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Original Article

MiR-155 inhibits glioma tumorigenesis through downregulation of FBXW7 expression

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Abstract: Background: MiR-155 is highly expressed in many malignant tumors with poor prognosis, however its role in glioma is largely unknown. In this study, we aims at investigating the clinical significance of miR-155, and further exploring the targeted regulation of F-box and WD repeat domain containing 7 (FBXW7) mRNA. Material/Methods: Forty-five serum samples, 66 tissues and paired adjacent noncancerous tissues from primary glioma patients were collected and used to detect the expression of miR-155 and FBXW7 mRNA by RT-qPCR. Protein expression of glioma cells were detected by western blot and the cell proliferation and apoptosis was also evaluated. Results: The expression of miR-155 was significantly increased in both serum and primary tissues from primary glioma patients, and enhanced miR-155 level was associated with tumor size, WHO grade and Karnofsky Performance Status (KPS). The Cox regression multivariate analysis showed that miR-155 was an independent prognostic factor of over survival for glioma patients. The gain and loss-function experiments revealed that miR-155 promoted cell proliferation and inhibited apoptosis. Furthermore, FBXW7 was identified as the direct target of miR-155. FBXW7 is a tumor suppressor gene through directly suppressing glioma cell growth and promoting apoptosis. Moreover, FBXW7 significantly abrogated the miR-155-induced cell proliferation and apoptosis effects. Conclusions: High level of miR-155 expression was correlated with enhanced malignant potential and poor prognosis of glioma patients. Furthermore, miR-155 could promote cell proliferation and suppress apoptosis through directly suppressing FBXW7 expression. Thus, miR-155 may be a novel prognostic biomarker and therapeutic target in glioma patients.

Keywords: miRNA-155, FBXW7, glioma, cell proliferation, apoptosis

Introduction

Glioma is one of the most common types of primary brain tumors in adults, and represents one of the most aggressive and lethal human cancer types [1]. Despite some advances in early detection, most of the patients are at advanced stages at the time of diagnosis, and the prognosis of these patients still remains poor [2]. There have been advancements in molecular mechanisms for the treatment of glioma, but the etiology of the disease is largely unknown [3]. Hence, it is crucial to identify the critical carcinogenic pathways and identify new and effective diagnostic and prognostic targets for this devastating disease.

MicroRNAs (miRNAs) are a class of short, endogenous, single-stranded RNAs that regulate gene expression. The expression of miRNAs is highly tissue and cell type specific, so is the functional significance of an expressed miRNA [4, 5]. MiRNAs are reported to play an important role in the pathogenesis of human cancers with disordered genome function [6, 7]. There is increasing evidence that miRNAs are involved in crucial biologic processes, including development, differentiation, apoptosis, and proliferation. Globally deregulation of miRNAs has been observed in human cancers, suggesting that altered miRNA expression most likely contributes to tumorigenesis [8].

MicroRNA-155 (miR-155) locates in region chromosome 21q21.3, which is known as B cell integration cluster (BIC) [9]. MiR-155 potential targets include the tumor suppressor genes, SOCS1 and APC, and the kinase WEE1, which blocks the activity of Cdc2 and prevents entry into mitosis [10, 11]. It has been shown that a

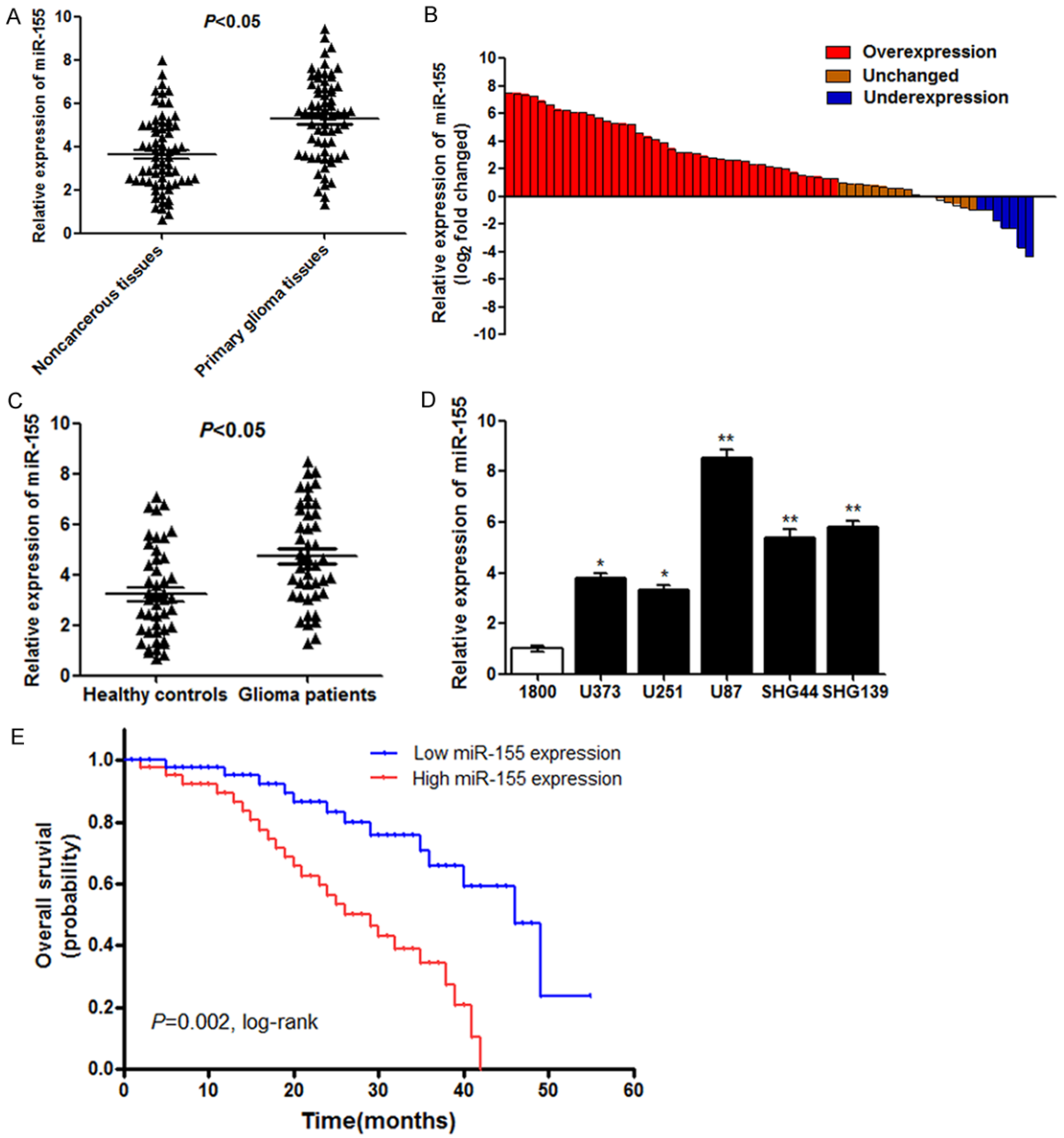


Figure 1. MiR-155 is up-regulated in glioma specimens and related to poor survival. A. RT-qPCR was used to detect the expression of miR-155 in 66 primary glioma tissues and noncancerous tissues. B. miR-155 expression level was analyzed by using RT-qPCR in 66 primary glioma tissues and expressed as $\log_2$ fold change (glioma/normal), and the $\log_2$ fold changes were presented as follows: >1, overexpression (41 cases); <1, underexpression (8 cases); the remainder were defined as unchanged (17 cases). C. RT-qPCR was used to determine the expression of miR-155 in 45 glioma serum samples and 30 healthy individuals. D. The expression level of miR-155 in five glioma cell lines and normal human astrocyte 1800 cell line was detected by using RT-qPCR method. E. Kaplan-Meier curves for overall survival were drawn according to miR-155 expression in 66 primary glioma tissues and were analyzed by using log-rank test. $^{*}P<0.05$, $^{**}P<0.01$.

high expression of miR-155 in glioma correlates with poor survival rates [12]. However, the specific role of miR-155 in glioma and the association with prognosis are largely unknown. F-box and WD repeat domain containing 7 (FBXW7) protein encodes a substrate adaptor for an E3 SCF ubiquitin ligase complex and negatively regulates the abundance of different oncoproteins [13]. Many of the FBXW7 targets are known oncogenes, thus these mutations

could promote tumorigenesis through multiple pathways. Based on oncology, clinical and basic research, the reduced expression or loss of function of FBXW7 has been frequently found in a variety of human cancers, with an overall mutation frequency of 6% [14]. Clinically, low expression of FBXW7 in human solid tumors such as glioma, colorectal cancer and gastric cancer is associated with a poor prognosis [15, 16].

Based on the prediction of target genes of miR-155, we hypothesized that there is a significant association between FBXW7 and miR-155. In this study, we aimed to investigate the potential role of miR-155 in glioma progression and further identify the direct target correlated with the malignant phenotype of glioma. Our results showed that miR-155 expression level was up-regulated in glioma and predicts poor survival. Furthermore, we identified and verified that FBXW7 is a functional target of miR-155, and miR-155 confers its effects through targeting FBXW7.

Material and methods

Clinical samples

Forty-five serum samples, 66 tissues and paired adjacent noncancerous tissues from primary glioma patients were collected at The Second Affiliated Hospital of Zhengzhou University between 2010 and 2014. Meanwhile, serum samples from 30 healthy volunteers were also collected in this study. All the patients were pathologically confirmed and the tissues were collected immediately after they were obtained during the surgical operation, and then stored at -80°C to prevent RNA loss. They were classified according to the WHO criteria and staged according to the tumor-node-metastasis (TNM) classification. Written informed consent from all the patients and research investigation was approved by the institutional research ethics committee of The Second Affiliated Hospital of Zhengzhou University.

Cell culture

Human glioma cell lines (U87 and U251) were purchased from the Shanghai Institute of Life Sciences Cell Resource Center, Shanghai, China. All cell lines were cultured in DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Biowest, France). All cell cultures were maintain at 37°C in a 5% CO_2, humidified incubator.

RNA oligoribonucleotides and cell transfection

The small interfering RNAs (siRNAs) that specifically target human miR-155 and FBXW7 mRNA were designated as anti-miR-155 and siFBXW7, respectively. The coding sequence of FBXW7 was amplified and then cloned into PCDNA3.1 vector, and was named as pFBXW7. The lentiviras vector containing anti-miR-155 plasmid (Lv-anti-miR-155) or miR-155 (Lv-miR-155) was amplified and cloned (Genechem Corporation, Shanghai, China). The negative control duplex (NC) for both miRNA mimics and siRNA, as well as the single standard negative control RNA for miRNA inhibitors (anti-NC), was not homologous to any human genome sequences. All RNA oligoribonucleotides were purchased from RiboBio (Guangzhou, China). The transfection of RNA oligoribonucleotides and plasmid was performed by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative real-time PCR (RT-qPCR)

Total RNA was isolated from glioma specimens or glioma cell lines using TRIzol reagent (Invitrogen). The amounts of miRNAs were quantified in duplicate by quantitative reverse transcription polymerase chain reaction (RT-PCR) by using the human TaqMan MicroRNA Assay Kits (Applied Biosystems, Foster City, CA, USA). U6 was used the internal control for miRNA detection. For FBXW7 mRNA detection, the cDNA was synthesized from 200 ng extracted total RNA using the SYBR Green master mixture and amplified by RT-qPCR. RT-qPCR was performed by using Light Cycler 480 SYBR Green I Master (Roche, Germany) and GAPDH was used as the control gene. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative quantification of gene expression levels. The premier sequences were as follows: FBXW7 (Forward): GGGAGCACTTTGCTGAAATC, (Reverse): CAGCAGCCACTTCTTGAAAC; GAPDH (Forward): GCACCGTCAAGGCTGAGAAC, (Reverse): ATGGTGGTGAAGACGCCAGT.

Cell apoptosis assay

Forty-eight hours after transfection, cells were harvested, stained with propidium iodide and

Table 1. Clinical characteristics of 66 patients and the expression of miR-155 in glioma tissues

Factors	Case	MiR-155 Median (range)	*P*
Gender			0.832
Male	36	3.17 (0.21-6.58)	
Female	30	3.24 (0.52-6.86)	
Age (years)			0.444
<60	32	3.09 (0.43-6.32)	
≥60	34	3.33 (0.46-5.43)	
Tumor size			0.005
<5 cm	35	4.02 (1.24-6.79)	
≥5 cm	31	2.47 (0.36-5.04)	
WHO grade			<0.001
I	12	2.04 (0.21-5.65)	
II	9	2.47 (0.68-6.10)	
III	19	3.78 (1.77-6.69)	
IV	26	4.39 (2.63-6.86)	
KPS			0.024
<90	48	2.76 (0.57-5.43)	
≥90	18	3.61 (1.35-6.86)	

KPS: Karnofsky Performance Status.

Table 2. Multivariate Cox proportional hazards regression model analysis for overall survival in glioma patients

Factors	Multivariate analysis		
	RR	95% CI	*P*
Gender	0.999	0.471-2.023	0.998
Age	1.953	0.837-4.326	0.094
Tumor size	1.792	1.026-3.022	0.026
WHO grade	3.678	1.321-8.947	0.011
KPS	2.358	0.773-3.965	0.127
MiR-155 expression	2.996	1.157-4.738	0.023

KPS: Karnofsky Performance Status.

anti-annexin-V antibody (Annexin V-FITC Apoptosis Detection kit, BD Biosciences, San Jose, CA, USA) following the manufacturer's protocol, and stained cells were detected by flow cytometry. The experiments for the apoptosis assay were performed at least three times.

Cell viability assay

Cell proliferation was quantified using the Cell Counting Kit-8 (CCK-8; Dojindo). Briefly, 100 μl of cells from the different transfection group were seeded onto a 96-well plate at a concentration of 2000 cells per well and were incubated at 37°C. At 72 h, the optical density was measured at 450 nm using a microtiter plate reader, and the rate of cell survival was expressed as the absorbance. The results represent the mean of three replicates under the same conditions.

Colony formation assay

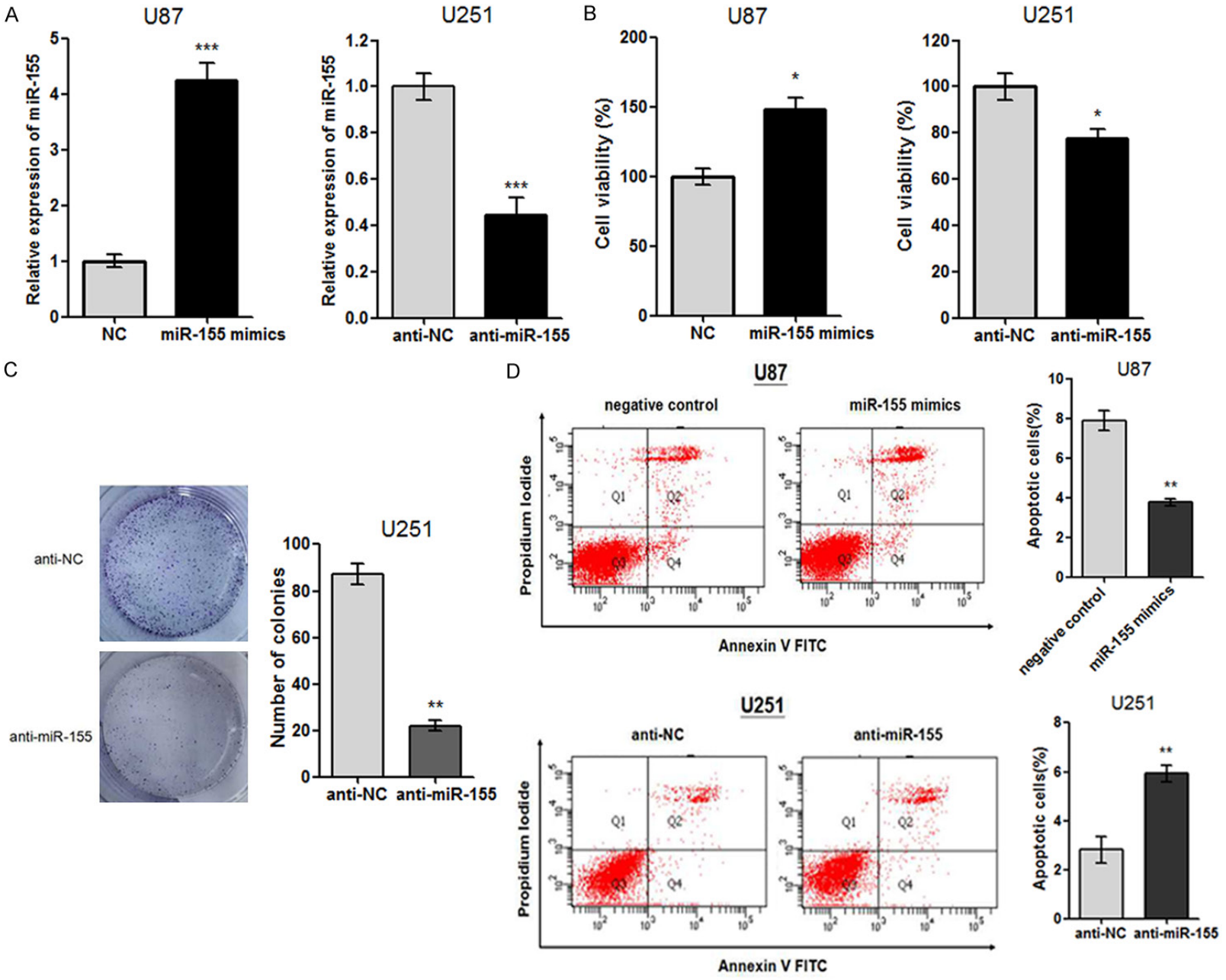
The transfected cells were placed in a fresh six-well plate and maintained in 1640 medium containing 10% FBS. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were manually counted.

Dual-luciferase reporter assay

The full-length FBXW7 3'-UTR was amplified by PCR and cloned downstream of the firefly luciferase gene in the pGL3 vector (Promega, USA). The vector was named wild-type 3'UTR. The GeneTailor Site-Directed Mutagenesis System (Invitrogen, USA) was used to perform site-directed mutagenesis of the miR-155 binding site in FBXW7 3'-UTR: the resultant was named mutant 3'-UTR. These cells were transfected with reporter plasmids and placed in 96-well plates. After incubating the cells for 48 h, the cells were harvested and assayed using the dual-luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions. Each experiment was performed in triplicate.

Western blot and antibodies

Cells were lysed in Laemmli-buffer containing 10% β-mercaptoethanol (Sigma-Aldrich). Equal amounts of cells were resolved by 10% SDS-PAGE. After blotting on a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) the membrane was blocked with 3% nonfat dry milk (Biorad) in TBS-T buffer. Then the membrane was incubated with a rabbit anti-human FBXW7 antibody (1:1000; Novas) and rabbit anti β-actin (1:2000, Santa Cruz, CA, USA). Finally the membrane was washed with TBS-T and incubated with the secondary antibody goat-anti-rabbit horseradish peroxidase-coupled and diluted 1:5000 (Biorad). The amount of detected protein was visualized by enhanced chemiluminescence (Amersham Biosciences, Freiburg, Germany) and autoradiography. Qu-


A
U87
Relative expression of miR-155
***
NC
miR-155 mimics
U251
Relative expression of miR-155
***
anti-NC
anti-miR-155
B
U87
Cell viability (%)
*
NC
miR-155 mimics
U251
Cell viability (%)
*
anti-NC
anti-miR-155
C
anti-NC
anti-miR-155
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Number of colonies
**
anti-NC
anti-miR-155
D
U87
negative control
miR-155 mimics
Propidium Iodide
Annexin V FITC
Q1
Q2
Q3
Q4
U87
Apoptotic cells(%)
**
negative control
miR-155 mimics
U251
anti-NC
anti-miR-155
Propidium Iodide
Annexin V FITC
U251
Apoptotic cells(%)
**
anti-NC
anti-miR-155

Figure 2. MiR-155 promotes cell proliferation and suppresses cell apoptosis *in vitro*. A. Glioma cells were transfected with miR-155 or anti-miR-155 and the relative expression of miR-155 was determined by using RT-qPCR after transfection for 48 h. B. CCK8 assay was performed to evaluate the glioma cell growth, and the relative cell viability was determined after transfection for 72 h. MiR-155 promoted U87 cell growth while anti-miR-155 suppressed U251 cell growth. C. Stable transfection of anti-miR-155 significantly suppressed colony formation capacity of U251 cells. D. MiR-155 inhibited cell apoptosis in U87 cells while anti-miR-155 significantly promoted cell apoptosis in U251 cells by flow cytometry assay. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

antification of autoradiography signals was carried out by densitometry using the ImageJ software (NIH, Bethesda, USA).

Statistical analysis

Statistical analyses were performed with GraphPad Prism 4.0 (GraphPad Software, La Jolla, California, USA). The differences between two groups were analyzed by the Mann-Whitney U-test. Correlation analyses were carried out using Spearman's rank correlation method. A log-rank test was used to analyze the statistical differences in survival as deduced from Kaplan-Meier curves. Cox proportional-hazard regression analysis was performed to calculate HR and 95% CI for each covariable. All differences were regarded as statistically significant when $P<0.05$.

Results

miR-155 is up-regulated in glioma specimens and related to poor survival

RT-qPCR was used to detect miR-155 expression levels in cell lines and clinical samples, normalized to U6. As shown in **Figure 1A**, miR-155 was sufficiently up-regulated in primary glioma tissues compared with primary noncancerous tissues. Besides, the glioma tissues in 62.1% (41 of 66) of cases had at least two-fold higher expression of miR-155 compared with noncancerous tissues (**Figure 1B**). Similarly, the serum miR-155 expression was also elevated in glioma patients compared with healthy individuals (**Figure 1C**). We also determined the miR-155 expression among glioma cell lines. As expected, the glioma cell lines showed elevated miR-155 expression when compared with that in the normal human astrocyte 1800 cell line (**Figure 1D**). The U87 and U251 cell lines were chosen for further study. We then determined the relationships between miR-155 expression and clinical pathological characters. As shown in **Table 1**, the expression level of miR-155 was significantly correlated with tumor size, Karnofsky Performance Status (KPS) and the WHO grade, while no significant correlations were observed between miR-155 expression and the common characteristics such as gender and age among 66 primary glioma tissues.

Furthermore, Kaplan-Meier survival analysis was performed to investigate the prognostic value of miR-155 in glioma patients. A median value of miR-155 (3.19) in 66 primary glioma tissues was used to divided these patients into a high and a low group. Our results indicated that patients with high miR-155 expression were associated with shorter overall survival compared with low miR-155 patients (**Figure 1E**). Moreover, Cox regression multivariate analysis was performed to determine if miR-155 expression was an independent predictor of overall survival for glioma patients. The results showed that high miR-155 was significantly associated with poor survival prognosis independent with other clinical covariates (**Table 2**). Collectively, these results suggest that miR-155 may function as a tumor-promoter gene and be an independent prognostic factor in glioma.

MiR-155 promotes cell proliferation and suppresses cell apoptosis in vitro

To explore the impact of miR-155 on cell survival, we transfected glioma cells with miR-155 mimics or anti-miR-155. As shown in **Figure 2A**, U87 cells exhibited significantly enhanced expression level of miR-155 after transfection of miR-155 mimics by RT-qPCR. Besides, miR-155 expression was dramatically silenced by anti-miR-155 in U251 cells. Cell proliferation was determined by using CCK8 assay. The results showed that miR-155 promoted U87 cell proliferation while anti-miR-155 suppressed U251 cell survival 48 h after transfection (**Figure 2B**). Moreover, the colony formation assay showed that the stable transfection of anti-miR-155 significantly suppressed colony formation capacity of U251 cells (**Figure 2C**). Furthermore, cell apoptosis was also per-

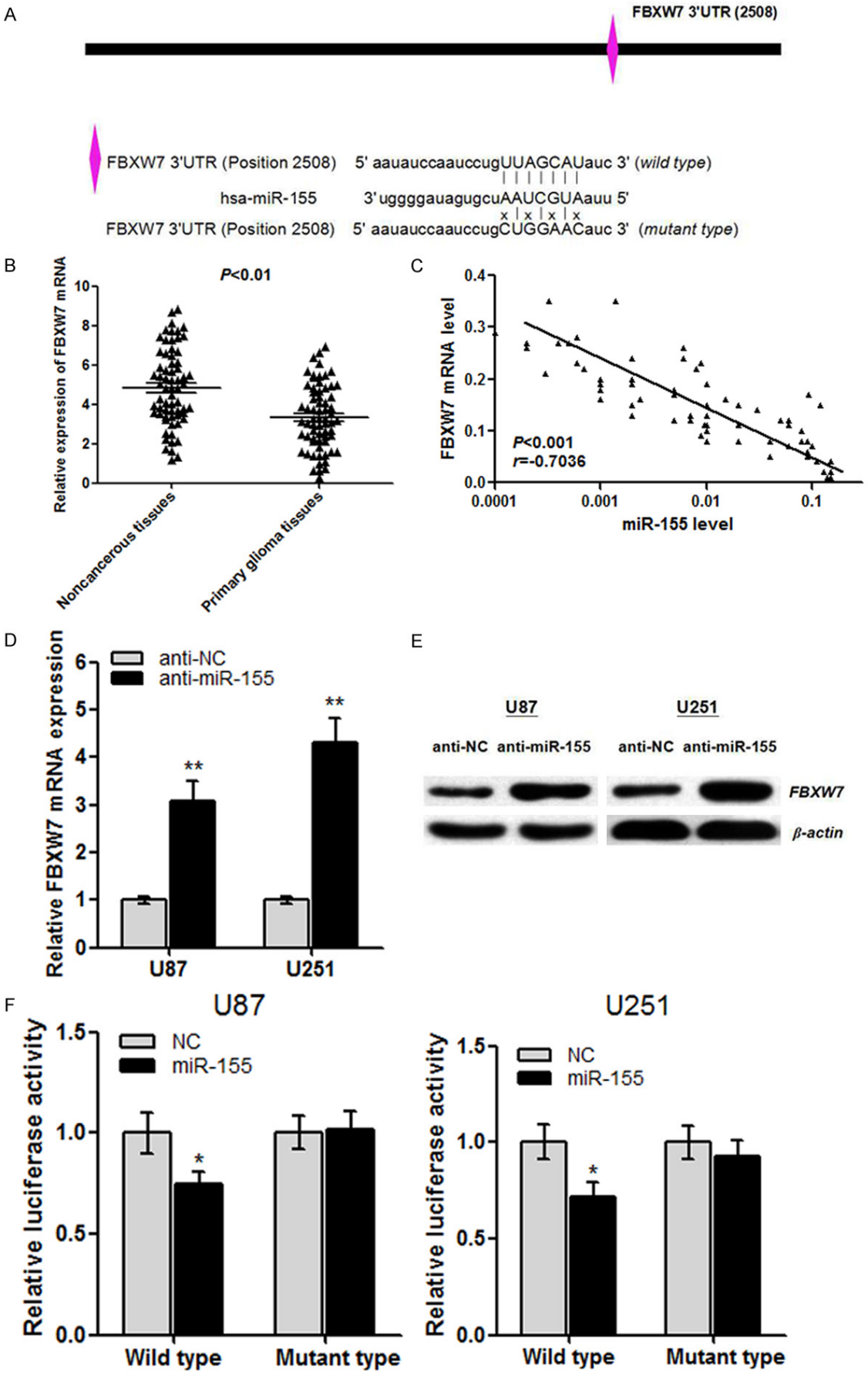
A
FBXW7 3'UTR (2508)
FBXW7 3'UTR (Position 2508) 5' aauauccaauccugUUAGCAUauc 3' (wild type)
hsa-miR-155 3' uggggauagugcuAAUCGUAauu 5'
FBXW7 3'UTR (Position 2508) 5' aauauccaauccugCUGGAACauc 3' (mutant type)
B
P<0.01
Relative expression of FBXW7 mRNA
Noncancerous tissues
Primary glioma tissues
C
FBXW7 mRNA level
P<0.001
r=-0.7036
miR-155 level
D
anti-NC
anti-miR-155
Relative FBXW7 mRNA expression
U87
U251
E
U87
U251
anti-NC anti-miR-155 anti-NC anti-miR-155
FBXW7
β-actin
F
U87
U251
NC
miR-155
Relative luciferase activity
Wild type
Mutant type

Figure 3. FBXW7 is identified as a direct target of miR-155 in glioma cells. A. Illustration of the putative predicted miR-155 binding sites in the FBXW7 3'UTR region. B. FBXW7 mRNA expression level was significantly decreased in primary glioma tissues compared with noncancerous tissues by RT-qPCR. C. FBXW7 mRNA is decreased following forced expression of miR-155 in primary glioma tissues. D. FBXW7 mRNA was up-regulated in glioma cell lines transfected with anti-miR-155. E. Western blots showing that anti-miR-155 increased FBXW7 protein expression in U87 and U251 cells. F. MiR-155 targets the wild-type but not the mutant 3'UTR of FBXW7 in U87 and U251 cells. $^{*}P<0.05$, $^{**}P<0.01$.

formed by using flow cytometry and the results showed that miR-155 inhibited cell apoptosis in U87 cells while anti-miR-155 significantly promoted cell apoptosis in U251 cells (**Figure 2D**). To sum up, we found that elevated miR-155 expression level promoted cell proliferation and suppressed cell apoptosis in glioma cells.

FBXW7 is identified as a direct target of miR-155 in glioma cells

To better understand the underlying regulatory way by which miR155 participated in glioma genesis and development, we sought to determine the gene targets that may account for these findings. FBXW7 mRNA was predicted to be the functional target of miR-155 by several bioinformatics programs including Targetscan and miRnada (**Figure 3A**). RT-qPCR showed that FBXW7 was upregulated in primary glioma tissues compared with noncancerous tissues (**Figure 3B**). Besides, a significant negative correlation was found between FBXW7 and miR-155 expression in 66 glioma tissues (**Figure 3C**). By using the gain and loss-function assay, we found that miR-155 knockdown significantly rescued both FBXW7 mRNA and protein expression in glioma cells (**Figure 3D** and **3E**).

Luciferase reporter assay was performed to explore the direct interaction between miR-155 and FBXW7 in glioma. Wild-type and mutant-type luciferase reporter plasmids were constructed according to this site as described in Methods. We observed that miR-155 significantly inhibited the luciferase activity compared with the negative control miRNA (**Figure 3F**), suggesting that miR-155 was able to interact directly with the 3'-UTR of FBXW7 mRNA. In addition, miR-155 did not inhibit the luciferase activity of the reporter vector containing mutant 3'-UTR of FBXW7 in the miR-155-binding site. Based on these results, we conclude that miR-155 specifically suppresses FBXW7 protein synthesis in glioma cells.

FBXW7 mediates miR-155-induced tumorigenesis in glioma cells

Based on the direct interaction between miR-155 and FBXW7 expression, we further investigated the functional regulation of miR-155 on FBXW7. U87 cells were transfected with siFBXW7 or siNC, and FBXW7 mRNA and protein expression levels were significantly down-regulated in cells transfected with siFBXW7 compared with siNC (**Figure 4A** and **4B**). Then CCK8 assay was performed to evaluate the effect of FBXW7 on glioma cell proliferation. As expected, FBXW7 silencing dramatically promoted cell growth compared with cells treated with siNC (**Figure 4C**). Moreover, the suppressed cell growth caused by miR-155 inhibition was significantly rescued by FBXW7 knockdown (**Figure 4D**). Furthermore, the Lv-anti-miR-155 induced cell apoptosis was reversed by siFBXW7 (**Figure 4E**).

On the other hand, the expression of FBXW7 in U251 cells was elevated by pFBXW7, and FBXW7 mRNA and protein expression were detected (**Figure 5A** and **5B**). Subsequently, CCK-8 assay was performed to investigate the effect of FBXW7 on the proliferation of glioma cells, **Figure 5C** showed that the proliferation abilities of the U251 cells decreased significantly after overexpression of FBXW7. Moreover, the enhanced cell growth induced by Lv-miR-155 was sufficiently suppressed after FBXW7 plasmid transfection (**Figure 5D**). Furthermore, the pFBXW7 also rescued the Lv-miR-155-induced suppression of cell apoptosis in U251 cells (**Figure 5E**). To sum up, these results suggest that FBXW7 is responsible for deregulation of cell viability induced by miR-155 in glioma cells.

Discussion

Gene expression is a complex cellular process that is tightly regulated at several levels to ensure proper gene dosage, and this process is dysregulated in human malignancies, leading

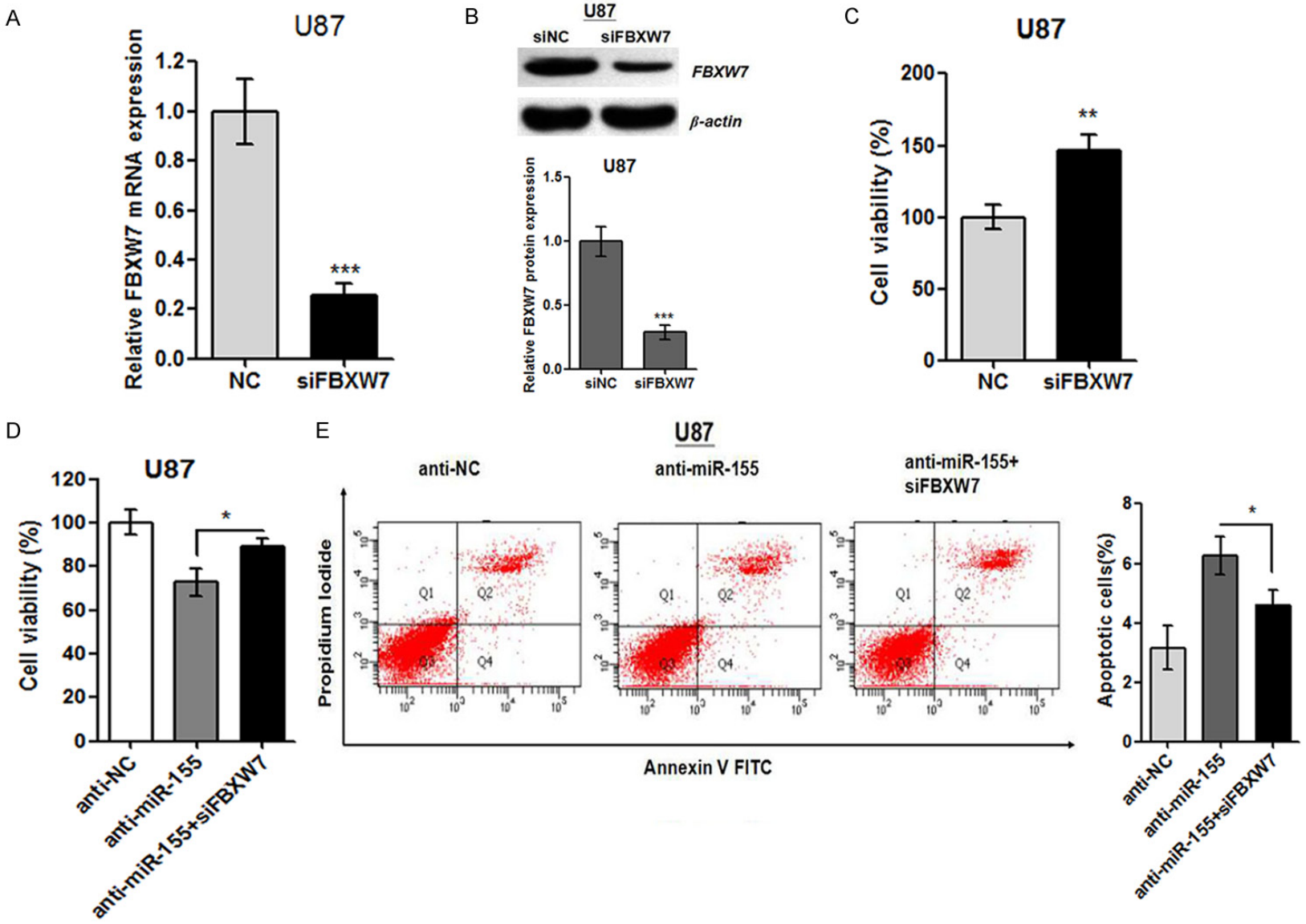

Figure 4. Si-FBXW7 abrogated the effects of miR-155 on glioma cell function. A. RT-qPCR showed that FBXW7 mRNA expression level was sufficiently suppressed by siFBXW7 transfection for 48 h in U87 cells. B. Western blotting showed that siFBXW7 also inhibited FBXW7 protein expression in U87 cells. C. CCK8 assay showed that siFBXW7 significantly suppressed cell proliferation of U87 cells. D. The suppressed cell growth caused by miR-155 inhibition was significantly rescued by FBXW7 knockdown. E. Anti-miR-155 induced cell apoptosis, and these effects were significantly abrogated by FBXW7 knockdown. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

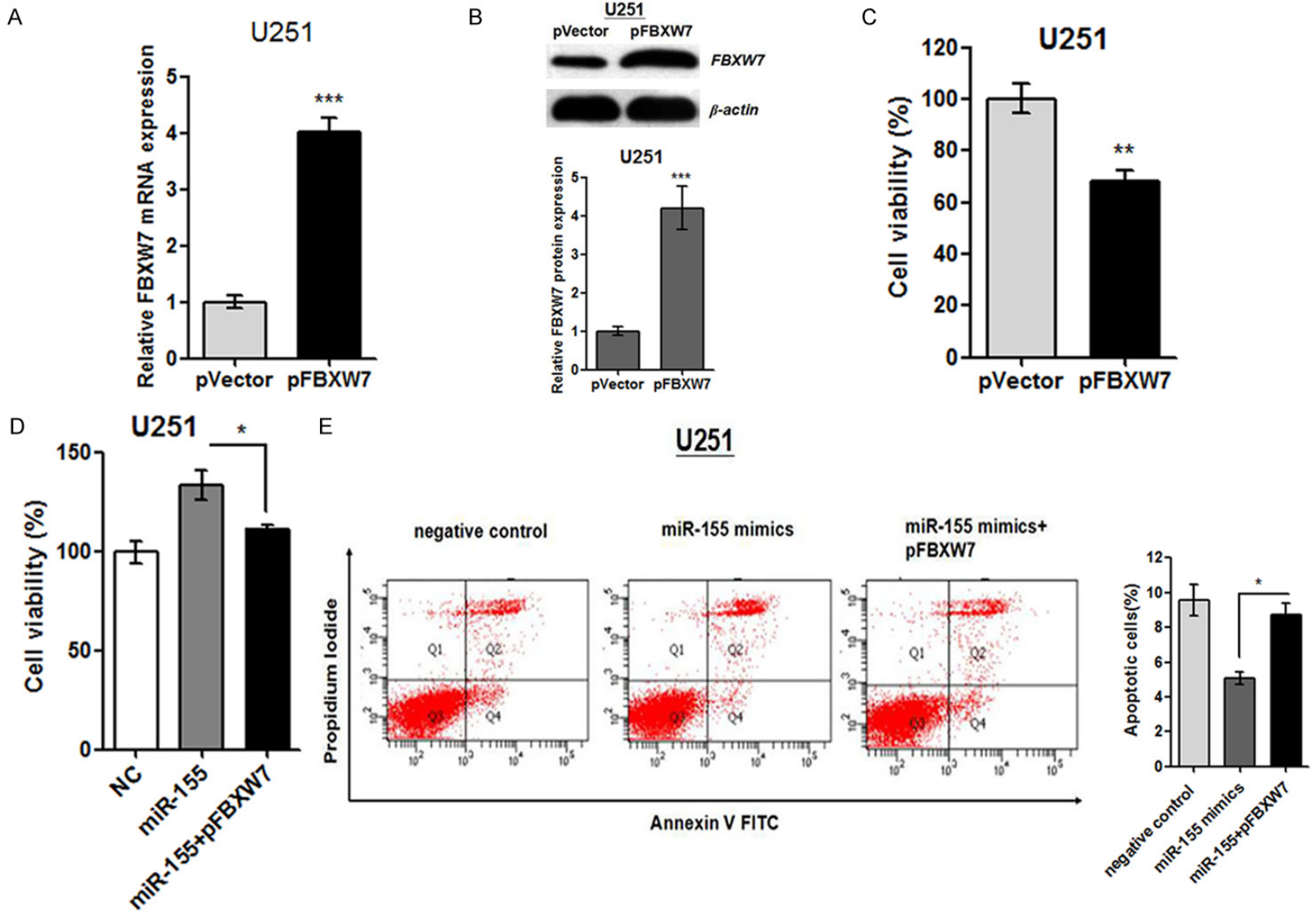

Figure 5. p-FBXW7 significantly reversed miR-155 effects on glioma cell function. A. RT-qPCR experiments showed that FBXW7 mRNA expression level was up-regulated after pFBXW7 transfection for 48 h in U251 cells. B. Western blotting showed that pFBXW7 also increased FBXW7 protein expression in U87 cells. C. CCK8 assay showed that pFBXW7 significantly promoted cell proliferation of U251 cells. D. The promoted cell growth caused by miR-155 was significantly reversed by pFBXW7 transfection. E. MiR-155 suppressed cell apoptosis, and this suppression were significantly reversed by FBXW7 overexpression. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

to overexpression of tumor-promoting genes, and downregulation of tumor suppressor genes. It is known that glioma is the most common intracranial tumor due to its high proliferation and poor apoptosis rate. Thus, it is of much importance to find and further understand the potential cellular process that is tightly regulated by special tumor-promoting genes. In this study, we aim to reveal the clinical role of miR-155 in human glioma and further investigate the potential regulatory mechanism by which miR-155 participate in glioma progression. Our data showed that the expression levels of miR-155 were significantly higher in glioma specimens than that in noncancerous brain tissues. Besides, miR-155 was significantly correlated with tumor size, WHO grade and KPS, and was an independent prognostic factor of overall survival for glioma patients. Furthermore, we demonstrated a novel regulate pathway by which miR-155 mediated tumorigenesis in glioma. MiR-155 suppresses cell viability through down-regulating FBXW7 expression and reversing FBXW7 functioning. These indicated that miR-155 could be a prognostic and therapeutic target in glioma patients.

MicroRNAs, acting as post-transcriptional regulators of gene expression, can regulate 30% protein coding genes in the human genome [6, 17]. Several studies have identified that miR-155 is an oncogene and involved in the development in early stages of malignancies [18]. Aberrant expression of miR-155 has been found in various cancers, including breast cancer and lymphoma [19, 20]. It is also reported that increased microRNA-155 may be a prognostic indicator in Graves' ophthalmopathy [21]. Recently, Sun et al demonstrated that overexpression of miR-155 predicts poor prognosis in glioma patients [12]. Consistent with previous studies, our results showed that miR-155 was sufficiently upregulated in glioma cells and specimens and the Cox regression multivariate analysis showed that miR-155 was an independent prognostic factor of over survival for glioma patients. Furthermore, the gain and loss-function experiments showed that miR-155 promoted cell proliferation and colony formation capacity of glioma cells. MiR-155 also inhibited glioma cell apoptosis. These results suggest that miR-155 may exert its oncogene role by promoting cell growth and suppressing apoptosis.

We subsequently investigated the underlying regulatory role of miR-155. It is widely accepted that microRNAs play essential roles in posttranscriptional regulation of gene expression. Previous studies indicated that inhibition of miR-155 induced upregulation of SOCS1 expression and subsequent inhibition of STAT3 in various malignant events [22]. Li found that miR-155 exerted suppressive functions in proliferation and induced apoptosis by upregulating BACH1 in renal cancer cells [23]. In our study, the target of miR-155 was predicted to explore the mechanism underlying the function of miR-155 in glioma. Our results established FBXW7 as a direct target of miR-155 by using the luciferase experiments. FBXW7 is a component of SCF (complex of SKP1, CUL1 and F-box-protein)-type ubiquitin ligases that targets several oncoproteins for ubiquitination and degradation by the proteasome [24]. Many observations indicate that FBXW7 involved cancer cell growth and tumor genesis. FBXW7 acts as a tumor suppressor involved in the degradation of substrates with oncogenic activity frequently overexpressed in breast cancer such as CyclinE, c-Myc and AURKA [25-27]. Our results showed that FBXW7 significantly suppressed glioma cell proliferation, and the suppressed cell growth caused by miR-155 inhibition was significantly reversed by FBXW7 knockdown. Similarly, the effect of FBXW7 on glioma cell apoptosis was also mediated by miR-155. These results suggest that FBXW7 is responsible for the effect of miR-155 on cell viability.

Conclusion

In conclusion, our data demonstrated for the first time that miR-155 is an independent prognostic factor for glioma patients survival. Moreover, we uncovered that miR-155 promoted glioma cell proliferation through down-regulation of FBXW7. Thus, miR-155 may be a novel prognostic biomarker and therapeutic target in glioma. Our results illustrated that the inhibition of the tumor promoter miR-155 might be useful and effective in the treatment of glioma cancer.

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Disclosure of conflict of interest

None.

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