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miR-372 Regulates Glioma Cell Proliferation and Invasion by Directly Targeting PHLPP2

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ABSTRACT

MicroRNAs are known to be involved in carcinogenesis and tumor progression in glioma. Recently, microRNA-372 (miR-372) has been proved to play a substantial role in several human cancers, but its functions in glioma remain unclear. In this study, we confirmed that miR-372 was commonly upregulated in glioma cell lines and tissues. Downregulation of miR-372 markedly inhibited cell proliferation and invasion and induced G1/S arrest and apoptosis. Consistently, the xenograft mouse model also unveiled the suppressive effects of miR-372 knockdown on tumor growth. Further studies revealed that miR-372 modulated the expression of PHLPP2 by directly targeting its 3′-untranslated region (3′-UTR) and that miR-372 expression was inversely correlated with PHLPP2 expression in glioma samples. Silencing of PHLPP2 could rescue the inhibitory effect of miR-372 inhibitor. Moreover, miR-372 knockdown suppressed the phosphorylation levels of the major components of PI3K/Akt pathway including Akt, mTOR, and P70S6K. Taken together, our results suggest that miR-372 functions as an oncogenic miRNA through targeting PHLPP2 in glioma. J. Cell. Biochem. 116: 225–232, 2015. © 2014 Wiley Periodicals, Inc.

KEY WORDS: miR-372; GLIOMA; PHLPP2; PROLIFERATION; INVASION

Glioblastoma (GBM) is the most common primary malignant brain tumors in adults [Wang et al., 2012a]. In spite of significant improvements in neurosurgery, radiotherapy, and chemotherapy, the median survival time of high-grade glioma patients has remained at 12–15 months over the past decade, and the cumulative 1-year survival rate remains lower than 30% [Wang et al., 2013]. Therefore, better understanding of the pathogenesis is essential for the development of new diagnostic and therapeutic strategies for glioma.

MicroRNAs (miRNAs), a class of small non-coding RNAs (21–23 nt in length), repress the expression of target genes at the post-transcriptional level by binding to their target mRNAs, thus influencing a variety of physiological and pathological processes, such as differentiation, proliferation, metabolism, apoptosis, and invasion [Wienholds and Plasterk, 2005; Hwang and Mendell, 2006; Kuhn et al., 2010]. Recently, a large body of evidence has shown that miRNAs have crucial roles in various human cancers. Several miRNA profiling studies in glioma have been performed, and a series of miRNAs including miR-9, miR-17, miR-21, miR-184, and miR-342–3p have been shown to be predictive biomarkers associated with glioma progression and clinical outcome [Rao et al., 2010; Kim et al., 2011; Wang et al., 2012b]. Moreover, miR-26a, miR-100, miR-155 and, miR-218 have been shown to exert tumor suppressive or oncogenic functions in glioma [Huse et al., 2009; Alrfaei et al., 2013; Ling et al., 2013; Tu et al., 2013]. Nevertheless, further knowledge about the molecular mechanisms of glioma miRNAs is required to provide deeper insights for better therapeutic opportunities for glioma patients.

In the present study, we investigated the potential function of miR-372 in the development and progression of glioma. We showed that miR-372 is significantly upregulated in glioma cell lines and clinical glioma tissues, compared to normal brain tissues. Down-regulation of miR-372 potently inhibited glioma cell proliferation and invasion by direct targeting PHLPP2, thus inhibiting the PI3K/Akt pathway. Our results provide a better understanding of the molecular mechanism by which miR-372 regulates glioma.

Conflict of interest: The authors have no conflict of interest.
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MATERIALS AND METHODS

TISSUE SAMPLES AND CELL LINES

Human glioma tissues and normal brain tissues were collected from Changhai Hospital (Shanghai, China). Informed consent was obtained from every patient, and this study was approved by the ethics committee of Second Military Medical University, Shanghai, China. Glioma cell lines (U87, LN229, LN18, and T98G) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science, Shanghai, China. All cells were maintained in DMEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum at 37°C in a humidified incubator containing 5% CO_2.

REAL-TIME QUANTITATIVE PCR

Total RNA was extracted from cells and tissues using the Trizol reagent (Invitrogen). cDNA was synthesized from 2 μg of total RNA using the Advantage RT-PCR kit (Clontech Laboratories Inc., Mountain View, California, USA). Quantitative PCR (qPCR) was then carried out with primers for miR-372 and PHLPP2 with SYBR in the 7500 HT real-time PCR System (Applied Biosystems, Carlsbad, CA) according to previously described methods [Kong et al., 2012]. PCR was done in triplicates. U6 and GAPDH were used as normalization controls and the relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

OLIGONUCLEOTIDES AND TRANSFECTION

The hsa-miR-372 inhibitor and negative control oligonucleotides were purchased from RiboBio (Guangzhou, Guangdong, China). PHLPP2 siRNA was purchased from Dharmacon (Lafayette, CO). Cells were transfected with the miR-372 inhibitor or PHLPP2 siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were collected 48 h after transfection.

CELL PROLIFERATION ASSAY

Glioma cells were seeded into 96-well plates at 2000 cells/well. After transfection with the miR-372 inhibitor or negative control, cell viability was assessed by MTT assay as previously described [Li et al., 2012].

BrdU INCORPORATION ASSAY

BrdU incorporation assay was performed according to a standard method as described previously [Li et al., 2013b]. Briefly, cells were seeded on coverslips in 24-well plates and cultured overnight. BrdU (10 μg/mL) was added to the culture medium for 1 h. Cells were immediately fixed in 4% paraformaldheyde for 10 min and stained with an anti-BrdUrd antibody (Upstate, Temecula, CA) following the manufacturer's instructions. The coverslips were counterstained with DAPI and imaged with a confocal laser-scanning microscope (Olympus FV1000, Tokyo, Japan). Results were expressed as the percentage of BrdU+ cells in DAPI+ cells. The experiment was performed independently three times for each cell line.

CELL APOPTOSIS ASSAY

Cell apoptosis analyses were performed using the Annexin V-FITC Kit (Beckman Colter, Boulevard Brea, CA) according to the manufacturer's protocol. In brief, cells were plated in 6-well plates and transfected with miR-372 inhibitor or negative control. Twelve-four hours after transfection, cells were stained with FITC-Annexin V and PI and then incubated at room temperature for 15 min in the dark. Flow cytometry was used to analyze the cell apoptosis. Data were evaluated with CellQuest software. The assays were performed in triplicate.

CELL CYCLE ANALYSIS

For cell-cycle analysis, cells were plated in 6-well plates at 2×10^5 per well and transfected with miR-372 inhibitor or negative control. Forty-eight hours posttransfection, cells were fixed in 70% ethanol and stained with 20 μg/mL propidium iodide (PI). The cell cycle distribution was analyzed on a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA). The assays were performed in triplicate.

INVASION ASSAY

The invasive ability of transfected cells was determined using 24-well transwell chambers coated with Matrigel (BD Pharmingen, San Jose, CA). Cells were cultured in serum-free medium containing 0.1% BSA to pre-starve for 24 h. Then cells (5×10^4) were seeded into the top chamber coated with Matrigel. After 24 h of incubation at 37°C, cells remaining in the top chamber were removed with cotton swabs. The invading cells on the bottom chamber surface were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The number of invading cells was calculated by counting three different fields of view using light microscopy.

LUCIFERASE ACTIVITY ASSAY

The PHLPP2 3′-UTR from 578 to 1227 bp was generated by PCR amplification and subcloned into the pGL3-basic luciferase reporter plasmid (Promega, Madison, WI). The corresponding mutant constructs were created by mutating the seed regions of the miR-372-binding sites. Glioma cell lines were seeded in 24-well plates and then cotransfected with appropriate reporter plasmid and miRNA using Lipofectamine 2000. Luciferase and renilla signals were measured 48 h after transfection using the Dual Luciferase Reporter Assay Kit (Promega). All experiments were performed in triplicate.

WESTERN BLOT ANALYSIS

Total protein was extracted from cell lines and the protein concentration was measured with the Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA). Proteins were separated by 10% SDS-PAGE gel and transferred to the nitrocellulose membrane (Bio-Rad). The membrane was first incubated with specific primary antibodies against PHLPP2 (Abcam, Cambridge, MA), then with secondary antibodies labeled with HRP and detected by ECL. GAPDH (Abcam) was used as a control.

IN VIVO TUMOR GROWTH ASSAY

All animal experiments were undertaken in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Scientific Investigation Board of Second Military Medical University, Shanghai, China. LN229 cells were

subcutaneously injected into the flanks of BALB/c nude mice (n = 6). When the tumor volume reached 100 mm^3, 200 pmol scramble oligo or miR-372 inhibitor was injected into xenograft tumor in multiple sites once every 2 days for 14 days and the animals were closely monitored for tumor growth. The tumor volume was calculated by using the formula: larger diameter × smaller diameter^2/2.

STATISTICAL ANALYSIS

All data are presented as the mean ± SD from at least three independent replicates. The significance of the differences between two groups was estimated with Student's t test. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

miR-372 IS UPREGULATED IN GLIOMA CELL LINES AND TISSUES

miR-372 expression was first evaluated by qPCR in four glioma cell lines (U87, U251, LN229. and T98G). As shown in Figure 1A, miR-372 was strongly upregulated in all analyzed cell lines, compared to normal brain tissues. Consistently, the average expression levels of miR-372 were markedly higher in glioma tissues than in normal brain tissues (Fig. 1B). These results indicate that miR-372 is abnormally overexpressed in human glioma cell lines and tissues.

DOWNREGULATION OF miR-372 INHIBITS PROLIFERATION OF GLIOMA CELLS IN VITRO AND IN VIVO

In order to investigate the function of miR-372 in glioma, we focused on whether silencing of miR-372 would inhibit proliferation of glioma cells. First U87 and LN229 cells were transiently transfected with miR-372 inhibitor (miR-372 I) or negative control (NC), and qPCR was used to demonstrate miRNA downregualtion (Fig. 2A). MTT assay displayed significant cell growth inhibition in miR-372 I transfectants compared with cells transfected with NC (Fig. 2B). Furthermore, we investigated whether miR-372 has any impact on cell cycle progression of glioma cells. As shown in Fig. 2C, downregulation of miR-372 decreased the percentage of U87 and LN229 cells in S phase and significantly increased the percentage of cells in G1 phase, which indicated that the growth-suppressive effect of miR-372 I was partly due to a G1-phase arrest. Consistently, we found that quantification of BrdU-positive cells was also dramatically reduced in miR-372-suppressing cells (Fig. 2D). To further examine the effect of miR-372 on glioma cell growth in vivo, LN229 cells were inoculated subcutaneously into the flank of nude mice. When the tumor volume reached 100 mm^3, miR-372 I or NC was injected into tumors in multiple sites once every 2 days for 14 days and the animals were closely monitored for tumor growth. As shown in Figure 2E and F, the tumors treated with miR-372 I grew more slowly than those treated with NC. Collectively, these results indicate that downregulation of miR-372 suppresses the proliferation of glioma cells in vitro and in vivo.

DOWNREGULATION OF miR-372 INHIBITS INVASION AND INDUCES APOPTOSIS IN GLIOMA CELLS

To determine whether miR-372 regulates glioma cell invasion, Transwell invasion assay was performed. As shown in Figure 3A, miR-372 downregulation significantly decreased invasion of U87 and LN229 cells (Fig. 3A). Additionally, we measured cell apoptosis in both cell lines and found that levels of cell apoptosis were elevated in cells exposed to miR-372 I versus the control cells (Fig. 3B).

PHLPP2 IS A DIRECT TARGET OF miR-372

To understand the underlying molecular mechanism by which miR-372 regulates glioma cell proliferation and invasion, we searched for miR-372 targets using different computational methods, such as TargetScan and miRanda. PH domain leucine-rich-repeats protein phosphatase 2 (PHLPP2) was identified and selected for further analysis because of its critical roles in suppressing tumor development and regulating cell proliferation and invasion [Molina et al., 2012; Li et al., 2014]. As illustrated in Figure 4A, complementary sequence of miR-372 was found in the 3′-UTR of PHLPP2 mRNA. A significant negative correlation

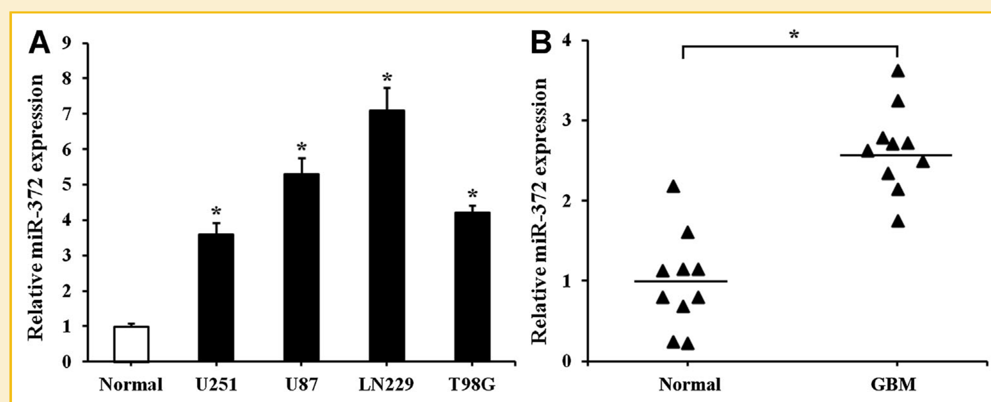

Fig. 1. miR-372 is upregulated in glioma cell lines and tissues. (A) Relative expression of miR-372 in four GBM cell lines and normal brain tissues was measured by qPCR. (B) Average expression level of miR-372 in human GBM specimens (n = 10) and normal brain tissues (n = 10). *$P < 0.05$.

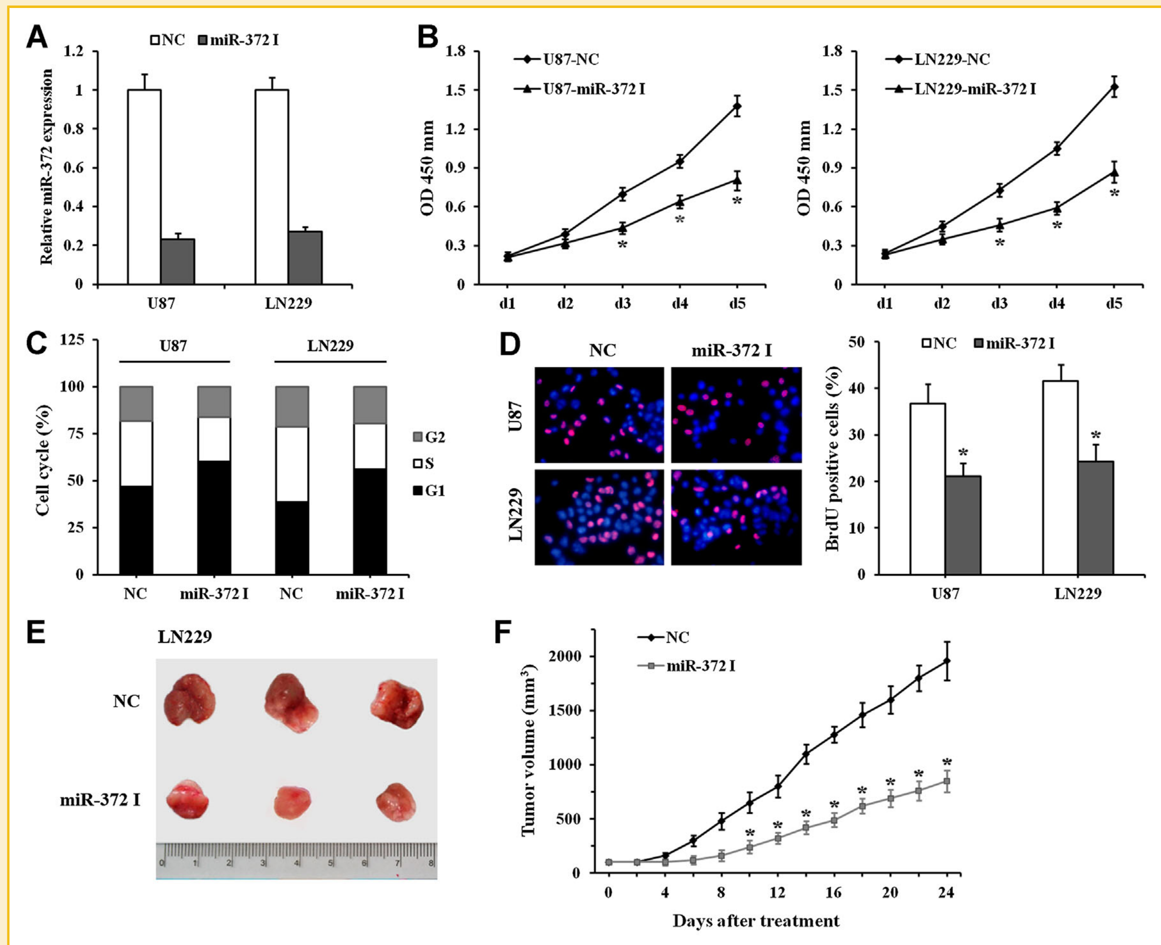

Fig. 2. Downregulation of miR-372 suppresses glioma cell proliferation in vitro and in vivo. (A) qPCR analysis of miR-372 expression in U87 and LN229 cells transiently transfected with miR-372 I or NC. (B) Cell viability was evaluated by MTT assay. miR-372 inhibitors (miR-372 I) significantly reduced cell growth of U87 and LN229 cells, compared to negative control (NC). (C) Flow cytometric analysis of the indicated cells. (D) Representative photos (left) and quantification (right) of BrdU-positive cells in the indicated cells. (E) Tumor volumes were measured every 2 days during treatment with miR-372 I or NC. $^{*}P < 0.05$.

between miR-372 levels and PHLPP2 mRNA expression was also observed (Fig. 4B). qPCR and Western blot analyses showed that inhibition of miR-372 increased the levels of PHLPP2 mRNA and protein in both U87 and LN229 cell lines (Fig. 4C). Then we performed luciferase reporter assay and the result showed that the luciferase activity of cells transfected with miR-372 I was significantly increased, whereas the mutation of the putative miR-372-binding sites in 3′-UTR region clearly abrogated the increase of the luciferase activity caused by miR-372 I transfection (Fig. 4D). Taken together, these results strongly suggest that PHLPP2 is a direct target of miR-372.

miR-372 REGULATES CELL PROLIFERATION AND INVASION BY TARGETING PHLPP2

To determine whether the inhibitory effect of miR-372 I was mediated by regulation of PHLPP2, we cotransfected LN229 cells with miR-372 I and PHLPP2 siRNA. Western blot confirmed that PHLPP2 siRNA significantly reduced the expression of PHLPP2 in cells transfected with miR-372 I (Fig. 5A). MTT assay showed that cell growth inhibition caused by miR-372 I was partially rescued by PHLPP2 siRNA (Fig. 5B). G1/S arrest induced by miR-372 inhibition was also abrogated by PHLPP2 siRNA (Fig. 5C). Similarly, the effect of miR-372 I on cell invasion and apoptosis was also antagonized by PHLPP2 siRNA (Fig. 5D and E). These data demonstrated that miR-372 regulates cell proliferation and invasion by targeting PHLPP2.

DOWNREGULATION OF miR-372 INHIBITS THE PI3K/Akt SIGNALING PATHWAY

Given that PHLPP could directly dephosphorylate and inactivate Akt [Molina et al., 2012], we further evaluated the effect of miR-372 I in the PI3K/Akt signaling pathway. The result showed that the phosphorylation levels of Akt, mTOR, and P70S6K, major components of the PI3K/Akt pathway, were decreased in miR-372-suppressing cells (Fig. 6A). Accordingly, the levels of these molecules and PHLPP2 were also reduced in the xenograft tumor tissues with

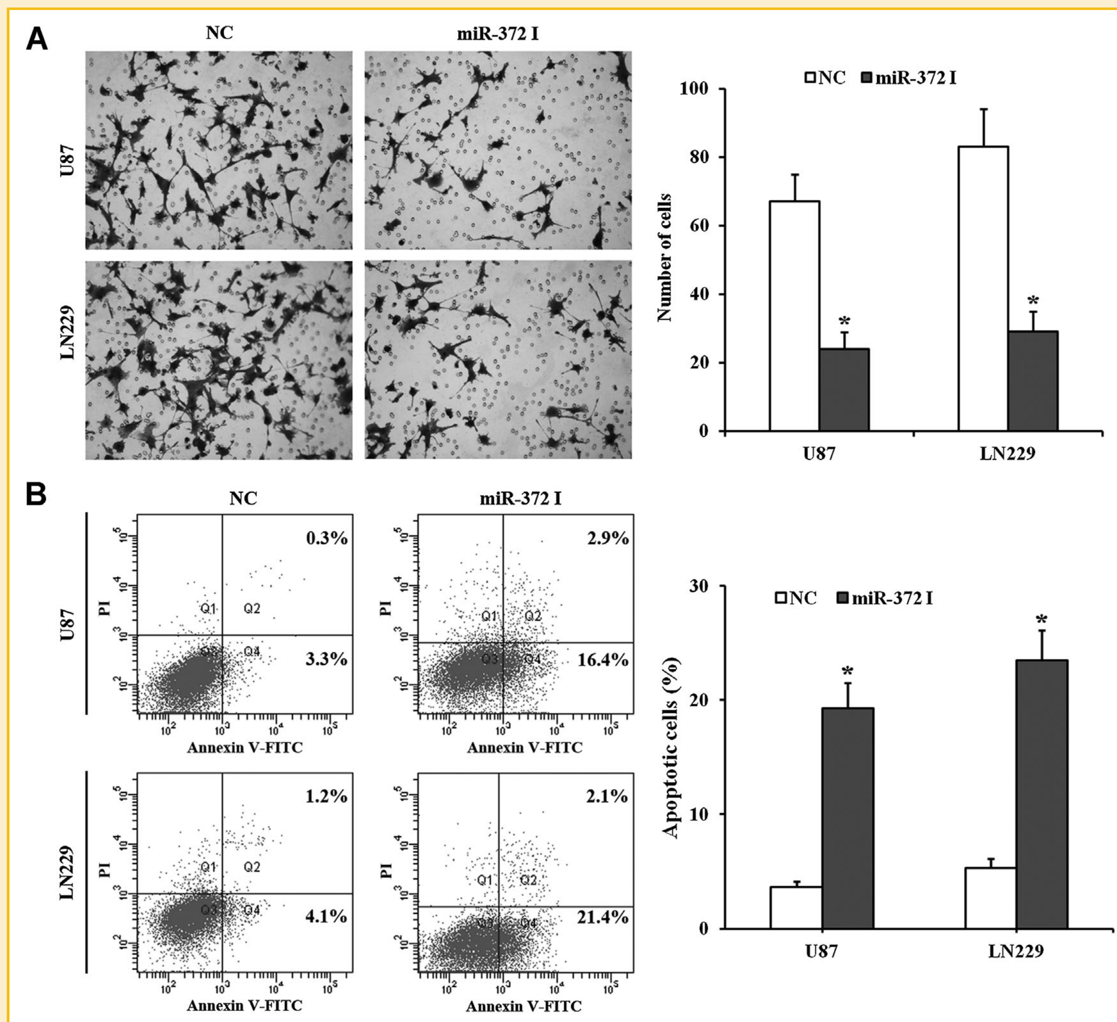

Fig. 3. Downregulation of miR-372 inhibits invasion and induces apoptosis in glioma cells. (A) Transwell invasion assay in U87 and LN229 cells treated with miR-372 I or NC. (B) Apoptotic ratios were analyzed by double staining with Annexin-V-FITC and PI in the indicated cells. *$P < 0.05$.

miR-372 I (Fig. 6B). These results demonstrate that miR-372 may be an important regulator of PI3K/Akt pathway.

DISCUSSION

It has been widely accepted that miRNAs are important players in human cancers, where they act as either oncogenes or tumor suppressors. The expression of miRNAs is remarkably deregulated in glioma, strongly suggesting that miRNAs are involved in the initiation and progression of this disease [Huse et al., 2009; Alrfaei et al., 2013; Ling et al., 2013; Tu et al., 2013]. In the present study, we investigated the biological role of miR-372 in glioma.

miR-372 belongs to the miR-371~373 cluster which also includes miR-93 and miR-302a. Several studies have reported that miR-372 is overexpressed and may play an oncogenic role in some cancers including hepatocellular carcinoma, gastric cancer, and colorectal cancer [Cho et al., 2009; Yamashita et al., 2012; Gu et al., 2013]. However, one report by Tian et al. showed that miR-372 is downregulated and acts as a tumor suppressor gene in cervical carcinoma [Tian et al., 2011]. In this study, we found that miR-372 expression was significantly upregulated in glioma cell lines tissues, which was in consistent with a previous report by Li and colleagues who showed that miR-372 was significantly upregulated in glioma tissues and the high miR-372 expression was significantly associated with the advanced pathological grade and prognosis of the patients [Li et al., 2013a]. Functional assays confirmed that miR-372 downregulation inhibited proliferation and invasion and induced G1/S arrest and apoptosis. Accordingly, the xenograft mouse model also unveiled the suppressive effects of miR-372 knockdown on tumor growth. These results suggest that miR-372 plays an important role in promoting carcinogenesis of gliomas.

Generally, miRNAs exert their effects via regulating specific target genes. Thus, we searched for candidate target genes of miR-372 using bioinformatic analysis and identified PHLPP2 as a theoretical target of miR-372. PHLPP2, together with PHLPP1,

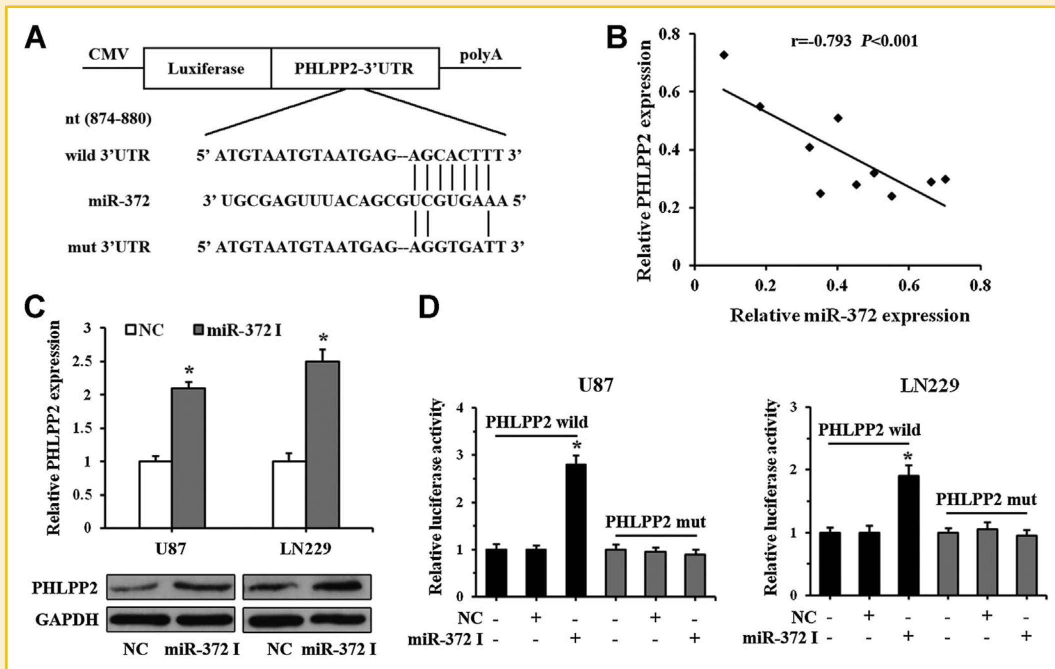

Fig. 4. PHLPP2 is a direct target of miR-372. (A) The diagram illustrates the construction of the luciferase reporter plasmids. (B) Spearman's correlation analysis between miR-372 and PHLPP2 mRNA levels in human GBM tissues. (C) PHLPP2 mRNA/protein levels were detected by qRT-PCR and western blot in U87 and LN229 cells transfected with miR-372 I or NC. (D) Luciferase assay was used to confirm the interaction of miR-372 with PHLPP2 in U87 and LN229 cells. $^*P < 0.05$.

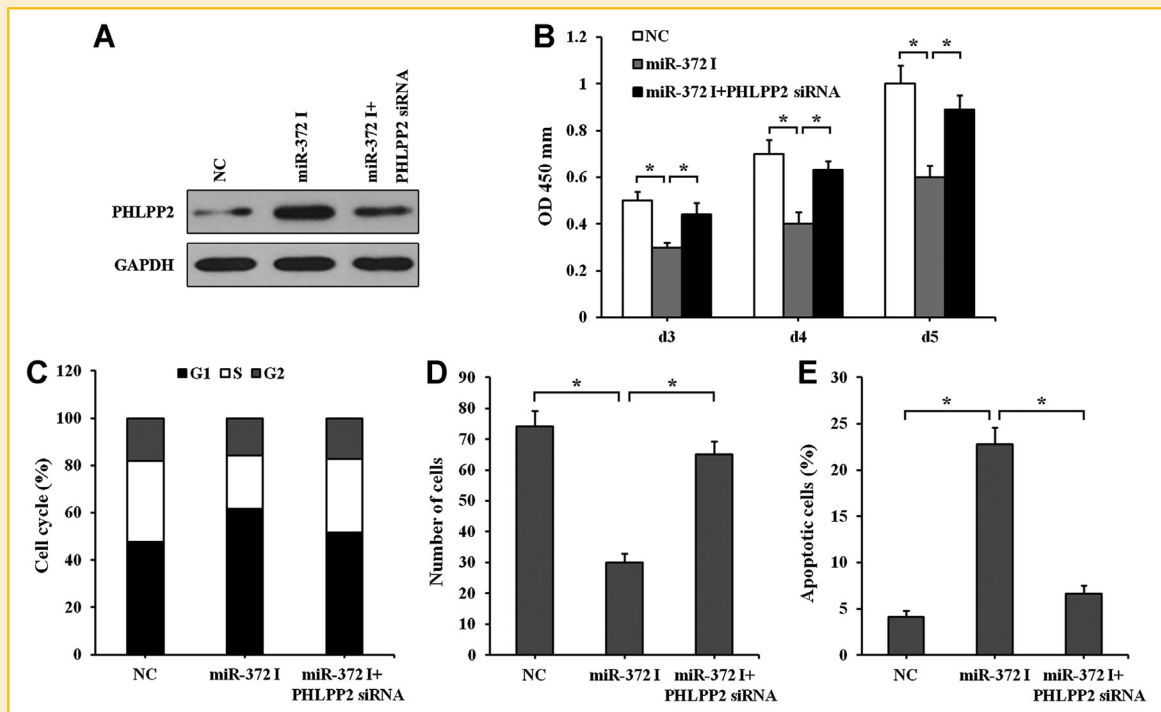

Fig. 5. PHLPP2 mediates the effects of miR-372. (A) Western blot analysis for PHLPP2 expression in LN229 cells cotransfected with miR-372 I and PHLPP2 siRNA. Cell growth rate (B), cell-cycle distribution (C), cell invasion (D) and apoptosis (E) in LN229 cells cotransfected with miR-372 I and PHLPP2 siRNA. $^*P < 0.05$.

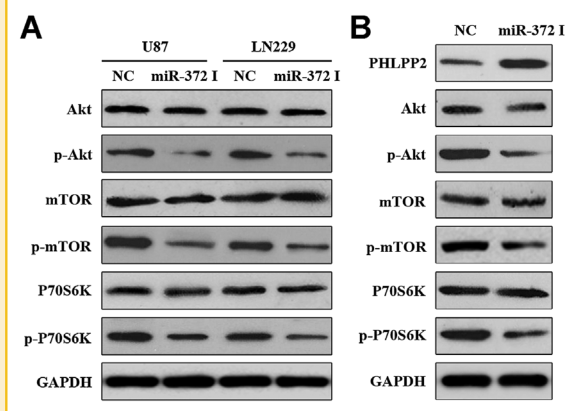

Fig. 6. The effect of miR-372 on the PI3K/Akt signaling pathway. (A) Western blot analysis with indicated antibodies in cultured U87 and LN229 cells transfected with miR-372 I or NC. (B) Western blot analysis with indicated antibodies in NC and miR-372 I-treated xenograft tumors.

belongs to a novel family of Ser/Thr protein phosphatases and plays central roles in maintaining cell survival suppression through negatively regulating the signaling pathways activated by AKT, PKC, MAPK, and Mst1 [Liao et al., 2013]. Convincing evidence indicates that PHLPP2 is downregulated in many types of malignant tumors including colon cancer, breast cancer, stomach cancer, pancreas cancer, and prostate cancer [Liu et al., 2009; Qiao et al., 2010; Nitsche et al., 2012; O'Neill et al., 2013]. Importantly, PHLPP2 has been reported to promote apoptosis and suppress tumor growth in glioblastoma cell lines [Gao et al., 2005; Molina et al., 2012]. However, the underlying molecular mechanisms that regulate the expression of PHLPP2 are still poorly understood. In this study, we identified PHLPP2 as a direct functional downstream target of miR-372 in glioma cells, and this finding is supported by the following observations: inhibition of miR-372 increased the luciferase reporter activity of wild-type 3′-UTR but not mutant 3′-UTR of PHLPP2; miR-372 inhibition increased PHLPP2 mRNA and protein expression, thus inhibiting the PI3K/Akt signaling pathway; the expression of miR-372 was inversely correlated with PHLPP2 expression in glioma specimens; more importantly, PHLPP2 silencing can partially reverse the inhibitory effects produced by miR-372 inhibition. These results suggested that miR-372 affects glioma cell proliferation and invasion through regulating PHLPP2 expression.

In summary, our results showed that miR-372 was overexpressed in glioma, and inhibition of miR-372 could suppress glioma cell proliferation and invasion partially by targeting PHLPP2. Our findings suggest that an inhibitory strategy against miR-372 might represent potential rationale for future prevention and treatment of human glioma.

REFERENCES

Alrfaei BM, Vemuganti R, Kuo JS. 2013. MicroRNA-100 Targets SMRT/NCOR2, Reduces Proliferation, and Improves Survival in Glioblastoma Animal Models. PLoS One 8:e80865.

Cho WJ, Shin JM, Kim JS, Lee MR, Hong KS, Lee JH, Koo KH, Park JW, Kim KS. 2009. MiR-372 regulates cell cycle and apoptosis of ags human gastric cancer cell line through direct regulation of LATS2. Mol Cells 28:521–527.

Gao T, Furnari F, Newton AC. 2005. PHLPP: A phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell 18:13–24.

Gu H, Guo X, Zou L, Zhu H, Zhang J. 2013. Upregulation of microRNA-372 associates with tumor progression and prognosis in hepatocellular carcinoma. Mol Cell Biochem 375:23–30.

Huse JT, Brennan C, Hambardzumyan D, Wee B, Pena J, Rouhanifard SH, Sohn-Lee C, le Sage C, Agami R, Tuschl T, Holland EC. 2009. The PTEN-regulating microRNA miR-26a is amplified in high-grade glioma and facilitates gliomagenesis in vivo. Genes Dev 23:1327–1337.

Hwang HW, Mendell JT. 2006. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 94:776–780.

Kim TM, Huang W, Park R, Park PJ, Johnson MD. 2011. A developmental taxonomy of glioblastoma defined and maintained by MicroRNAs. Cancer Res 71:3387–3399.

Kong KL, Kwong DL, Chan TH, Law SY, Chen L, Li Y, Qin YR, Guan XY. 2012. MicroRNA-375 inhibits tumour growth and metastasis in oesophageal squamous cell carcinoma through repressing insulin-like growth factor 1 receptor. Gut 61:33–42.

Kuhn AR, Schlauch K, Lao R, Halayko AJ, Gerthoffer WT, Singer CA. 2010. MicroRNA expression in human airway smooth muscle cells: Role of miR-372 in regulation of airway smooth muscle phenotype. Am J Respir Cell Mol Biol 42:506–513.

Liao WT, Li TT, Wang ZG, Wang SY, He MR, Ye YP, Qi L, Cui YM, Wu P, Jiao HL, Zhang C, Xie YJ, Wang JX, Ding YQ. 2013. MicroRNA-224 promotes cell proliferation and tumor growth in human colorectal cancer by repressing PHLPP1 and PHLPP2. Clin Cancer Res 19:4662–4672.

Li C, Nie H, Wang M, Su L, Li J, Yu B, Wei M, Ju J, Yu Y, Yan M, Gu Q, Zhu Z, Liu B. 2012. MicroRNA-409-3p regulates cell proliferation and apoptosis by targeting PHF10 in gastric cancer. Cancer Lett 320:189–197.

Li G, Zhang Z, Tu Y, Jin T, Liang H, Cui G, He S, Gao G. 2013a. Correlation of microrna-372 upregulation with poor prognosis in human glioma. Diagn Pathol 8:1.

Li HP, Zeng XC, Zhang B, Long JT, Zhou B, Tan GS, Zeng WX, Chen W, Yang JY. 2013b. MiR-451 inhibits cell proliferation in human hepatocellular carcinoma through direct suppression of IKK-β. Carcinogenesis 34:2443–2451.

Ling N, Gu J, Lei Z, Li M, Zhao J, Zhang HT, Li X. 2013. MicroRNA-155 regulates cell proliferation and invasion by targeting FOXO3a in glioma. Oncol Rep 30:2111–2118.

Liu J, Weiss HL, Rychahou P, Jackson LN, Evers BM, Gao T. 2009. Loss of PHLPP expression in colon cancer: role in proliferation and tumorigenesis. Oncogene 28:994–1004.

Li X, Stevens PD, Liu J, Yang H, Wang W, Wang C, Zeng Z, Schmidt MD, Yang M, Lee EY, Gao T. 2014. PHLPP is a negative regulator of RAF1, which reduces colorectal cancer cell motility and prevents tumor progression in mice. Gastroenterology 146:1301–1312.

Molina JR, Agarwal NK, Morales FC, Hayashi Y, Aldape KD, Cote G, Georgescu MM. 2012. PTEN, NHERF1 and PHLPP form a tumor suppressor network that is disabled in glioblastoma. Oncogene 31:1264–1274.

Nitsche C, Edderkaoui M, Moore RM, Eibl G, Kasahara N, Treger J, Grippo PJ, Mayerle J, Lerch MM, Gukovskaya AS. 2012. The phosphatase PHLPP1 regulates Akt2, promotes pancreatic cancer cell death, and inhibits tumor formation. Gastroenterology 142:377–387.

O'Neill AK, Niederst MJ, Newton AC. 2013. Suppression of survival signalling pathways by the phosphatase PHLPP. FEBS J 280:572–583.

Qiao M, Wang Y, Xu X, Lu J, Dong Y, Tao W, Stein J, Stein GS, Iglehart JD, Shi Q, Pardee AB. 2010. Mst1 is an interacting protein that mediates PHLPPs' induced apoptosis. Mol Cell 38:512–523.

Rao SA, Santosh V, Somasundaram K. 2010. Genome-wide expression profiling identifies deregulated miRNAs in malignant astrocytoma. Mod Pathol 23:1404–1417.

Tian RQ, Wang XH, Hou LJ, Jia WH, Yang Q, Li YX, Liu M, Li X, Tang H. 2011. MicroRNA-372 is down-regulated and targets cyclin-dependent kinase 2 (CDK2) and cyclin A1 in human cervical cancer, which may contribute to tumorigenesis. J Biol Chem 286:25556–25563.

Tu Y, Gao X, Li G, Fu H, Cui D, Liu H, Jin W, Zhang Y. 2013. MicroRNA-218 inhibits glioma invasion, migration, proliferation, and cancer stem-like cell self-renewal by targeting the polycomb group gene Bmi1. Cancer Res 73:6046–6055.

Wang H, Lu YT, Luo L, Li WS, Liang CF, He HY, Ba YY. 2013. MicroRNA-195 inhibits the proliferation of human glioma cells by directly targeting cyclin D1 and cyclin E1. PLoS One 8:e54932.

Wang L, Shi M, Hou S, Ding B, Liu L, Ji X, Zhang J, Deng Y. 2012a. MiR-483–5p suppresses the proliferation of glioma cells via directly targeting ERK1. FEBS Lett 586:1312–1317.

Wang Q, Li P, Li A, Jiang W, Wang H, Wang J, Xie K. 2012b. Plasma specific miRNAs as predictive biomarkers for diagnosis and prognosis of glioma. J Exp Clin Cancer Res 31:97.

Wienholds E, Plasterk RH. 2005. MicroRNA function in animal development. FEBS Lett 579:5911–5922.

Yamashita S, Yamamoto H, Mimori K, Nishida N, Takahashi H, Haraguchi N, Tanaka F, Shibata K, Sekimoto M, Ishii H, Doki Y, Mori M. 2012. MicroRNA-372 is associated with poor prognosis in colorectal cancer. Oncology 82: 205–212.