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MiR-1224-5p acts as a tumor suppressor by targeting CREB1 in malignant gliomas

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Abstract The dysregulation of miR-1224-5p has been reported in several human cancers. However, the expression and function of miR-1224-5p in glioma remains unknown. The aim of our study was to investigate the effect of miR-1224-5p on glioma cells and to determine its functional signaling mediators. Using 198 glioma samples within the Chinese Glioma Genome Atlas expression dataset, we demonstrated that miR-1224-5p expression is decreased in high-grade gliomas when compared with low-grade gliomas. Differential miR-1224-5p expression in 50 randomly selected samples was verified by in situ hybridization. The expression of miR-1224-5p was shown to positively correlate with overall survival in 82 glioblastoma patients. Exogenous expression of miR-1224-5p in glioma cells suppressed proliferation and invasion and promoted apoptosis. Target prediction algorithms identified a consensus miR-1224-5p recognition site in the 3′UTR of the cAMP response element-binding protein (CREB1) gene, and this sequence was shown to directly confer miR-1224-5p repression in luciferase reporter assays. Furthermore, exogenous miR-1224-5p expression was shown to down-regulate CREB1, as well as its downstream target genes matrix metalloproteinase-9 and B-cell lymphoma-2. Conversely, over-expression of CREB1 reversed the effect of miR-1224-5p on the proliferation, invasion, and apoptosis of glioma cells. These data indicate that miR-1224-5p may inhibit tumor-associated activity in malignant gliomas by targeting CREB1.

Keywords MiR-1224-5p · CREB1 · Glioma · Cell proliferation · Invasion · Apoptosis

Jin Qian, Rui Li, and Ying-Yi Wang contributed equally to this work.

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Introduction

Malignant glioma is one of the most common forms of neural malignancy with high morbidity and mortality. Despite recent advances in surgery and adjuvant therapy, the prognosis and tumor recurrence in glioblastoma patients have not been significantly improved [1–3]. Growing evidence suggests that the progression of glioblastoma depends on the rate of cell proliferation and apoptosis, and one of the reasons for recurrence of glioblastoma is the widespread diffuse invasion of tumor cells into brain tissues [4]. Thus, understanding the key molecular mechanism underlying gliomagenesis is crucial for developing novel and effective therapeutic strategies.

MicroRNAs (miRNAs) are single-stranded, short, noncoding RNAs involved in many biological processes including tumorigenesis [5, 6]. Accumulating evidences show that miRNAs modulate cell proliferation, apoptosis, invasion, and metastasis in human cancers [7–10]. The

dysregulation of miRNAs has been observed in various types of human malignant tumors, including lymphoma, colorectal cancer, lung cancer, breast cancer, liver cancer and glioblastoma [11–16]. Recently, abnormal expression of miR-1224-5p has been reported in several human cancers [17–19]. However, little is known about the expression and function of miR-1224-5p in gliomas.

cAMP response element-binding protein (CREB1) is a nuclear transcription factor that expressed abnormally in many cancers, including non-small cell lung carcinoma, breast cancer, acute myeloid leukemia, and gliomas [20–23]. As a potent oncogene, it regulates the proliferation, survival, apoptosis, invasion, and metastasis of tumor cells [24, 25]. Previous studies have shown that CREB1 is regulated by miR-200b and that it suppresses cell growth in human malignant glioma [26]. However, the mechanism of CREB1 in glioma and the potential regulation by other miRNAs warrant further investigation.

In this study, we determined that miR-1224-5p is downregulated in glioma tissue, and its expression serves as a protective factor for the prognosis of glioblastoma patients. We demonstrated that exogenous expression of miR-1224-5p in glioblastoma cells inhibits tumor proliferation, decreases invasion capability, and promotes cell apoptosis. Furthermore, we showed that miR-1224-5p directly targets the 3′UTR of CREB1 to regulate its expression and downstream signaling proteins. Our findings provide the first evidence of a role for miR-1224-5p as a tumor suppressor in glioma mediated by targeting CREB1. Based on these findings, miR-1224-5p could provide a novel therapeutic target and survival predictor for glioma patients.

Materials and methods

Samples and data analysis

Data on miR-1224-5p expression from 198 glioma samples (WHO II, $n = 63$; WHO III, $n = 44$; WHO IV, $n = 91$) and data on miR-1224-5p-related survival from 82 glioblastoma tissues (low expression, $n = 41$; high expression, $n = 41$) were downloaded from the Chinese Glioma Genome Atlas (CGGA, http://www.cgcg.org.cn/). The data were further analyzed using one ANOVA, Kaplan–Meier survival analysis, and log-rank testing.

In situ hybridization

Fifty glioma tissues (10 grade II, 20 grade III and 20 grade IV) were randomly selected for verification of differential miR-1224-5p expression. In situ hybridization was processed according to the manufacturer's instructions using antisense locked nucleic acid (LNA)-modified probes (Boster, Wuhan, China). The probe sequence of miR-1224-5p was as follows: 5′-CACTCCTGAGCCCTC-CACC-3. Each sample was examined separately by two pathologists.

Cell lines and culture

Human U251 and U87 glioblastoma cell lines were purchased from the Chinese Academy of Sciences Cell Bank. All cells were grown in Dulbecco's Modified Eagle's medium (Gibco, USA) supplemented with 10 % fetal bovine serum (FBS) (Invitrogen) in an incubator at 37 °C with 5 % CO_2.

Oligonucleotides and cell transfection

Oligonucleotides were chemically synthesized by Gene Pharma (Shanghai, China), based on the following sequences: hsa-miR-1224-5p mimic (miR-1224-5p), 5′-GUGAGGACUCGGGAGGUGG-3′; scrambled miRNA as a negative control (NC), 5′-UUCUCCGAACGUGUCAC-GUTT-3′. The CREB1 cDNA construct in pReceiver-M02 plasmid (CREB1) lacks its 3′UTR and was obtained from Fulengen (Guangzhou, China). Oligonucleotides were transfected into U251 and U87 glioblastoma cells at different concentrations using Lipofectamine 2000 (Invitrogen) for 6 h.

Cell proliferation assay

U87 and U251 glioma cells were seeded at 5,000 cells per well in 96-well plates (six replicate wells per condition) and cultured overnight. The cells were transfected with miR-1224-5p mimic or a scrambled control construct. The proliferation of the cells was measured every 24 h after transfection using a Cell Counting Kit-8 (CCK-8) (Beyotime, China) according to the manufacturer's instructions. The cell viability was determined at 450 nm absorbance using an enzyme-linked immunosorbent assay plate reader.

In vitro invasion assay

Cell invasion was assessed using transwell assays. The upper chambers were pre-incubated for 3 h to form a layer of Matrigel (BD Biosciences). Transfected cells were plated at a density of 3×10^4 cells per well in the upper chambers filled with serum-free medium. The lower chamber was filled with 20 % FBS as a chemo-attractant. After incubating for 24 h, cells remaining in the upper chamber were carefully removed with a cotton swab, while invading cells at the bottom well were fixed with 3 % paraformaldehyde, stained with crystal violet, counted, and

photographed in three independent fields per well (×100 magnification).

Apoptosis assay

U251 and U87 cells were plated into 6-well plates and transfected with oligonucleotides. The apoptosis ratio was analyzed 48 h after transfection using an Annexin V-FITC apoptosis detection kit (BD Biosciences, USA) according to the manufacturer's instructions. Annexin V-FITC and propidium iodide (PI) double staining was used to evaluate the percentages of apoptosis. Cells without annexin V and PI were used as controls. Cells that were Annexin V+ and PI− were considered apoptotic. Assessment of apoptosis was repeated in triplicate.

Luciferase reporter assay

For the luciferase reporter assay, the 3′UTR fragment of CREB1 containing the putative binding site of miR-1224-5p was sub-cloned into a pGL3 luciferase reporter vector. The 3′UTR of CREB1 without the putative miR-1224-5p binding sequences was used as a mutant (Mut) (Invitrogen). U251 and U87 glioma cells were seeded into 24-well plates. Once the cells had reached 60–70 % confluence, they were co-transfected with miR-1224-5p and pGL3-CREB1-3′UTR-Luc. Luciferase activity was measured with the Dual-Luciferase Reporter Assay System 48 h after transfection, according to the manufacturer's protocols (Promega, Madison, USA).

Western blotting

After transfection of oligonucleotides, proteins were extracted from cells with RIPA lysis buffer (KenGEN, China) and quantified using a BCA Protein Assay Kit (Beyotime, China). Total protein lysates were separated by SDS-PAGE. The separated proteins were transferred to PVDF membranes (Millipore, USA). The membranes were blocked in 5 % nonfat milk and incubated with CREB1 (1:400, SantaCruz, USA), Bcl-2 (1:1,000, Bioworld, USA), and MMP9 (1:1,000, CST, USA) primary antibodies, followed by incubation with HRP-conjugated secondary antibody (1:2,000, SantaCruz, USA). GAPDH was tested as a control (1:5,000, Bioworld, USA).

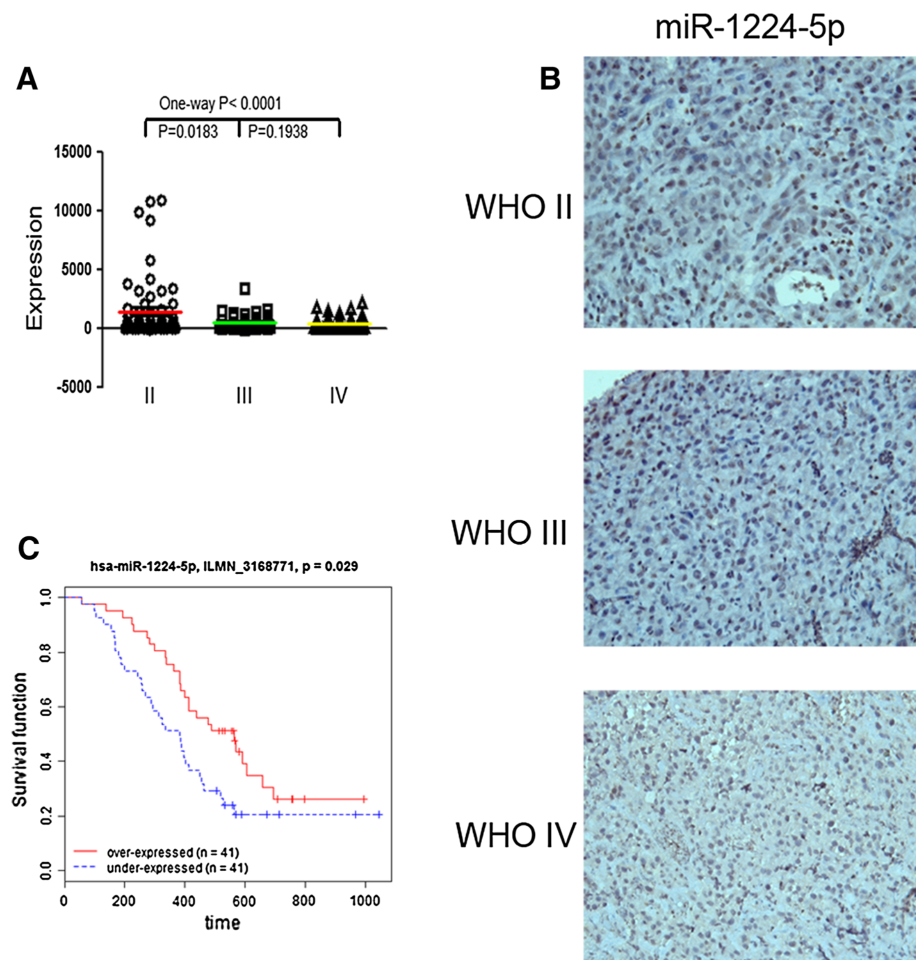

Fig. 1 The expression of miR-1224-5p in gliomas and its association with survival in glioblastomas. **a** The levels of 1224-5p were analyzed by one-way ANOVA using data from 198 glioma tissues in the CGGA glioma dataset (WHO II, $n = 63$; WHO III, $n = 44$; WHO IV, $n = 91$). The expression in WHO II samples was statistically greater than in either WHO III samples or WHO IV samples, but the expression in WHO III and WHO IV was not statistically different. **b** In situ hybridization confirmed that the levels of miR-1224-5p are decreased in high-grade glioma tissues compared with low-grade glioma tissues (×400 magnification). Results are representative of the results from 50 glioma samples. **c** Kaplan–Meier survival curves according to the expression of miR-1224-5p in 82 glioblastoma samples from the CGGA dataset. Results suggest that low expression of miR-1224-5p confers a poor prognosis in glioma patients

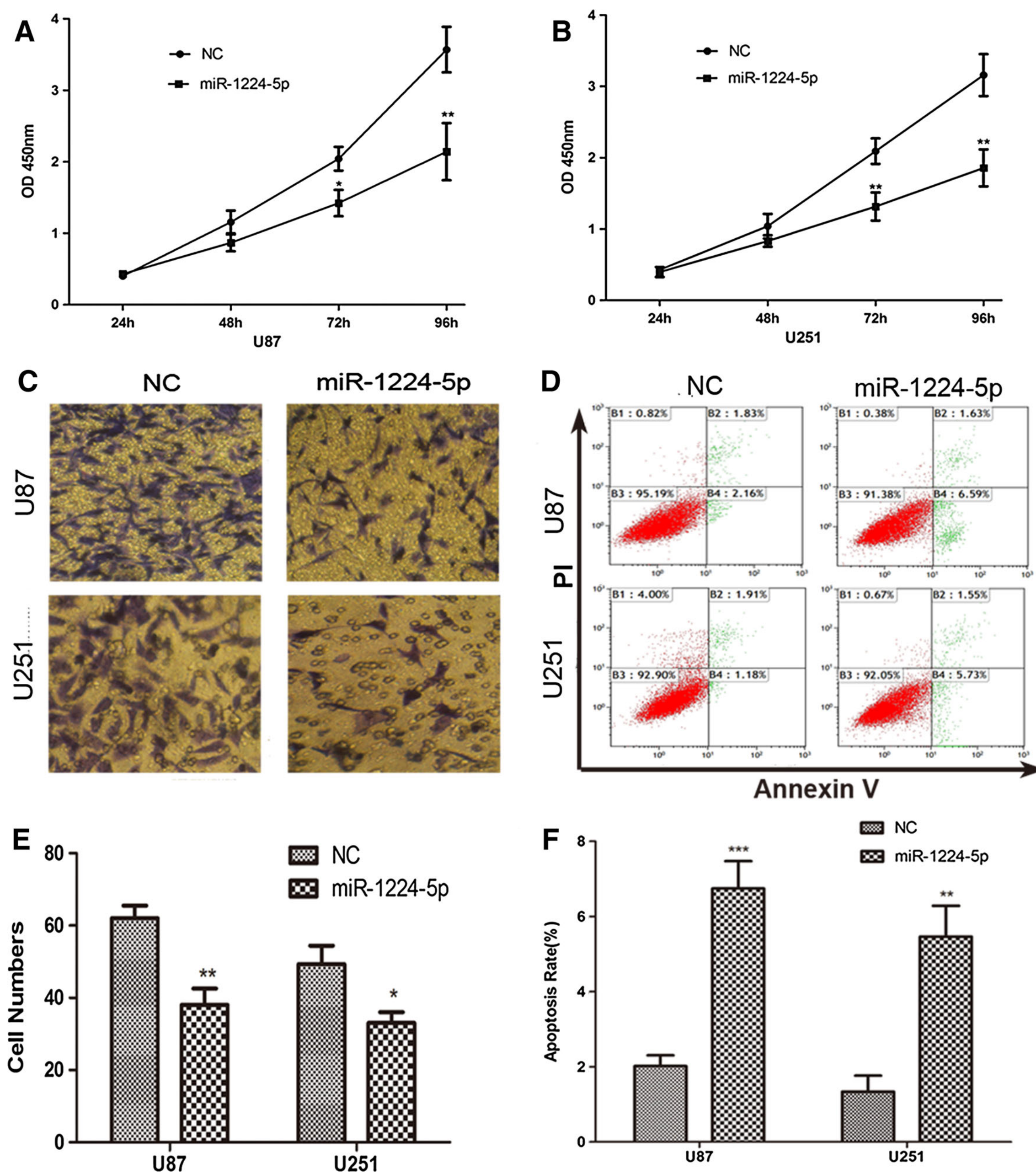

Fig. 2 Effect of miR-1224-5p on the proliferation, invasiveness, and apoptosis of glioma cell lines. **a**, **b** Effect of miR-1224-5p expression on the proliferation of glioma U87 (**a**) and U281 (**b**) cell lines was measured by CCK8 assay at a timecourse after transfection with negative control oligonucleotide (NC) or miR-1224-5p mimic (miR-1224-5p). The mean ± SD of six wells is shown. **c**, **d** The effect of miR-1224-5p on glioma cells invasion was assessed by in vitro transwell invasion assay. Representative results are shown in (**c**), and the mean + SD of the cell numbers in triplicate well is plotted in (**d**). **e**, **f** Effect of miR-1224-5p on cell apoptosis in glioma cell lines was assessed by flow cytometry after Annexin V/PI staining. Representative results are shown in (**e**), and the mean + SD of the apoptosis rate (Annexin V +/PI-cells) of triplicates is shown in (**f**). *$P < 0.05$;**$P < 0.01$, ***$P < 0.001$

Statistical analysis

Kaplan–Meier survival analysis was used to estimate the survival distributions, and the log-rank test was used to assess the statistical significance between stratified survival groups. Data were analyzed by the Student's *t*-test and one-way ANOVA, using SPSS 13.0 software package. $P < 0.05$ was considered significant. All experiments were performed three times.

Results

MiR-1224-5p is down-regulated in glioma tissues and confers a protective prognosis to glioma patients

To determine whether miR-1224-5p expression may serve as a prognostic factor in glioma, the expression of mature miR-1224-5p was analyzed in a series of 198 different grade glioma samples from the CGGA. As shown in Fig. 1a, high-grade (WHO III and IV) gliomas demonstrated a significant decrease in mean miR-1224-5p transcript levels compared to the levels in low-grade (WHO II) gliomas ($P < 0.05$). The lower levels of miR-1224-5p in high-grade gliomas in comparison with low-grade gliomas were verified by in situ hybridization of 50 tissue samples (Fig. 1b). Furthermore, the correlation between miR-1224-5p expression and overall survival was measured through Kaplan–Meier survival curve analysis with a log-rank comparison. The group with low miR-1224-5p expression was associated with poor survival relative to the high-expression group ($n = 41$ each group; $P = 0.02$) (Fig. 1c). These results demonstrate that miR-1224-5p is down-regulated in glioblastoma patients.

Critical role of miR-1224-5p in glioma cells

To explore the biological function of miR-1224-5p in glioma cells, a commercially synthesized miR-1224-5p mimic was transfected into U251 glioma cells. Over-expression of miR-1224-5p markedly attenuated cell proliferation as determined by CCK8 assay (Fig. 2a, b). Furthermore, up-regulation of miR-1224-5p significantly decreased cell invasion potential compared with the NC, both in U251 and U87 cells (Fig. 2c, d). More apoptotic cells were observed in U251 and U87 glioma cells treated with the miR-1224-5p mimic as compared to the NC as assessed by Annexin V/PI staining and flow cytometry (Fig. 2e, f). These results suggest that miR-1224-5p plays an important role in proliferation, invasion, and apoptosis of glioma cells.

CREB1 is a direct target of miR-1224-5p

To determine the mechanism of action of miR-1224-5p in glioma cells, we performed an miRNA target search using TargetScan and miRanda. A highly conserved putative recognition sequence of miR-1224-5p was identified in the 3′UTR of CREB1 (Fig. 3a), suggesting that CREB1 is a potential target of miR-1224-5p. To determine if this site is targeted by miR-1224-5p, we created plasmids containing wild-type (WT) and Mut CREB1 3′UTRs. Over-expression of miR-1224-5p suppressed the luciferase activity of the WT plasmid, though no significant change was detected in luciferase activity of the Mut plasmid in U87 or U251 cells (Fig. 3b). Consistently, Western blot analysis showed that transfection of miR1224-5p reduced the expression of endogenous CREB1 in glioma cells. Furthermore, the protein expression of the CREB1 target genes, B-cell lymphoma-2 (Bcl-2, which is associated with apoptosis), and matrix metalloproteinases-9 (MMP9, which is associated with invasion) were also decreased after transfection of miR-

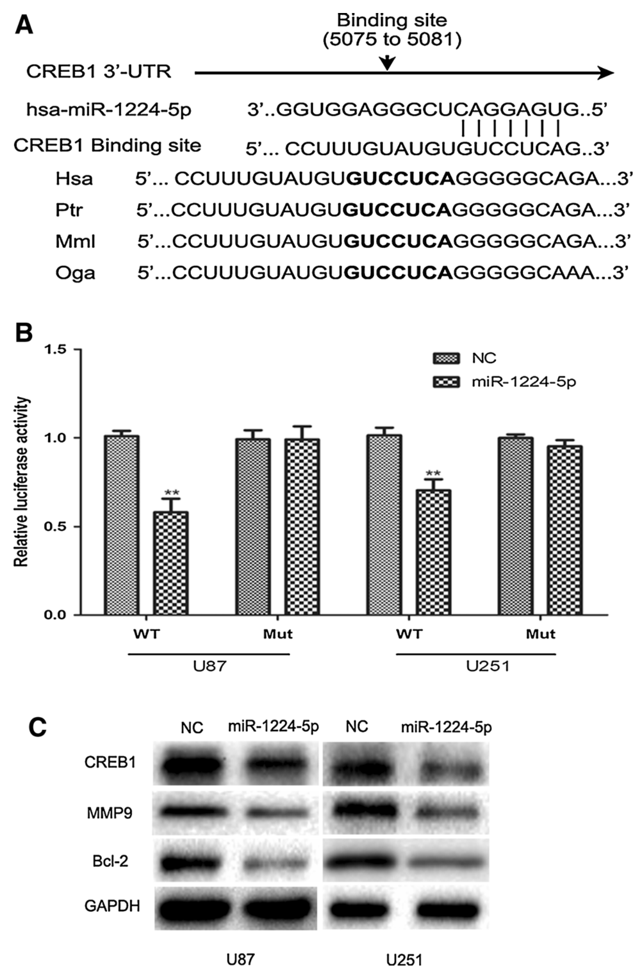

Fig. 3 MiR-1224-5p directly targets CREB1. **a** Putative binding sites of miR-1224-5p within the 3′UTR of CREB1 and four additional genes, as predicted by TargetScan and miRanda. **b** Transfected miR-1224-5p mimic (miR-1224-5p), but not the negative control (NC) oligonucleotide down-regulated the luciferase activity of a wild-type CREB1 3′UTR (WT) reporter construct in U87 and U251 cells. As a control, the activity of the mutant CREB1 3′UTR (Mut) reporter was not down-regulated by miR-1224-5p. Values represent the mean + SD of triplicates and were normalized to 1.0 in the NC transfection samples. $^{**}P < 0.01$. **c** Over-expression of miR-1224-5p decreased CREB1, MMP9 and Bcl-2 expression in U87 and U251 cells. Protein expression was assessed by Western blotting 24 h after transfection with NC or miR-1224-5p oligonucleotides. GAPDH was tested as a loading control. Results are representative of three independent experiments

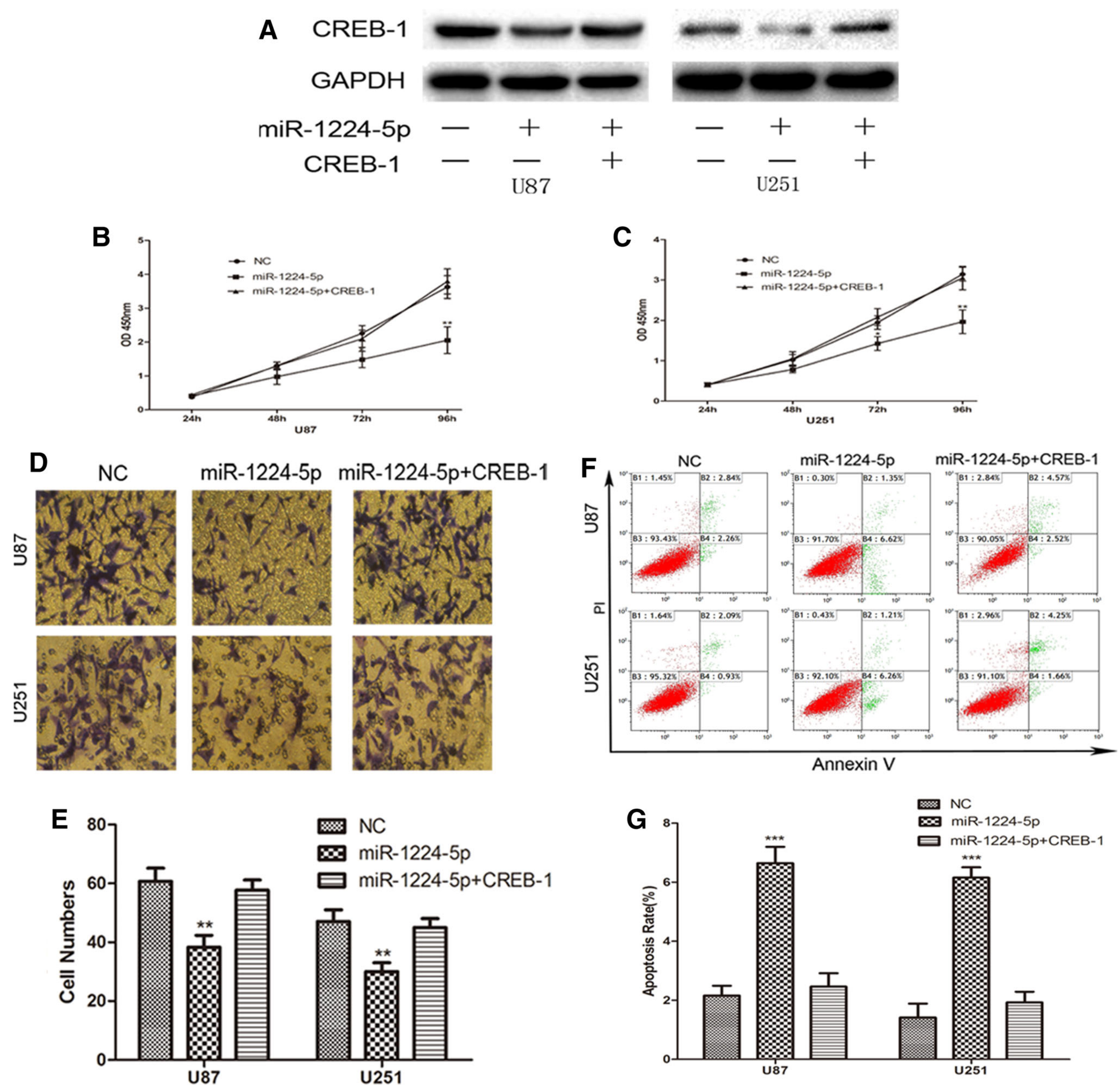

Fig. 4 Expression of CREB1 abrogates miR-1224-5p biological function of glioma cell lines. **a** Western blots to assess CREB1 expression after transfection of U87 and U251 cells with miR-1224-5p mimic and/or CREB1 expression plasmid. GAPDH was tested as a loading control. **b**, **c** Expression of CREB1 reversed the miR-1224-5p-induced inhibition of proliferation as assessed by CCK-8 assay for U87 cells (**b**) and U251 cells (**c**). Results represent the mean + SD of six wells. **d**, **e** Expression of CREB1 reversed the miR-1224-5p-induced inhibition of invasiveness as assessed by transwell invasion assay. Images (**d**) and mean + SD cell numbers (**e**) from triplicate well for a representative experiment are shown. **f**, **g** Expression of CREB1 reversed the miR-1224-5p-induced apoptosis as assessed by Annexin/PI staining and flow cytometry. Results from one representative replicate (**f**) and the mean + SD of triplicates (**g**) are shown. Results are representative of three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$

1224-5p (Fig. 3c). These data provide evidence that miR-1224-5p can directly modulate CREB1 expression by binding to the 3′UTR of CREB1 mRNA, which modulates downstream MMP9 and Bcl-2 expression in glioma cells [27–29] (Fig. S).

Expression of CREB1 overrides miR-1224-5p biological function

To determine the functional consequence of the targeting of CREB1 by miR-1224-5p, we transfected miR-1224-5p

together with a CREB1 expression plasmid into U87 and U251 cells followed by functional assays. The expression of CREB1 protein was confirmed by Western blotting (Fig. 4a). Importantly, expression of CREB1 rescued the effects of miR-1224-5p on proliferation, invasion, and apoptosis of U87 and U251 glioma cells (Fig. 4b–g). These results indicate that CREB1 is a critical target of miR-1224-5p in regulating glioma cell biological function.

Discussion

MiRNAs play an important role in regulating various physiological and pathological events at the post-transcriptional level [30]. Growing evidence indicates that miRNAs target multiple genes involving almost all aspects of cancer biology [31]. The investigation of miRNAs and their mRNA target genes and pathways, therefore, provides an effective approach to further our understanding of carcinogenesis. Recent studies have shown that many miRNAs function as tumor suppressors and are down-regulated in diverse human cancers [32, 33]. Several independent research groups have reported that miR-1224-5p is down-regulated in colorectal cancer, rectal cancer, and lung cancer [17–19]. However, the expression and function of miR-1224-5p in malignant glioma remains unclear. In the present study, by analyzing data from the CGGA, we found that the expression of miR-1224-5p is decreased in high-grade gliomas compared to low-grade gliomas. We confirmed the differential expression of miR-1224-5p by in situ hybridization. Furthermore, survival analysis suggested that miR-1224-5p may serve as an independent prognostic factor in glioma patients. The up-regulation of miR-1224-5p dramatically repressed glioma cell proliferation and invasion and promoted cell apoptosis, which is consistent with its potential role as a tumor suppressor in glioma. These data suggest that miR-1224-5p might play a critical role in the development of glioma as a tumor suppressor miRNA.

In a search of functional targets of miR-1224-5p, we identified CREB1 as a miR-1224-5p-regulated gene in glioma cells. CREB1 is a transcription factor containing a 43-kDa basic/leucine zipper structure that has a central role in regulating the expression of multiple target genes [25]. It can be activated through phosphorylation via a number of kinases pathways, including the protein kinase A (PKA), protein kinase C (PKC), Ca^{2+}/calmodulin-dependent kinase (CaM kinases), p90 ribosomal S6 kinase, and extracellular signal-regulated kinases (ERK1/2) signaling pathways [34, 35]. CREB1 has been classically studied in the physiology of nerve or contractile cells [36, 37]. Accumulating evidence suggests that CREB1 is a proto-oncogenic transcription factor that promotes tumorigenesis in many cancers [24, 38]. Knockdown of CREB1 suppresses the malignant progression of human mesotheliomas by reducing the expression of Bcl2, c-FOS, MMP9, and MMP13 [25]. Recently, some miRNAs involved in the dysregulation of CREB1 have been identified in human gliomagenesis [26, 39]. Here, we demonstrated that CREB1 is a direct target of miR-1224-5p in glioma cells through a recognition site in its 3′UTR. Consistent with the functional regulation of CREB1 by miR-1224-5p, Bcl2 and MMP9 were down-regulated upon miR-1224-5p expression. Exogenous up-regulation of CREB1 largely reversed the effects of miR-1224-5p on cell proliferation, invasion and apoptosis. These findings indicate that CREB1 is regulated by miR-1224-5p in glioma cells, and that CREB1 subsequently directly or indirectly modulates its target genes to control the cell proliferation, invasion, and apoptosis of gliomas.

In summary, we have shown that miR-1224-5p is down-regulated in malignant glioma and acts as a tumor suppressor in glioma by targeting CREB1, thus regulating cell proliferation, invasion, and apoptosis. We also provide evidence that low levels of miR-1224-5p expression are significantly associated with poor overall survival. Consequently, miR-1224-5p may function as an intrinsic regulator in human gliomagenesis and a potential predictor of survival in this devastating disease. As our functional data were largely derived from cell lines, further studies should be focused on the miR-1224-5p/CREB1 molecular network within the clinical context, as well as the targeting of the miR-1224-5p/CREB1 axis as a potential therapeutic application.

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References

1. Clarke J, Butowski N, Chang S (2010) Recent advances in therapy for glioblastoma. Arch Neurol 67:279–283. doi:10.1001/archneurol.2010.5
2. Saika K, Katanoda K (2011) Comparison of time trends in brain and central nervous system cancer mortality (1990–2006) between countries based on the WHO mortality database. Jpn J Clin Oncol 41:304–305. doi:10.1093/jjco/hyr004
3. Yan W, Zhang W, Jiang T (2011) Oncogene addiction in gliomas: implications for molecular targeted therapy. J Exp Clin Cancer Res 30:58. doi:10.1186/1756-9966-30-58

4. Tate MC, Aghi MK (2009) Biology of angiogenesis and invasion in glioma. Neurotherapeutics 6:447–457. doi:10.1016/j.nurt.2009.04.001
5. Yue J, Tigyi G (2006) MicroRNA trafficking and human cancer. Cancer Biol Ther 5:573–578
6. Zimmerman AL, Wu S (2011) MicroRNAs, cancer and cancer stem cells. Cancer Lett 300:10–19. doi:10.1016/j.canlet.2010.09.019
7. Zhang CM, Zhao J, Deng HY (2013) MiR-155 promotes proliferation of human breast cancer MCF-7 cells through targeting tumor protein 53-induced nuclear protein 1. J Biomed Sci 20:79. doi:10.1186/1423-0127-20-79
8. Wang XF, Shi ZM, Wang XR, Cao L, Wang YY, Zhang JX, Yin Y, Luo H, Kang CS, Liu N, Jiang T, You YP (2012) MiR-181d acts as a tumor suppressor in glioma by targeting K-ras and Bcl-2. J Cancer Res Clin Oncol 138:573–584. doi:10.1007/s00432-011-1114-x
9. Zhang C, Zhang J, Hao J, Shi Z, Wang Y, Han L, Yu S, You Y, Jiang T, Wang J, Liu M, Pu P, Kang C (2012) High level of miR-221/222 confers increased cell invasion and poor prognosis in glioma. J Transl Med 10:119. doi:10.1186/1479-5876-10-119
10. Krishnan K, Steptoe AL, Martin HC, Pattabiraman DR, Nones K, Waddell N, Mariasegaram M, Simpson PT, Lakhani SR, Vlassov A, Grimmond SM, Cloonan N (2013) MiR-139-5p is a regulator of metastatic pathways in breast cancer. RNA. doi:10.1261/rna.042143.113
11. Spaccarotella E, Pellegrino E, Ferracin M, Ferreri C, Cuccuru G, Liu C, Iqbal J, Cantarella D, Taulli R, Provero P, Di Cunto F, Medico E, Negrini M, Chan WC, Inghirami G, Piva R (2013) STAT3-mediated activation of microRNA cluster 17–92 promotes proliferation and survival of ALK positive anaplastic large cell lymphoma. Haematologica. doi:10.3324/haematol.2013.088286
12. Tokarz P, Blasiak J (2012) The role of microRNA in metastatic colorectal cancer and its significance in cancer prognosis and treatment. Acta Biochim Pol 59:467–474
13. Garofalo M, Jeon YJ, Nuovo GJ, Middleton J, Secchiero P, Joshi P, Alder H, Nazaryan N, Di Leva G, Romano G, Crawford M, Nana-Sinkam P, Croce CM (2013) MiR-34a/c-dependent PDGFR-alpha/beta downregulation inhibits tumorigenesis and enhances TRAIL-induced apoptosis in lung cancer. PLoS One 8:e67581. doi:10.1371/journal.pone.0067581
14. Feliciano A, Castellvi J, Artero-Castro A, Leal JA, Romagosa C, Hernandez-Losa J, Peg V, Fabra A, Vidal F, Kondoh H, Ramon YCS, Lleonart ME (2013) MiR-125b acts as a tumor suppressor in breast tumorigenesis via Its novel direct targets ENPEP, CK2-alpha, CCNJ, and MEGF9. PLoS One 8:e76247. doi:10.1371/journal.pone.0076247
15. Kishikawa T, Otsuka M, Yoshikawa T, Ohno M, Takata A, Shibata C, Kondo Y, Akanuma M, Yoshida H, Koike K (2013) Regulation of the expression of the liver cancer susceptibility gene MICA by microRNAs. Sci Rep 3:2739. doi:10.1038/srep02739
16. Rao SA, Arimappamagan A, Pandey P, Santosh V, Hegde AS, Chandramouli BA, Somasundaram K (2013) MiR-219-5p inhibits receptor tyrosine kinase pathway by targeting EGFR in glioblastoma. PLoS One 8:e63164. doi:10.1371/journal.pone.0063164
17. Mosakhani N, Lahti L, Borze I, Karjalainen-Lindsberg ML, Sundstrom J, Ristamaki R, Osterlund P, Knuutila S, Sarhadi VK (2012) MicroRNA profiling predicts survival in anti-EGFR treated chemorefractory metastatic colorectal cancer patients with wild-type KRAS and BRAF. Cancer Genet 205:545–551. doi:10.1016/j.cancergen.2012.08.003
18. Della Vittoria Scarpati G, Falcetta F, Carlomagno C, Ubezio P, Marchini S, De Stefano A, Singh VK, D'Incalci M, De Placido S, Pepe S (2012) A specific miRNA signature correlates with complete pathological response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 83:1113–1119. doi:10.1016/j.ijrobp.2011.09.030
19. Nymark P, Guled M, Borze I, Faisal A, Lahti L, Salmenkivi K, Kettunen E, Anttila S, Knuutila S (2011) Integrative analysis of microRNA, mRNA and aCGH data reveals asbestos- and histology-related changes in lung cancer. Genes Chromosom Cancer 50:585–597. doi:10.1002/gcc.20880
20. Park JK, Park SH, So K, Bae IH, Yoo YD, Um HD (2010) ICAM-3 enhances the migratory and invasive potential of human non-small cell lung cancer cells by inducing MMP-2 and MMP-9 via Akt and CREB. Int J Oncol 36:181–192
21. Zhang M, Xu JJ, Zhou RL, Zhang QY (2013) cAMP responsive element binding protein-1 is a transcription factor of lysosomal-associated protein transmembrane-4 beta in human breast cancer cells. PLoS One 8:e57520. doi:10.1371/journal.pone.0057520
22. Pigazzi M, Manara E, Baron E, Basso G (2009) miR-34b targets cyclic AMP-responsive element binding protein in acute myeloid leukemia. Cancer Res 69:2471–2478. doi:10.1158/0008-5472.CAN-08-3404
23. Perry C, Sklan EH, Soreq H (2004) CREB regulates AChE-R-induced proliferation of human glioblastoma cells. Neoplasia 6:279–286. doi:10.1593/neo.3424
24. Sakamoto KM, Frank DA (2009) CREB in the pathophysiology of cancer: implications for targeting transcription factors for cancer therapy. Clin Cancer Res 15:2583–2587. doi:10.1158/1078-0432.CCR-08-1137
25. Shukla A, Bosenberg MW, MacPherson MB, Butnor KJ, Heintz NH, Pass HI, Carbone M, Testa JR, Mossman BT (2009) Activated cAMP response element binding protein is overexpressed in human mesotheliomas and inhibits apoptosis. Am J Pathol 175:2197–2206. doi:10.2353/ajpath.2009.090400
26. Peng B, Hu S, Jun Q, Luo D, Zhang X, Zhao H, Li D (2013) MicroRNA-200b targets CREB1 and suppresses cell growth in human malignant glioma. Mol Cell Biochem 379:51–58. doi:10.1007/s11010-013-1626-6
27. Van Themsche C, Mathieu I, Parent S, Asselin E (2007) Transforming growth factor-beta3 increases the invasiveness of endometrial carcinoma cells through phosphatidylinositol 3-kinase-dependent up-regulation of X-linked inhibitor of apoptosis and protein kinase c-dependent induction of matrix metalloproteinase-9. J Biol Chem 282:4794–4802. doi:10.1074/jbc.M608497200
28. Liu G, Ding W, Neiman J, Mulder KM (2006) Requirement of Smad3 and CREB-1 in mediating transforming growth factor-beta (TGF beta) induction of TGF beta 3 secretion. J Biol Chem 281:29479–29490. doi:10.1074/jbc.M600579200
29. Perianayagam MC, Madias NE, Pereira BJ, Jaber BL (2006) CREB transcription factor modulates Bcl2 transcription in response to C5a in HL-60-derived neutrophils. Eur J Clin Invest 36:353–361. doi:10.1111/j.1365-2362.2006.01637.x
30. Mendell JT (2005) MicroRNAs: critical regulators of development, cellular physiology and malignancy. Cell Cycle 4:1179–1184
31. Hwang HW, Mendell JT (2007) MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 96(Suppl):R40–R44
32. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR (2005) MicroRNA expression profiles classify human cancers. Nature 435:834–838. doi:10.1038/nature03702
33. Xi JJ (2013) MicroRNAs in cancer. Cancer Treat Res 158:119–137. doi:10.1007/978-3-642-31659-3_5
34. Mayr B, Montminy M (2001) Transcriptional regulation by the phosphorylation-dependent factor CREB. Nat Rev Mol Cell Biol 2:599–609. doi:10.1038/35085068
35. Shaywitz AJ, Greenberg ME (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular

signals. Annu Rev Biochem 68:821–861. doi:10.1146/annurev.biochem.68.1.821

36. Majumder S, Varadharaj S, Ghoshal K, Monani U, Burghes AH, Jacob ST (2004) Identification of a novel cyclic AMP-response element (CRE-II) and the role of CREB-1 in the cAMP-induced expression of the survival motor neuron (SMN) gene. J Biol Chem 279:14803–14811. doi:10.1074/jbc.M308225200
37. Alvarez-Lopez C, Cernuda-Cernuda R, Paniagua MA, Alvarez-Viejo M, Fernandez-Lopez A, Garcia-Fernandez JM (2004) The transcription factor CREB is phosphorylated in neurons of the piriform cortex of blind mice in response to illumination of the retina. Neurosci Lett 357:223–226. doi:10.1016/j.neulet.2003.12.099
38. Thway K, Fisher C (2012) Tumors with EWSR1-CREB1 and EWSR1-ATF1 fusions: the current status. Am J Surg Pathol 36:e1–e11. doi:10.1097/PAS.0b013e31825485c5
39. Tan X, Wang S, Zhu L, Wu C, Yin B, Zhao J, Yuan J, Qiang B, Peng X (2012) cAMP response element-binding protein promotes gliomagenesis by modulating the expression of oncogenic microRNA-23a. Proc Natl Acad Sci USA 109:15805–15810. doi:10.1073/pnas.1207787109