 4) medulloblastomas. *J Hematol Oncol* 2019;12:29.
2 Northcott PA, Robinson GW, Kratz CP, *et al*. Medulloblastoma. *Nat Rev Dis Primers* 2019;5:11.
3 Besharat ZM, Sabato C, Po A, *et al*. Low expression of miR-466f-3p sustains epithelial to mesenchymal transition in sonic hedgehog medulloblastoma stem cells through Vegfa-Nrp2 signaling pathway. *Front Pharmacol* 2018;9:1281.
4 Sheng J, Wang L, Han Y, *et al*. Dual roles of protein as a template and a sulfur provider: a general approach to metal sulfides for efficient photothermal therapy of cancer. *Small* 2018;14:1702529.
5 Subramaniam S, Jeet V, Clements JA, *et al*. Emergence of microRNAs as key players in cancer cell metabolism. *Clin Chem* 2019;65:1090–101.
6 Chen Y, Gao D-Y, Huang L. In vivo delivery of miRNAs for cancer therapy: challenges and strategies. *Adv Drug Deliv Rev* 2015;81:128–41.
7 Rynkeviciene R, Simiene J, Strainiene E, *et al*. Non-coding RNAs in glioma. *Cancers* 2018;11:17.
8 Garg N, Vijayakumar T, Bakhshinyan D, *et al*. MicroRNA regulation of brain tumour initiating cells in central nervous system tumours. *Stem Cells Int* 2015;2015:141793
9 Shalaby T, Fiaschetti G, Baumgartner M, *et al*. Significance and therapeutic value of miRNAs in embryonal neural tumors. *Molecules* 2014;19:5821–62.
10 Vidal DO, Marques MMC, Lopes LF, *et al*. The role of microRNAs in medulloblastoma. *Pediatr Hematol Oncol* 2013;30:367–78.
11 Panwalkar P, Moiyadi A, Goel A, *et al*. Mir-206, a cerebellum enriched miRNA is downregulated in all medulloblastoma subgroups and its overexpression is necessary for growth inhibition of medulloblastoma cells. *J Mol Neurosci* 2015;56:673–80.
12 Yang SY, Choi SA, Lee JY, *et al*. miR-192 suppresses leptomeningeal dissemination of medulloblastoma by modulating cell proliferation and anchoring through the regulation of DHFR, integrins, and CD47. *Oncotarget* 2015;6:43712–30.
13 Thor T, Künkele A, Pajtler KW, *et al*. MiR-34a deficiency accelerates medulloblastoma formation in vivo. *Int J Cancer* 2015;136:2293–303.
14 Jin Y, Xiong A, Zhang Z, *et al*. MicroRNA-31 suppresses medulloblastoma cell growth by inhibiting DNA replication through minichromosome maintenance 2. *Oncotarget* 2014;5:4821–33.
15 Singh SV, Dakhole AN, Deogharkar A, *et al*. Restoration of miR-30a expression inhibits growth, tumorigenicity of medulloblastoma cells accompanied by autophagy inhibition. *Biochem Biophys Res Commun* 2017;491:946–52.
16 Musa J, Aynaud M-M, Mirabeau O, *et al*. MYBL2 (B-Myb): a central regulator of cell proliferation, cell survival and differentiation involved in tumorigenesis. *Cell Death Dis* 2017;8:e2895.
17 Wei T, Weiler SME, Tóth M, *et al*. YAP-dependent induction of UHMK1 supports nuclear enrichment of the oncogene MYBL2 and proliferation in liver cancer cells. *Oncogene* 2019;38:5541–50.
18 Chen J, Chen X. MYBL2 is targeted by miR-143-3p and regulates breast cancer cell proliferation and apoptosis. *Oncol Res* 2018;26:913–22.
19 Raschellà G, Cesi V, Amendola R, *et al*. Expression of B-Myb in neuroblastoma tumors is a poor prognostic factor independent from MYCN amplification. *Cancer Res* 1999;59:3365–8.
20 López-Ochoa S, Ramírez-García M, Castro-Sierra E, *et al*. Analysis of chromosome 17 miRNAs and their importance in medulloblastomas. *Biomed Res Int* 2015;2015:717509
21 Yang Y, Cui H, Wang X. Downregulation of EIF5A2 by miR-221-3p inhibits cell proliferation, promotes cell cycle arrest and apoptosis in medulloblastoma cells. *Biosci Biotechnol Biochem* 2019;83:400–8.
22 Xu X-H, Zhang S-J, Hu Q-B, *et al*. Effects of microRNA-494 on proliferation, migration, invasion, and apoptosis of medulloblastoma cells by mediating

c-myc through the p38 MAPK signaling pathway. *J Cell Biochem* 2018. doi:10.1002/jcb.27559. [Epub ahead of print: 10 Oct 2018].
23 Li Y, Jiang T, Shao L, *et al*. Mir-449a, a potential diagnostic biomarker for WNT group of medulloblastoma. *J Neurooncol* 2016;129:423–31.
24 Gao Y, Li P, Liu Z, *et al*. Expression levels of vascular endothelial cell growth factor and microRNA-210 are increased in medulloblastoma and metastatic medulloblastoma. *Exp Ther Med* 2015;10:2138–44.
25 Leichter AL, Sullivan MJ, Eccles MR, *et al*. MicroRNA expression patterns and signalling pathways in the development and progression of childhood solid tumours. *Mol Cancer* 2017;16:15.
26 Shirjang S, Mansoori B, Asghari S, *et al*. MicroRNAs in cancer cell death pathways: apoptosis and necroptosis. *Free Radic Biol Med* 2019;139:1–15.
27 Zhang Y-H, Jin M, Li J, *et al*. Identifying circulating miRNA biomarkers for early diagnosis and monitoring of lung cancer. *Biochim Biophys Acta Mol Basis Dis* 2020;1866:165847.
28 Jadideslam G, Ansarin K, Sakhinia E, *et al*. Diagnostic biomarker and therapeutic target applications of miR-326 in cancers: a systematic review. *J Cell Physiol* 2019;234:21560–74.
29 Hummel R, Maurer J, Haier J. MicroRNAs in brain tumors : a new diagnostic and therapeutic perspective? *Mol Neurobiol* 2011;44:223–34.
30 Wang N, Xiang X, Chen K, *et al*. Targeting of NT5E by miR-30b and miR-340 attenuates proliferation, invasion and migration of gallbladder carcinoma. *Biochimie* 2018;146:56–67.
31 Wang J, Jiao Y, Cui L, *et al*. miR-30 functions as an oncomiR in gastric cancer cells through regulation of P53-mediated mitochondrial apoptotic pathway. *Biosci Biotechnol Biochem* 2017;81:119–26.
32 Shi M, Mu Y, Zhang H, *et al*. MicroRNA-200 and microRNA-30 family as prognostic molecular signatures in ovarian cancer: a meta-analysis. *Medicine* 2018;97:e11505.
33 Li Q, Zhang X, Li N, *et al*. miR-30b inhibits cancer cell growth, migration, and invasion by targeting homeobox A1 in esophageal cancer. *Biochem Biophys Res Commun* 2017;485:506–12.
34 Xiong Y, Wang Y, Wang L, *et al*. MicroRNA-30b targets snail to impede epithelial-mesenchymal transition in pancreatic cancer stem cells. *J Cancer* 2018;9:2147–59.
35 Tian S-B, Yu J-C, Liu Y-Q, *et al*. MiR-30b suppresses tumor migration and invasion by targeting EIF5A2 in gastric cancer. *World J Gastroenterol* 2015;21:9337–47.
36 Liu Z, Wei X, Zhang A, *et al*. Long non-coding RNA HNF1A-AS1 functioned as an oncogene and autophagy promoter in hepatocellular carcinoma through sponging hsa-miR-30b-5p. *Biochem Biophys Res Commun* 2016;473:1268–75.
37 Liu Y, Zhou Y, Gong X, *et al*. MicroRNA-30a-5p inhibits the proliferation and invasion of gastric cancer cells by targeting insulin-like growth factor 1 receptor. *Exp Ther Med* 2017;14:173–80.
38 Liu W, Li H, Wang Y, *et al*. MiR-30b-5p functions as a tumor suppressor in cell proliferation, metastasis and epithelial-to-mesenchymal transition by targeting G-protein subunit α-13 in renal cell carcinoma. *Gene* 2017;626:275–81.
39 Tanno B, Negroni A, Vitali R, *et al*. Expression of insulin-like growth factor-binding protein 5 in neuroblastoma cells is regulated at the transcriptional level by c-Myb and B-Myb via direct and indirect mechanisms. *J Biol Chem* 2002;277:23172–80.
40 Sala A. B-MYB, a transcription factor implicated in regulating cell cycle, apoptosis and cancer. *Eur J Cancer* 2005;41:2479–84.
41 Martinez I, Cazalla D, Almstead LL, *et al*. Mir-29 and miR-30 regulate B-Myb expression during cellular senescence. *Proc Natl Acad Sci U S A* 2011;108:522–7.
42 Zhang K, Fu G, Pan G, *et al*. Demethylzeylasteral inhibits glioma growth by regulating the miR-30e-5p/MYBL2 axis. *Cell Death Dis* 2018;9:1035.