Int J Clin Exp Pathol 2018;11(9):4493-4502
www.ijcep.com /ISSN:1936-2625/IJCEP0082004

Original Article

MicroRNA-142-3p inhibits proliferation and induces apoptosis by targeting the high-mobility group box 1 via the Wnt/β-catenin signaling pathway in glioma

Chong Li, Shiyu Feng, Ling Chen

Department of Neurosurgery, Chinese PLA General Hospital, Beijing, China

Received July 2, 2018; Accepted July 30, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: Background: Glioma is one of the most common brain tumors. Copious microRNAs have been identified as critical regulators in the development of glioma. MicroRNA-142-3p (miR-142-3p) has been reported as a tumor suppressor in some malignancies. However, the roles and molecular mechanisms of miR-142-3p in the development of glioma are poorly defined. Methods: An RT-qPCR assay was carried out to detect expressions of miR-142-3p and high-mobility group box 1 (HMGB1) mRNA. A bioinformatic analysis and a luciferase reporter assay were used to explore the interaction between miR-142-3p and HMGB1 3'UTR. A Western blot assay was performed to examine protein expression of HMGB1, c-myc, cleaved caspase-3, and β-catenin. Cell proliferative ability was assessed by an MTS assay. The cell apoptotic rate was measured using flow cytometry via the double-staining of Annexin V-FITC and propidium iodide (PI). Results: MiR-142-3p expression was remarkably reduced in glioma tissues. Mechanical analyses showed that HMGB1 was a target of miR-142-3p. Functional investigations revealed that miR-142-3p suppressed proliferation and induced apoptosis by targeting HMGB1 in glioma cells. Moreover, miR-142-3p inactivated Wnt/β-catenin signaling and activated caspase-3 signaling by targeting HMGB1 in glioma cells. Conclusion: MiR-142-3p inhibits proliferation and induces apoptosis by targeting HMGB1 via the Wnt/β-catenin signaling pathway in glioma cells, providing a deep exploration into the roles and molecular basis of miR-142-3p in the proliferation and apoptosis of glioma cells and highlighting the therapeutical values of miR-142-3p and HMGB1 for glioma.

Keywords: MicroRNA-142-3p, high-mobility group box 1, Wnt/β-catenin, glioma

Introduction

Glioma, one of the most common primary brain tumors, accounts for about 26.5% of brain and other CNS tumors and 80.7% of malignant tumors in the United States [1]. Brain and other CNS tumors are an enormous threat for human health and life, with an estimated 200 new diagnoses and 41 deaths each day in the United States [2]. The median survival time for patients with low-grade glioma is approximately 7 years [3], while the average survival time for patients with high-grade glioma declines to almost 15 months [4]. Although great advances and exciting breakthroughs have been made in the treatment of glioma over the past decades, the prognosis-especially for high-grade glioma-remains very poor, highlighting the urgency to explore glioma pathogenesis [4, 5].

MicroRNAs (miRNAs), a group of small non-coding RNAs without the protein-coding potential, function as guide molecules in RNA silencing [6]. Recently, emerging miRNAs have been identified as potential oncogenes or tumor suppressors in the development of malignancies, including glioma [7, 8]. MicroRNA-142-3p (miR-142-3p), generated by the guide strand of miR-142 hairpin, has been reported as a tumor suppressor in some malignancies, such as colon cancer [9], non-small-cell lung cancer [10], and osteosarcoma [11]. For instance, miR-142-3p suppresses cell invasion by targeting integrin alpha V and some cytoskeleton-associated molecules in breast cancer [12]. The ectopic expression of miR-142-3p induces the reduction of cell proliferative and invasive capacities by inhibiting the expression of the frizzled 7 receptor (FZD7) in cervical cancer [13]. MiR-142-3p also has been demonstrated to be

Table 1. Relationship between miR-142-3p expression and clinicopathological features of gliomas

Characteristics	N	miR-142-3p expression		X^2	*P*
		High	Low		
Gender					
Male	33	18	15	0.041	0.523
Female	27	15	12		
Age				0.589	0.487
> 50	39	21	18		
≤ 50	21	11	10		
Tumor size (cm)					
< 3	36	16	20	4.856	0.023
≥ 3	24	18	6		
WHO grade					
I-II	34	30	4	58.632	< 0.001
III-IV	26	3	23		

abnormally expressed in glioma tissues, cells, and the serum of glioma patients [14, 15]. Also, miR-142-3p could inhibit murine glioma growth and extend its median survival time *in vivo* [15, 16]. However, the roles and molecular mechanisms of miR-142-3p in the development of glioma have not been well studied until now.

In the present study, we demonstrated that miR-142-3p inhibited proliferation and promoted apoptosis by targeting the high-mobility group box 1 (HMGB1) via the Wnt/β-catenin signaling pathway in glioma.

Materials and methods

Tissue samples and cell culture

Glioma tissues and adjacent normal tissues were obtained from 60 untreated glioma patients before their surgical resection in the Neurosurgery Department of the Chinese PLA General Hospital. The clinicopathological features of the glioma patients are provided in **Table 1**. Written informed consents were obtained from every patient before the experiments started. Also, our study was performed with the approval of the Ethics Committee of the Chinese PLA General Hospital.

U251 and U87 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Invitrogen) in a 5% CO_2 incubator at 37°C.

Reagents, plasmid construction and transfection

An MiR-142-3p mimic (miR-142-3p) and a scramble control (miR-control), an miR-142-3p inhibitor (Anti/miR-142-3p) and its negative control (Anti-NC), and a small interference RNA (siRNA) targeting HMGB1 (siRNA/HMGB1) were obtained from GenePharma Co. Ltd. (Suzhou, China). A full-length fragment of the HMGB1 coding region was synthetically produced by PCR and then constructed into a pcDNA3.1 vector (Invitrogen) to generate a pcDNA/HMGB1 overexpression plasmid. All oligonucleotides or plasmids were transfected into U251 and U87 cells using the Lipofectamine™ 2000 transfection reagent (Invitrogen) following the instructions recommended by the manufacturer.

RT-qPCR assay

Total RNA was extracted from glioma tissues and cells using TRIzol reagent (Invitrogen) according to the protocols of manufacturer. For expression analyses of HMGB1 and β-actin, RNA was reversely transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen) and random primers. Then, cDNA was used as a template to measure the levels of HMGB1 and β-actin by SYBR™ Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). β-actin was used to normalize the expression of HMGB1. Also, a TaqMan real-time PCR kit (Applied Biosystems, Carlsbad, CA) and TaqMan MicroRNA Assay primers (Applied Biosystems) for miR-142-3p and RNU6B were used to determine miR-142-3p levels with RNU6B as an internal control. Quantitative primers for HMGB1 and β-actin were presented as follows: HMGB1, 5'-GATCCCAATGCACCCAAGAG-3' (forward) and 5'-GGGCGATACTCAGAGCAGAAGA-3' (reverse); β-actin, 5'-CCTGGCACCCAGCACAAT-3' (forward) and 5'-GCCGATCCACACGGAGTACT-3' (reverse).

Western blot assay

Total proteins were extracted from glioma cells using a RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Rockford, IL, USA) containing a protease inhibitor cocktail (Sigma-

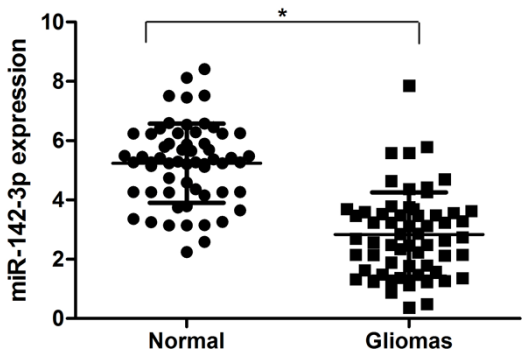

Figure 1. MiR-142-3p expression was downregulated in glioma tissues. RT-qPCR assay was performed to detect the expression of miR-142-3p in 60 pairs of glioma tissues (Gliomas) and adjacent normal tissues (Normal). *$P < 0.05$.

Aldrich, St. Louis, MO, USA) and quantified using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). Then, forty micrograms of proteins of each sample were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). Next, the membranes were subjected to the incubation overnight at 4°C with primary antibodies against HMGB1 (#6893, 1: 1000, Cell Signaling Technology, Inc., Danvers, MA, USA), c-myc (#13987, 1: 1000, Cell Signaling Technology), cleaved caspase-3 (#9661, 1: 1000, Cell Signaling Technology), β-catenin (#8480, 1: 1000, Cell Signaling Technology) and β-actin (#8457, 1:2000, Cell Signaling Technology). Afterwards, the membranes were probed with goat anti-rabbit IgG H&L (HRP) secondary antibody (ab205718, 1:10000, Abcam, Cambridge, UK). Finally, chemoluminescent signals were visualized by a Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific) and quantified using Quantity One software Version 4.1.1 (Bio-Rad Laboratories).

Luciferase reporter assay

An HMGB1 3'UTR sequence containing predicted miR-142-3p binding sites was synthetically produced by PCR and constructed into the luciferase reporter vector psiCHECK-2 (Promega, Madison, WI, USA) to obtain the HMGB1 3'UTR WT reporter. Also, an HMGB1 3'UTR MUT reporter containing mutant miR-142-3p binding sites was generated using a KOD-plus-mutagenesis kit (Toyobo, Osaka, Japan) referring to the protocols of the manufacturers. Then, an HMGB1 3'UTR WT or HMGB1 3'UTR MUT reporter were transfected into U251 and U87 cells along with miR-control or miR-142-3p. At 48 h upon transfection, luciferase activities were measured using a Dual-Luciferase® Reporter Assay System (Promega) with renilla luciferase activity as a normalization standard to normalize the firefly luciferase activity.

MTS assay

Cell proliferative ability was assessed using a MTS Cell Proliferation Assay Kit (Colorimetric) (Abcam) according to the instructions of manufacturers. Briefly, cells were seeded into a 96-well microtiter plate with a final volume of 200 μl/well and incubated overnight. On the next day, the cells were transfected with corresponding oligonucleotides or plasmids. At 48 h upon transfection, 20 μl/well of MTS reagent was added into each well. After incubation for another 2 h at 37°C, cell absorbance was measured at the wavelength of 490 nm.

Cell apoptosis assay

Cell apoptotic rates were determined using an AnnexinV-FITC/PI Apoptosis Detection Kit (Yeasen technology, Shanghai, China). Briefly, collected cells were re-suspended in a 100 μl 1 × binding buffer and then stained with a 5 μl Annexin V-FITC and 10 μl PI staining solution for 15 min. Finally, the cell apoptotic rate was measured using flow cytometry (BD Biosciences, Franklin Lakes, New Jersey, USA).

Statistical analysis

Data was obtained from at least three timeindependent experiments and analyzed using GraphPad Prism software (La Jolla, CA, USA). All results were presented as the mean ± the standard deviation. Student's *t* test was used to compare the difference between the two groups. $P < 0.05$ was considered statistically significant.

Results

MiR-142-3p expression was downregulated in glioma tissues

Firstly, the expression patterns of miR-142-3p in the 60 pairs of glioma tissues (Gliomas) and the adjacent normal tissues (Normal) were

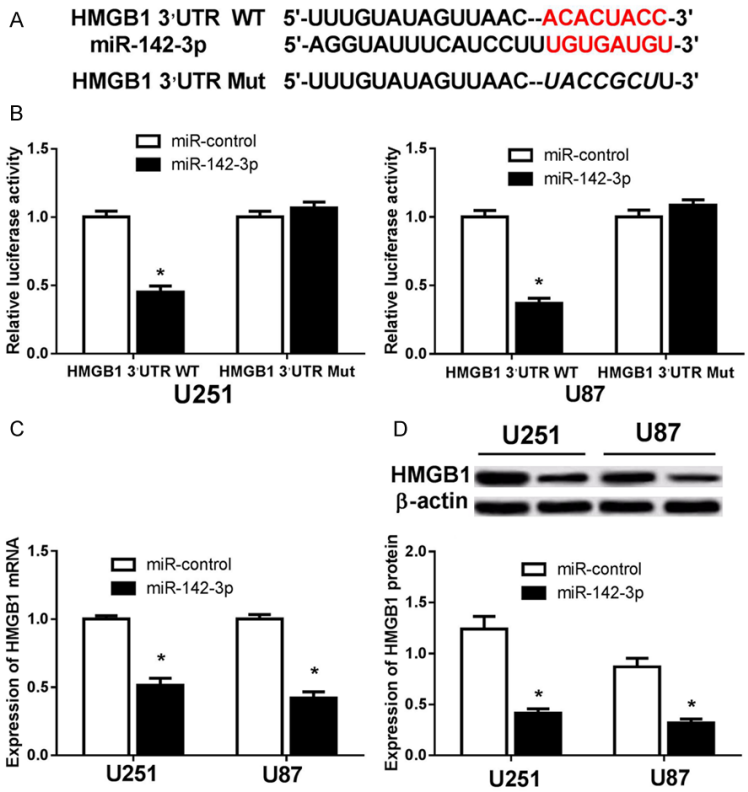

Figure 2. HMGB1 was a target of miR-142-3p. (A) Predicted binding sites between miR-142-3p and HMGB1 3'UTR, and mutant sites in the HMGB1 3'UTR MUT reporter. (B) U251 and U87 cells were co-transfected with HMGB1 3'UTR WT or the HMGB1 3'UTR MUT reporter and miR-control or miR-142-3p. At 48 h after transfection, relative luciferase activity was determined using a Dual-Luciferase® Reporter Assay System. (C and D) U251 and U87 cells were transfected with miR-control or miR-142-3p. At 48 h upon transfection, HMGB1 mRNA (C) and protein (D) levels were measured by RT-qPCR and western blot assays, respectively. *$P < 0.05$.

examined by an RT-qPCR assay. The results showed that the miR-142-3p level was markedly reduced in the glioma tissues (n = 60) as compared to the adjacent normal tissues (n = 60) (**Figure 1**), hinting that miR-142-3p might be implicated in the pathogenesis of glioma.

HMGB1 was a target of miR-142-3p

A bioinformatical analysis by the TargetScan website showed that HMGB1 is a potential target of miR-142-3p (**Figure 2A**). To further verify this prediction, the effect of miR-142-3p on the luciferase activity of the HMGB1 3'UTR WT or HMGB1 3'UTR MUT reporter was firstly determined in U251 and U87 cells. The results showed that the introduction of miR-142-3p induced a conspicuous reduction in luciferase activity of the HMGB1 3'UTR WT reporter in U251 and U87 cells (**Figure 2B**). Moreover, miR-142-3p had no obvious influence on luciferase activity of the HM-GB1 3'UTR MUT reporter in U251 and U87 cells (**Figure 2B**). In other words, these results suggest that miR-142-3p could interact with HMGB1 3'UTR through putative binding sites. Following RT-qPCR and Western blot assays, it was further disclosed that the ectopic expression of miR-142-3p inhibited HMGB1 expression at the mRNA (**Figure 2C**) and protein (**Figure 2D**) levels in U251 and U87 cells. In summary, these data indicated that HMGB1 is a target of miR-142-3p in U251 and U87 cells.

MiR-142-3p inhibited proliferation and induced apoptosis by targeting HMGB1 in U251 and U87 cells

A Western blot assay further demonstrated that the enforced expression of HMGB1 partly abrogated the inhibitory effect of miR-142-3p on HMGB1 expression in U251 and U87 cells (**Figure 3A**). Also, the inhibition of miR-142-3p by anti/miR-142-3p induced an increase of HMGB1 protein levels in U251 and U87 cells, while the promotive effect of anti/miR-142-3p on HMGB1 expression was weakened by siRNA/HMGB1-mediated HMGB1 silencing (**Figure 3B**). MTS assay revealed that miR-142-3p overexpression suppressed the proliferation of U251 and U87 cells (**Figure 3C**). Also, a notable elevation of the apoptotic rate was observed in U251 and U87 cells following the upregulation of miR-142-3p (**Figure 3E**). In other words, the ectopic expression of miR-142-3p induced apoptosis in U251 and U87 cells. Restoration experiments further demonstrated that the overexpression of HMGB1 alleviated miR-142-3p-mediated anti-proliferation (**Figure 3C**) and pro-apoptosis (**Figure 3E**) effects, as evidenced by improved proliferation capacity and reduced apoptotic rate in miR-142-3p-transfected U251 and U87 cells following the upregulation of HMGB1 (**Figure 3C**, **3E**).

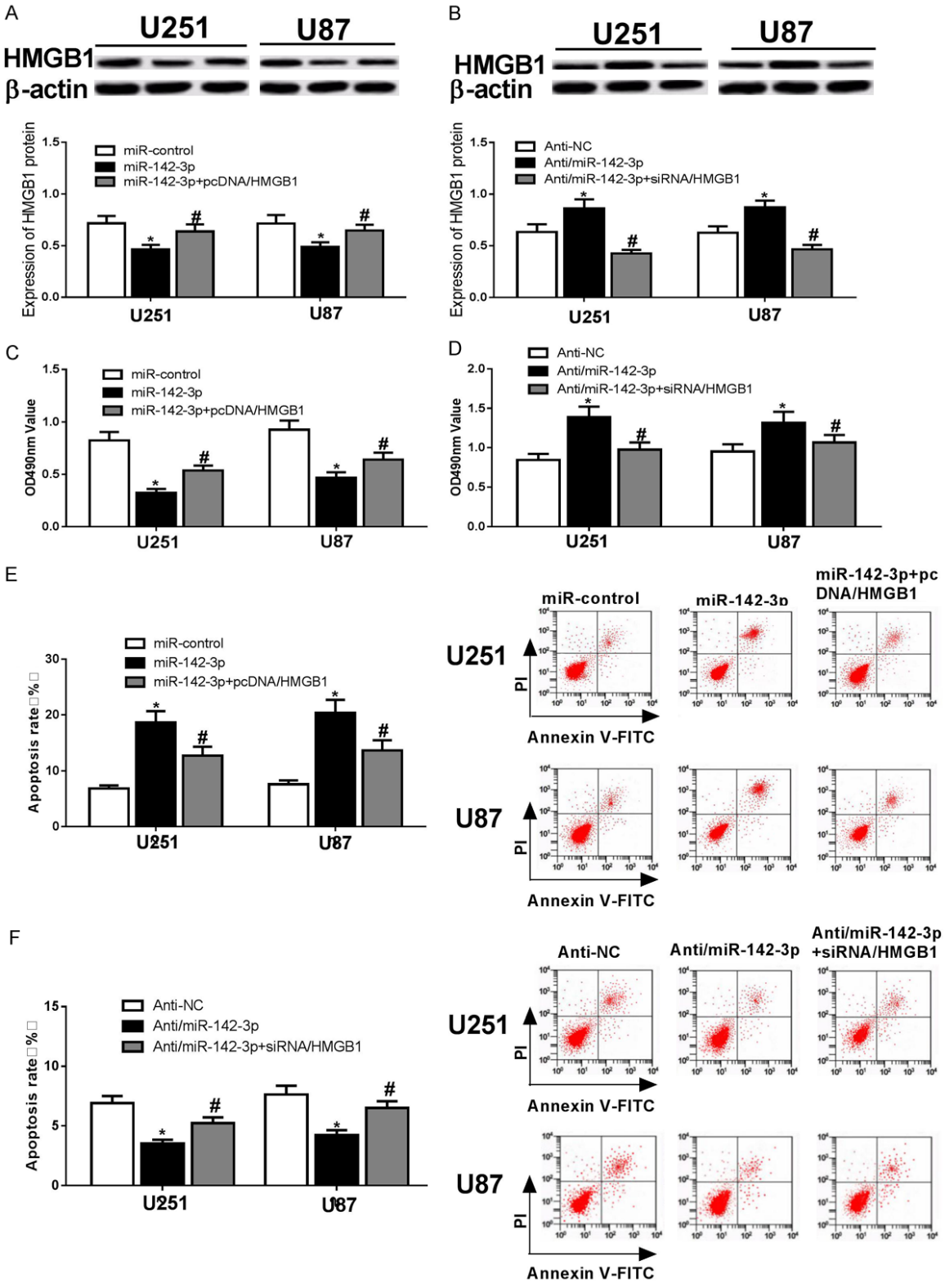

Figure 3. MiR-142-3p inhibited proliferation and induced apoptosis by targeting HMGB1 in U251 and U87 cells. (A-F) U251 and U87 cells were transfected with miR-control, miR-142-3p, miR-142-3p + pcDNA/HMGB1, Anti-miR-NC, Anti/miR-142-3p, or Anti/miR-142-3p + siRNA/HMGB1. At 48 h after transfection, the HMGB1 protein level was examined by a Western blot assay (A and B), cell proliferative ability was measured by an MTS assay (C and D), and the cell apoptotic rate was determined using flow cytometry via the double-staining of Annexin V-FITC and PI (E and F). $*P < 0.05$. $*\#P < 0.05$.

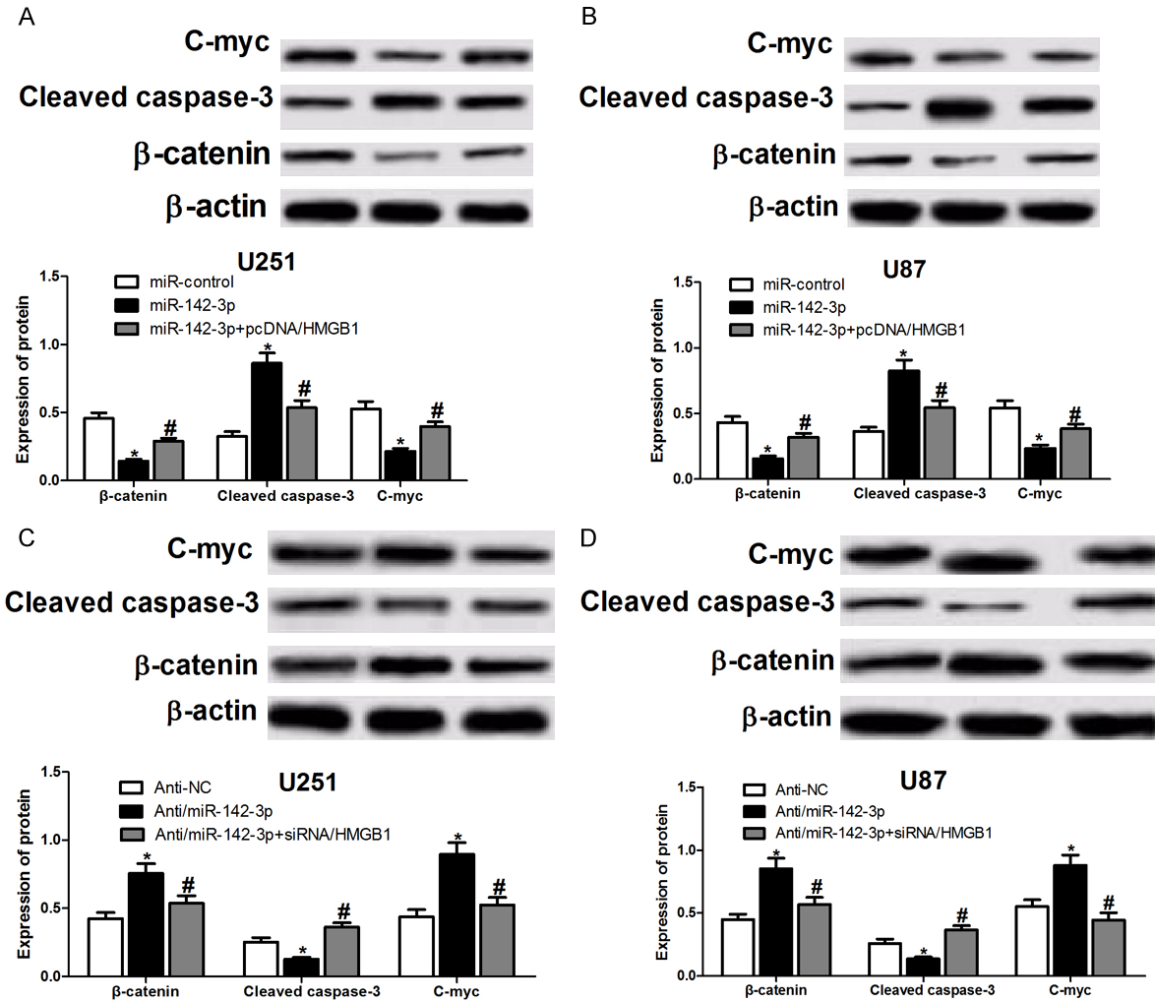

Figure 4. MiR-142-3p inactivated Wnt/β-catenin signaling and activated caspase-3 signaling by targeting HMGB1 in U251 and U87 cells. A-D. U251 and U87 cells were transfected with miR-control, miR-142-3p, miR-142-3p + pcDNA/HMGB1, Anti-miR-NC, Anti/miR-142-3p, or Anti/miR-142-3p + siRNA/HMGB1. At 48 h post transfection, a Western blot assay was performed to examine the protein levels of c-myc, β-catenin and cleaved caspase-3. *#$P < 0.05$.

Conversely, the deficiency of miR-142-3p resulted in an increase of cell proliferative ability (**Figure 3D**) and the reduction of the cell apoptotic rate (**Figure 3F**) in U251 and U87 cells. Additionally, the depletion of HMGB1 undermined the miR-142-3p inhibitor-induced proliferation increase and apoptosis reduction in U251 and U87 cells (**Figure 3D**, **3F**). In summary, these data manifested that miR-142-3p inhibited proliferation and induced apoptosis by targeting HMGB1 in U251 and U87 cells.

MiR-142-3p inactivated Wnt/β-catenin signaling and activated caspase-3 signaling by targeting HMGB1 in U251 and U87 cells

Next, the effect of miR-142-3p alone or along with HMGB1 on Wnt/β-catenin and caspase-3 signaling was further examined. The results showed that miR-142-3p overexpression notably reduced c-myc and β-catenin protein levels, and markedly increased the cleaved caspase-3 level in U251 and U87 cells (**Figure 4A**, **4B**). In other words, miR-142-3p inhibited the activation of Wnt/β-catenin signaling and induced the activation of caspase-3 signaling in U251 and U87 cells. Moreover, we further demonstrated that HMGB1 upregulation rescued the miR-142-3p-mediated Wnt/β-catenin signaling inactivation and caspase-3 activation in U251 and U87 cells, as evidenced by the increase of c-myc and β-catenin levels and the reduction of the cleaved caspase-3 level in miR-142-3p-transfected cells following the overexpression of HMGB1 (**Figure 4A**, **4B**). Inversely, the inhibi-

tion of miR-142-3p resulted in the upregulation of c-myc and β-catenin levels, and the downregulation of cleaved caspase-3 levels in U251 and U87 cells, while this effect was counteracted by HMGB1 knockdown (**Figure 4C**, **4D**). In summary, these results indicated that miR-142-3p inactivated Wnt/β-catenin signaling and activated caspase-3 signaling by targeting HMGB1 in U251 and U87 cells.

Discussion

Glioma can induce serious functional impairment for patients and bring about a massive burden for their families and society [17, 18]. Studies on the molecular basis of glioma carcinogenesis have contributed to the discovery of some key genetic events and the invention of novel therapeutic approaches [19]. MiR-142-3p has been reported to be involved in regulating multiple biological processes such as autophagy [20], neuronal differentiation [21], and aerobic glycolysis [22]. Moreover, miR-142-3p expression was found to be drastically downregulated in glioma tumors, glioma cell lines, and glioblastoma-infiltrating macrophages compared to healthy donor monocytes [15]. However, Dong *et al.* pointed out that the miR-142-3p level was strikingly increased in the serum of glioblastoma patients compared with healthy controls [14]. In the present study, we demonstrated that miR-142-3p was expressed lower in glioma tissues relative to adjacent normal tissues.

It is well known that miRNAs can negatively regulate gene expression by binding with 3'UTR of target mRNAs and inducing target degradation or/and translational repression [23]. Hence, a biological prediction by the TargetScan online website was performed to search for potential targets of miR-143-3p. Among candidate targets, HMGB1 was selected due to its significant roles in human health and diseases including cancers [24]. Following a luciferase reporter assay, RT-qPCR and western blot assays further revealed that HMGB1 was a target of miR-142-3p in glioma cells, which was in line with a previous study [25]. Also, HMGB1 as a target of miR-142-3p also has been validated in other neoplasms such as non-small-cell cancer [10] and osteosarcoma [26]. Additionally, our study showed that miR-142-3p suppressed proliferation and induced apoptosis by targeting HMGB1 in U251 and U87 cells.

HMGB1 is a member of HMGB family, which also contains HMGB2, HMGB3 and HMGB4 [27]. It is well documented that HMGB1 plays a vital role in multiple biological processes such as inflammation, immunity, proliferation, senescence and death [24]. Moreover, the dual paradoxical roles of HMGB1 have been noted in some cancers including glioma [28, 29]. For instance, intracellular HMGB1 behaves as a tumor suppressor, while extracellular HMGB1 functions as an oncogene in pancreatic cancer [29]. Also, tumor-derived HMGB1 induced the activation of endogenous TLR-2 signaling and initiated T cell-dependent glioma regression [30]. Conversely, some studies have found that HMGB1 promotes proliferation, migration, invasion and inhibited apoptosis in glioma cells [31, 32].

It was reported that the Wnt/β-catenin signaling played vital roles in glioma progression, including initiation, proliferation, and invasion [33]. Moreover, Isobe *et al.* demonstrated that miR-142 knockdown inhibited the tumorigenicity of human breast cancer stem cells partly by inactivating the Wnt/β-catenin signaling pathway [34]. Also, miR-142-3p overexpression promoted osteoblast differentiation by activating Wnt/β-catenin signaling [35]. Additionally, some studies discovered that HMGB1 facilitated β-catenin expression in acute lung injury models [36], malignant mesothelioma cells [37], and bronchial epithelial cells [38]. Moreover, an earlier study showed that Wnt signaling facilitated tumorigenesis by suppressing c-Myc-induced apoptosis [39]. In a word, this evidence hinted that the Wnt/β-catenin signaling was closely linked with the functions of the miR-142-3p/HMGB1 axis in glioma. A prior study pointed out that the proteolytic inactivation of caspase 3 inhibited tumorigenicity in U87 glioma xenografts [40]. Moreover, high cleaved caspase-3 level was positively associated with a better prognosis of glioma patients [41]. Furthermore, HMGB1 could induce apoptosis by activating caspase-3 in hepatocyte [42], pancreatic and colorectal cells [43]. Hence, the effect of miR-142-3p alone or along with HMGB1 on Wnt/β-catenin and caspase-3 signaling was examined in glioma cells. The results showed that miR-142-3p inactivated Wnt/β-catenin signaling and activated caspase-3 signaling by targeting HMGB1 in glioma cells.

Collectively, our study showed that miR-142-3p inhibited proliferation and induced apoptosis by targeting HMGB1 and by inactivating the Wnt/β-catenin signaling and activating caspase-3 signaling in glioma, deepening our understanding of the roles and molecular basis of the miR-142-3p/HMGB1 axis in the development of glioma and providing some candidate targets for the intervention and treatment of glioma.

Disclosure of conflict of interest

None.

Address correspondence to: Chong Li, Department of Neurosurgery, Chinese PLA General Hospital, 28 Fuxing Rd, Haidian District, Beijing 100853, China. Tel: +86-18611847670; E-mail: badlc3011@126.com

References

[1] Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. Neuro Oncol 2017; 19: v1-v88.

[2] Kruchko C, Ostrom QT, Gittleman H, Barnholtz-Sloan JS. The CBTRUS story: providing accurate population-based statistics on brain and other central nervous system tumors for everyone. Neuro Oncol 2018; 20: 295-298.

[3] Claus EB, Walsh KM, Wiencke JK, Molinaro AM, Wiemels JL, Schildkraut JM, Bondy ML, Berger M, Jenkins R, Wrensch M. Survival and low-grade glioma: the emergence of genetic information. Neurosurg Focus 2015; 38: E6.

[4] Stupp R, Brada M, van den Bent MJ, Tonn JC, Pentheroudakis G. High-grade glioma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2014; 25 Suppl 3: iii93-101.

[5] Khansur EM, Shah AH, Lacy K, Kuchakulla M, Komotar RJ. Novel imunotherapeutics for the treatment of glioblastoma: the last decade of research. Cureus 2018; 10: e2130.

[6] Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol cell Biol 2014; 15: 509-524.

[7] Barbato S, Solaini G, Fabbri M. MicroRNAs in oncogenesis and tumor suppression. Int Rev Cell Mol Biol 2017; 333: 229-268.

[8] Beyer S, Fleming J, Meng W, Singh R, Haque SJ, Chakravarti A. The role of miRNAs in angiogenesis, invasion and metabolism and their therapeutic implications in giomas. Cancers (Basel) 2017; 9.

[9] Shen WW, Zeng Z, Zhu WX, Fu GH. MiR-142-3p functions as a tumor suppressor by targeting CD133, ABCG2, and Lgr5 in colon cancer cells. J Mol Med (Berl) 2013; 91: 989-1000.

[10] Xiao P, Liu WL. MiR-142-3p functions as a potential tumor suppressor directly targeting HMGB1 in non-small-cell lung carcinoma. Int J Clin Exp Pathol 2015; 8: 10800-10807.

[11] Xu G, Wang J, Jia Y, Shen F, Han W, Kang Y. MiR-142-3p functions as a potential tumor suppressor in human osteosarcoma by targeting HMGA1. Cell Physiol Biochem 2014; 33: 1329-1339.

[12] Schwickert A, Weghake E, Bruggemann K, Engbers A, Brinkmann BF, Kemper B, Seggewiß J, Stock C, Ebnet K, Kiesel L, Riethmüller C, Götte M. microRNA miR-142-3p inhibits breast cancer cell invasiveness by synchronous targeting of WASL, integrin Alpha V, and additional cytoskeletal elements. PLoS One 2015; 10: e0143993.

[13] Deng B, Zhang Y, Zhang S, Wen F, Miao Y, Guo K. MicroRNA-142-3p inhibits cell proliferation and invasion of cervical cancer cells by targeting FZD7. Tumour Biol 2015; 36: 8065-8073.

[14] Dong L, Li Y, Han C, Wang X, She L, Zhang H. miRNA microarray reveals specific expression in the peripheral blood of glioblastoma patients. Int J Oncol 2014; 45: 746-756.

[15] Xu S, Wei J, Wang F, Kong LY, Ling XY, Nduom E, Gabrusiewicz K, Doucette T, Yang Y, Yaghi NK, Fajt V, Levine JM, Qiao W, Li XG, Lang FF, Rao G, Fuller GN, Calin GA, Heimberger AB. Effect of miR-142-3p on the M2 macrophage and therapeutic efficacy against murine glioblastoma. J Natl Cancer Inst 2014; 106.

[16] Chiou GY, Chien CS, Wang ML, Chen MT, Yang YP, Yu YL, Chien Y, Chang YC, Shen CC, Chio CC, Lu KH, Ma HI, Chen KH, Liu DM, Miller SA, Chen YW, Huang PI, Shih YH, Hung MC, Chiou SH. Epigenetic regulation of the miR142-3p/interleukin-6 circuit in glioblastoma. Mol Cell 2013; 52: 693-706.

[17] Lucas MR. The impact of chemo brain on the patient with a high-grade glioma. Adv Exp Med Biol 2010; 678: 21-25.

[18] Jacobs DI, Kumthekar P, Stell BV, Grimm SA, Rademaker AW, Rice L, Chandler JP, Muro K, Marymont M, Helenowski IB, Wagner LI, Raizer JJ. Concordance of patient and caregiver reports in evaluating quality of life in patients with malignant gliomas and an assessment of caregiver burden. Neurooncol Pract 2014; 1: 47-54.

[19] Reifenberger G, Wirsching HG, Knobbe-Thomsen CB, Weller M. Advances in the molecular

genetics of gliomas - implications for classification and therapy. Nat Rev Clin Oncol 2017; 14: 434-452.

[20] Lu Y, Gao J, Zhang S, Gu J, Lu H, Xia Y, Zhu Q, Qian X, Zhang F, Zhang C, Shen H, Hippen KL, Blazar BR, Lu L, Wang X. miR-142-3p regulates autophagy by targeting ATG16L1 in thymic-derived regulatory T cell (tTreg). Cell Death Dis 2018; 9: 290.

[21] Oh Y, Park J, Kim JI, Chang MY, Lee SH, Cho YH, Hwang J. Lin28B and miR-142-3p regulate neuronal differentiation by modulating Staufen1 expression. Cell Death Differ 2018; 25: 432-433.

[22] Hua S, Liu C, Liu L, Wu D. miR-142-3p inhibits aerobic glycolysis and cell proliferation in hepatocellular carcinoma via targeting LDHA. Biochem Biophys Res Commun 2018; 496: 947-954.

[23] Towler BP, Jones CI, Newbury SF. Mechanisms of regulation of mature miRNAs. Biochem Soc Trans 2015; 43: 1208-1214.

[24] Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, Huang J, Yu Y, Fan XG, Yan Z, Sun X, Wang H, Wang Q, Tsung A, Billiar TR, Zeh HJ, Lotze MT, Tang D. HMGB1 in health and disease. Mol Aspects Med 2014; 40: 1-116.

[25] Zhang R, Jin H, Lou F. The long non-coding RNA TP73-AS1 interacted with miR-142 to modulate brain glioma growth through HMGB1/RAGE pathway. J Cell Biochem 2018; 119: 3007-3016.

[26] Liu K, Huang J, Ni J, Song D, Ding M, Wang J, Huang X, Li W. MALAT1 promotes osteosarcoma development by regulation of HMGB1 via miR-142-3p and miR-129-5p. Cell Cycle 2017; 16: 578-587.

[27] Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cell Biol 1999; 19: 5237-5246.

[28] He SJ, Cheng J, Feng X, Yu Y, Tian L, Huang Q. The dual role and therapeutic potential of high-mobility group box 1 in cancer. Oncotarget 2017; 8: 64534-64550.

[29] Cebrian MJ, Bauden M, Andersson R, Holdenrieder S, Ansari D. Paradoxical role of HMGB1 in pancreatic cancer: tumor suppressor or tumor promoter? Anticancer Res 2016; 36: 4381-4389.

[30] Curtin JF, Liu N, Candolfi M, Xiong W, Assi H, Yagiz K, Edwards MR, Michelsen KS, Kroeger KM, Liu C, Muhammad AK, Clark MC, Arditi M, Comin-Anduix B, Ribas A, Lowenstein PR, Castro MG. HMGB1 mediates endogenous TLR2 activation and brain tumor regression. PLoS Med 2009; 6: e10.

[31] Bassi R, Giussani P, Anelli V, Colleoni T, Pedrazzi M, Patrone M, Viani P, Sparatore B, Melloni E, Riboni L. HMGB1 as an autocrine stimulus in human T98G glioblastoma cells: role in cell growth and migration. J Neurooncol 2008; 87: 23-33.

[32] Zhang J, Liu C, Hou R. Knockdown of HMGB1 improves apoptosis and suppresses proliferation and invasion of glioma cells. Chin J Cancer Res 2014; 26: 658-668.

[33] Nager M, Bhardwaj D, Canti C, Medina L, Nogues P, Herreros J. Beta-catenin signalling in glioblastoma multiforme and glioma-initiating cells. Chemother Res Pract 2012; 2012: 192362.

[34] Isobe T, Hisamori S, Hogan DJ, Zabala M, Hendrickson DG, Dalerba P, Cai S, Scheeren F, Kuo AH, Sikandar SS, Lam JS, Qian D, Dirbas FM, Somlo G, Lao K, Brown PO, Clarke MF, Shimono Y. miR-142 regulates the tumorigenicity of human breast cancer stem cells through the canonical WNT signaling pathway. Elife 2014; 3.

[35] Hu W, Ye Y, Zhang W, Wang J, Chen A, Guo F. miR1423p promotes osteoblast differentiation by modulating Wnt signaling. Mol Med Rep 2013; 7: 689-693.

[36] Zhou M, Zhang Y, Chen X, Zhu J, Du M, Zhou L, Wang W, Sun G. PTEN-Foxo1 signaling triggers HMGB1-mediated innate immune responses in acute lung injury. Immunol Res 2015; 62: 95-105.

[37] Yang H, Pellegrini L, Napolitano A, Giorgi C, Jube S, Preti A, Jennings CJ, De Marchis F, Flores EG, Larson D, Pagano I, Tanji M, Powers A, Kanodia S, Gaudino G, Pastorino S, Pass HI, Pinton P, Bianchi ME, Carbone M. Aspirin delays mesothelioma growth by inhibiting HMGB1-mediated tumor progression. Cell Death Dis 2015; 6: e1786.

[38] Chen YC, Statt S, Wu R, Chang HT, Liao JW, Wang CN, Shyu W, Lee CC. High mobility group box 1-induced epithelial mesenchymal transition in human airway epithelial cells. Sci Rep 2016; 6: 18815.

[39] You Z, Saims D, Chen S, Zhang Z, Guttridge DC, Guan KL, MacDougald OA, Brown AM, Evan G, Kitajewski J, Wang CY. Wnt signaling promotes oncogenic transformation by inhibiting c-Myc-induced apoptosis. J Cell Biol 2002; 157: 429-440.

[40] Feng X, Yu Y, He S, Cheng J, Gong Y, Zhang Z, Yang X, Xu B, Liu X, Li CY, Tian L, Huang Q. Dying glioma cells establish a proangiogenic microenvironment through a caspase 3 dependent mechanism. Cancer Lett 2017; 385: 12-20.

[41] Kobayashi T, Masumoto J, Tada T, Nomiyama T, Hongo K, Nakayama J. Prognostic significance of the immunohistochemical staining of

cleaved caspase-3, an activated form of caspase-3, in gliomas. Clin Cancer Res 2007; 13: 3868-74.

[42] Gwak GY, Moon TG, Lee DH, Yoo BC. Glycyrrhizin attenuates HMGB1-induced hepatocyte apoptosis by inhibiting the p38-dependent mitochondrial pathway. World J Gastroenterol 2012; 18: 679-684.

[43] Tang D, Kang R, Cheh CW, Livesey KM, Liang X, Schapiro NE, Benschop R, Sparvero LJ, Amoscato AA, Tracey KJ, Zeh HJ, Lotze MT. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. Oncogene 2010; 29: 5299-5310.