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ORIGINAL ARTICLE

MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a

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Abstract MicroRNAs are ~21nt single-stranded RNAs and function as regulators of gene expression. Previous studies have shown that microRNAs play crucial roles in tumorigenesis by targeting the mRNAs of oncogenes or tumor suppressors. Here we show that brain-enriched miR-128 is down-regulated in glioma tissues and cell lines when compared to normal brain tissues. Overexpression of miR-128 in glioma cells inhibited cell proliferation. A bioinformatics search revealed a conserved target site within the 3′ untranslated region (UTR) of E2F3a, a transcription factor that regulates cell cycle progression. The protein levels of E2F3a in gliomas and normal brain tissues were negatively correlated to the expression levels of miR-128 in these tissues. Overexpression of miR-128 suppressed a luciferase-reporter containing the E2F3a-3′UTR and reduced the level of E2F3a protein in T98G cells. Moreover, knocking down of E2F3a had similar effect as overexpression of miR-128, and overexpression of E2F3a can partly rescue the proliferation inhibition caused by miR-128. Taken together, our study demonstrates that miR-128 can inhibit proliferation of glioma cells through one of its targets, E2F3a.

Keywords miR-128 · E2F3a · Glioma · Proliferation

Introduction

MicroRNAs (miRNAs) are a class of endogenously expressed small noncoding RNAs which are usually 18–24 nucleotides long and regulate gene expression post-transcriptionally [1]. Hundreds of miRNAs have been found both in prokaryote and eukaryote, and many of them have been shown to have function in cell proliferation [2, 3], differentiation [4, 5], and apoptosis [6, 7]. Recent studies have demonstrated an important role for miRNAs in tumorigenesis. They function as tumor suppressors or oncogenes by regulating the expression of their target genes [8]. Interestingly, miR-128 was found to be down-regulated in gliomas [9]. However, the molecular details of miR-128's involvement in glioma development remain unclear.

The members of E2F family are essential for cell proliferation and apoptosis, e.g., E2F1–3 can promote S-phase entry [10]. Despite their functional redundancy, E2F3a has unique roles in cell division [11], and it has been reported that the level of E2F3a has a crucial role in the development of human bladder and prostate cancers [12]. More recently, several miRNAs such as miR-34a and miR-20a have been reported to negatively regulate E2F3a or have an auto-regulatory feed-back loop with E2F3a; disruption of this balance plays an important role in tumorigenesis [13–15]. In this study, we demonstrate that

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miR-128 is down-regulated in glioma tissues and cell lines. Moreover, both bioinformatics prediction and experimental results indicate that miR-128 can target E2F3a and inhibit cell proliferation in glioma.

Materials and methods

Human tissue samples All human normal brain and glioma tissue samples were obtained from the Department of Neurosurgery, Beijing Tiantan Hospital. All samples were classified according to the third edition on the histological typing of tumors of the nervous system published by WHO in 2000. Informed consents for the use of their samples were obtained from all patients before surgery.

Cell lines and culture conditions Human glioma cell lines T98G, A172, and U87 were purchased from American Type Culture Collection (ATCC) and cultured according to the guidelines recommended by ATCC. U251 cell line (from Cell Center of Peking Union Medical College) and 293ET cell line (kindly provided by Dr. Chengyu Jiang, Peking Union Medical College) were cultured in minimum essential medium and Iscove's Modified Dulbecco's Medium supplemented with 10% fetal bovine serum, respectively. All cells were maintained at 37°C under an atmosphere of 5% CO_2–95% air.

RNA extraction and real-time quantification of miRNAs by stem-loop reverse transcriptase polymerase chain reaction Total RNA was extracted from cells with the Trizol Reagent (Invitrogen) according to the manufacturer's instructions. Stem-loop reverse transcriptase polymerase chain reaction (RT-PCR) for mature miR-128 was performed as described previously [16]. The sequence of the stem-loop reverse transcription (RT) primer of miR-128 was 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAGA-3′. The sequence of the stem-loop RT primer for U6 snRNA was 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAATA-3′. Real-time PCR was performed using SYBR-green-containing PCR kit (Takara) according to the manufacture's instructions, and RNA input was normalized to the level of human U6 snRNA. The sequences of the forward and reverse primers for human miR-128 were 5′-CGCGCTCACAGTGAACCG-3′ and 5′-GTGCAGGGTCCGAGGT-3′, respectively. The sequences of the forward and reverse primers for human U6 snRNA were 5′-GCGCGTCGTGAAGCGTTC-3′ and 5′-GTGCAGGGTCCGAGGT-3′, respectively.

MiRNA and siRNA transfection Mature miR-128 and siRNA were transfected into T98G using lipofectamine 2000 (Invitrogen), with miRNA at a final concentration of 100 nM and siRNA at a final concentration of 200 nM. One microgram or 0.25 μg of plasmids were used per well in 24-well plate or 96-well plate when co-transfection was performed.

Databases and bioinformatics analysis The miRNA sequence was analyzed using miRBase (http://microrna.sanger.ac.uk/sequences/), and the target gene information of miR-128 was available at Human microRNA targets (http://www.microrna.org/mammalian/index.html).

Construction of E2F3a 3′untranslated region reporter plasmid and miRNA overexpression plasmid The E2F3a 3′ untranslated region (UTR; accession number: NM 001949) was amplified from the genomic DNA of human normal brain tissues using the following primers: forward 5′-AAACAATGCCAGGGTGTCTC-3′, reverse 5′-TAGCCATTTCGTGTGTGAGC-3′. For its mutagenesis, the sequence complementary to the binding site of miR-128 in its 3′UTR (CACTGTG) was replaced by ACGACAC. The wild-type and mutated 3′UTRs of E2F3a were cloned downstream of luciferase using the Xho I and Xba I site of the pcDNA3.1-luc vector. For E2F3a expression constructs, mRNA was isolated from adult human normal brain tissue and reversed transcribed, and the full-length cDNA was first amplified using the following primers: forward 5′-CGAGAGATGAGAAAGGGAATCCAG-3′, reverse 5′-GAGAGTTCACACGAAGCATAATCAAC-3′, and then cloned into the pCDEF-FN and pCDEF-MN vectors using SfiI site (Shanghai Genomics Inc.). For miR-128 expression construct, a 337-base pair-genomic sequence spanning mature miR-128 was cloned into pcDNA3.1 with the following primers: forward 5′-GATTTTAGGTTTACAAAGCCCTAGCTGT-3′, reverse 5′-CTAATCCCTATTTCTGAGTATGATGCATGA-3′. The sequence of mature miR-128 (UCACAGUGAACCGGUCUCUUUU) and the control sequence were provided by Genechem.

Luciferase assay Twenty-four hours before transfection, 293ET cells were plated in a 24-well plate. Plasmids of empty pcDNA3.1-luc/miR-128-pcDNA3.1-luc (1 μg), WT/mutant 3′UTR (0. μg), and pRL-TK (0.1 μg) were transfected into 293ET cells using the Vigorous transfection reagent [17]. Luciferase assays were performed 48 h after transfection using the Dual Luciferase Reporter Assay System (Promega, USA).

Dimethyl thiazolyl diphenyl tetrazolium (MTT) assay To evaluate the effect of miR-128 and E2F3a on the propagation of T98G cells, cells were seeded at a density of 3,000 cells per well containing 100 μl medium in 96-well plate for 24 h and then transfected with miR-128/siRNA/miRNA and plasmids as described above. Every 24 h post transfection, 10 μl of dimethyl thiazolyl diphenyl

tetrazolium (MTT) reagent was added to a well, and the incubation continued for 4 h. Then medium was removed, and 100 μl dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan by pipetting up and down several times; the absorbance, with a test wavelength of 570 nm and a reference wavelength of 630 nm, was measured to obtain sample signal. Wells without cells (DMSO alone) were used as blanks.

BrdU incorporation Cells were incubated with culture media containing BrdU at a dilution of 1:10,000 (10 μM) for 30 min before being fixed in 4% paraform. The fixed cells were incubated in 0.2% TritonX-100 for 10 min at room temperature, 1 M HCl for 10 min on ice, 2 M HCl at 37°C, 0.1 M $Na_2B_4O_7$ for 12 min at room temperature before being incubated with anti-BrdU antibody (Cat. Sc-879, Santa Cruz, USA) diluted 1:100 in blocking buffer] 3% bovine serum albumin/phosphate-buffered saline (PBS)] for 3 h at room temperature. The cells were subsequently washed in PBS and incubated with Fluorescein (FITC)-conjugated Affinipure Goat-Anti Mouse IgG H+L (Zhongshan, China) at 1:50 for 2 h at room temperature. Then 1 μg/ml DAPI was applied for 2 min to stain the cells. Images were captured on a Nikon microscope (TE2000S, NIKON, Japan) at a total magnification of ×200 and processed using image acquisition Nis-Elements BR (NIKON, Japan). Subsequent graphic processing was done using the Photoshop CS2 software

Protein extraction and western blot Total protein was extracted with lysis buffer (150 mM NaCl, 1% NP40 and 50 mM Tris–HCl, pH 8.0) supplemented with protease inhibitors (2 μg/ml leupeptin, 2 μg/ml pepstain, 2 μg/ml aprotinin, and 2 μg/ml PMSF). After being lysed on ice for 30 min, the lysate was centrifuged at 12,000 rpm for 20 min, and the supernatant was collected for experiments. Protein concentration was determined using the Bradford method. Lysate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and the gel was transferred onto nitrocellulose membrane. The protein was probed with rabbit anti-human E2F3a (Cat. sc-879, Santa Cruz, USA) or mouse anti-human β-actin antibody (Cat. A5441, Sigma, USA).

Immunohistochemistry Immunohistochemical analyses of E2F3a were done using formalin-fixed, frozen section specimens of glioma and normal brain tissues from 19 patients. The sections were soaked in antigen retrieval citra solution (1.8 mM citric acid, 8.2 mM natrium citricum, pH 6.0) at 95°C for 10 min and then incubated with anti-E2F3a antibody at 4°C for 16 h. Staining was done using a diaminobenzidine staining kit (Zhongshan, China). Three independent pathologists analyzed the results.

Results

MiR-128 is down-regulated in human glioma tissues and cell lines

To confirm the expression level of miR-128 in glioma and normal brain tissues as reported previously [9], total RNA was extracted from nine primary glioma samples, which contain three grade II, three grade III, and three grade IV glioma tissues, four glioma cell lines and two normal brain tissues, and then real-time PCR was performed to analyze the expression profile. The results showed that expression of miR-128 was down-regulated in both glioma tissues and cell lines compared to normal brain tissues (Student's *t* test, $p<0.01$; Fig. 1). However, we did not find any correlation between the down-regulation of miR-128 and chromatin modification status. Treatment of 5-aza-2′-deoxycytidine (5-Aza-CdR) and/or the histone deacetylase inhibitor trichostatin A (TSA) did not affect the expression level of miR-128 in T98G cells (data not shown).

Overexpression of miR-128 inhibits cellular proliferation in T98G cells

To examine the role of miR-128 in proliferation, miR-128 was overexpressed in T98G cells. Real-time RT-PCR

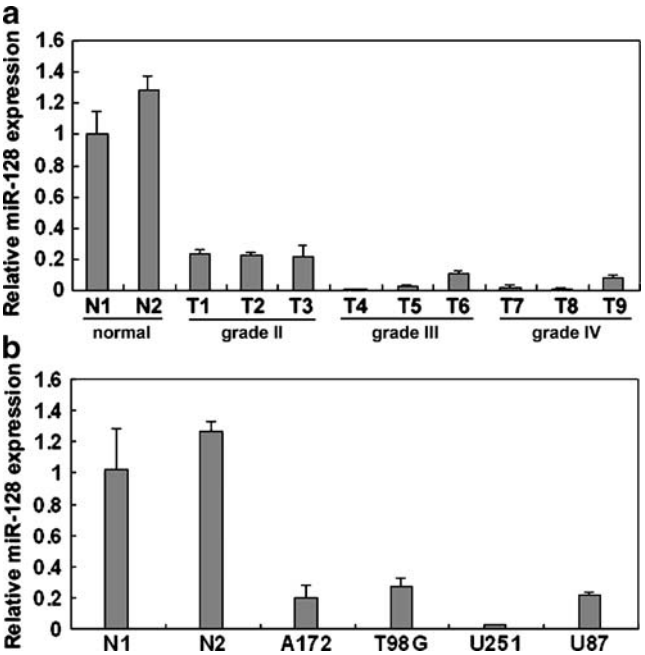

Fig. 1 MiR-128 was down-regulated in glioma tissues and cell lines compared to normal brain tissues. **a** Real-time PCR analysis of miR-128 in two normal brain tissues (*N1*, *N2*) and nine glioma tissues (*T1–T3 grade II*, *T4–T6 grade III*, and *T7–T9 grade IV*). **b** Real-time PCR analysis of miR-128 in two normal brain tissues (*N1*, *N2*) and four glioma cell lines (*A172*, *T98G*, *U251*, *U87*). RNA input was normalized by human U6 snRNA

analysis showed that the transfection was effective (Fig. 2a). T98G cells transfected with miR-128 had a reduction in metabolically active cells compared to those transfected with scrambled negative control. The reduction was detected after 2 days of transfection, and it became more apparent on day 4 by MTT assay (Student's t test, $p<0.05$; Fig. 2b).

To address whether the decrease in cell number following overexpression of miR-128 was due to anti-proliferative or apoptotic response, BrdU incorporation was monitored in miR-128-overexpressing cells. In contrast to the negative control, the percentage of BrdU-incorporated cells was reduced by nearly 15% in T98G cells after transfection with miR-128 for 48 h (Student's t test, $p<0.05$; Fig. 2c, d). But the Hoechst staining showed that cells were not undergoing apoptosis (data not shown).

MiR-128 negatively targets E2F3a in 3′UTR

As predicted by bioinformatics, there were more than 1,000 targets of miR-128. In order to identify its real target genes, the potential expression levels of eight putative target genes that are thought to be involved in cells proliferation or neural differentiation were measured using a luciferase reporter assay. These include E2F3, TCF20, LTBP-1, JAG1, DCX, Sox11, BMI1, and CrkL. Each one of these genes has a near-perfect binding site for miR-128 in their 3′ UTRs, as the 3′UTR of E2F3a shown in Fig 3a. The 3′ UTRs of the above genes were cloned downstream of a luciferase reporter, and luciferase activites were measured in 293ET cells. However, among these predicted target genes, no significant activities were observed except for E2F3a. Cells transfected with miR-128 showed a reduction of 40% in luciferase activity compared to those transfected with empty vectors (Student's t test, $p<0.05$). When the hypothesized miR-128 binding site in the 3′UTR of E2F3a mRNA was mutated, luciferase activity was restored close to the control level (Fig. 3b). Overexpression of miR-128 in T98G cells resulted in down-regulation of E2F3a at post-transcriptional level as assessed by western blot (Fig. 3c).

To investigate whether miR-128 affects the E2F3a expression in vivo, the protein level of E2F3a in glioma and normal brain tissues were determined by western blot (Fig. 3d) and immunohistochemistry (Fig. 4). Western blot and immunohistochemistry assay revealed an inverse

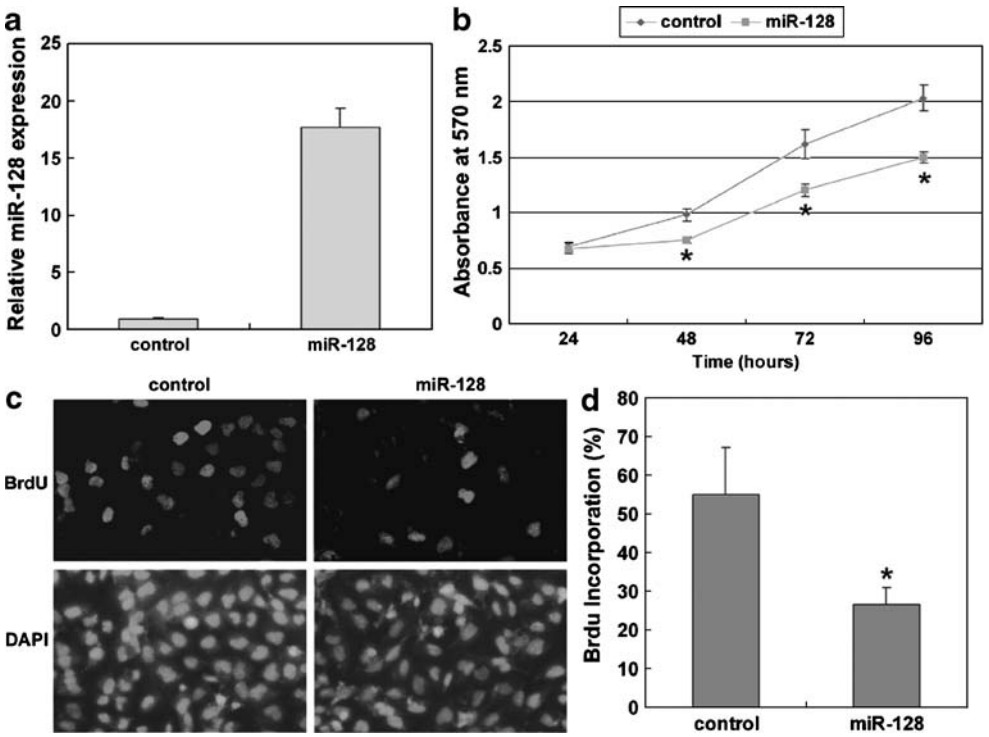

Fig. 2 Overexpression of miR-128 inhibited the propagation of T98G cells. **a** Real-time PCR analysis of miR-128 in T98G cells after transfection with mature miR-128 or negative control for 48 h. Mature miR-128 (UCACAGUGAACCGGUCUCUUUU, 100 nM) and negative control (ACGUGACACGUUCGGAGAA, 100 nM) were transfected into T98G using Lipofectamine 2000 (Invitrogen). **b** MTT assay of T98G cells after treatment with negative control or miR-128 as above. The viable cell number was determined as the value of the absorbance at 570 nm with a reference wavelength of 630 nm. Values represent means ± standard deviation (SD) for four wells. *Asterisk* $p\leq0.05$, all compared with control. **c** BrdU incorporation assessed by fluorescent immunohistochemistry after transfection with control or miR-128 for 48 h. The cells were incubated with culture media containing BrdU at a dilution of 1:10,000 (10 μM) for 30 min before fixing in 4% paraform; anti-BrdU dilution was 1:100. **d** Cell proliferation rate as revealed by BrdU incorporation, experiments were repeated at least three times, and more than three visual fields were counted each time. *Columns* Mean of three independent experiments. *Asterisk* $p\leq0.05$, compared with control

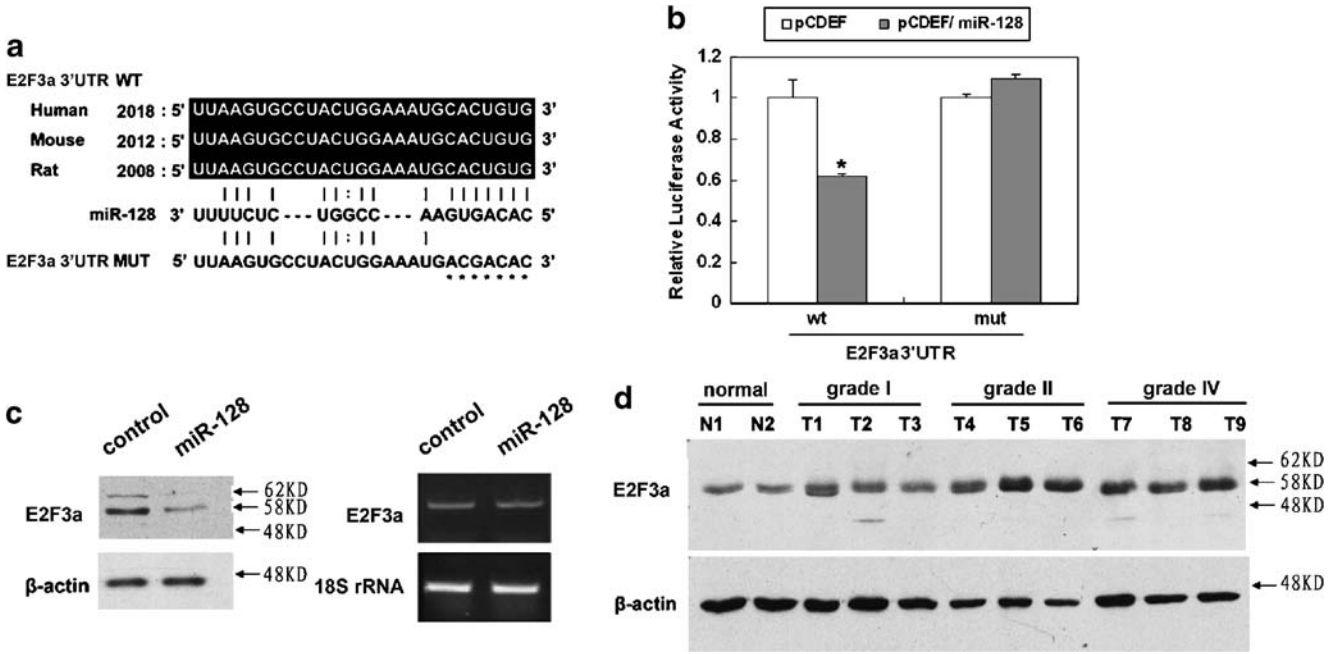

Fig. 3 E2F3a is a target of miR-128. **a** The target site of miR-128 in the 3′-UTR of E2F3a is completed conserved in human, mouse, and rat as predicted by Miranda software (http://www.microrna.org/mammalian/index.html). The mutant sequence is identical to the wild-type sequence except for mutations at the 3′ end of target site showed by *asterisk*. **b** Luciferase assays in 293ET cells. The reporter constructs, including wild-type or mutant sequence, were cotransfected with miR-128. Relative luciferase activities were normalized with the Renilla luciferase activities. Construction and transfection methods were previously reported [17]. Values are expressed as percentages of relative luciferase activity of pcDNA3.1-luc plasmid. *Columns* Mean of three independent experiments. *Asterisk* $p \leq 0.05$, compared with empty plasmids. **c** Western blot and RT-PCR. T98G cells were transfected with negative control or miR-128 for 48 h as above. Cell lysates were immunoblotted for E2F3a (*left*), and RT-PCR was to detect the mRNA level of E2F3a (*right*). β-actin was used as a loading control (1:3,000) in western blot, while 18s-rRNA was used as a loading control in RT-PCR. In western blot, 1:1,000 of anti-human E2F3a was used (the raw data of the western blots are shown in the figures found in the Electronic supplementary material). **d** Protein levels of E2F3a in two normal brain tissues (*N1*, *N4*) and nine glioma tissues (*T1–T3 grade II*, *T4–T6 grade III*, and *T7–T9 grade IV*). β-actin was used as a loading control

correlation between miR-128 and E2F3a protein levels. It showed that, in grade III and grade IV glioma tissues, the down-regulation of miR-128 was more drastic than that in grade II glioma tissues, while the protein levels of E2F3a in grade III and grade IV glioma tissues were higher than those in grade II glioma tissues. In immunohistochemistry staining, higher percentage and level of nuclear E2F3a were found in glioma tissues than in normal brain tissues. No

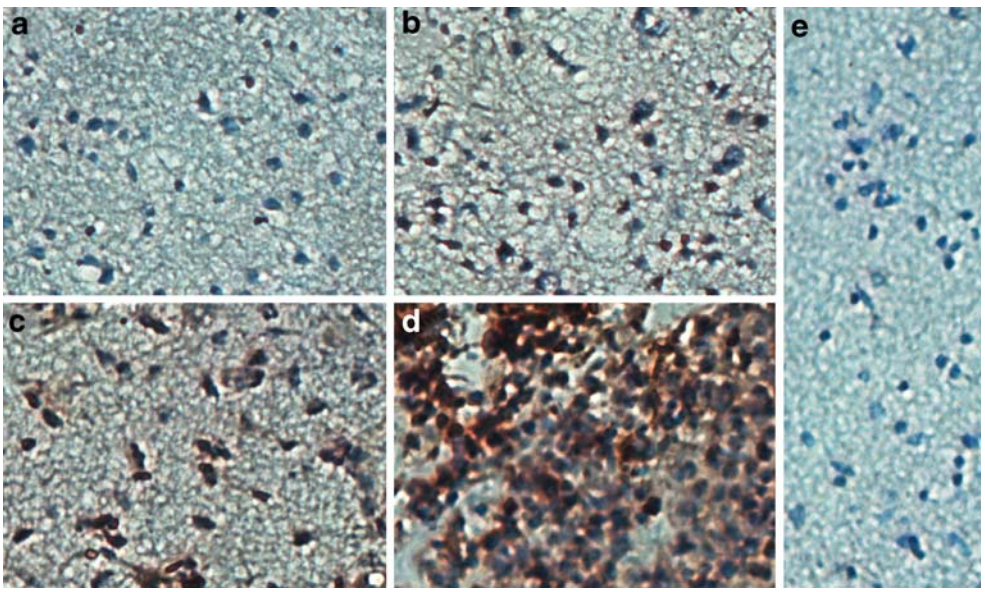

Fig. 4 Immunohistochemical staining of E2F3a protein in glioma and normal brain tissues Immunohistochemical analyses were done using anti-human E2F3a antibodies. **a** Normal brain tissue. **b** Grade II glioma. **c** Grade III glioma. **d** Grade IV glioma. **e** There was no staining with normal rabbit IgG. Original magnification, ×200

staining was observed using control rabbit IgG. There was no correlation between the level of miR128 and the mRNA levels of E2F3a in glioma tissues and cell lines (data not shown).

Knockdown of E2F3a in T98G cells resulted in similar phenotype as overexpression of miR-128

Although E2F3a has been widely reported to be involved in tumorigenesis, there is no report on its role in gliomagenesis. To investigate the role of E2F3a in glioma development, we synthesized a small interfering RNA (siRNA) which can specifically target E2F3a as previously reported [12]. After transfection for 48 h, siRNA-mediated knockdown of E2F3a reduced its protein level by least 90% compared to that in control samples (Fig. 5a). Both MTT and BrdU incorporation assay showed that the proliferation rate and the percentage of S-phase cells were reduced after E2F3asi treatment (Student's t test, $p<0.05$; Fig. 5b–d), which was similar to the result from overexpressing miR-128. This supports the hypothesis that miR128 inhibits proliferation of glioma cells by negatively regulating the expression of E2F3a at protein level.

Overexpression of E2F3a rescued the effect of miR-128 overexpression

To further test the hypothesis that the phenotype as a result of miR-128 overexpression was due to the down-regulation of E2F3a, miR-128 and E2F3a were co-transfected into T98G cells. As shown in Fig. 6b,c, 48 h after miR-128 transfection, there were fewer S-phase cells than those in the control experiment; however, if the cells were co-transfected with miR-128 and E2F3a, the percentage of BrdU incorporation rose close to the control level (Student's t test, $p<0.05$). The variations in transfection efficiency were confirmed by western blot to be insignificant (Fig. 6a). It must be pointed out that, although the percentage of S-phase cells increased after being co-transfected with miR-128 and E2F3a, the active cell number did not increase as determined by MTT assay because more cell death was also observed (data not shown).

Discussion

It is known that miRNAs can function as tumor suppressors and oncogenes, and they are referred to as "oncomirs" [8]. MiRNA abnormalities are thought to play broad roles in cancer genesis. A more direct link between miRNA function and oncogenesis is supported by studies examining the expression of miRNAs in clinical samples [18, 19]. The profiling of miRNA expression showed that most of them are down-regulated in tumors compared to normal tissues [20], like let-7 in lung cancers [21] and miR-127 in human bladder cancers [22]. MiR-128 was found having lower expression level in glioblastoma. We detected the expression of miR-128 in tumor samples as well as in glioma cell lines

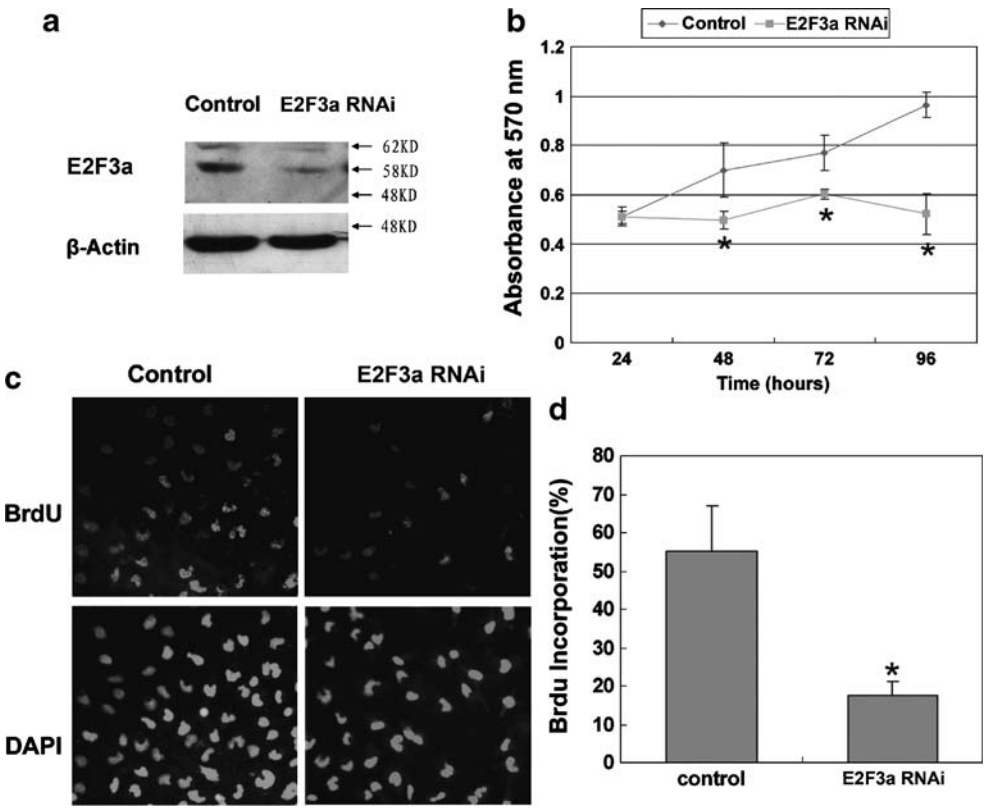

Fig. 5 SiRNA-mediated knockdown of E2F3a resulted in similar phenotypes as overexpressing miR-128 in T98G cells. **a** Western blot of E2F3a expression in T98G cells following treatment with E2F3a siRNA or negative controls. SiRNA (AACCTA GAAGGACCGTTTGTG, 50 nM) and control (ACGUGA CACGUUCGGAGAA, 50 nM) were transfected into T98G cell for 48 h using Lipofectamine 2000. **b** MTT assay of T98G cells after transfection with negative control or E2F3aSi as above. Values represent means ± SD for four wells. *Asterisk* $p\leq0.05$, all compared with control. **c** BrdU incorporation assessed by fluorescent immunohistochemistry after transfection with negative control or E2F3aSi for 48 h as above. **d** Proliferation rate counted from **c**. *Bars* indicate the standard errors of the mean from three repeated experiments. *Asterisk* $p\leq0.05$, compared with control

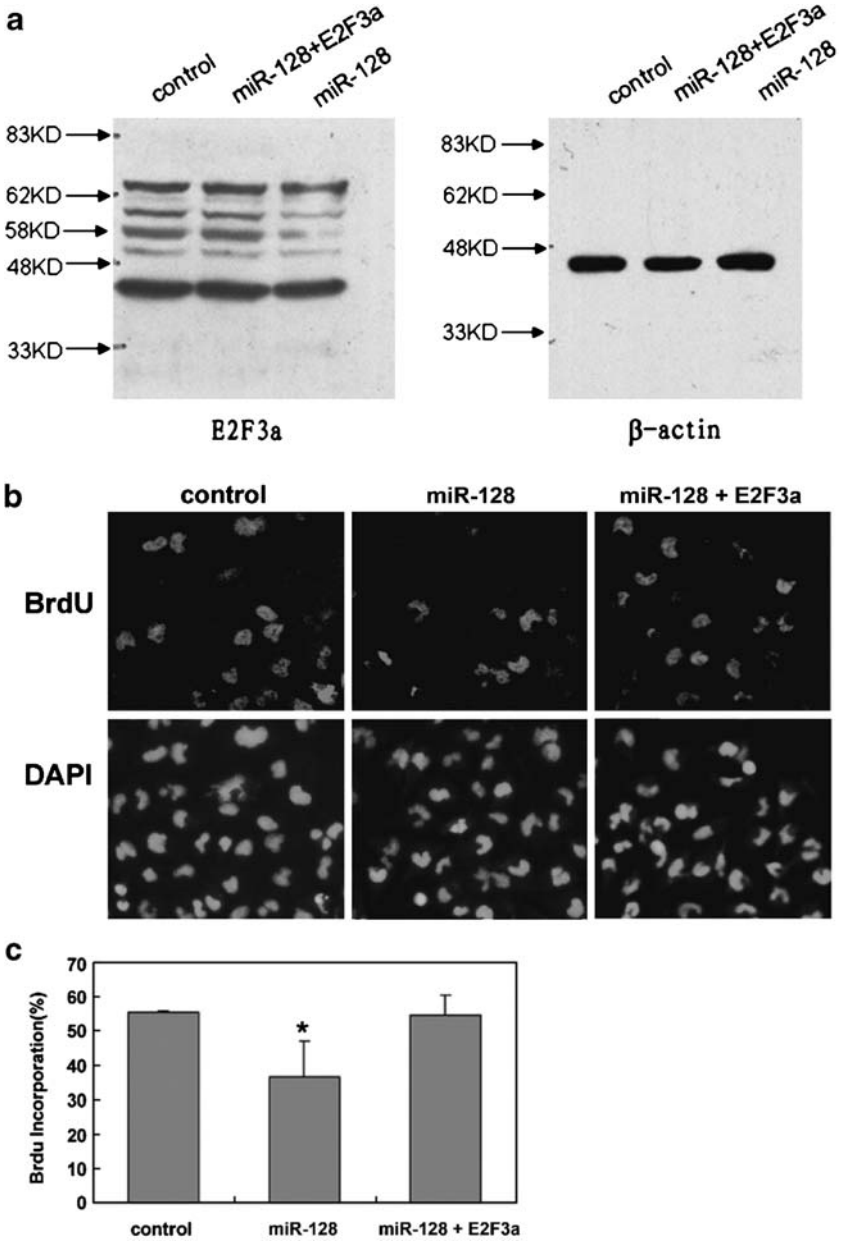

Fig. 6 E2F3a rescued the phenotype induced by miR-128. **a** Western blot of E2F3a expression in T98G cells following transfection with control, miR-128, or miR-128 and E2F3a for 48 h. When co-transfected, 100 μM miR-128 and 4 μg E2F3a-pCDEF-MN per well were used in a six-well-plate. **b** BrdU incorporation at 48 h posttransfection. E2F3a resulted in higher BrdU incorporation. When co-transfected, 100 μM miR-128 and 0.8 μg E2F3a-pCDEF-MN per well were used in a 24-well-plate. **c** Proliferation rate counted from **a**. *Bars* indicate the standard errors of the mean from three repeated experiments. *Asterisk* $p \leq 0.05$, compared with control

and found that its down-regulation compared to levels in normal brain tissues exists not only in glioblastoma but also in grade II and grade III gliomas.

The regulation of miRNA expression is not yet fully understood. So far, there are three major regulatory mechanisms. Some miRNAs are located at sites of the genome that are frequently deleted or rearranged in cancers [23], as miR-34a in neuroblastoma cells [24], while some of them are epigenetically regulated. Yoshimasa Saito et al. found that 17 out of 313 human miRNAs were up-regulated more than 3-fold by simultaneous treatment of T24 cells with chromatin-modifying drugs 5-aza-20-deoxycytidine and 4-phenylbutyric acid [22]. Besides these two regulation mechanisms, it has also been demonstrated that miRNA expression can be regulated by signal transduction pathway, such as Stat3-mediated induction of miR21 in interleukin-6-dependent survival of multiple myeloma cells [25]. In our experiment, we checked only the possibility of epigenetic regulation on miR-128; however, no induction of miR-128 was found after the treatment of T98G cells with 5-Aza-CdR or/and TSA. It has been reported that there exists a negative feed-back loop between E2Fs and the miR-17–92 cluster [13]; whether there also exists a regulatory feed-back loop between E2F3 and miR-128 or other regulation mechanism such as the one demonstrated for miR-21 expression needs to be further investigated.

Identification of miRNA target genes is still a great challenge. Computational algorithms showed more than

1,000 targets of miR-128. Considering that miR-128 overexpression can inhibit proliferation of glioma cells, several predicted target genes associated with tumogenesis or cell proliferation were subjected to a luciferase reported assay. The results showed that, among these chosen genes, only E2F3a was negatively regulated by miR-128. More evidences showed E2F3a is expressed at higher levels in glioma than those in normal brain tissues. There is, however, a limitation within our data interpretation regarding the correlated expression of miR-128 and E2F3a since we used glioma and normal brain tissues to detect their expression levels. Nevertheless, brain is a mixture of different cell types; detection of miR-128 and E2F3a by other methods may be needed, such as isolating specific glia cell type from tissues by laser capture microdissection technique.

E2F3a has been found to control cell cycle progression. Previous studies have showed that the E2F3a gene was amplified and overexpressed and that E2F3a modulated cellular proliferation rate in human bladder and prostate cancer cells [12].Thus, we posit that the inhibition of proliferation by miR-128 might in part be mediated through negatively regulating E2F3a. E2F3b has a same 3′UTR as that of E2F3a; whether E2F3b has a role in gliomagenesis needs to be further examined.

Our results showed that overexpressed miR-128 only partly inhibited the cell proliferation, and this inhibition is to a lesser extent than that induced by knocking down E2F3a, suggesting that there may be miRNAs other than miR-128 that also participate in the development of glioma. To date, several miRNAs have been reported to regulate E2F3a, such as miR-17-92, miR-34a, and miR-210 [26]. Whether these miRNAs are involved in the formation of glioma remains an open question.

One interesting point we observed in this study is that the down-regulation of miR-128 and the up-regulation of E2F3a in glioma are both more significant in grade III and grade IV than those in grade II. Whether miR-128 and E2F3a can be used as molecular markers for the classification of grade II and III glioma requires more evidence.

The nucleotide sequences around the target site of miR-128 are highly conserved among different species (Fig. 3a); therefore, regulation by miR-128 may also occur in species other than human. This implies that miR-128 may exert different regulatory activities in different species and in different biological events such as tumorigenesis or neural differentiation. Since miR-128 is brain-enriched, we wonder whether it has a role in neuronal differentiation. Several genes known to be involved in neuronal differentiation such as Sox11 and BMI-1 were selected and tested for expression level using luciferase as a reporter gene in 293 ET cells. However, the measured luciferase activities were quite low. We reasoned that these luciferase assays might need to be performed in cells of neuronal origin.

In conclusion, the results presented here suggest that miR-128 can inhibit the proliferation of glioma cells through negatively regulating one of its targets, E2F3a, which is highly expressed in glioma and important for cell cycle progession.

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