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Original Article

MicroRNA-26b-3p/ANTXR1 signaling modulates proliferation, migration, and apoptosis of glioma

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Abstract: Glioma is a common malignant human brain tumor. The progression of glioma is associated with dysregulation of various microRNAs. Previous studies have demonstrated that microRNA-26b-3p (miR-26b-3p) is correlated with the pathogenesis of various tumors, but the functional role of miR-26b-3p and its underlying mechanisms in glioma are not clear. Here, we found that overexpression of miR-26b-3p repressed cell migration and proliferation and promoted apoptosis. In contrast, the opposite effects were observed when miR-26b-3p was inhibited in glioma cells. Anthrax toxin receptor 1 (ANTXR1) was confirmed to be a downstream molecule of miR-26b-3p. Reintroduction of ANTXR1 with an ORF region rescued the suppressive effects of miR-26b-3p on proliferation and migration, and inhibited the apoptosis of glioma cells. Moreover, the downstream target of miR-26b-3p, ANTXR1, was increased in glioma tissues and was inversely correlated with miR-26b-3p. MiR-26b-3p and ANTXR1 were correlated with the severity of glioma. Taken together, these results demonstrate that miR-26b-3p is a critical modulator of glioma via its downstream molecule, ANTXR1. Further, the miR-26b-3p/ANTXR1 axis may serve as a treatment or diagnostic target in glioma.

Keywords: MiR-26b-3p, glioma, ANTXR1, migration, proliferation

Introduction

Glioma is a common and highly malignant type of brain tumor that can originate from various types of cells. The prognosis of glioma patients is poor, despite developments in the treatment of glioma, patients have a very short median survival time [1-3]. Therefore, further research is needed to understand the pathogenesis and mechanisms underlying the development of glioma, and new diagnostic and therapeutic strategies are critical.

MicroRNAs are non-coding small RNAs that regulate various cellular processes, such as migration, apoptosis, and proliferation. The regulative mechanisms include the prohibition of translation or induction of degradation of mRNAs [4-6]. Many human tumors, including glioma, exhibit altered miRNA expression, which positively or negatively regulates tumor development [7-11]. In particular, several studies have shown that multiple miRNAs are associated with brain tumors and diseases [12-14].

Recent studies showed that miR-26b-3p regulates the progression of several cancers [15-17]. Further, miR-26b-3p has been shown to inhibit cell invasion and proliferation through various mechanisms [18, 19]. However, the role of miR-26b-3p in various glioma cell behaviors and the targets of miR-26b-3p in glioma require further study.

In this study, we performed a series of experiments and assays and found that miR-26b-3p is a tumor suppressor and exerts its effects via a novel downstream molecule, ANTXR1. Moreover, miR-26b-3p and ANTXR1 were inversely correlated in clinical samples and were correlated with the severity of glioma malignancy.

Materials and methods

Cell culture and human tissues

Human glioblastoma cells (A172, T98G, and LN229), astrocytoma cells (U251 and SHG44), and HEK293T cells were obtained from the iCell company (iCell Bioscience Inc., Shanghai, China). Glioma cells were maintained in complete medium. The complete medium comprised RPMI 1640 (Hyclone, USA) with 10% fetal bovine serum (FBS, GIBCO). Cells were cultured in an incubator with 5% CO_2 at 37°C.

In total, 551 glioma tissues derived from glioma patients in the TCGA database (http://cancergenome.nih.gov/) were studied. Recorded data for each sample included the sample name, expression value, and status (glioma or normal). MicroRNA expression profiles for 107 glioma samples were also downloaded from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn), and expression profiles were compared between low-grade glioma and high-grade glioma. Moreover, the Department of Neurosurgery at the First Affiliated Hospital of Zunyi Medical College (Zunyi, Guizhou, China) provided us sixteen paired glioma tissues. All tissues were collected for analysis of the expression of ANTXR1 and miR-26b-3p and were stored at -80°C. All subjects provided written informed consent, and the study was approved by the institutional ethics committee.

Transfection and miRNA oligoes

MiR-26b-3p mimic was used for overexpression of miR-26b-3p and inhibitors used for knockdown of miR-26b-3p were fully designed and obtained from RiboBio (Guangzhou, Guangdong, China). Lipofectamine 2000 (Thermo Fisher, Waltham, MA, USA) was used to transfect constructed vectors, miR-26b-3p mimic, miR-26b-3p inhibitor, or negative control (NC) into glioma cells. After incubation for 8 h at 37°C, the medium was replaced by the complete medium according to the instructions.

Dual luciferase assay

The ANTXR1 3'-UTR region that potentially binds with miR-26b-3p was amplified by PCR and cloned into the psiCHECK™-2 reporter plasmid. Briefly, psiCHECK™-2 reporter plasmids and miR-26b-3p mimics were co-transfected into HEK293 and U251 cells in 96-well plates. After incubation for 48 h, the luciferase activities of glioma cells were determined using a Dual-Luciferase Reporter Assay Kit (Promega, Fitchburg, WI, USA).

RNA extraction and real time RT-PCR

Trizol reagent (Thermo Fisher., USA) was used to isolate the RNA of human specimens and cultured cells. 1 μg total RNA was reversely transcribed to cDNA according to the All-in-One™ miRNA qRT-PCR Detection Kit (QP015, GeneCopoeia, Rockville, USA) instructions. The following real-time PCR aimed to detect the expression levels of miR-26b-3p in glioma cells and tissues. U6 was amplified as an internal control. Primers used in this analysis were purchased from Genecopoeia. The $2^{-\Delta\Delta Ct}$ analysis method was used to determine the relative level of miR-26b-3p. These experiments were carried out in triplicate.

Western blot assay

After washing with PBS, RIPA buffer and proteinase inhibitors were added to the tubes containing glioma tissues and cultured cells. After lysis of these samples, the bicinchoninic acid (BCA) method (Thermo Fisher, Waltham, USA) was used to quantify the protein concentration of each sample. Equal amounts of protein samples within each group were added into 10% SDS-PAGE gels and were separated for 1-2 h. Then, the gels were blotted to PVDF membranes (EMD Millipore, Billercia, MA, USA) and blocked through incubation in 5% non-fat milk at room temperature for 1 h. Anti-ANTXR1 monoclonal antibody (ANTXR1, 15091-1-AP, 1:1000; Proteintech, Wuhan, China) or anti-actin primary antibody (Santa Cruz, CA) was used to incubate the membranes overnight at 4°C. On the second day, the membranes were washed and immersed in horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Then, enhanced chemiluminescence (Pierce, Rockford, IL) was used to detect the protein signals of the membranes.

Proliferation analysis

A Cell Counting Kit-8 (Dojindo, Tokyo, Japan) was used to assess the proliferation of glioma cells in vitro. 1×10^3 glioma cells and 100 μl of

medium were placed into 96-well plates. The medium was replaced with 90 μl RPMI 1640 and 10 μl CCK-8 solution each day during the following week. Absorbance at 450 nm for each group was detected after incubation for 3 h.

Migration assay

Cellular migration was examined using a transwell chamber (BD Biosciences, CA, USA). The chamber included two compartments: a lower chamber and an upper chamber. First, 500 μl of complete medium was added into the lower chamber to stimulate migration of glioma cells. The upper chamber contained 250 μl RPMI 1640 medium (serum-free) with 1.0×10^5 cells. After incubation for 24 h or 48 h, a cotton swab was used to remove the glioma cells within the upper chamber. Glioma cells within the lower chamber were considered to be cells derived from the upper chamber. Then, we fixed these cells using methanol for 1 h. Finally, glioma cells were stained with hematoxylin for 30 min. Migration of glioma cells within each group was quantified as the average number of stained cells in five random 20 × magnification visual fields.

Apoptosis assay in vitro

The apoptosis of transfected glioma cells was examined according to previously described procedures [14].

Immunohistochemistry

Human tissue microarray of 125 glioma patients was purchased from Shanghai Outdo Biotech Co., Ltd (Shanghai, China). Immunohistochemistry was performed using the following antibody: rabbit anti-human ANTXR1, 15091-1-AP, 1:500 (Proteintech, Wuhan, China). Finally, DAB solution (DAKO, Santa Clara, USA) and hematoxylin staining solution (Sigma, Shanghai, China) were used to visualize the glioma tissue microarray.

Statistical analysis

In this study, student's *t*-test was carried out for comparison between two independent groups. One-way ANOVA followed by least significance difference (LSD) analysis was used for three-group comparisons. The association between the expression of miR-26b-3p and ANTXR1 was assessed with a Pearson correlation test. Two-way ANOVA followed by Tukey's post hoc analysis was used to analyze the glioma cell proliferation test. The results are expressed as mean ± SEM. SPSS 16.0 software was used to analyze all data, and $P<0.05$ was regarded as statistically significant.

Results

MiR-26b-3p prohibits tumor proliferation in glioma cells

To explore the role of miR-26b-3p in glioma development, we examined endogenous expression levels of miR-26b-3p in several glioma cell lines. The results showed that the levels of miR-26b-3p in T98G and SHG44 cells were very low, whereas U251 and LN229 exhibited strong expression of miR-26b-3p (**Figure 1A**).

To address the functional role of miR-26b-3p in glioma cell behaviors, miR-26b-3p mimic and its negative control were transfected into SHG44 and T98G cells in vitro. QRT-PCR measured the successful overexpression of miR-26b-3p in these cells (**Figure 1B**). We used the CCK-8 assay to assess cell viability. The results showed that the proliferation of glioma cells was decreased in miR-26b-3p overexpressing cells (**Figure 1C**). To explore if miR-26b-3p knockdown can reverse this, U251 and LN229 cells were introduced with miR-26b-3p inhibitor. The results indicated successful inhibition of miR-26b-3p (**Figure 1D**). The CCK-8 assay revealed that miR-26b-3p inhibitor repressed the proliferation of glioma cells (**Figure 1E**).

MiR-26b-3p inhibits glioma migration in vitro

Next, we ascertained if miR-26b-3p affects the migration of T98G and SHG44 cells in vitro. Our results from the migration assay indicated that ectopic expression of miR-26b-3p in T98G and SHG44 cells significantly decreased cell migration (**Figure 2A**, **2B**). To explore if miR-26b-3p knockdown can reverse this, U251 and LN229 cells were introduced with miR-26b-3p inhibitor. Then, we assessed the functional role of miR-26b-3p inhibitor on the migration of U251 and LN229 cells. The results revealed enhanced the migration of U251 and LN229 cells after knockdown of miR-26b-3p (**Figure 2C**, **2D**).

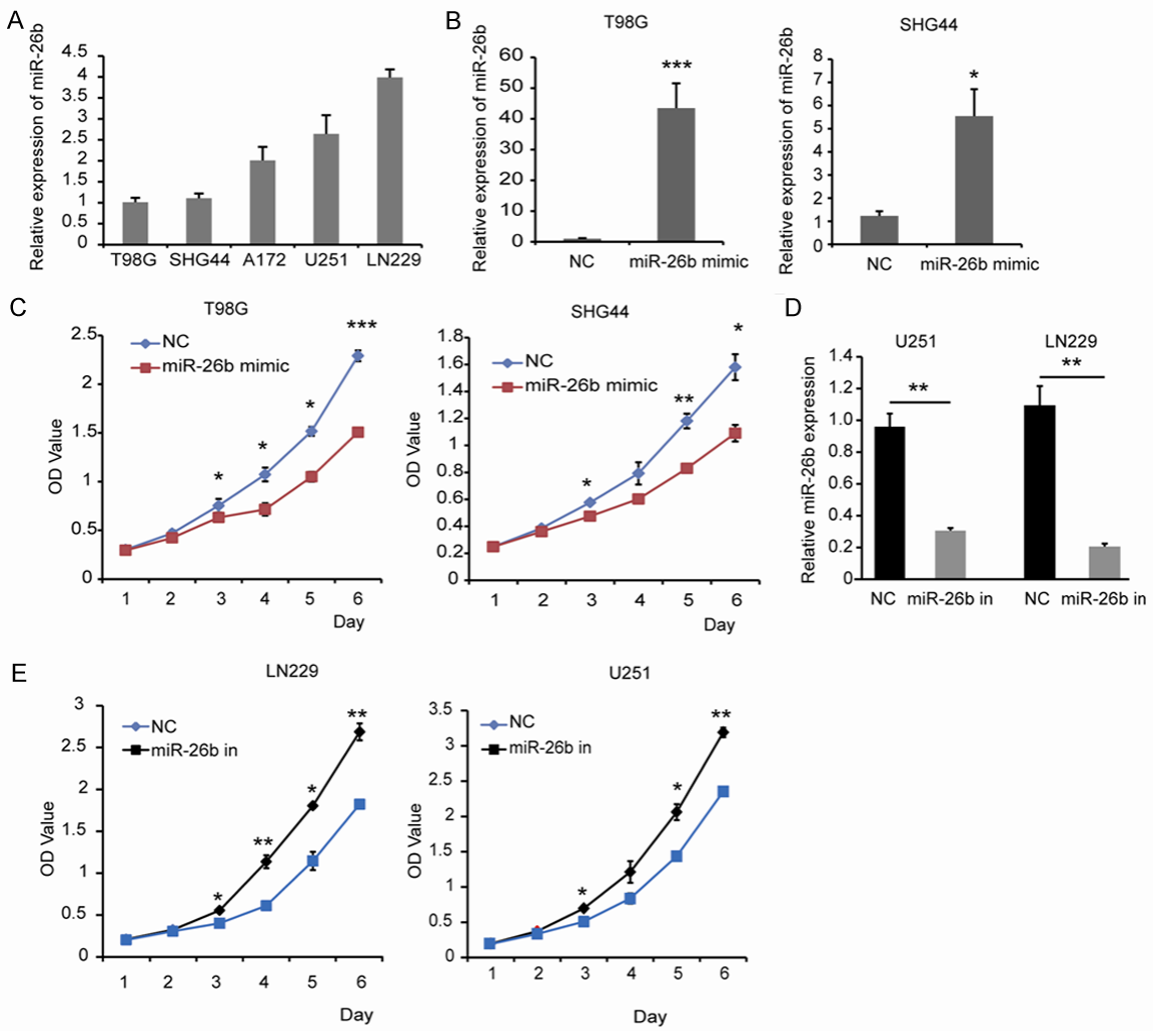

Figure 1. MiR-26b-3p prohibits tumor proliferation in glioma cells. A. Relative expression of miR-26b-3p in various glioma cells. B. Ectopic expression of miR-26b-3p in T98G and SHG44 cells by qRT-PCR. C. MiR-26b-3p inhibited the proliferation of T98G and SHG44 cells in vitro. D. Expression of miR-26b-3p detected by qPCR was suppressed in LN229 and U251 cells transfected with miR-26b-3p inhibitor. E. CCK-8 assay revealed that knockdown of miR-26b-3p promoted the proliferation of LN229 and U251 cells. *P<0.05, **P<0.01, ***P<0.001.

MiR-26b-3p enhances glioma cell apoptosis

Flow cytometry indicated that overexpression of miR-26b-3p resulted in enhanced apoptosis of T98G and SHG44 cells (**Figure 3A**, **3B**). On the other hand, flow cytometry revealed that U251 and LN229 cells transfected with miR-26b-3p inhibitor exhibited reduced apoptosis (**Figure 3C**, **3D**). Taken together, the results so far indicate that miR-26b-3p inhibits tumor growth and migration and promotes apoptosis in glioma cells. Thus, miR-26b-3p plays a tumor-suppressive role in glioma development.

MiR-26b-3p directly binds to and regulates ANTXR1

To evaluate the mechanism by which miR-26b-3p modulates glioma development, potential miR-26b-3p downstream molecules were explored via multiple prediction algorithms (miRBase, PicTar, and TargetScan). Our prediction results showed that ANTXR1 was a possible functional downstream target of miR-26b-3p according to conserved molecule sequences in the region of the ANTXR1 3'UTR (**Figure 4A**). To further analyze whether miR-26b-3p directly targets ANTXR1, we performed a luciferase

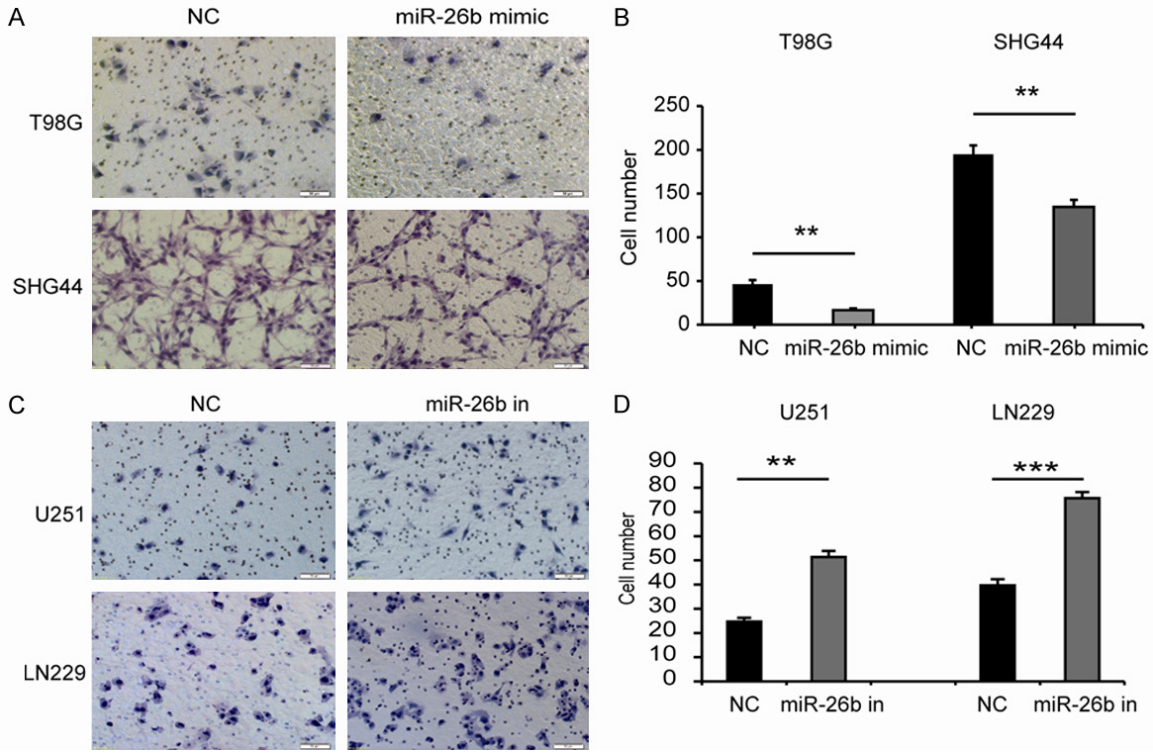

Figure 2. MiR-26b-3p inhibits glioma migration in vitro. (A, B) Migration assay revealed that miR-26b-3p inhibited the migration of SHG44 and T98G cells. Representative images (A) and statistical results (B) are shown. (C, D) Transwell assays revealed that miR-26b-3p inhibitor increased the migration of LN229 and U251 cells. Data reflects the average of five random fields. **P<0.01, ***P<0.001.

reporter assay in vitro. The results indicated that miR-26b-3p repressed the luciferase activities of the ANTXR1 3'UTR reporter plasmid in U251 and HEK293T cells (**Figure 4B**). We then analyzed if miR-26b-3p mimics and inhibitors affected the protein levels of ANTXR1 in glioma cells. Clear decreases in the levels of ANTXR1 protein were observed after miR-26b-3p overexpression in T98G and SHG44 cells, whereas the inhibition of miR-26b-3p increased the expression levels of ANTXR1 (**Figures 4C**, **4D** and S1). Thus, these findings indicate that miR-26b-3p can modulate the expression of ANTXR1 in glioma cells.

ANTXR1 abolishes miR-26b-3p-induced suppressive effects on proliferation, apoptosis, and migration of glioma cells

The above results indicated that miR-26b-3p targets ANTXR1 and regulates the expression of ANTXR1 in glioma cells. Thus, we then explored if ectopic expression of ANTXR1 could reverse the cell behaviors of SHG44 and T98G cells transfected with miR-26b-3p mimic. Since miR-26b-3p overexpression reduced the expression of ANTXR1, ANTXR1 overexpressing vectors with an ORF region were introduced into SHG44 and T98G cells transfected with miR-26b-3p mimic. Next, cell proliferation, cell apoptosis and transwell migration assays were performed. The results of the rescued experiments indicated that restoration of ANTXR1 significantly abolished the suppression of proliferation ability induced by miR-26b-3p (**Figure 5A**). Meanwhile, ANTXR1 induced increased migration ability (**Figure 5B**). Moreover, ANTXR1 prohibited glioma cell apoptosis in cells transfected with miR-26b-3p (**Figure 5C**). Taken together, these results show that miR-26b-3p affects proliferation, migration, and apoptosis via downstream ANTXR1.

MiR-26b-3p is downregulated and has a negative expression pattern compared with ANTXR1 in glioma tissues

To address the role of miR-26b-3p in clinical glioma patients, miR-26b-3p expression was determined in 16 paired glioma tissues using

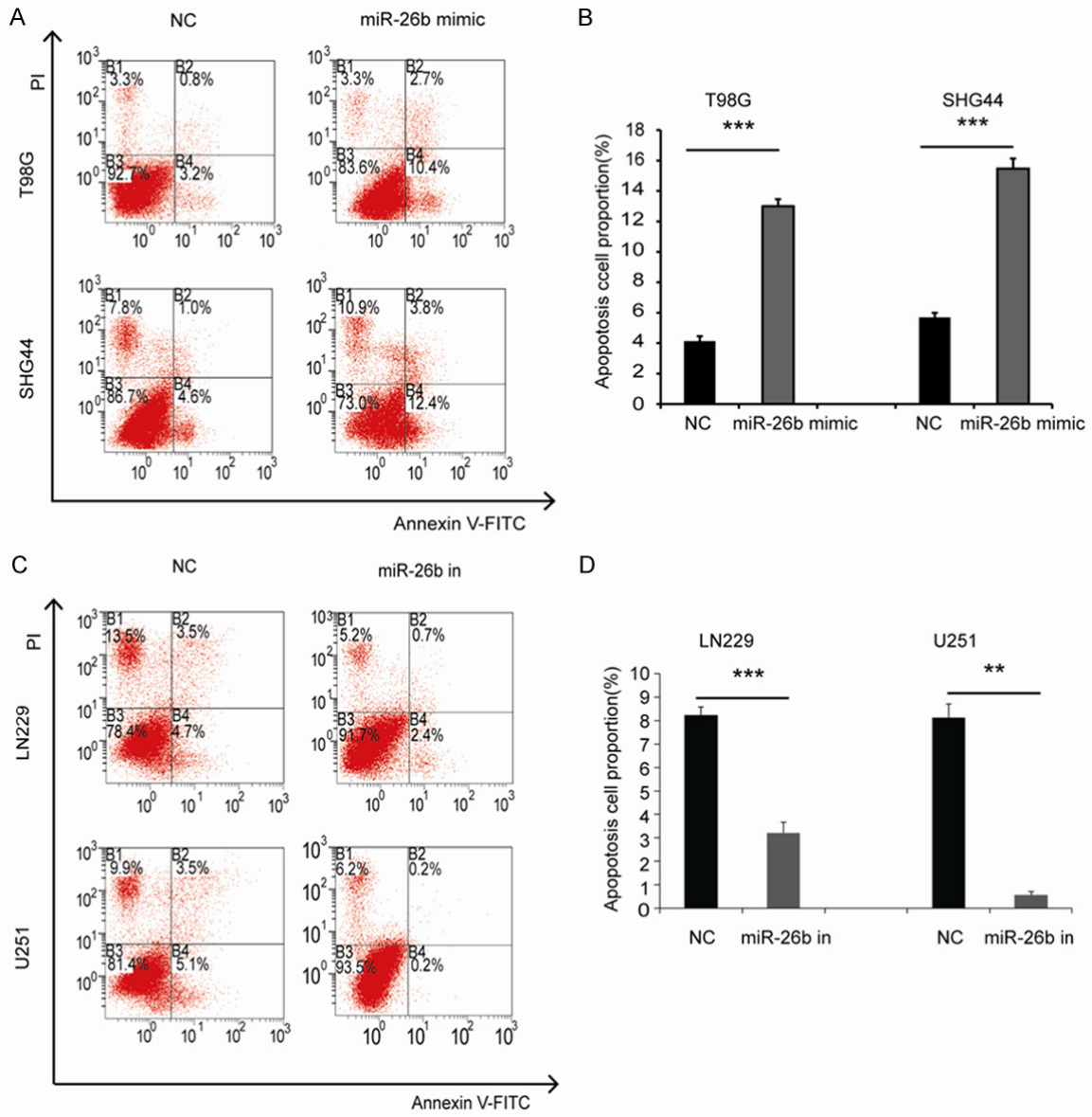

Figure 3. MiR-26b-3p enhances glioma cell apoptosis. A, B. MiR-26b-3p mimic promoted the apoptosis of SHG44 and T98G cells by flow cytometry. C, D. Suppression of miR-26b-3p reduced the apoptosis of LN229 and U251 cells. Data reflects the average of triplicate experiments. **P<0.01, ***P<0.001.

qRT-PCR. The expression of miR-26b-3p in clinical tissues was lower than their adjacent specimens (**Figure 6A**). Meanwhile, ANTXR1 expression in human clinical glioma tissues was assessed by Western blotting. The results showed that ANTXR1 was upregulated in glioma specimens compared with control tissues (**Figure 6B**). Moreover, an inverse correlation between the expression of miR-26b-3p and the target gene, ANTXR1, in glioma specimens was revealed by linear regression analysis (**Figure 6C**).

MiR-26b-3p and ANTXR1 are correlated with the severity of glioma

Given that miR-26b-3p was downregulated in our glioma tissues, we explored whether miR-26b-3p was correlated with glioma severity in our glioma patients. Transcription levels of miR-26b-3p in the CGGA database were analyzed and the results showed that miR-26b-3p levels in WHO III gliomas were significantly reduced compared with WHO II gliomas (**Figure 7A**). Moreover, the expression of miR-26b-3p in

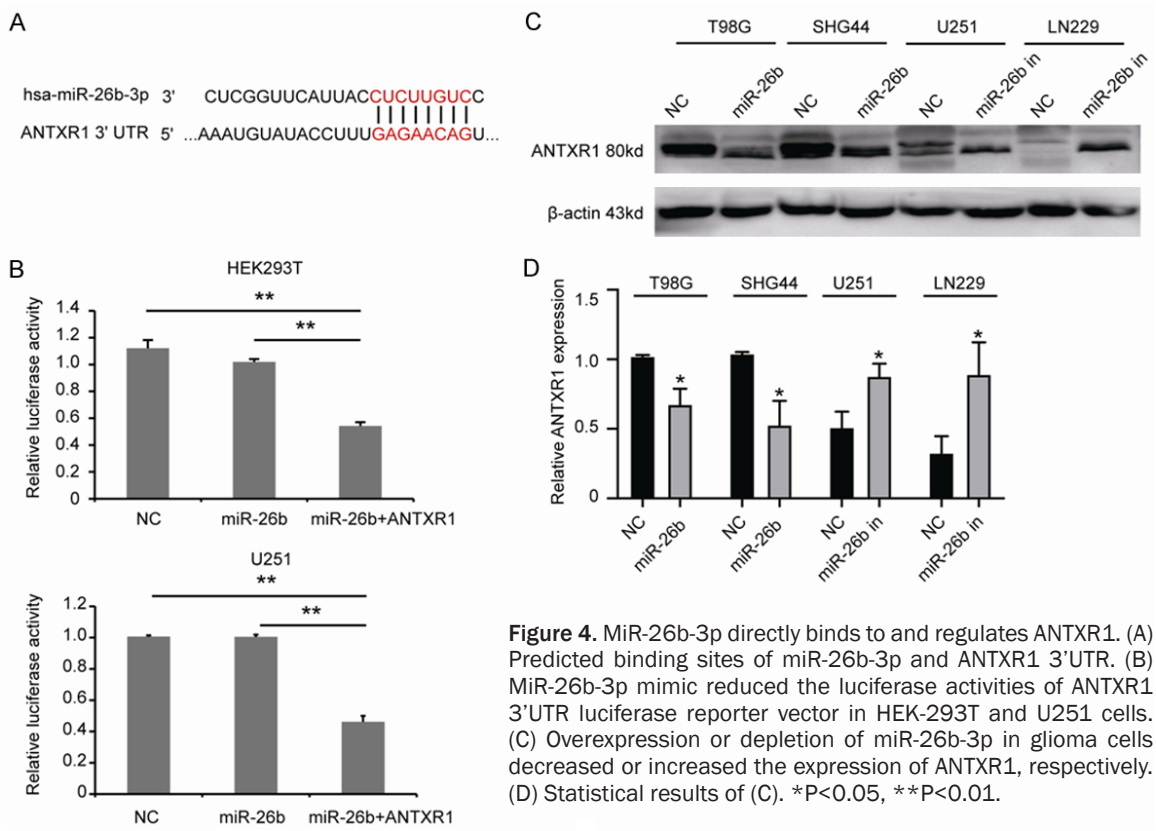

Figure 4. MiR-26b-3p directly binds to and regulates ANTXR1. (A) Predicted binding sites of miR-26b-3p and ANTXR1 3'UTR. (B) MiR-26b-3p mimic reduced the luciferase activities of ANTXR1 3'UTR luciferase reporter vector in HEK-293T and U251 cells. (C) Overexpression or depletion of miR-26b-3p in glioma cells decreased or increased the expression of ANTXR1, respectively. (D) Statistical results of (C). *P<0.05, **P<0.01.

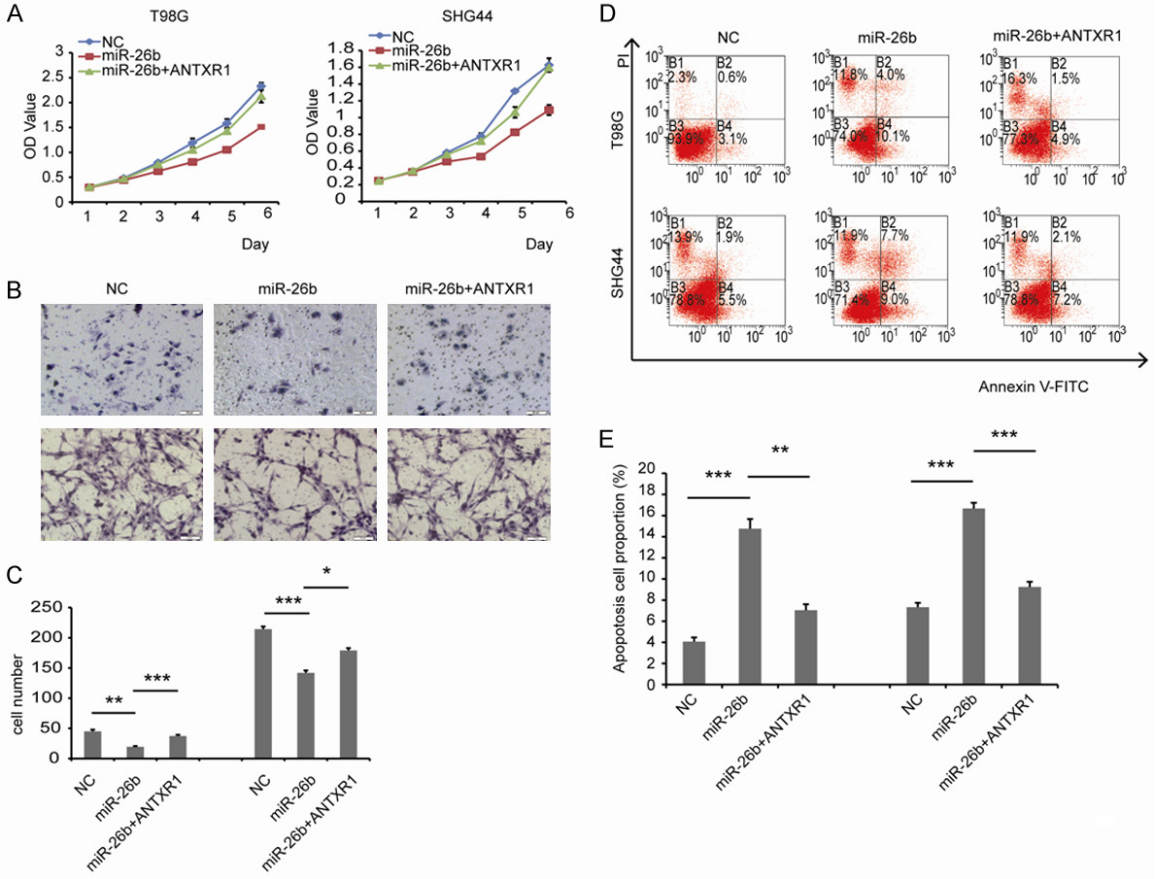

Figure 5. ANTXR1 abolishes miR-26b-3p-induced suppressive effects on proliferation, apoptosis and migration of glioma cells. A. Ectopic expression of ANTXR1 reversed miR-26b-3p-induced inhibition of cell proliferation in SHG44 and T98G cells. Glioma cell viability was determined by CCK-8 assay following culture for 6 days. B, C. Transwell assays indicated that ANTXR1 blocked miR-26b-3p-induced effects on cell migration. Original magnification, × 200. D, E. ANTXR1 decreased the glioma cell apoptosis induced by miR-26b-3p by the analysis of flow cytometry. *P<0.05, **P<0.01, ***P<0.001.

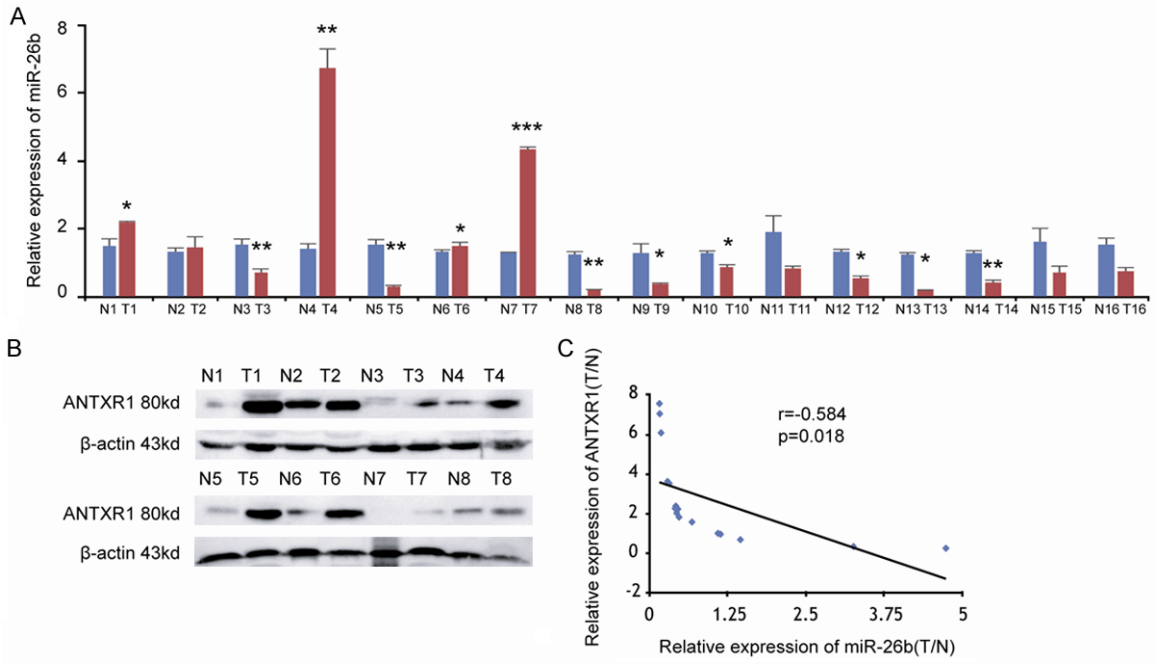

Figure 6. MiR-26b-3p is downregulated and has a negative expression pattern compared with ANTXR1 in glioma tissues. A. Expression of miR-26b-3p was decreased in human glioma specimens compared with control samples. B. Results of Western blot showed increased expression of ANTXR1 in human glioma tissues. β-actin served as the internal control. C. Pearson's correlation analysis of the expression of miR-26b-3p and ANTXR1 in clinical glioma tissues. *P<0.05, **P<0.01, ***P<0.001.

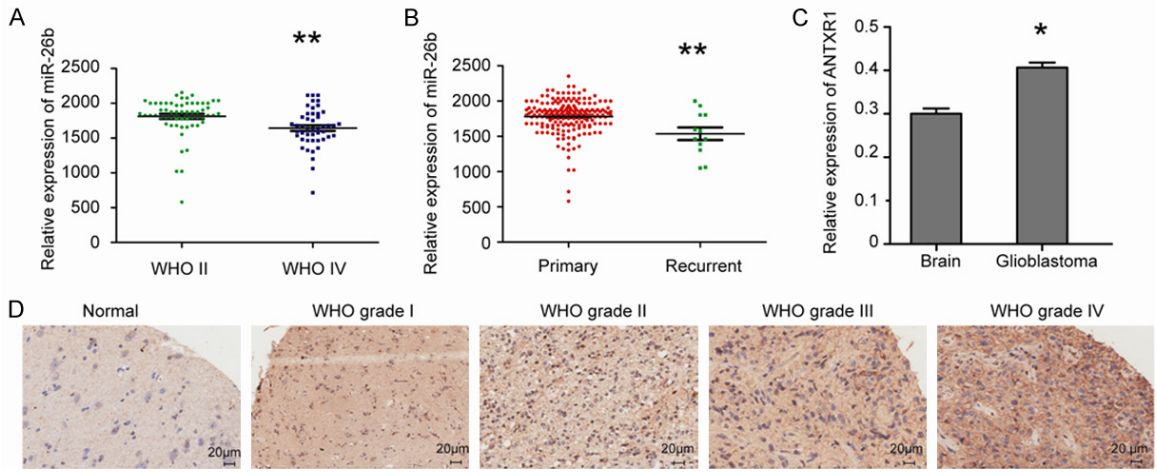

Figure 7. MiR-26b-3p and ANTXR1 are correlated with the severity of glioma. A. Data from the CGGA database showed decreased expression of miR-26b-3p in high-grade glioma specimens compared with low-grade tissues (n=107). B. Reduced expression of miR-26b-3p was also found in patients diagnosed with recurrent glioma in the CGGA database (n=198). C. The expression of ANTXR1 was upregulated in glioma tissues derived from the TCGA database (n=551). D. The expression of ANTXR1 in non-cancerous brain tissues, low-grade glioma tissues and high-grade glioma tissues was assessed by immunohistochemistry (n=125). *P<0.05, **P<0.01.

patients with recurrent gliomas was remarkably decreased compared to patients with the primary gliomas (**Figure 7B**).

In addition, the TCGA database was analyzed to confirm the expression pattern of ANTXR1 in glioma patients (**Figure 7C**). Immunohistochemistry (IHC) was utilized to further elucidate the potential role of ANTXR1 in the severity of glioma. The IHC results revealed increased expression of ANTXR1 in high-grade gliomas (**Figure 7D**). Taken together, these data suggest that miR-26b-3p and ANTXR1 are correlated with the severity of glioma patients.

Discussion

Over the past few years, many studies have shown that microRNAs play roles in various tumors, including lung cancer, gastric cancer, and large cell lymphoma [20, 21]. In fact, microRNAs can prohibit tumor progression [22] or promote the development of tumors [23]. Previous studies have shown that miR-26b-3p is closely linked with breast cancer, intrahepatic cholangiocarcinoma, colorectal cancer, and laryngeal cancer [15-17, 24], serving as a tumor suppressor or oncogene. However, the functional role of miR-26b-3p and its underlying mechanism in the development of glioma remain to be fully explored.

Here, we revealed that miR-26b-3p prohibits glioma development in vitro through various cellular processes such as migration, proliferation, and apoptosis. MicroRNAs can regulate these processes through their downstream molecules. In this study, we confirmed that ANTXR1 is a target gene of miR-26b-3p. ANTXR1 is closely related to the Wnt/β-catenin signaling pathway [25], which regulates various tumor cell processes, including cell proliferation and migration [26-29]. Along with ANTXR1, microRNAs have been shown to exert their anti-tumor roles through Wnt/β-catenin signaling pathway [30], with a finding which fits with the results of this study.

We also assessed the expression of miR-26b-3p and ANTXR1 in vitro. MiR-26b-3p inversely regulated the expression of ANTXR1. Thus, miR-26b-3p and ANTXR1 displayed opposite expression patterns. We also found an inverse correlation between miR-26b-3p and the target gene, ANTXR1, in glioma tissues. In general, the results of this study indicate that miR-26b-3p acts as a glioma suppressor through regulation of ANTXR1.

Although the present results from online databases and clinical glioma tissue samples indicate that ANTXR1 is upregulated in glioma, the functional role of ANTXR1 in glioma progression remains unclear. Our vitro results and clinical data suggest that ANTXR1 may act as an oncogene in glioma progression and may be correlated with the severity of glioma malignance.

Taken together, these findings indicate that miR-26b-3p/ANTXR1 signaling may be a novel modulator of glioma development. Thus, miR-26b-3p/ANTXR1 could act as a novel diagnostic or treatment target in glioma.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Ohgaki H and Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathol 2005; 109: 93-108.

[2] Ohgaki H and Kleihues P. Population-based studies on incidence, survival rates, and genetic alterations in astrocytic and oligodendroglial gliomas. J Neuropathol Exp Neurol 2005; 64: 479-489.

[3] Wen PY and Kesari S. Malignant gliomas in adults. N Engl J Med 2008; 359: 492-507.

[4] Asangani IA, Rasheed SA, Nikolova DA, Leupold JH, Colburn NH, Post S and Allgayer H.

MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. Oncogene 2008; 27: 2128-2136.

[5] Chai C, Song LJ, Han SY, Li XQ and Li M. MicroRNA-21 promotes glioma cell proliferation and inhibits senescence and apoptosis by targeting SPRY1 via the PTEN/PI3K/AKT signaling pathway. CNS Neurosci Ther 2018; 24: 369-380.

[6] Xu J, Ma B, Chen G, Wei D, Li L and Hu W. MicroRNA-622 suppresses the proliferation of glioma cells by targeting YAP1. J Cell Biochem 2018; 119: 2492-2500.

[7] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 2005; 353: 1793-1801.

[8] Nakao K, Miyaaki H and Ichikawa T. Antitumor function of microRNA-122 against hepatocellular carcinoma. J Gastroenterol 2014; 49: 589-593.

[9] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC and Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103: 2257-2261.

[10] Zheng J, Wang J, Jia Y, Liu T, Duan Y, Liang X and Liu L. microRNA-211 promotes proliferation, migration, and invasion ability of oral squamous cell carcinoma cells via targeting the bridging integrator 1 protein. J Cell Biochem 2019; 120: 4644-4653.

[11] Xu X, Bao Z, Liu Y, Ji J and Liu N. MicroRNA-98 attenuates cell migration and invasion in glioma by directly targeting Pre-B cell leukemia homeobox 3. Cell Mol Neurobiol 2017; 37: 1359-1371.

[12] Lawler S and Chiocca EA. Emerging functions of microRNAs in glioblastoma. J Neurooncol 2009; 92: 297-306.

[13] Wang S, Yin Y and Liu S. Roles of microRNAs during glioma tumorigenesis and progression. Histol Histopathol 2019; 34: 213-222.

[14] Lu GF, You CY, Chen YS, Jiang H, Zheng X, Tang WW, Wang XY, Xu HY and Geng F. MicroRNA-671-3p promotes proliferation and migration of glioma cells via targeting CKAP4. Onco Targets Ther 2018; 11: 6217-6226.

[15] Meng C, Liu Y, Shen Y, Liu S, Wang Z, Ye Q, Liu H, Liu X and Jia L. MicroRNA-26b suppresses autophagy in breast cancer cells by targeting DRAM1 mRNA, and is downregulated by irradiation. Oncol Lett 2018; 15: 1435-1440.

[16] Fan F, Lu J, Yu W, Zhang Y, Xu S, Pang L and Zhu B. MicroRNA-26b-5p regulates cell proliferation, invasion and metastasis in human intrahepatic cholangiocarcinoma by targeting S100A7. Oncol Lett 2018; 15: 386-392.

[17] Fan D, Lin X, Zhang F, Zhong W, Hu J, Chen Y, Cai Z, Zou Y, He X, Chen X, Lan P and Wu X. MicroRNA 26b promotes colorectal cancer metastasis by downregulating phosphatase and tensin homolog and wingless-type MMTV integration site family member 5A. Cancer Sci 2018; 109: 354-362.

[18] Li YP, Dai WM, Huang Q, Jie YQ, Yu GF, Fan XF, Wu A and Mao DD. Effects of microRNA-26b on proliferation and invasion of glioma cells and related mechanisms. Mol Med Rep 2017; 16: 4165-4170.

[19] Chen ZG, Zheng CY, Cai WQ, Li DW, Ye FY, Zhou J, Wu R and Yang K. MiR-26b mimic inhibits glioma proliferation in vitro and in vivo suppressing COX-2 expression. Oncol Res 2019; 27: 147-155.

[20] Esquela-Kerscher A and Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

[21] Petrovic N and Ergun S. miRNAs as potential treatment targets and treatment options in cancer. Mol Diagn Ther 2018; 22: 157-168.

[22] Cao Y, Song J, Ge J, Song Z, Chen J and Wu C. MicroRNA-100 suppresses human gastric cancer cell proliferation by targeting CXCR7. Oncol Lett 2018; 15: 453-458.

[23] Pritchard CC, Cheng HH and Tewari M. MicroRNA profiling: approaches and considerations. Nat Rev Genet 2012; 13: 358-369.

[24] Tian L, Zhang J, Ren X, Liu X, Gao W, Zhang C, Sun Y and Liu M. Overexpression of miR-26b decreases the cisplatin-resistance in laryngeal cancer by targeting ATF2. Oncotarget 2017; 8: 79023-79033.

[25] Abrami L, Kunz B, Deuquet J, Bafico A, Davidson G and van der Goot FG. Functional interactions between anthrax toxin receptors and the WNT signalling protein LRP6. Cell Microbiol 2008; 10: 2509-2519.

[26] Anastas JN and Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer 2013; 13: 11-26.

[27] Gerlach JP, Emmink BL, Nojima H, Kranenburg O and Maurice MM. Wnt signalling induces accumulation of phosphorylated beta-catenin in two distinct cytosolic complexes. Open Biol 2014; 4: 140120.

[28] Giles RH, van Es JH and Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003; 1653: 1-24.

[29] Klaus A and Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008; 8: 387-398.

[30] Xu Y, Ge K, Lu J, Huang J, Wei W and Huang Q. MicroRNA-493 suppresses hepatocellular carcinoma tumorigenesis through down-regulation of anthrax toxin receptor 1 (ANTXR1) and R-Spondin 2 (RSPO2). Biomed Pharmacother 2017; 93: 334-343.

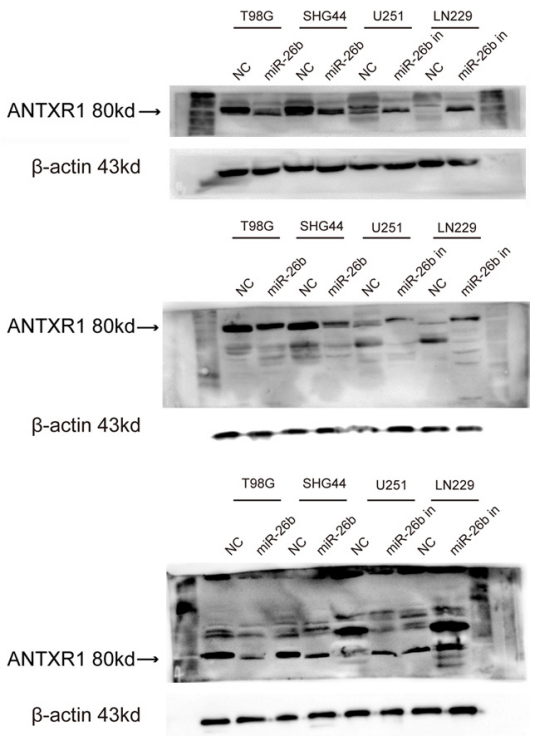

Figure S1. The western images of **Figure 4**.