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MiR-140 promotes the progression of intracranial aneurysms by targeting *BCL2L2*

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To investigate the role of miR-140/BCL2L2 axis on the formation of intracranial aneurysms. The expression of miR-140 in the serum of patients with intracranial aneurysms and healthy volunteers was detected. CCK-8 assay and Annexin V-FITC/PI double staining flow cytometry were used to evaluate the effect of miR-140 knockdown on the proliferation and apoptosis of human brain vascular smooth muscle cells (HBVSMCs). Meanwhile, the relationship between miR-140 and *BCL2L2* was examined. MiR-140 was found to be upregulation in intracranial aneurysm patients. MiR-140 knock-out significantly inhibited the apoptosis of HBVSMCs and promoted cell proliferation. *BCL2L2* was a direct target gene of miR-140 and suppressed its expression. Knockdown of miR-140 alleviates the development of intracranial aneurysms. MiR-140/BCL2L2 axis promotes the progression of intracranial aneurysms by regulating apoptosis of HBVSMCs. Therefore, miR-140 is a potential therapeutic target for intracranial aneurysms.
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Introduction

Intracranial aneurysm is a common cerebrovascular disease, which is one of the biggest challenges faced by clinicians. Intracranial aneurysm is a local enlargement of the arterial wall caused by the destruction of the inner elastic plate and the media, which is manifested as local dilatation or cerebral artery enlargement [1]. It has been reported that 1–6% of the world's population is affected by intracranial aneurysms [2]. Intracranial aneurysm is at risk of rupture bleeding at any time, resulting in high mortality of spontaneous subarachnoid hemorrhage and ruptured cerebral hemorrhage. Despite the development of neural intervention and microscopy, the disability rate and the mortality rate of intracranial aneurysms are still as high as 30–40% [3]. At present, the occurrence of intracranial aneurysm is related to genetic, hemodynamic and inflammatory factors, which lead to protrusion and eventual rupture of the arterial wall [1]. However, the molecular mechanism behind these events is still unclear, so a better understanding of the physiopathology of intracranial aneurysms is needed to improve the detection and treatment strategy.

Studies have shown that miRNAs play a regulatory role in the formation and rupture of intracranial aneurysms [2]. MiRNA is a small noncoding RNA molecule, approximately 22 nucleotides in length, that is an important regulator in a variety of biological processes. At present, research on miRNAs is still concentrated in the field of tumor, and many miRNAs have been found to be tumor biomarkers, which can participate in the diagnosis, treatment and prognosis evaluation of tumor. MiR-140 has been shown to be associated with various cancers. Overexpression of miR-140 in colorectal cancer and prostate cancer cells inhibits migration and invasion [4]. The level of miR-140 is downregulated in esophageal cancer, suggesting that miR-140 may play an antineoplastic role in a certain type of tumor [5]. In addition, miR-140 was also found to be an inhibitor in gastric cancer, and breast cancer [6,7]. It has been confirmed that miR-140 is not only abnormally expressed in tumors, but also that the expression level of miR-140-5p is low after ischemic stroke. However, the overexpression of miR-140-5p can inhibit angiogenesis after ischemic stroke and has a protective effect on ischemic stroke-induced injury. Song believed that high miR-140-5p expression can be used as a potential candidate drug for developing ischemic treatment strategies. There are few descriptions of the mechanism of miR-140 in the occurrence and development of intracranial aneurysms. The research on miR-140 may provide a hypothetical mechanism for a better understanding of intracranial aneurysms. This study was designed to focus on the role of miRNAs in the pathogenesis of intracranial aneurysms.

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BCL-2 is an important regulatory factor of cell death, which is located in the outer membrane of mitochondria. It controls the release of mitochondrial cytochrome c oxidase and inhibits the function of pro-apoptotic genes. BCL2L2 belongs to the BCL-2 family, which plays an anti-apoptotic regulatory role and reduces apoptosis under cytotoxic conditions [8]. Excessive apoptosis in vascular smooth muscle cells (VSMCs) may be an important mechanism for the formation and rupture of intracranial aneurysms. Feng found that hemodynamic abnormalities, as the causative factor of VSMC apoptosis, were related to the activation of caspase-9 by the BCL-2-mediated mitochondrial pathway and were all involved in the early formation of the intracranial aneurysm [9]. In clinical samples, the expression of miR-193 in the villi of patients with recurrent abortion is obviously upregulated, which can inhibit the proliferation activity of human trophoblast HTR-8/SVneo and promote the occurrence of apoptosis. Molecular studies have shown that its potential target gene is *BCL2L2*, which is negatively correlated and plays a role in regulating the apoptosis of trophoblasts [10]. Therefore, we explored the regulatory mechanism of the miR-140/*BCL2L2* molecular axis in intracranial aneurysms.

Materials and methods

Clinical samples, cells and primary reagents

There were 25 patients with unruptured intracranial aneurysms and 20 healthy volunteers. They were enrolled in our hospital from January 2020 to May 2021. These patients were admitted to the hospital because of an intracranial aneurysm found in physical examination, and they were suspected to be intracranial aneurysm clinically. After the diagnosis was confirmed by CTA or MRA examination of the brain, DSA examination of the brain was completed in 10 cases. Clinical and biochemical test data and imaging examination data were complete. Exclusion criteria include history of brain diseases such as intracranial aneurysm rupture, secondary cerebral hemorrhage and cerebral infarction, severe craniocerebral injury, other tumors, diseases of blood circulation system, dysfunction of heart, liver and kidney and other important organs. Among the 25 patients with intracranial aneurysms, there were 17 male and 8 female, with an age distribution ranging from 35 to 61 years old, and the average age (47.82 ± 6.53) years old. Among the 20 healthy volunteers, 12 were male and 8 were female, aged 33–59 years old, with an average age of (46.11 ± 5.12) years old. According to the location, 25 aneurysms were distributed in 10 cases (40%) of anterior communicating artery, 6 cases (24%) of the junction between the internal carotid artery and posterior communicating artery, 4 cases (16%) of the bifurcation of the middle cerebral artery and 5 cases (21%) of the basilar artery. The clinical symptoms of these 25 patients with unruptured aneurysms included dizziness (80%), mild headache (50%), transient visual decline (20%) and facial numbness (16%). The study was approved by the hospital ethics committee and all subjects signed informed consent. Human cerebrovascular smooth muscle cells (HBVSMCs type: 1100) were provided by ScienCell Co., Ltd, San Diego, California, USA. Cell lines were cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in an incubator at 37°C and 5% CO_2. MiR-140 inhibitor/mimics and *BCL2L2* overexpression vector were provided by Shanghai Gemar Co., Ltd, China. DMEM and FBS were purchased from Biological Industries in the USA. The CCK-8 kit was purchased from Wuhan Huamei Biological Engineering Co., Ltd., China. Annexin V-FITC/PI detection kit was purchased from eBioscience (San Diego, California, USA). A miRcute/plasma miRNA extraction and separation kit was purchased from Beijing, Tiangen Biochemical Technology (Beijing, China) Co., Ltd. SYBR premix Ex TaqTM quantitative PCR kit was purchased from TaKaRa, Tokyo, Japan. Hiper Fect transfection was purchased from Promega Co., Ltd, Madison, Wisconsin, USA. The dual-luciferase reporter kit and reporter vector were purchased from Promega. Human renal epithelial cell line 293T packaged cells were purchased from Promega. The protein extraction kit and SDS-PAGE gel rapid preparation kit were both purchased from Bio-Rad (Hercules, California, USA). Western blot antibodies sheep-resistant rabbit IgG (H+L) were purchased from CST Co., Ltd, Boston, Massachusetts, USA.

Cell transfection

Dense monolayers of cells appeared in culture flasks, they were digested with 0.25% trypsin. When the intercellular junction became loose, the cells were blown into a single-cell suspension for split charging culture. The cryopreservation solution was prepared in advance and the HBVSMCs were adjusted to a 1×10^6–6×10^6/mL cell suspension. The specific cryopreservation process was as follows: placing at 4°C for 30–60 min, refrigerator at −20°C for 30 min, then −80°C overnight, and finally liquid nitrogen for long-term storage. Normal cultured HBVSMCs were selected for passage, and the density of HBVSMCs was adjusted to 50 000/mL after being counted and inoculated into 96-well plates. Cells in the logarithmic phase were selected for the grouping experiment. For cell identification and determination of the undifferentiated state, we chose to inoculate 5000 cells/well in a 24-well plate and added 0.01% acridine orange dye to a dried slide containing HBVSMCs. The nuclei can be observed to have a yellow-green complete oval under an inverted fluorescence microscope. Followed by transfection with miR-140 mimics/inhibitors and pcDNA-BCL2L2. The transfection method was performed according to the instructions of the Hiper Fect transfection agent, and the transfection effect was observed under a fluorescence microscope 48 h later.

Table 1 Primer sequence

Primer	Sequence
miR-140	F: 5′-TGCGGCAGTGGTTTTACCCTATG-3′
	R: 5′-CCAGTGCAGGGTCCGAGGT-3′
BCL2L2	F: 5′-CTTGGTCTTGTTGTGAGTATGC-3′
	R: 5′-TGGAGCCGATGGTCTAGTC-3′
U6	F: 5′-TGCGGGTGCTCGCTTCGGCAGC-3′
	R: 5′-CCAGTGCAGGGTCCGAGGT-3′
GAPDH	F: 5′-AACGGATTTGGTCGTATTG-3′
	R: 5′-GGAAGATGGTGATGGGATT-3′

Treatment of HBVSMCs with H_2O_2

After treatment of HBVSMCs with 0, 50, 100, 150, 200, 250 and 300 mol/L H_2O_2 for 6 h, the cells were divided into groups A to G according to the H_2O_2 concentration gradient, and other treatment factors were consistent among the groups to simulate the brain smooth muscle cell injury model of intracranial aneurysms. After treatment, the culture medium was changed to continue to be cultured and used for follow-up experiments.

The expression level of miR-140 was measured by qRT-PCR

Serum samples were collected and centrifuged to obtain the supernatant, which was filtered through a 0.2-μm filter membrane. Five hundred microliters were transferred to a 30-kDa ultrafiltration tube, and 3000 ×g was centrifuged to approximately 100 μL. Subsequently, miRNA from exosomes was extracted according to the instructions. Total RNA was extracted by Trizol in HBVSMCs transfected with miR-140 mimics/inhibitors and pcDNA-BCL2L2. Subsequently, a NanoDrop was used to detect the concentration and purity of RNA, which was reverse transcribed to prepare cDNA. Then, 2 μL reverse transcription products were used for PCR detection, with U6 and GAPDH as internal controls. Subsequently, a PCR reaction system was established according to the kit instructions: 2 μL reverse transcription products, 10 μL SYBR Green Mix, upstream and downstream primers, 0.5 μL each. Thermal cycle parameters of PCR: 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, 45 cycles. The $2^{-\Delta\Delta Ct}$ method was used to calculate the results. The experiment was repeated three times in all. The primer sequences used in the article are shown in Table 1.

Western blotting was used to detect the expression levels of *BCL2L2* and apoptosis-related proteins

After protein extraction, the protein was isolated by SDS-PAGE and the membrane was transferred. The protein was blocked in 5% bovine serum protein for 1 h, followed by the addition of a primary antibody (the dilution concentration was 1:2000) and overnight at 4°C. The membrane was washed three times, for 5 min each time, followed by the addition of a secondary antibody (the dilution concentration was 1:5000), and incubation in the greenhouse for 1 h. After washing the membrane three times, a luminescent reagent was added to develop the protein. GAPDH was used as the internal control, and Image J software was used to analyze the gray level of the target band.

CCK-8 was used to detect the proliferation activity of HBVSMCs

HBVSMCs in the logarithmic growth phase were inoculated in 96 well plates, and each well contained 1×10^4 cells and 100 μL medium. One hour before cell incubation, there were 10 μL CCK-8 solution was added to each well, and the optical density (D) value at 450 nm was measured by a microplate reader for 1–4 h in the incubator.

Flow cytometry to detect apoptosis of HBVSMCs

HBVSMCs in the logarithmic growth phase were washed twice with PBS, mixed with precooled with 500 μL buffer and 5 μL Annexin V-FITC, and were incubated at room temperature for 15 min in the dark, followed by 2.5 μL PI staining for 5 min before the operation. After that, cell apoptosis was detected on the machine.

Dual-Luciferase Report Gene System verification of the targeting relationship between miR-140 and BCL2L2

A total of 293 T cells were transfected and miR-140 was expressed according to the kit instructions. Cells were collected after 48 h of culture at 37°C in a 5% CO_2 incubator. A Dual-Luciferase Report gene detection kit was used to detect three replicate samples.

Statistical analysis

SPSS 22.0 statistical software was used. Measurement data are expressed $\overline{x} \pm s$, and a t test was used to compare the mean between the two groups. GraphPad Prism 8 drew relevant pictures of the experimental data. The result $P < 0.05$ was considered statistically significant.

Result

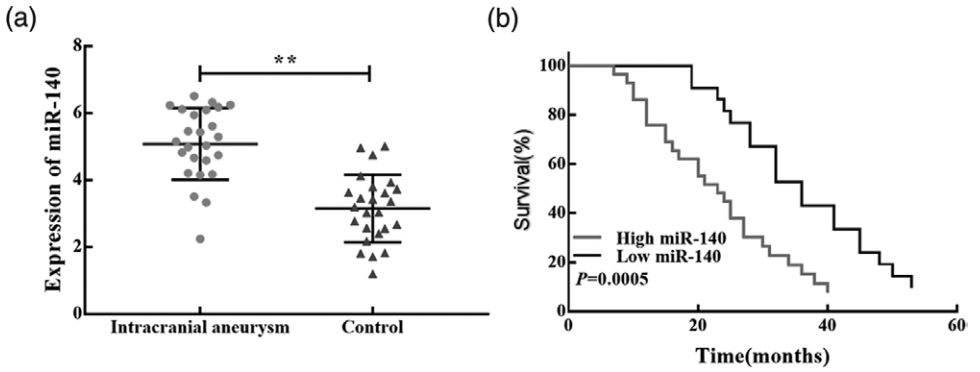
Expression level of miR-140 in the serum of intracranial aneurysms

qRT-PCR showed that the expression level of miR-140 in the serum of patients with intracranial aneurysms was significantly higher than that of healthy control group, as shown in Fig. 1a. Kaplan–Meier survival curve analysis showed that patients with intracranial aneurysms whose miR-140 expression is upregulated have low survival rate and poor prognosis, as shown in Fig. 1b.

The effect of miR-140 on the proliferation and apoptosis of HBSMCS under the action of H_2O_2

qRT-PCR results showed that concentrations of H_2O_2 could significantly promote the expression level of miR-140 in HBVSMCs, as shown in Fig. 2a. Meanwhile, the transfection efficiency of miR-140 measured by

Fig. 1



Expression of miR-140 in the serum of patients with intracranial aneurysm. (a) The expression level of miR-140 was measured by qRT-PCR, and the content comparison between the experimental group and the control group was $(5.07\times10^9 \pm 3.14\times10^9)$/μL vs. $(3.13\times10^9 \pm 2.08\times10^9)$/μL, $P=0.003$. (b) We selected a 5-year period as the endpoint of the survival analysis and calculated monthly survival rates. The higher miR-140 group could predict the patient's adverse outcomes and a lower survival rate ($P=0.005$).

qRT-PCR showed that, compared with the control group, transfection of miR-140 inhibitor significantly inhibited the expression of miR-140, and the transfection of miR-140 mimics significantly increased the expression level of miR-140 in HBVSMCs, as shown in Fig. 2b. The CCK-8 assay showed that HBVSMCs induced with H_2O_2 for 300 mol/L after miR-140 knockdown were significantly more active than those in the H_2O_2 group, while the results of miR-140 overexpression were the opposite, as shown in Fig. 2c. Annexin V - FITC/PI double staining flow cytometry confirmed that, compared with the H_2O_2 group, overexpression of miR-140 significantly promoted the apoptosis level of HBVSMCs treated with H_2O_2, but knockdown of miR-140 significantly reduced the apoptosis level of HBVSMCs treated with H_2O_2, as shown in Fig. 2d.

miR-140 specifically regulates the expression of BCL2L2

The target gene prediction of miR-140 in the Target Scan bioinformatics database showed that miR-140 could bind to the 3′UTR of BCL2L2, as shown in Fig. 3a. The results of the Dual-Luciferase Report Gene System showed that the luciferase activity was significantly reduced after the transfer of miR-140 mimic in BCL2L2 wild-type cells (WT group), while the luciferase activity was not significantly changed after the transfer of miR-140 mimic into BCL2L2 mutant cells (MUT group), as shown in Fig. 3b. Meanwhile, western blot results showed that knockdown of miR-140 significantly promoted the expression level of BCL2L2 protein ($P=0.001$), as shown in Fig. 3c. Analysis results of WB original band and image J about Fig. 3c can be found in WB outcome analysis S1 in attached Supplementary Materials, Supplemental Digital Content 1, *http:///links.lww.com/WNR/A682*.

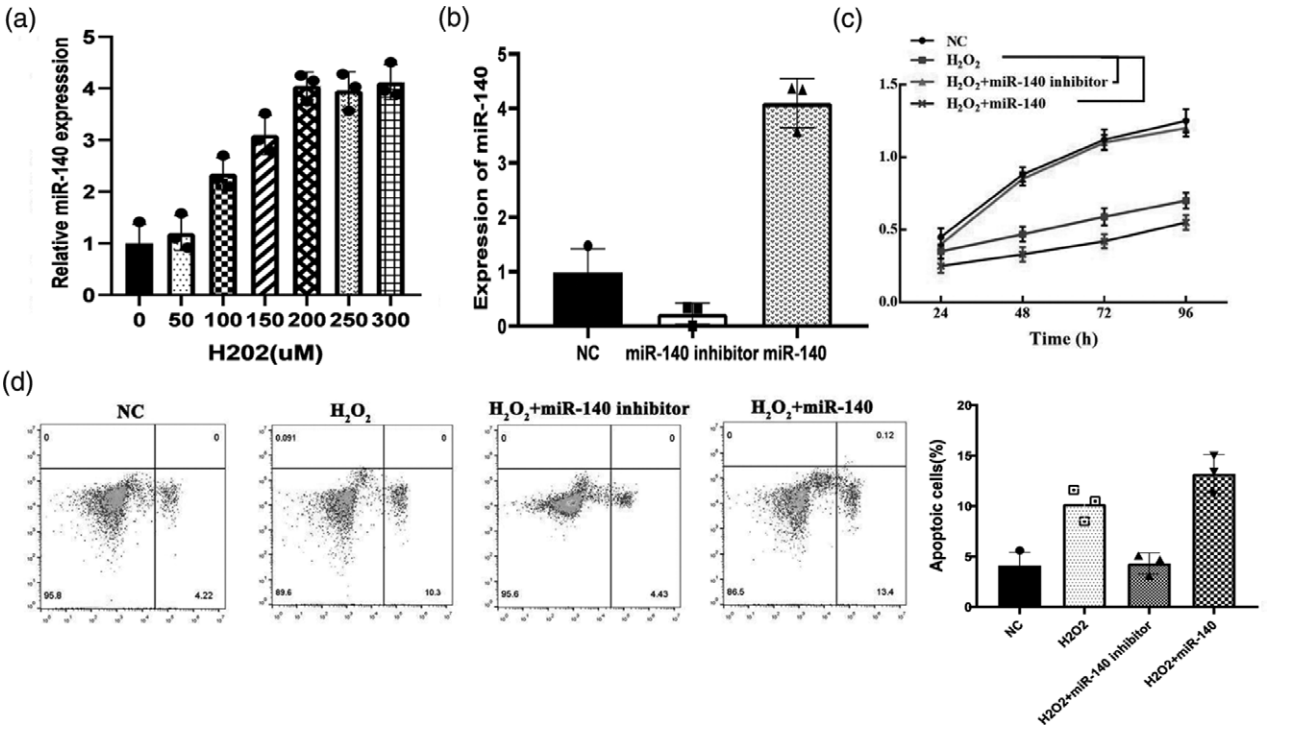
The effect of miR-140 on the proliferation and apoptosis of HBVSMCs by targeting BCL2L2

Western blot results showed that compared with the control group, H_2O_2 significantly decreased the expression level of BCL2L2 protein ($P=0.045$). Overexpression of BCL2L2 significantly downregulated the inhibitory effect of H_2O_2 on the expression of BCL2L2 protein ($P=0.04$), while the expression level of BCL2L2 protein in HBSMCS cells after overexpression of the miR-140 and BCL2L2 genes was lower than that of the control group ($P=0.03$), and there was no significant difference from the H_2O_2 group, as shown in Fig. 4a. The CCK-8 test results showed that compared with the H_2O_2 group, the overexpression of the *BCL2L2* gene significantly downregulated the inhibitory effect of H_2O_2 on the proliferation activity of HBVSMCs ($P=0.04$), while the overexpression of the miR-140 and *BCL2L2* genes showed no significant difference from the H_2O_2 group, as shown in Fig. 4b. Annexin V-FIT/PI double staining flow optometry showed that, compared with the H_2O_2 group, the overexpression of BCL2L2 significantly downregulated H_2O_2-induced apoptosis of HBVSMCs ($P=0.001$), but the apoptosis level of HBVSMCs after the overexpression of miR-140 and *BCL2L2* genes was not significantly different from that of the H_2O_2 only group ($P>0.05$), as shown in Fig. 4c. Analysis results of WB original band and image J about (Fig. 4a) can be found in WB outcome analysis S2 in attached Supplementary Materials, Supplemental Digital Content 1, *http:///links.lww.com/WNR/A682*.

Discussion

Cerebral aneurysm is the abnormal bulging of cerebral artery wall. The blood flows in the artery and keeps hitting the wall. The weak place may bulge to form an aneurysm, similar to a balloon. Middle-aged, smokers, women with, genetic history, alcoholism and

Fig. 2

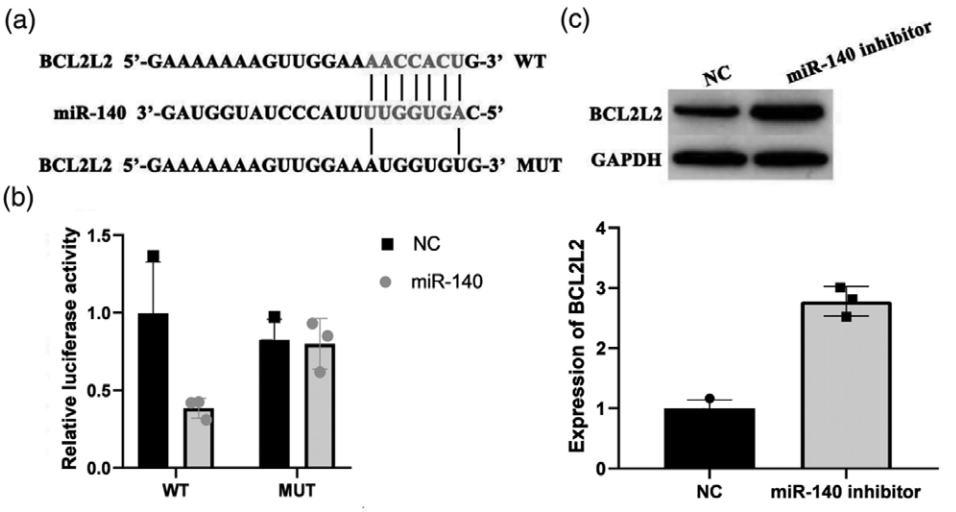


Effect of miR-140 on the proliferation and apoptosis of HBVSMCs. (a) After treatment of HBVSMCs with a series of concentration gradients of H_2O_2 for 6 h, we found that the level of miR-140 reached the maximum in 300 mol/L H_2O_2 conditions, (P=0.038). Therefore, 300 mol/L H_2O_2 was selected for the follow-up experiment. (b) miR-140 inhibitor/mimics have a dual effect on the concentration of miR-140 (miR-140 inhibitor group, P=0.002; miR-140 mimics group, P=0.003). (c) The CCK-8 assay showed that the concentration of miR-140 had an obvious effect on cell proliferation after H_2O_2 (300 mol/L) treatment (miR-140 inhibitor group, P=0.005; miR-140 mimics group, P=0.03). (d) Annexin V-FITC/PI double staining flow cytometry showed that overexpression/knockdown of miR-140 affected the apoptosis level of target cells (miR-140 mimics group P=0.003; miR-140 inhibitor group, P=0.005). HBVSMCs, human brain vascular smooth muscle cells.

hypertension patients are more likely to grow or rupture aneurysms [2]. Cerebral vasospasm, oculomotor nerve paralysis and spontaneous cerebral hemorrhage are the main clinical manifestations and complications of intracranial aneurysms. Clinical treatment of intracranial aneurysms mainly includes vascular intervention and tracheotomy clipping. Early detection of an unruptured intracranial aneurysm allows for regular monitoring and preventive microsurgical treatment to prevent future ruptures. One of the ways to find early aneurysms is to increase our understanding of the underlying mechanisms of aneurysm development. It has been proved that the apoptosis and proliferation damage of human cerebral vascular smooth muscle cells was related to the development of intracranial aneurysms [11]. H_2O_2 can decrease the proliferation of HBVSMCs and increase apoptosis. MiRNA is essential to the homeostasis of VSMCs, playing an important role in aneurysm formation, and is a promising biomarker in the treatment of intracranial aneurysms. MiRNA is involved in the development of intracranial aneurysms and is related to the regulation of proliferation and apoptosis of VSMCs [12].

More and more studies have confirmed the abnormal expression of microRNAs in intracranial aneurysm tissues [13], and these abnormal expressions of microRNAs mediate the occurrence and development of aneurysms [14]. Wang *et al.* have shown that miR-29a can be used as a molecular marker for the diagnosis of intracranial aneurysm development and rupture [15]. Luo *et al.* found that the knockdown of miR-9 inhibits the development of intracranial aneurysms by promoting the proliferation of VSMCs and inhibiting cell apoptosis [16]. There are two similar structures miR-140-5p and miR-140-3p, and their abundances are roughly equal. It is convincing that miR-140 is closely related to many kinds of tumors. The expression level of miR-140-3 is low in ERα^+ breast cancer because ERα^+ can bind to the miR-140-3p promoter of the estrogen-responsive element and inhibit its expression [17]. MiR-140-5p also inhibits the development of bile duct cell carcinoma by upregulating the target tumor-promoting gene septin2 [18]. Therefore, the first step of our study was to determine the possible relationship between miR-140 and HBVSMCs. Contrary to the main solid tumors, our results confirmed that the high expression of miR-140 in the serum of intracranial

Fig. 3



Effect of miR-140 on BCL2L2 expression. (a) Target gene base sequences of the BCL2L2 WT group, miR-140 group, and BCL2L2 MUT group. (b) The Dual-Luciferase Report Gene System verified the targeted binding relationship between BCL2L2 and miR-140 (MUT group, $P=0.001$) (c) Western blot analysis showed that miR-140 inhibitor targeting promoted the expression of BCL2L2 protein ($P=0.002$), and GAPDH was used as the internal control. HBVSMCs, human brain vascular smooth muscle cells.

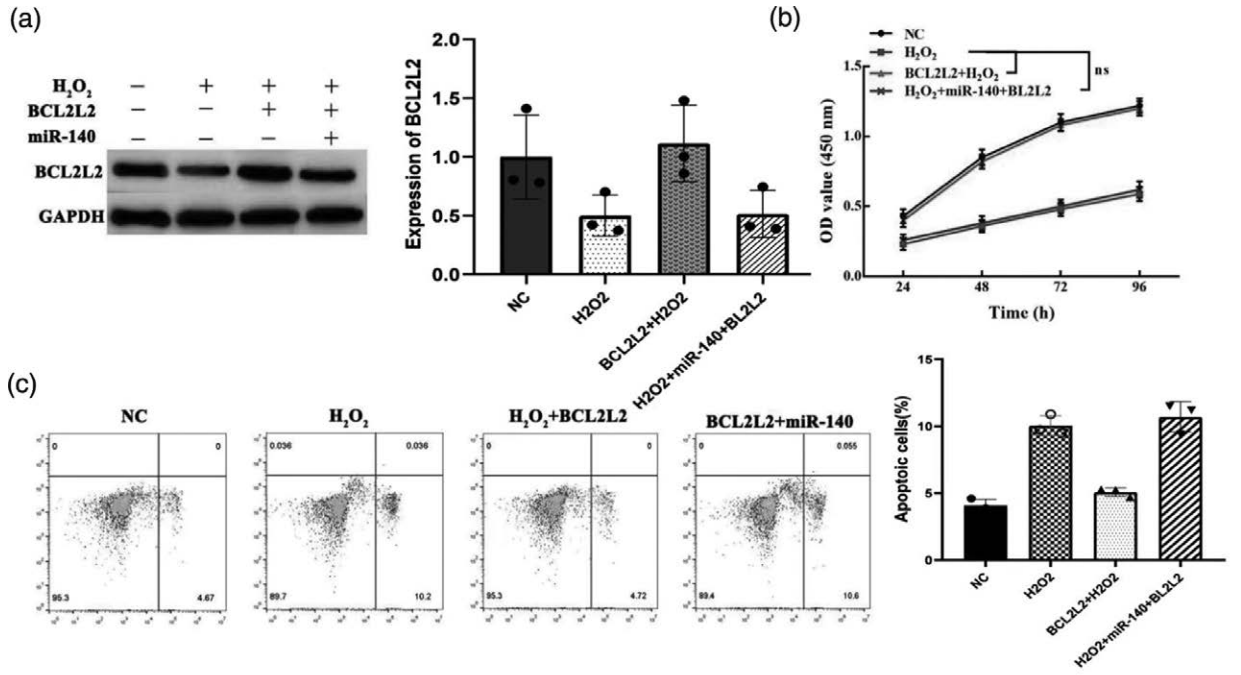
aneurysms was higher than that in healthy people. Meanwhile, cell proliferation activity, CCK-8 assay and Annexin V-FITC/PI double-staining flow cytometry confirmed that high expression of miR-140 promoted the apoptosis of vascular wall smooth muscle cells.

MiRNA can bind to the 3′UTR noncoding region of miRNA in complementary or partially complementary ways, thus mediating the degradation of mRNA or inhibiting its translation and negatively regulating the participation of downstream targets in physiological processes [19]. Our study verified that BCL2L2 is the downstream target gene of miR-140, overexpression of BCL2L2 can negatively inhibit HBVSMCs apoptosis and promote cell proliferation. It has been found that BCL2L2, as a member of the Bcl-2 family of anti-apoptotic-related proteins, is widely involved in the regulation of apoptosis [20]. The Bcl family, represented by Bak and bax, contains BH 3 domain. Under the induction of several activators such as NOVA, it promotes the apoptosis of the target cell through the conformational changes of protein subunits, phosphorylation, fragmentation effect and other mechanisms. BCL2L2 can resist the dissolving effect of bax and bak and other proapoptotic molecules on the mitochondrial membrane, and maintain the stability of the cell structure [21].

Li showed that miR-140-5p was downregulated in patients with chronic myeloid leukemia, and overexpression of miR-140-5p promoted the apoptosis of CML cells [22]. MiR-140 may inhibit the proliferation of AML cells by targeting DNAJC3-AS1 [23]. Salidroside is the active component of Rhodiola Rosea, which has antitumor effects on many cancers. Salidroside plays a role in the treatment of CML by regulating miR-140-5p to inhibit the wnt5a/β-catenin signaling pathway [24]. Wang *et al.* have confirmed that BCL2L2 is the target gene of miR-212-5p. Overexpression of BCL2L2 can reverse the inhibitory effect of GL-1 extracted from Glycyrrhizae Radixon the proliferation, migration and invasion of thyroid tumor cells and the expression of Cyclin D1 and MMP-2 by targeting the miR-212-5p/BCL2L2 axis [25]. The relationship between miR140 and BCL2L2 has not been found before, and our study first expounded the biological role of miR-140/BCL 2L 2 axis in aneurysms.

Although surgical treatment of an aneurysm is effective, intraoperative injury and subsequent complications have to be considered. For occasionally found intracranial aneurysms, especially those with a diameter of less than 5 mm, it is a difficult problem in clinical practice how to balance the risk of surgery and the risk of natural rupture to maximize the patients' benefits. Recent studies have suggested that aneurysms that do not match the surgical indications may be controlled by drugs. However, at present, there are no drug targeting miR-140/BCL2L2 axis to treat intracranial aneurysms. As mentioned above, GL-1 can regulate BCL2L2 and salidroside can regulate miR-140, both of which have antitumor effects. In the future, we can try to extract effective components from traditional Chinese medicine, which can target and regulate miR-140/BCL2L2 axis to inhibit the growth of intracranial aneurysms. These breakthroughs will provide new ideas for research on drugs for the prevention and treatment of intracranial aneurysms.

Fig. 4



Effect of miR-140 on the proliferation and apoptosis of HBVSMCs by targeting BCL2L2. (a) Different experimental treatments have confirmed that the expression of BCL2L2 protein is affected by the feedback of H_2O_2-regulated HBVSMCs, while miR-140 can also negatively regulate the expression of the target gene *BCL2L2*. (b) The CCK-8 assay showed that overexpression of *BCL2L2* could basically eliminate the effect of H_2O_2 on HBVSMCs proliferation, and *BCL2L2* gene was directly regulated by miR-140. (c) Annexin V-FITC/PI double staining and flow cytometry showed that overexpression of the *BCL2L2* gene reduced the effect of H_2O_2 on HBVSMCs apoptosis. HBVSMCs, human brain vascular smooth muscle cells.

Conclusion

We found that the expression of miR-140 increased in patients with intracranial aneurysms. BCL2L2 can significantly promote the proliferation of HBVSMCs and inhibit apoptosis by negatively regulating miR-140, thus controlling the occurrence of intracranial aneurysms. Future research should focus on using miR-140 as a molecular marker of unruptured intracranial aneurysms, and developing drugs, especially traditional Chinese medicine, to control the occurrence and development of intracranial aneurysms.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. All patients have signed informed consent.

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The datasets used and analyzed during the current study is available from the corresponding author upon reasonable request.

Graduate student J.D. finished the majority of the experiments and the article. Graduate student K.N. conceived part of the ideas of the article. Graduate student D.L. conducted the data analysis. Professor D.W. and R.W. were responsible for the collection and enrolment of cases and controls. Professor J.G. provided experimental scheme recommendations. At the same time, it needs to be added that J.D., the first author of the article, is responsible for the revision and writing of the article. All authors have read and approved the final article.

Conflicts of interest

There are no conflicts of interest.

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