

RESEARCH

Open Access



Hsa_circ_0004872 alleviates meningioma progression by sponging miR-190a-3p/PTEN signaling

Yongkai Huang¹, Zhihui Wu², Zewei Peng¹, Anmin Liu³, Wen Yuan¹, Deqing Han¹ and Junmin Peng^{4*}

Abstract

Background Meningioma, the most prevalent intracranial tumor, possesses a significant propensity for malignant transformation. Circular RNAs (circ-RNAs), a class of non-coding RNAs, have emerged as crucial players in tumorigenesis. This study explores the functional relevance of hsa_circ_0004872, a specific circ-RNA, in the context of meningioma.

Methods Molecular structure and stability of hsa_circ_0004872 were elucidated through PCR identification. Meningioma cell proliferation and apoptosis were assessed using the CCK-8 assay and flow cytometry, respectively. Gene and protein expression were analyzed via qRT-PCR and western blot. Molecular interactions were confirmed through dual-luciferase reporter gene and RIP assays.

Results Hsa_circ_0004872, derived from exons 2 to 4 of the host gene MAPK1, demonstrated enhanced stability compared to its host MAPK1. Clinical data described that hsa_circ_0004872 was reduced in meningioma tissues and cell lines, and negatively correlated to poor survival rate of meningioma patients. Overexpression of hsa_circ_0004872 exhibited inhibitory effects on cell proliferation and promotion of apoptosis in vitro. Subsequent investigations unveiled a direct interaction between hsa_circ_0004872 and miR-190a-3p, leading to the activation of the PI3K/AKT signaling pathway through targeting PTEN. Notably, miR-190a-3p silence accelerated the apoptosis and proliferation inhibition of meningioma cells by inactivating PTEN/PI3K/AKT signaling, while miR-190a-3p overexpression showed an opposite effect, which greatly reversed the anti-tumor effects of hsa_circ_0004872 overexpression.

Conclusion In summary, our findings highlighted the intricate role of hsa_circ_0004872 in meningioma, shedding light on the regulatory mechanisms involving circ-RNAs in tumor progression. This positions hsa_circ_0004872 as a potential key regulatory factor in meningioma with implications for future therapeutic interventions.

Highlights

- Hsa_circ_0004872 exhibits low expression in meningioma, correlating with a diminished survival rate among patients.
- Overexpression of hsa_circ_0004872 impedes meningioma cell proliferation while enhancing apoptosis.
- Hsa_circ_0004872 acts as a negative regulator of miR-190a-3p by functioning as a miRNA sponge.

*Correspondence:

Junmin Peng
pjm22peng@163.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

- Upregulation of miR-190a-3p counteracts the anti-tumor effects induced by hsa_circ_0004872 overexpression in vitro.
- Inhibition of miR-190a-3p reduces cell proliferation and heightens apoptosis by targeting the PTEN/PI3K/AKT signaling pathway.

Keywords Meningioma, hsa_circ_0004872, miR-190a-3p, PTEN/PI3K/AKT pathway

Introduction

Meningioma, constituting 36.6% of all primary central nervous system (CNS) malignancies [1], poses a significant health challenge. Classified into benign (Grade I), atypical (Grade II), and anaplastic (Grade III) tumors according to the 2021 WHO CNS tumor grading guidelines [2], malignant meningiomas (Grade II/III) exhibit high recurrence rates and dismal prognoses, profoundly impacting patient survival and quality of life [3]. Understanding the etiology of malignant meningioma is crucial for devising innovative treatment strategies.

It was well known that non-coding RNAs are the most of the transcription products of the human genome [4]. Circular RNAs (circRNAs) refer to a class of non-coding RNAs with closed loop structure [5]. It has been widely described that circRNAs are involved in the progression of human malignant tumors [6]. As proof, Chen et al. demonstrated that circRNA_0000285 expression was highly expressed in cervical cancer tissues, and its knockdown significantly suppressed cervical cancer cell growth and migration [7]. In addition, circ_102231 was significantly upregulated in gastric cancer tissues, and its silencing suppressed gastric cancer cell proliferation and invasion [8]. Hsa_circ_0004872 (circMAPK1), derived from exons 2~4 of the mitogen-activated protein kinase 1 (MAPK1) gene [9]. Several studies have emphasized the function of hsa_circ_0004872 in tumor biology. For instance, hsa_circ_0004872 was found to be downregulated in oral squamous cell carcinoma, and its overexpression inhibited the proliferation, cell vitality and invasion of oral squamous cells [10]. However, the regulatory role of hsa_circ_0004872 in meningioma progression remains unexplored, necessitating further investigation.

CircRNAs, known for their role as competing endogenous RNAs (ceRNAs), function by sequestering microRNAs (miRNAs) and modulating their binding to target genes [11]. MiRNAs refer to non-coding RNAs with a length of about 22 nts, which are involved in various biological processes [12]. MiRNAs dysregulation is an important inducer for the pathogenesis and development of meningioma [13]. For instance, miRNA-145 was down-regulated in atypical and anaplastic meningiomas and could negatively regulate meningioma cell proliferation [14]. Recent focus has turned to miR-190a-3p in cancer research. Studies by Zhou et al. revealed elevated expression of miR-190a-3p in glioma tissues and cells. Its knockdown suppressed glioma cell proliferation while promoting

apoptosis [15]. Conversely, in glioblastoma, miR-190a-3p facilitated tumorigenesis as a downstream target of LINC00657 [16]. These findings collectively underscore the cancer-promoting role of miR-190a-3p. However, the functions of miR-190a-3p in meningioma, as well as its regulatory relationship with hsa_circ_0004872, remain largely unexplored.

In the present study, we unraveled the inhibitory role of hsa_circ_0004872 in meningioma progression by negatively regulating miR-190a-3p expression, subsequently modulating the phosphatase and tensin homolog deleted on chromosome ten (PTEN)/phosphoinositide-3 kinase (PI3K)/protein kinase B (AKT) pathway. The findings contribute to the theoretical foundation for developing novel therapeutic strategies for meningioma.

Materials and methods

Cell culture

Human normal meningotheial cells (MECs, HUM-iCell-n002, iCell, Shanghai, China) and meningioma cells (IOMM-Lee, CRL-3370, ATCC, VA, USA), BEN-MEN-1 (GD-C00515294S, Sgdbio, Shanghai, China), CH157-MN (SR90102R, Xrshbio, Shanghai, China) and HBL-52 (GD-C00516386S, Sgdbio) were cultured in Dulbecco's Modified Eagle's medium (DMEM, Gibco, MD, USA) containing 10% fetal bovine serum (FBS, Gibco) at 37 °C with 5% CO₂.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

TRIzol reagent (Thermo Fisher Scientific, MA, USA) was used to extract total RNAs from meningioma tissues and cell lines. Then, the cDNA was synthesized using the cDNA synthesis kit (Toyobo, Tokyo, Japan) and subsequently used for qRT-PCR assay using SYBR (Thermo Fisher Scientific). The relative expressions were normalized by GAPDH or U6 and calculated by the $2^{-\Delta\Delta CT}$ method. The primers used in the study were listed as follows (5'-3'):

hsa_circ_0004872 (F): ACCTACTGCCAGAGAACCCT.
 hsa_circ_0004872 (R): CAGGTTGGAAGGCTTGAGGT.
 miR-190a-3p (F): CTAGGAGCTTCAGTAACGTTA.
 miR-190a-3p (R): GAATTCGAATCGGTAACAGCT.
 PTEN (F): CGACGGGAAGACAAGTTCAT.
 PTEN (R): AGGTTTCCTCTGGTCTCTGGT.
 GAPDH (F): CCAGGTGGTCTCCTCTGA.
 GAPDH (R): GCTGTAGCCAAATCGTTGT.
 U6 (F): GTGCAGGGTCCGAGGT.

U6 (R): CTCGCTTCGGCAGCACAA.

Identification of hsa_circ_0004872

To confirm the specificity of the hsa_circ_0004872, the PCR products of hsa_circ_0004872 was amplified by divergent primer (F: 5'-TGTTGAATTCCAAGCTCTGCTTA-3'; R 5'-TGTTGAATTCCAAGCTCTGCTTA-3') or convergent primer (F: 5'-ACCTACTGCCAGAGAACCCT-3'; R 5'-CAGGT TGGAAAGGCTTGAGGT-3'), and followed by separated on 1% agarose gel. The genomic DNA (gDNA) and cDNA were used as a PCR template. To further validate the stability of hsa_circ_0004872, IOMM-Lee and CH157-MN cells were exposed to RNase R (4U/mg, Epicentre Technologies, WI, USA) for 30 min at 37 °C or treated with 5 mg/ml actinomycin D (Sigma-Aldrich, MO, USA) for 0 h, 4 h, 8 h, 12 and 24 h. Afterwards, the total RNA was extracted as described above, and the abundance of hsa_circ_0004872 and liner MAPK1 gene were evaluated by qRT-PCR.

Clinical sample collection

A total of 30 malignant meningioma tissues, along with paired adjacent normal tissues, were post-operatively collected from meningioma patients at Zhuzhou Hospital affiliated with Xiangya Medical College. Immediately following collection, the tumor and adjacent tissues were flash-frozen and stored in liquid nitrogen. The study protocol underwent rigorous review and approval by the Ethics Committee of Zhuzhou Hospital affiliated with Xiangya Medical College (Approval No. 2018K0345). All participants in the study provided informed consent.

Cell transfection

Overexpression plasmids, including hsa_circ_0004872 (pcDNA3.1-circ), miR-190a-3p mimics/inhibitor, and their respective negative controls (pcDNA3.1), were procured from GenePharma (Shanghai, China). Transfection was carried out using Lipofectamine™ 3000 (Invitrogen, CA, USA) following the manufacturer's instructions. Transfection efficiency was assessed using qRT-PCR.

Western blot

Proteins were extracted using RIPA lysis buffer (Beyotime, Shanghai, China), and their concentrations were quantified with a BCA kit (Beyotime). Equal amounts of total proteins were separated by 12% SDS-PAGE and subsequently transferred onto a PVDF membrane (Millipore, Boston, MA, USA). The membranes were incubated overnight with specific antibodies, including B-cell lymphoma-2 (Bcl-2) (#26593-1-AP, Proteintech, Chicago, USA), Bax (#250599-2-Ig, Proteintech), Caspase3 (#19677-1-AP, Proteintech), PTEN (ab267787, Abcam, Cambridge, MA, USA), PI3K (#60225-1-Ig, Proteintech), p-PI3K (Abcam,

ab278545), AKT (#10176-2-AP, Proteintech), p-AKT (#28731-1-AP, Proteintech), and GAPDH (#10494-1-AP, Proteintech). Subsequently, the blots were hybridized with secondary antibodies (Abcam, ab7090 or ab6728) for 60 min. Blot visualization was performed using a GEL imaging system (Bio-Rad, CA, USA), and the results were analyzed with ImageJ software.

Subcellular fractionation

Nuclear and cytoplasmic RNA isolation was conducted using the PARISTM kit (Austin, TX, USA). Subsequently, RNA extraction followed the previously described method. Quantification of RNA samples was performed through qRT-PCR analysis, as outlined above. U6 served as a nuclear control, while GAPDH was utilized as a cytoplasmic control.

Cell proliferation assay

Cell proliferation of meningioma cells was measured by using a cell counting kit-8 (CCK-8) assay (CCK-8, Dojin, Japan). Briefly, IOMM-Lee and CH157-MN cells were cultured in 24-well plates (2×10^3 cells/well) for 24 h and incubated with 10 μ L of CCK-8 solution at 37 °C for 3 h. Absorbance was analyzed at 450 nm with a microplate spectrophotometer (Biotek, Beijing, China).

Cell apoptosis assay

Annexin V-FITC Apoptosis Detection Kit (Invitrogen) was used to detect apoptosis. After the corresponding treatments, IOMM-Lee and CH157-MN cells were harvested and washed with PBS solution. Then, cells were re-suspended in 500 μ L of 1X Annexin-binding buffer and then incubated with 10 μ L Annexin V-FITC and 5 μ L PI stain for 10 min. Samples were immediately analyzed using flow cytometry (BD, NJ, USA).

Dual-luciferase reporter gene assay

Hsa_circ_0004872/PTEN 3'-UTR fragments containing miR-190a-3p WT/MUT binding sites were amplified by PCR and inserted into pmirGLO reporter plasmids (Promega, WI, USA). Cells were co-transfected with circ_0004872-WT/PTEN-WT or circ_0004872-MUT/PTEN-MUT plasmids along with miR-190a-3p mimics or mimics NC using Lipofectamine™ 3000 (Invitrogen). Luciferase activity was assessed using a dual-luciferase reporter assay system (Promega).

RNA immunoprecipitation (RIP) assay

The RIP assay was conducted using a RIP kit (Millipore). Briefly, cells were lysed with a complete RIP lysis buffer. Cell extracts were incubated with IgG (Abcam, ab172730) or Ago2 (Abcam, ab186733) antibodies at 4 °C overnight. Subsequently, the precipitated RNA was extracted as described above, and the expressions of

hsa_circ_0004872 and miR-190a-3p were examined using qRT-PCR.

In vivo experiments

IOMM-Lee cells were transfected with pcDNA3.1 or pcDNA3.1-circ_0004872, or co-transfected with pcDNA3.1-circ_0004872 and miR-190a-3p mimics. After transfection, cells were trypsinized, washed, and resuspended in PBS. Twenty male BALB/c nude mice (8-week-old, LABORATORY Animal Co, Ltd., Hunan, China) were randomly assigned to four groups: sham group, pcDNA3.1 group, pcDNA3.1-circ_0004872 group, and pcDNA3.1-circ_0004872+miR-190a-3p mimics group (n=5 per group). A 0.2 mL cell suspension containing 4×10^6 cells was injected subcutaneously into the flank of each mouse. Tumor volumes were determined by measuring length (l) and width (w) and calculating volume (V) as follows: $V = lw^2/2$. After 21 days, mice were euthanized, and tumor tissues were collected. The animal studies were approved by Zhuzhou Hospital affiliated to Xiangya Medical College (No.2018K0345).

Statistical analysis

All data were obtained from three independent experiments. Statistical analysis was performed using SPSS 19.0 (IBM, Armonk, NY) and expressed as means \pm SD. Between-group differences and multi-group comparisons were determined using Student's t-test and one-way ANOVA, respectively. P values less than 0.05 were considered significant.

Results

Hsa_circ_0004872 was lowly expressed in meningioma and negatively correlated patient poor prognosis

Hsa_circ_0004872 is a tumor suppressor gene in various human malignant tumors [10, 17], while the expression and the role of hsa_circ_0004872 in meningioma remain unknown. Hsa_circ_0004872 was generated by circularization of exons 2 to 4 of the MAPK1 gene according to circinteractome (<https://circinteractome.nia.nih.gov/index.html>) (Fig. 1A and S1A). To characterize the specificity of hsa_circ_0004872, we designed divergent and convergent primers to obtain the PCR amplification products, then the existence of hsa_circ_0004872 was further verified by 1% agarose gel electrophoresis. The results showed that hsa_circ_0004872 was amplified only from cDNA but not gDNA (Fig. S1B). Moreover, after digestion by Rnase R, it was observed that RNase R treatment could significantly reduce MAPK1 mRNA expression, but no significant change was observed in hsa_circ_0004872 expression (Fig. S1C). Similarly, after Actinomycin D treatment, the half-life of hsa_circ_0004872 was obviously longer than that of linear MAPK1 (Fig. S1D), revealing that hsa_circ_0004872 was more stable than linear MAPK1

mRNA. Subsequently, qRT-PCR showed that the level of hsa_circ_0004872 in human meningioma tissues was significantly higher than that in the normal group (Fig. 1B). Kaplan-Meier plotter analysis further showed that the high level of hsa_circ_0004872 was associated with good relapse free survival among meningioma patients (Fig. 1D).

Hsa_circ_0004872 overexpression inhibited proliferation and promoted the apoptosis in vitro

To further study the potential function of hsa_circ_0004872 in the development of meningioma in vitro, hsa_circ_0004872 overexpression was induced in IOMM-Lee and CH157-MN cells. The transfection results were shown in Fig. 2A, hsa_circ_0004872 expression in IOMM-Lee and CH157-MN cells was significantly increased by pcDNA3.1-circ_0004872 transfection. Additionally, transfection with pcDNA3.1-circ_0004872 notably elevated linear MAPK1 mRNA expression, but no significant changes were observed in MAPK protein levels (Fig. S2A-B). Subsequent CCK8 assay results demonstrated that hsa_circ_0004872 overexpression led to a significant inhibition of human meningioma cell proliferation (Fig. 2B). Flow cytometry analysis revealed that upregulation of hsa_circ_0004872 promoted human meningioma cell apoptosis (Fig. 2C). In line with these findings, hsa_circ_0004872 overexpression significantly increased the expressions of pro-apoptotic proteins (Bax and Cleaved-caspase 3) while inhibited the expression of the anti-apoptotic protein (Bcl2) (Fig. 2D). These results collectively underscored the tumor-suppressive role of hsa_circ_0004872 in the development of meningioma.

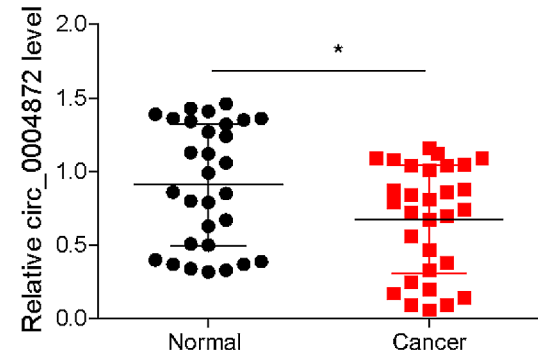
Hsa_circ_0004872 directly targeted to miR-190a-3p

As shown in Fig. 3A, the cytoplasmic localization of hsa_circ_0004872 in meningioma cells was prominently illustrated. Utilizing the Circinteractome database, the RNA sequence of hsa_circ_0004872 was acquired, revealing a discernible binding site between hsa_circ_0004872 and miR-190a-3p through sequence analysis and comparison (Fig. 3B). Subsequent findings from the dual-luciferase reporter gene assay underscored that miR-190a-3p mimics significantly reduced the luciferase activity presented by WT-circ_0004872, while exhibiting no significant effect on that of MUT-circ_0004872 (Fig. 3C). Meanwhile, RIP results displayed that hsa_circ_0004872 and miR-190a-3p were both enriched in the Ago2 antibody pull-down complex (Fig. 3D). Moreover, miR-190a-3p expression in IOMM-Lee and CH157-MN cells was notably diminished upon hsa_circ_0004872 overexpression (Fig. 3E). A parallel observation indicated an upregulation of miR-190a-3p in human meningioma tissues compared to the normal group (Fig. 3F). Notably, a negative correlation was discerned between miR-190a-3p and hsa_circ_0004872

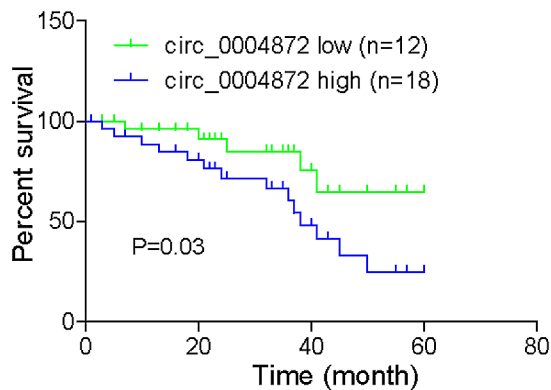
A

CircRNA ID	hsa_circ_0004872	Location	chr22:22153300-22162135
Genomic Length	8835 bp	Spliced Seq Length	490 bp
Best Transcript	NM_002745 Primers	Gene Symbol	MAPK1
Samples	Hs68, RNase, Hs68_control, Nhek, K562, Huvec, Hepg2, Helas3, H1hesc, Gm12878, Bj, Ag04450, A549, Sknshra, MCF7, diencephalon, cerebellum, occipital_lobe, frontal_cortex, parietal_lobe, temporal_lobe	Study	Jeck2013, Rybak2015, Salzman2013
GenomicSeq	hsa_circ_0004872	Mature Seq	hsa_circ_0004872

B



C



D

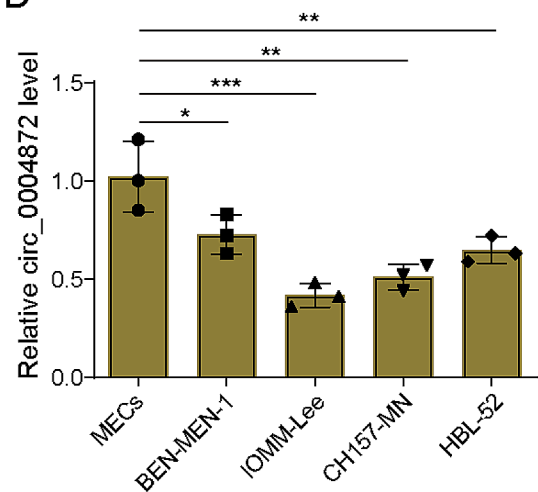


Fig. 1 Hsa_circ_0004872 was lowly expressed in meningioma and negatively correlated patient poor prognosis. **(A)** The basic information about hsa_circ_0004872 obtained from the Circinteractome database was presented. **(B)** Expression levels of hsa_circ_0004872 in tumor and adjacent tissues from meningioma patients was detected qRT-PCR ($n=30$). **(C)** Kaplan-Meier analysis showing the correlation between hsa_circ_0004872 expression and the survival rate of meningioma patients. **(D)** Detection of hsa_circ_0004872 expression in meningioma cells (IOMM-Lee, BEN-MEN-1, CH157-MN, and HBL-52) and human normal meningothelial cells (MECs) using qRT-PCR. Data are presented as mean \pm SD from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

expressions in clinical samples (Fig. 3G). Additionally, miR-190a-3p exhibited higher expression in IOMM-Lee and CH157-MN cells relative to MECs (Fig. 3H). In summary, these findings collectively affirmed that hsa_circ_0004872 inhibited miR-190a-3p expression in meningioma by acting as a sponge for miR-190a-3p.

miR-190a-3p negatively regulated the PTEN/PI3K/AKT signaling pathway

PTEN/PI3K/AKT is an important pathway for regulating the signal transduction of various biological processes such as cell apoptosis, metabolism, cell proliferation and cell growth [18]. Herein, it was predicted that miR-190a-3p had a potential binding site to PTEN (Fig. 4A). Dual-luciferase reporter gene assay results demonstrated

that miR-190a-3p overexpression significantly diminished the luciferase activity associated with WT-PTEN, while displaying no notable impact on MUT-PTEN (Fig. 4B). Additionally, qRT-PCR findings indicated that miR-190a-3p mimics transfection led to an elevation in miR-190a-3p expression and a concurrent reduction in PTEN expression in meningioma cells (Fig. 4C). Western blot assay also demonstrated that miR-190a-3p overexpression reduced PTEN protein level but increased p-PI3K and p-AKT levels in meningioma cells (Fig. 4D). Collectively, these findings underscored the functional interplay between miR-190a-3p and the PTEN/PI3K/AKT signaling pathway, positioning the latter as the downstream pathway influenced by miR-190a-3p in meningioma.

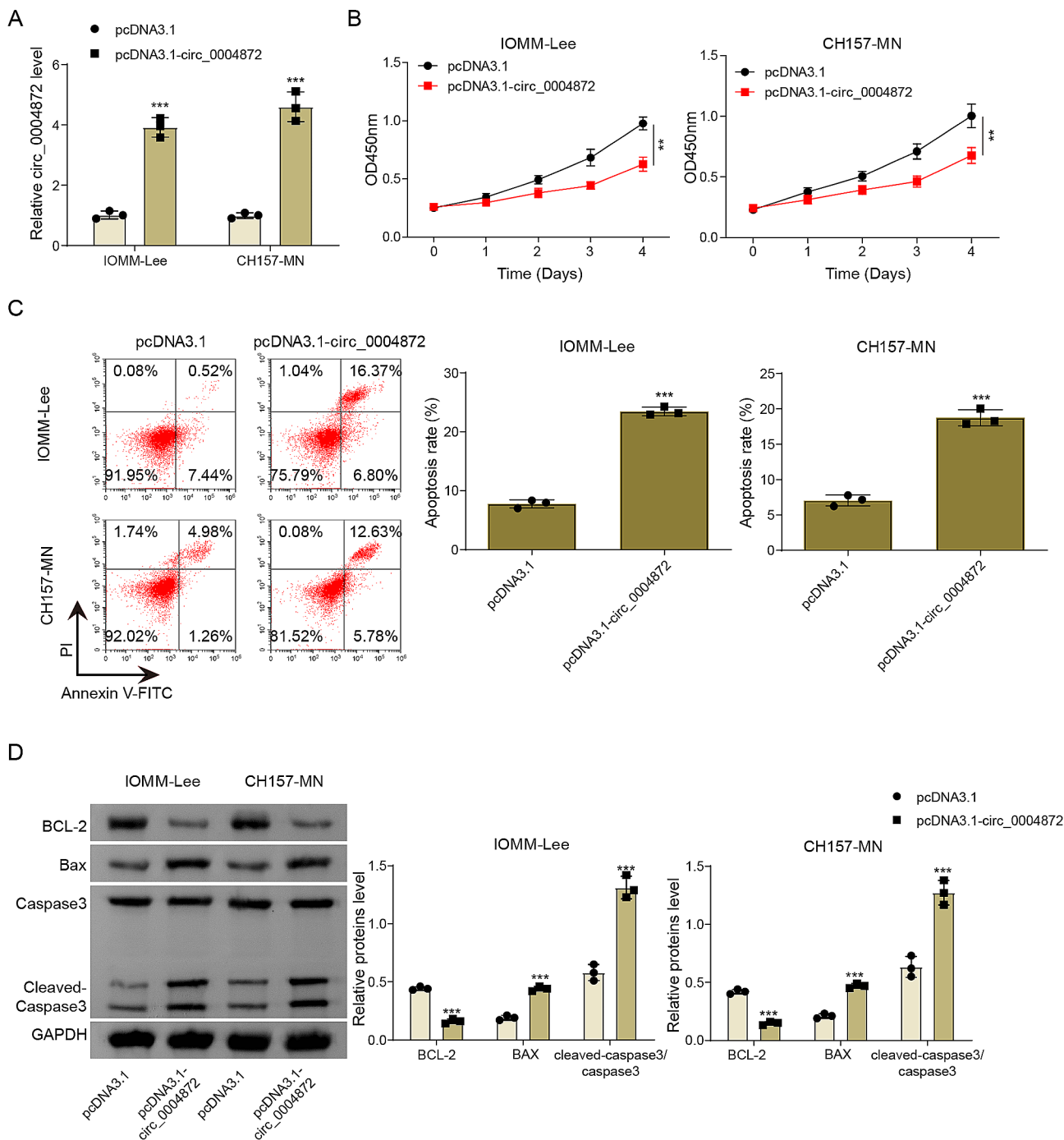


Fig. 2 Hsa_circ_0004872 overexpression inhibited proliferation and promoted the apoptosis in vitro. IOMM-Lee and CH157-MN cells were transfected with either pcDNA3.1 or pcDNA3.1-circ_0004872. **(A)** hsa_circ_0004872 expression was evaluated by qRT-PCR. **(B)** Cell viability was assessed using the CCK8 assay. **(C)** Flow cytometry analysis determined cell apoptosis. **(D)** Protein levels of Bax, Bcl-2, Cleaved-caspase3, and Caspase3 were examined by Western blot. Data are presented as mean ± SD from at least three independent experiments. **p* < 0.05, ***p* < 0.01, ****p* < 0.001

MIR-190a-3p inhibition acted as a tumor-suppressing effect by modulating PTEN/PI3K/AKT pathway

To comprehensively investigate the impact of miR-190a-3p and PTEN/PI3K/AKT pathway in meningioma, we induced miR-190a-3p knockdown in meningioma cells and combined with PTEN inhibitor (SF1670) treatment.

The transfection results were shown in Fig. 5A, miR-190a-3p inhibitor transfection significantly reduced miR-190a-3p expression in IOMM-Lee and CH157-MN cells. In addition, as shown in Fig. 5B, miR-190a-3p knockdown increased PTEN protein level and reduced p-PI3K and p-AKT levels in meningioma cells, while these effects

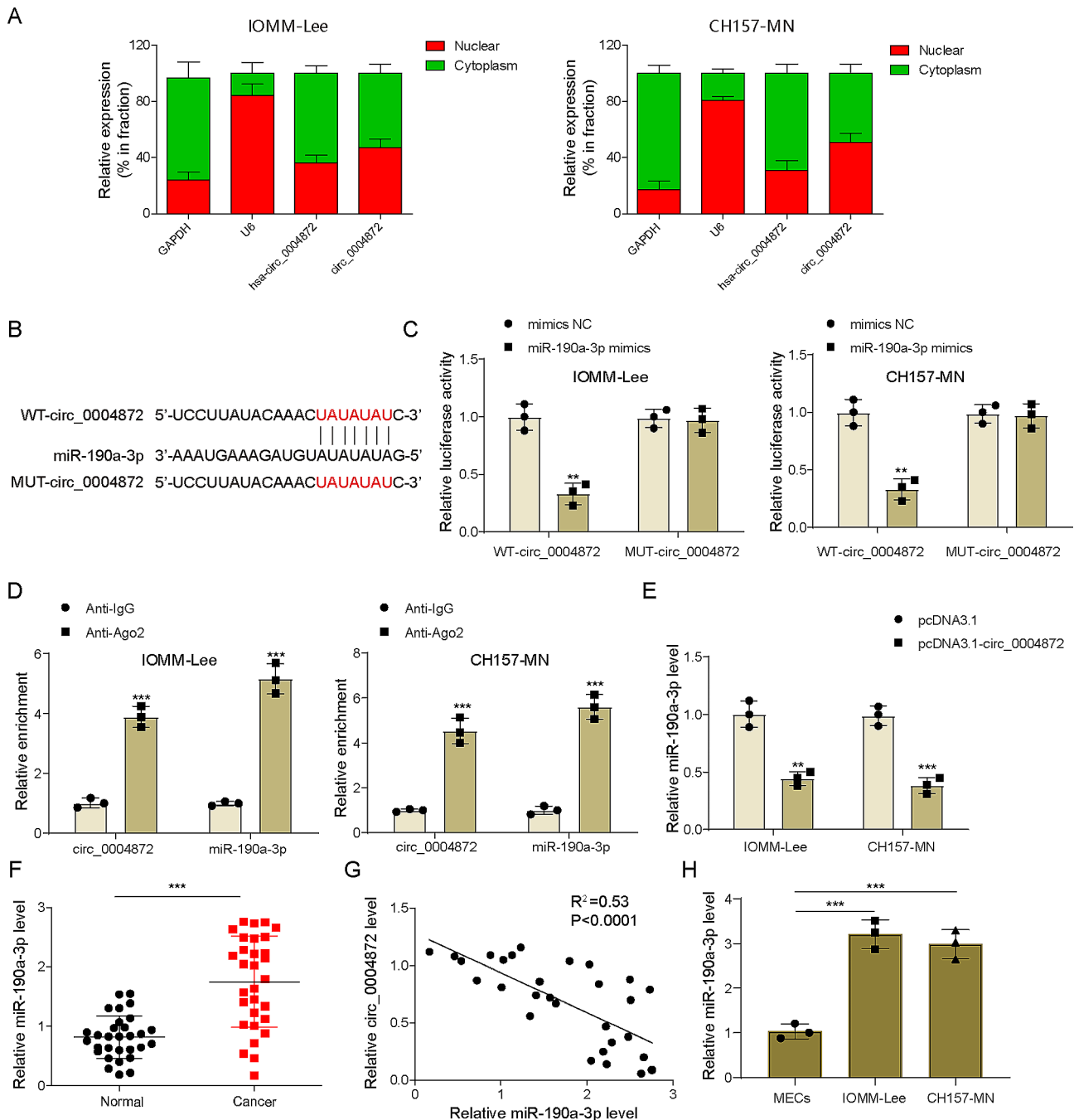


Fig. 3 Hsa_circ_0004872 directly targets miR-190a-3p. **(A)** The subcellular localization of hsa_circ_0004872 was determined by qRT-PCR. **(B)** The potential binding site between hsa_circ_0004872 and miR-190a-3p. **(C-D)** The interaction between hsa_circ_0004872 and miR-190a-3p was assessed by dual-luciferase reporter gene and RIP assays. **(E)** MiR-190a-3p expression in IOMM-Lee and CH157-MN cells after pcDNA3.1 or pcDNA3.1-circ_0004872 transfection was measured by qRT-PCR. **(F)** MiR-190a-3p expression in tumor and adjacent tissues from meningioma patients was detected by qRT-PCR ($n=30$). **(G)** Correlation analysis between hsa_circ_0004872 and miR-190a-3p expressions in clinical samples using Pearson's correlation. **(H)** MiR-190a-3p expression in meningioma cells (IOMM-Lee and CH157-MN) and human normal meningeothelial cells (MECs) was determined by qRT-PCR. Data are presented as mean \pm SD from at least three independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

were reversed by SF1670 treatment. Functional experiments subsequently revealed that miR-190a-3p knock-down restrained meningioma cell viability but promoted apoptosis. Importantly, these effects were abrogated by

PTEN inhibition (Fig. 5C-D). Moreover, miR-190a-3p silencing significantly enhanced the protein levels of Bax and cleaved-caspase3 while concurrently inhibiting Bcl2 level (Fig. 5E). Significantly, these alterations were effectively

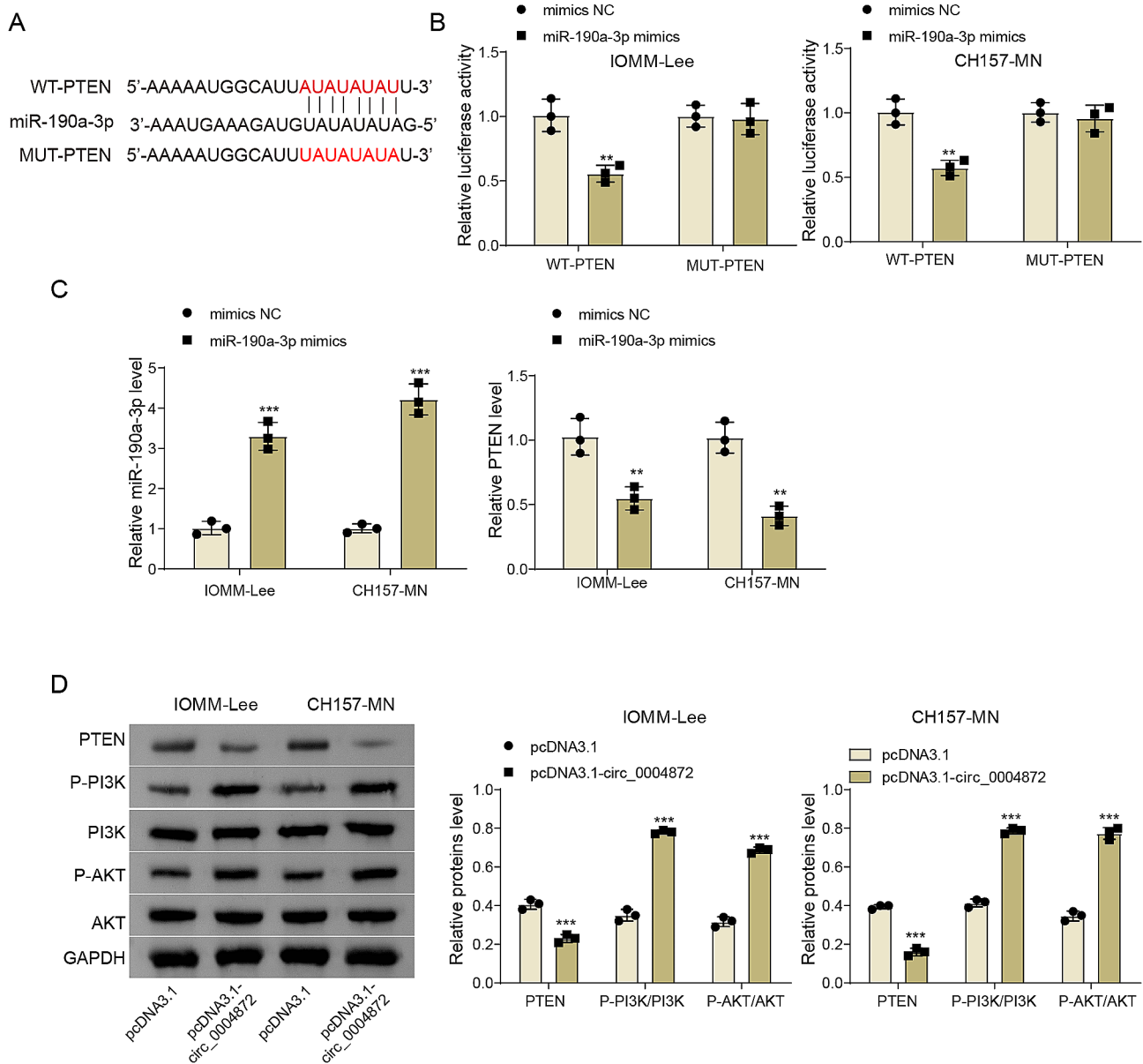


Fig. 4 miR-190a-3p negatively regulated the PTEN/PI3K/AKT signaling pathway. **(A)** The potential binding site between PTEN and miR-190a-3p. **(B)** Dual-luciferase reporter gene assay showing the interaction between PTEN and miR-190a-3p. **(C)** MiR-190a-3p and PTEN expressions in IOMM-Lee and CH157-MN cells after mimics NC or miR-190a-3p mimics transfection were detected using qRT-PCR. **(D)** Western blot was employed to detect PTEN, PI3K, p-PI3K, AKT and p-AKT protein level in cells after mimics NC or miR-190a-3p mimics transfection. Data are presented as mean \pm SD from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

counteracted following SF1670 treatment (Fig. 5E). To sum up, miR-190a-3p knockdown inhibited malignant behaviors of human meningioma cells by regulating the PTEN/PI3K/AKT pathway.

miR-190a-3p overexpression partially eliminated the antitumor effect mediated by hsa_circ_0004872 overexpression

To unravel the intricate interplay between hsa_circ_0004872 and miR-190a-3p in orchestrating the development of human meningioma, both

hsa_circ_0004872 and miR-190a-3p were co-overexpressed in human meningioma cells. It was observed that miR-190a-3p overexpression greatly reversed the inhibitory effect of hsa_circ_0004872 overexpression on meningioma cell viability (Fig. 6A). Additionally, the pro-apoptotic roles of hsa_circ_0004872 overexpression in meningioma cells were significantly abrogated upon miR-190a-3p overexpression (Fig. 6B). Further insights from western blot analysis elucidated that miR-190a-3p overexpression strikingly reverted the inhibitory impact of hsa_circ_0004872 upregulation on Bcl2 level

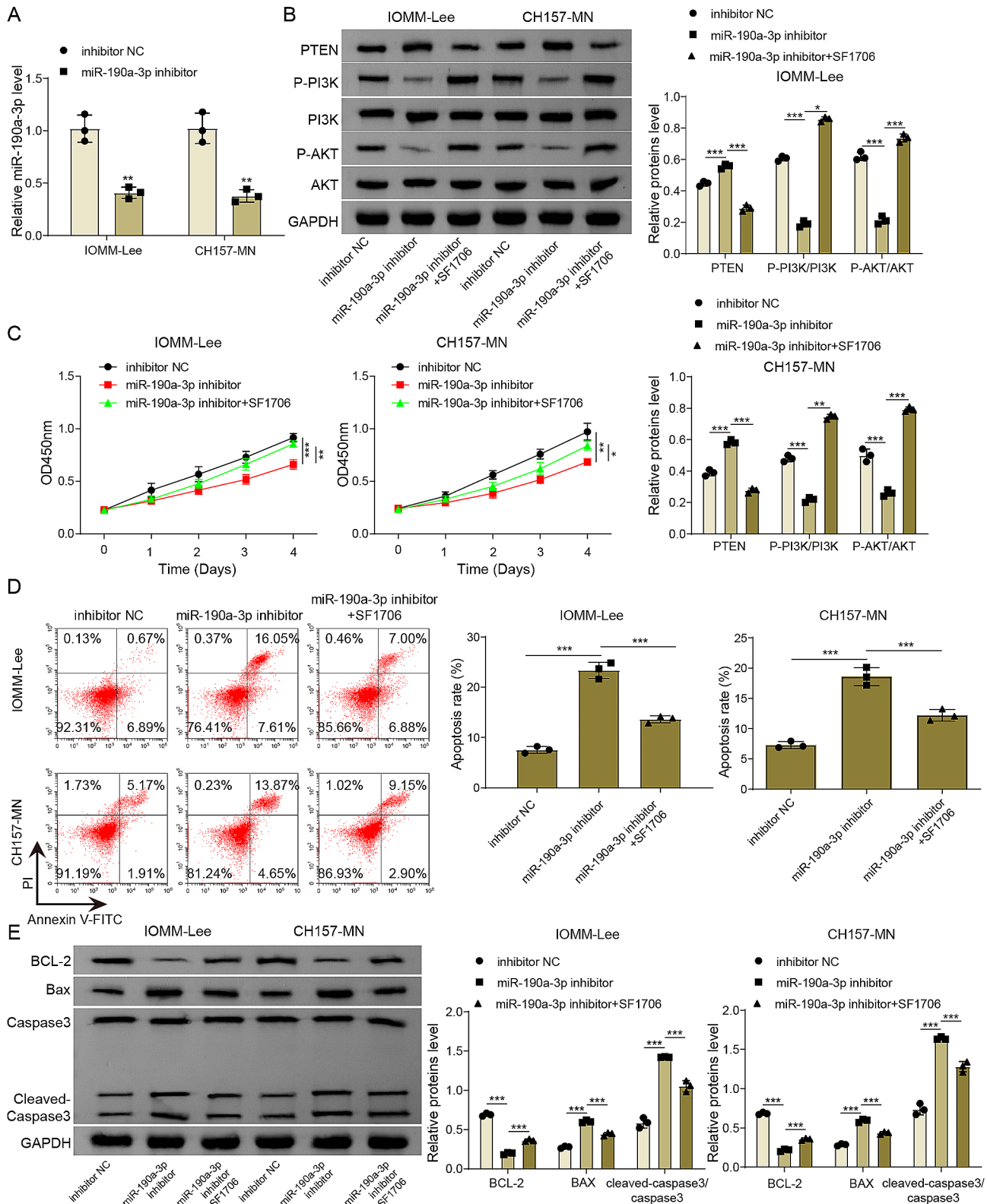


Fig. 5 MiR-190a-3p inhibition acted as a tumor-suppressing effect by modulating PTEN/PI3K/AKT pathway. **(A)** IOMM-Lee and CH157-MN cells were transfected with inhibitor NC or miR-190a-3p inhibitor, and miR-190a-3p expression in cells was detected using qRT-PCR. IOMM-Lee and CH157-MN cells were transfected with miR-190a-3p inhibitor or inhibitor NC combined with PTEN inhibitor (SF1670) treatment. **(B)** PTEN, PI3K, p-PI3K, AKT and p-AKT protein level were examined using western blot. **(C)** Cell viability was assessed using CCK8 assay. **(D)** Cell apoptosis was detected by flow cytometry. **(E)** Western blot was adopted to measure Bax, Bcl-2, Cleaved-caspase3, and Caspase3 protein levels. Data are presented as mean \pm SD from at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

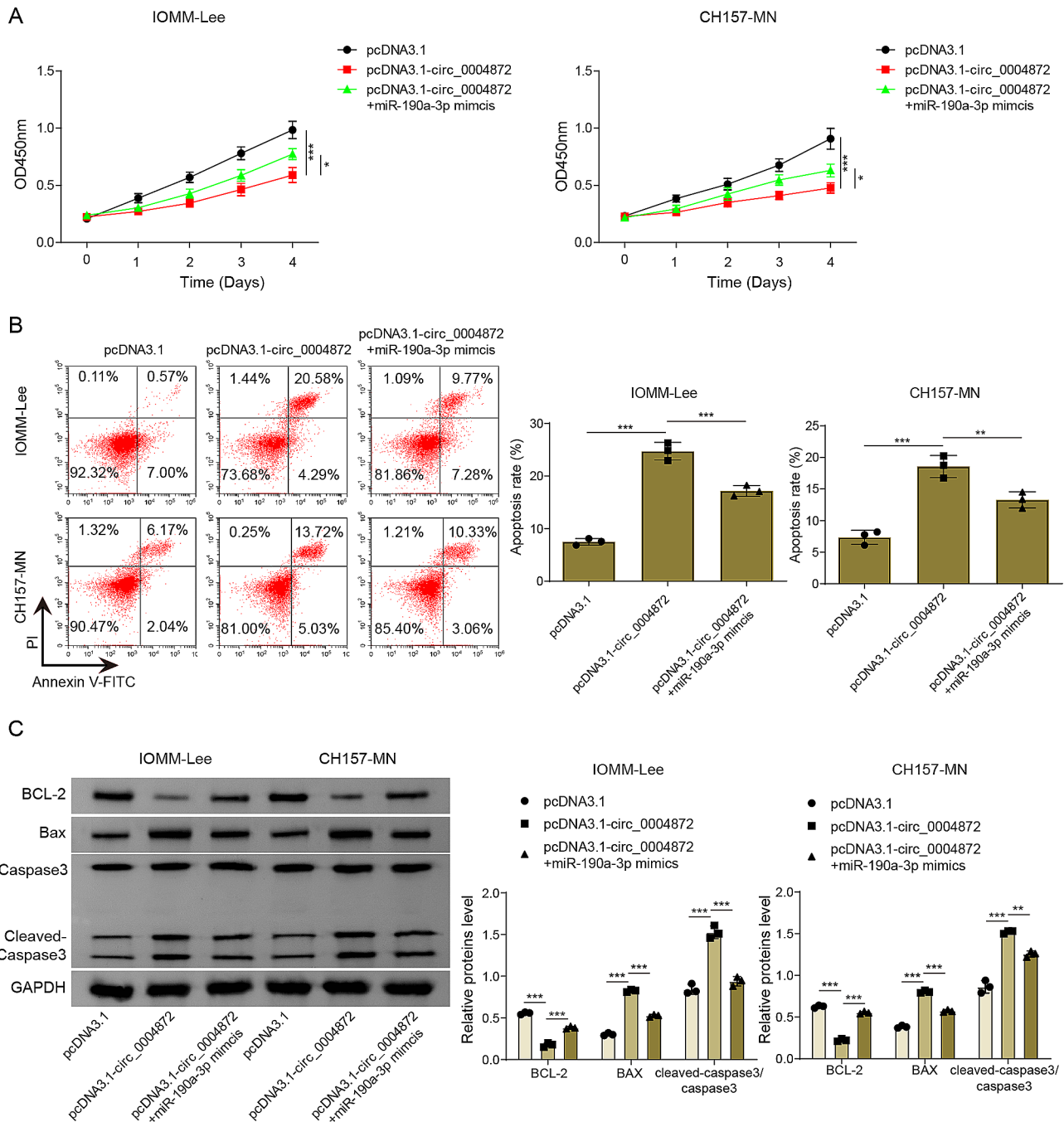


Fig. 6 miR-190a-3p overexpression partially eliminated the antitumor effect mediated by hsa_circ_0004872 overexpression. IOMM-Lee and CH157-MN cells were transfected with pcDNA3.1, pcDNA3.1-circ_0004872 or co-transfected with pcDNA3.1-circ_0004872 plus miR-190a-3p mimics. **(A)** Cell viability was assessed using CCK8 assay. **(B)** Cell apoptosis was detected by flow cytometry. **(C)** Bax, Bcl-2, Cleaved-caspase3 and Caspase3 protein levels in cells were examined by Western blot. Data are presented as mean ± SD from at least three replicate experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

in meningioma cells (Fig. 6C). Simultaneously, it negated the promotional effects of hsa_circ_0004872 upregulation on Bax and Cleaved-caspase 3 levels (Fig. 6C). In conclusion, hsa_circ_0004872 overexpression inhibited human meningioma cell proliferation and promoted the apoptosis by inhibiting miR-190a-3p expression.

Hsa_circ_0004872 overexpression repressed tumor growth in vivo by inhibiting miR-190a-3p

Next, the function of has_circ_000482 in meningioma was further confirmed in vivo by constructing a Xenograft tumor model. As shown in Fig. 7A-C, in comparison to the pcDNA3.1 group, hsa_circ_0004872 overexpression exerted a profound inhibitory effect on tumor growth.

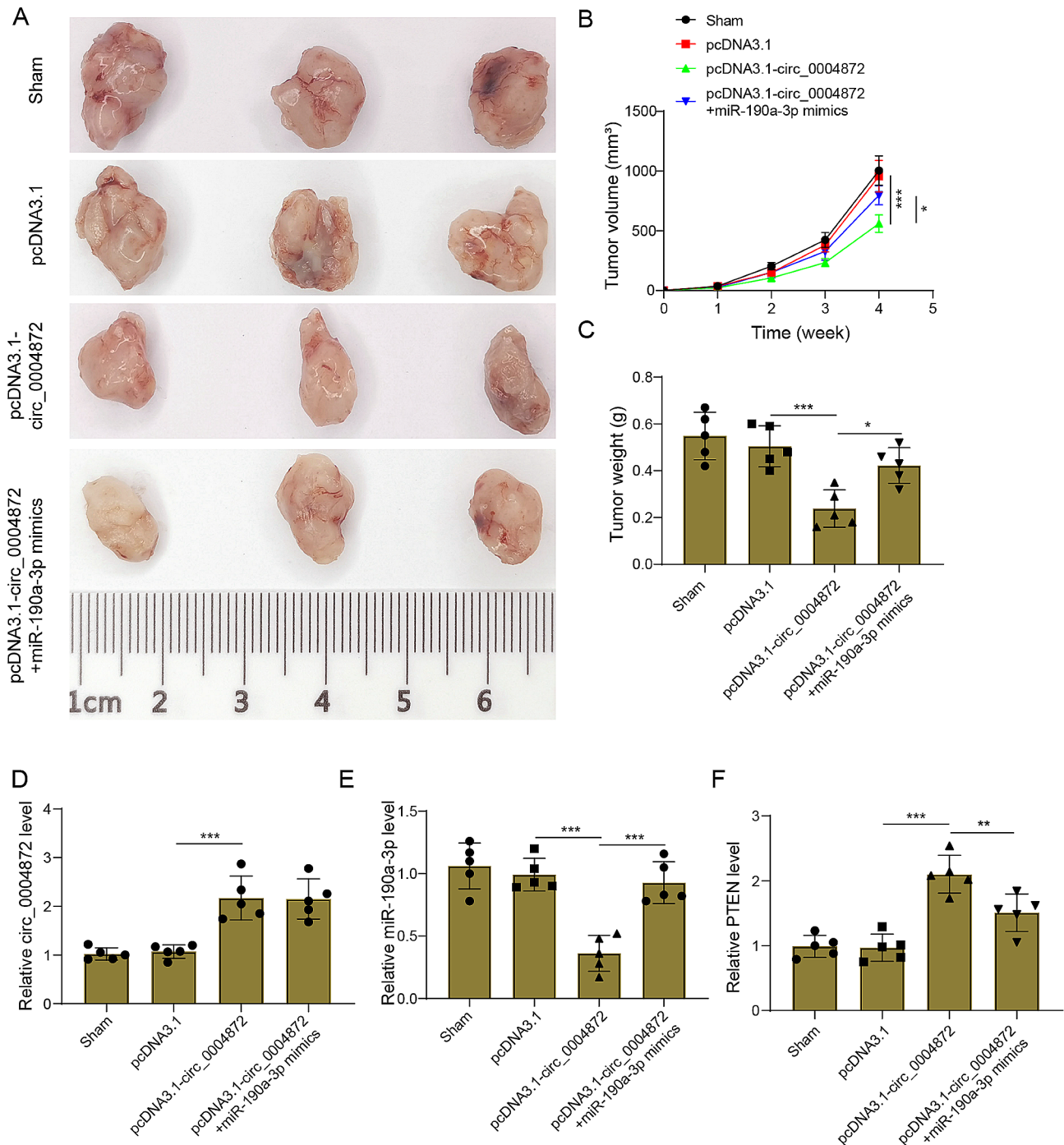


Fig. 7 Hsa_circ_0004872 overexpression represses tumor growth in vivo by inhibiting miR-190a-3p. Nude mice were used to construct a Xenograft tumor model by subcutaneously injecting IOMM-Lee cells with different treatments (pcDNA3.1, pcDNA3.1-circ_0004872, or pcDNA3.1-circ_0004872 plus miR-190a-3p mimics). (A-C) Tumor tissues were collected, and images, tumor volume, and weight were measured. (D-F) The expressions of hsa_circ_0004872 (D), miR-190a-3p (E), and PTEN (F) in tumor tissues were examined by qRT-PCR. Data are presented as mean ± SD (n=5). *p < 0.05, **p < 0.01, ***p < 0.001

Intriguingly, this anti-tumor impact of hsa_circ_0004872 upregulation was conspicuously reversed upon concurrent overexpression of miR-190a-3p (Fig. 7A-C). Furthermore, pcDNA3.1-circ_0004872 transfection significantly elevated the expressions of both hsa_circ_0004872 and PTEN, while concurrently diminishing miR-190a-3p

expression (Fig. 7D-F). Conversely, co-transfection with miR-190a-3p mimics resulted in a marked increase in miR-190a-3p expression and a corresponding reduction in PTEN expression, albeit with no significant effect on hsa_circ_0004872 expression (Fig. 7D-F). In conclusion,

hsa_circ_0004872 upregulation could suppress meningioma tumor growth in vivo by inhibiting miR-190a-3p.

Discussion

Meningioma, the most prevalent intracranial tumor, exhibits a substantial incidence rate [19]. Despite the majority of meningiomas being categorized as benign, a concerning 90% of these tumors progress to malignancy [20]. While advances in meningioma treatment have transpired, the prognosis for malignant meningioma patients remains suboptimal [21]. Over the past decades, circRNAs have emerged as pivotal regulators in the development of various human malignancies. However, their specific role in the progression of meningiomas has remained elusive. This study unveiled a novel revelation, demonstrating that the upregulation of hsa_circ_0004872 served as a potent inhibitor of meningioma progression. This inhibitory effect was mediated through the intricate regulation of the miR-190a-3p/PTEN/PI3K/AKT pathway. The findings shed light on a previously undiscovered dimension of circRNA involvement in meningioma development.

Circular RNA (circRNA) stands out as a distinctive class of endogenous, single-stranded closed RNA molecules known for their stable structure, high conservation, and widespread expression across multiple species [22]. It has been widely revealed that circRNAs are involved in the initiation and development of human cancers by regulation of various biological processes, such as cell proliferation, invasion, and apoptosis [23]. As proof, circ_102958 was increased in GC tumor tissue, and its high level was positively correlated to the TNM stage and shorter survival [24]. In addition, it was previously illustrated that hsa_circ_001783 knockdown markedly inhibited breast cancer cell proliferation and invasion [25]. One notable circRNA, hsa_circ_0004872, is derived from the reverse splicing of MAPK1 exons 2, 3, and 4. MAPK1 plays an important role in regulating the proliferation, invasion, and migration of cancer cells [26]. Previous studies have demonstrated the antitumor effects of hsa_circ_0004872, including its inhibition of gastric cancer cell proliferation and invasion, ultimately leading to improved survival rates [27]. During our experiments, transfection with pcDNA3.1-circ_0004872 notably elevated linear MAPK1 mRNA expression, albeit without a corresponding change in MAPK protein level. This intriguing finding may be attributed to the partial base sequence overlap between hsa_circ_0004872 and linear MAPK1. Considering that hsa_circ_0004872 shares a part of the base sequence with linear MAPK1, but it is only a fragment, which does not contain the complete linear MAPK1 mRNA structure, such as translation initiation site. This may be the reason why hsa_circ_0004872 overexpression did not promote the expression of its MAPK1 protein. Notably, our data

revealed elevated hsa_circ_0004872 level in meningioma tissues and cells, and its heightened expression correlated with favorable relapse-free survival among meningioma patients. Functional experiments demonstrated that hsa_circ_0004872 overexpression exerted inhibitory effects on human meningioma cell proliferation and tumor growth while promoting apoptosis. This groundbreaking study, to our knowledge, marked the first investigation into the functional role of hsa_circ_0004872 in meningioma. Furthermore, our findings suggested that the diminished expression of hsa_circ_0004872 may serve as a potential marker for poor prognosis in meningioma. This discovery holds promise for the development of innovative clinical diagnostic techniques, offering new avenues for improved prognostication in meningioma.

The intricate world of circRNAs encompasses a myriad of regulatory functions, including the modulation of transcription, splicing, stability, and translation of cytoplasmic mRNA. Operating as versatile players, circRNAs can interfere with signaling pathways through specific interactions with DNA, RNA, and proteins, relying on their subcellular localization [28]. A well-documented role of circRNAs involves acting as 'sponges' for miRNAs, engaging in competitive binding to neutralize the impact of miRNAs on mRNA translation [29]. Our investigation into the function of hsa_circ_0004872 in meningioma unveiled a compelling regulatory network, positioning hsa_circ_0004872 as a miRNA sponge, particularly for miR-190a-3p. Subsequent nuclear-cytoplasmic separation experiments affirmed the cytoplasmic localization of hsa_circ_0004872, reinforcing the hypothesis that its mode of action in meningioma involves miRNA sequestration. This pioneering study introduced a novel ceRNA regulatory network in meningioma, with hsa_circ_0004872 orchestrating its effects by sponging miR-190a-3p. miR-190a-3p, the predominant mature form of miR-190, assumed a crucial role in various diseases, particularly in the context of malignant tumors [30]. The role of miR-190a-3p in different cancers can be dual, either promoting or suppressing tumorigenic processes. For example, miR-190a-3p exhibited elevated levels in glioma, where it stimulated proliferation and migration by targeting YOD1 [15]. Moreover, miR-190a-3p was decreased in cervical cancer, and play as a suppressor to repress invasion by targeting KLF6 [31]. Despite the wealth of knowledge on miR-190a-3p in diverse malignancies, its function in meningioma remains elusive. Our study bridged this knowledge gap by revealing that miR-190a-3p was markedly upregulated in meningioma tissues, and its expression was negatively correlated with hsa_circ_0004872 expression. Our results subsequently revealed that hsa_circ_0004872 operated as the sponge of miR-190a-3p to negatively regulate miR-190a-3p expression in meningioma cells. In addition, as expected, miR-190a-3p overexpression reversed

the inhibitory effect of hsa_circ_0004872 overexpression on meningioma cell malignant behaviors. Collectively, these findings underscored the pivotal role of the hsa_circ_0004872/miR-190a-3p regulatory axis in steering the progression of malignant meningioma. This axis shed light on the intricate molecular mechanisms at play but also holds promise as a potential diagnostic and prognostic marker for meningioma.

PTEN, a dual-function protein and lipid phosphatase, stands as a formidable tumor suppressor, wielding a pivotal role in constraining tumor cell proliferation [32]. Its inactivation has been entwined with the genesis and progression of diverse human malignancies, spanning from colon cancer to lung cancer [33, 34]. The crux of PTEN's functionality lies in its ability to negate the effects of PI3K, with its primary substrate being PIP3. The dysregulation of PTEN unleashes a cascade of events, notably the hyperactivation of the PI3K/AKT signaling pathway, a phenomenon associated with the accelerated progression of malignant tumors, including meningioma [35, 36]. Our investigation delved into the intricate dynamics of the miR-190a-3p/PTEN relationship in meningioma cells, revealing a direct negative regulation exerted by miR-190a-3p on PTEN expression. This interaction extended its impact to the downstream effectors of the PTEN/PI3K/AKT pathway, with miR-190a-3p overexpression elevating p-PI3K and p-AKT levels in meningioma cells. Importantly, the consequential inhibitory effects on malignant behaviors of meningioma cells due to miR-190a-3p knockdown were effectively reversed by PTEN inhibition. The broader context of this regulatory network encompasses the hsa_circ_0004872/miR-190a-3p/PTEN axis, where hsa_circ_0004872 acted as an orchestrator, influencing the expression and function of miR-190a-3p, which in turn governed the PTEN/PI3K/AKT signaling pathway. These findings provided a nuanced understanding of the intricate functions and potential mechanisms underlying the hsa_circ_0004872/miR-190a-3p/PTEN network in meningioma. This comprehensive elucidation of the ceRNA mechanisms at play not only enhanced our understanding of the molecular underpinnings of meningioma but also positioned this regulatory axis as a potential source of novel biomarkers for prognostic evaluation in meningioma patients.

In summary, this pioneering study shed light on the previously unexplored role of hsa_circ_0004872 in thwarting the progression of malignant meningioma. Through its intricate regulatory dance with the miR-190a-3p/PTEN/PI3K/AKT axis, hsa_circ_0004872 emerged as a potent suppressor of malignant behaviors in meningioma cells. These discoveries deepen our comprehension of the molecular intricacies underpinning meningioma, and further provides a solid theoretical foundation for the

development of innovative approaches in the battle against malignant meningioma.

Abbreviations

CNS	Central nervous system
circRNAs	Circular RNAs
MAPK1	Mitogen-activated protein kinase 1
ceRNAs	Competing endogenous RNAs
miRNAs	MicroRNAs
PTEN	Phosphatase and tensin homolog deleted on chromosome ten
PI3K	Phosphoinositide-3 kinase
AKT	Protein kinase B
CCK-8	Cell counting kit-8
RIP	RNA Immunoprecipitation
qRT-PCR	Quantitative real-time polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-024-12084-1>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5

Acknowledgements

The authors acknowledge the support provided by the Hunan Provincial Joint Fund and the Hunan Provincial Natural Science Foundation of China (2020JJ6105) for this study.

We would like to give our sincere gratitude to the reviewers for their constructive comments.

Author contributions

Conception and design of study: Yongkai Huang
Acquisition of data: Zhihui Wu, Anmin Liu, Deqing Han
Analysis and interpretation of data: Zewei Peng, Wen Yuan, Junmin Peng
Drafting the manuscript: Zhihui Wu
Revising the manuscript critically for important intellectual content: Yongkai Huang
All authors reviewed the manuscript.

Funding

This study was supported by Hunan Provincial Joint Fund and Hunan Provincial Natural Science Foundation of China (2020JJ6105).

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declarations

Conflict of interest

All authors agree with the presented findings, have contributed to the work, and declare no conflict of interest.

Ethical approval and consent to participate

This study protocol was reviewed and approved by the review of Ethics Committee of Zhuzhou Hospital affiliated to Xiangya Medical College [No. 2018 K 0345] and all participants signed informed consent. The study was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Author details

¹Neurosurgery Department, Zhuzhou Hospital Affiliated to Xiangya Medical College, Central South University, 412000 Zhuzhou, Hunan Province, China

²Surgery Department, Zhuzhou Hospital Affiliated to Xiangya Medical College, Central South University, 412000 Zhuzhou, Hunan Province, China

³Emergency Department, Zhuzhou Hospital Affiliated to Xiangya Medical College, Central South University, 412000 Zhuzhou, Hunan Province, China

⁴Department of Anesthesiology, Zhuzhou Hospital Affiliated to Xiangya Medical College, Central South University, 412000 Zhuzhou, Hunan Province, China

Received: 12 July 2023 / Accepted: 4 March 2024

Published online: 18 March 2024

References

- Buerki RA, et al. An overview of meningiomas. *Future Oncol.* 2018;14(21):2161–77.
- Louis DN, et al. The 2021 WHO classification of tumors of the Central Nervous System: a summary. *Neuro Oncol.* 2021;23(8):1231–51.
- Ding Y, et al. LncRNA-IMAT1 promotes Invasion of meningiomas by suppressing KLF4/hsa-miR22-3p/Snai1 pathway. *Mol Cells.* 2022;45(6):388–402.
- Yan H, Bu P. Non-coding RNA in cancer. *Essays Biochem.* 2021;65(4):625–39.
- Memczak S, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature.* 2013;495(7441):333–8.
- Chen L, Shan G. CircRNA in cancer: fundamental mechanism and clinical potential. *Cancer Lett.* 2021;505:49–57.
- Chen RX, et al. Circular RNA circRNA_0000285 promotes cervical cancer development by regulating FUS. *Eur Rev Med Pharmacol Sci.* 2019;23(20):8771–8.
- Yuan G, et al. Upregulated circRNA_102231 promotes gastric cancer progression and its clinical significance. *Bioengineered.* 2021;12(1):4936–45.
- Xu K, et al. Annotation and functional clustering of circRNA expression in rhesus macaque brain during aging. *Cell Discov.* 2018;4:48.
- Dai Y, Zhu Y, Xu H. circ_0004872 inhibits proliferation, invasion, and glycolysis of oral squamous cell carcinoma by sponged miR-424-5p. *J Clin Lab Anal.* 2022;36(7):e24486.
- Song H, et al. Construction of a circRNA-Related ceRNA Prognostic Regulatory Network in breast Cancer. *Onco Targets Ther.* 2020;13:8347–58.
- Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. *Dis Model Mech.* 2021. 14(4).
- Wang L, et al. The biological and diagnostic roles of MicroRNAs in meningiomas. *Rev Neurosci.* 2020;31(7):771–8.
- Kliese N, et al. miRNA-145 is downregulated in atypical and anaplastic meningiomas and negatively regulates motility and proliferation of meningioma cells. *Oncogene.* 2013;32(39):4712–20.
- Zhou L, et al. miR-190a-3p promotes Proliferation and Migration in Glioma cells via YOD1. *Comput Math Methods Med.* 2021;2021:p3957738.
- Chu L, et al. Long intergenic non-coding LINC00657 regulates tumorigenesis of glioblastoma by acting as a molecular sponge of miR-190a-3p. *Aging.* 2019;11(5):1456–70.
- Ma C, et al. Circular RNA hsa_circ_0004872 inhibits gastric cancer progression via the miR-224/Smad4/ADAR1 successive regulatory circuit. *Mol Cancer.* 2020;19(1):157.
- Carnero A, et al. The PTEN/PI3K/AKT signalling pathway in cancer, therapeutic implications. *Curr Cancer Drug Targets.* 2008;8(3):187–98.
- Dijkstra BM, et al. SSTR-2 as a potential tumour-specific marker for fluorescence-guided meningioma surgery. *Acta Neurochir (Wien).* 2018;160(8):1539–46.
- Li T, et al. LINC00702/miR-4652-3p/ZEB1 axis promotes the progression of malignant meningioma through activating Wnt/ β -catenin pathway. *Biomed Pharmacother.* 2019;113:108718.
- Sicking J, et al. The evolution of cranial meningioma surgery—a single-center 25-year experience. *Acta Neurochir (Wien).* 2018;160(9):1801–12.
- Li Y, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res.* 2015;25(8):981–4.
- Zhu M, et al. Circular BANP, an upregulated circular RNA that modulates cell proliferation in colorectal cancer. *Biomed Pharmacother.* 2017;88:138–44.
- Jiang L, et al. Bach1-induced suppression of angiogenesis is dependent on the BTB domain. *EBioMedicine.* 2020;51:102617.
- Liu Z et al. *Circular RNA hsa_circ_001783 regulates breast cancer progression via sponging miR-200c-3p* *Cell Death Dis.* 2019. 10(2): p. 55.
- Kamiyama M, Naguro I, Ichijo H. Vivo gene manipulation reveals the impact of stress-responsive MAPK pathways on tumor progression. *Cancer Sci.* 2015;106(7):785–96.
- Jiang T, et al. A novel protein encoded by circMAPK1 inhibits progression of gastric cancer by suppressing activation of MAPK signaling. *Mol Cancer.* 2021;20(1):66.
- Misir S, Wu N, Yang BB. Specific expression and functions of circular RNAs. *Cell Death Differ.* 2022;29(3):481–91.
- Liu Z, et al. Novel circRNA_0071196/miRNA-19b-3p/CIT axis is associated with proliferation and migration of bladder cancer. *Int J Oncol.* 2020;57(3):767–79.
- Yu Y, Cao XC. Mir-190-5p in human diseases. *Cancer Cell Int.* 2019;19:257.
- Su Y, et al. Long non-coding RNA ZFAS1 regulates cell proliferation and invasion in cervical cancer via the miR-190a-3p/KLF6 axis. *Bioengineered.* 2022;13(2):3840–51.
- Jiang BH, Liu LZ. PI3K/PTEN signaling in angiogenesis and tumorigenesis. *Adv Cancer Res.* 2009;102:19–65.
- Zununi Vahed S, et al. Leuconostoc mesenteroides-derived anticancer pharmaceuticals hinder inflammation and cell survival in colon cancer cells by modulating NF- κ B/AKT/PTEN/MAPK pathways. *Biomed Pharmacother.* 2017;94:1094–100.
- Yang Z, et al. Modulation of NF- κ B/miR-21/PTEN pathway sensitizes non-small cell lung cancer to cisplatin. *PLoS ONE.* 2015;10(3):e0121547.
- EI-Habr EA, et al. Complex interactions between the components of the PI3K/AKT/mTOR pathway, and with components of MAPK, JAK/STAT and Notch-1 pathways, indicate their involvement in meningioma development. *Virchows Arch.* 2014;465(4):473–85.
- Pain M, et al. Multiple meningiomas in a patient with Cowden Syndrome. *J Neurol Surg Rep.* 2016;77(3):e128–33.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.