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MFI2-AS1 regulates the aggressive phenotypes in glioma by modulating MMP14 via a positive feedback loop

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have vital functions during the progression of malignant tumors. Nevertheless, the precise function of lncRNA MFI2 Antisense RNA 1 (MFI2-AS1) in glioma remains not well elaborated.

PATIENTS AND METHODS: The quantitative Real Time PCR (qPCR) assay was used to assess the level of MFI2-AS1, matrix metalloproteinase 14 (MMP14), and miR-485-5p in glioma tissues and cell lines. The growth of glioma cell was analyzed using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The migration and invasion of glioma cell were investigated using wound healing and transwell invasion analysis. The apoptosis of glioma cell was detected using flow cytometry. The expression of MMP14 was determined by Western blotting. The growth of glioma cell *in vivo* was assessed using the xenograft model.

RESULTS: We showed that MFI2-AS1 was markedly overexpressed in glioma cells and clinical tissues. The higher level of MFI2-AS1 is related to the poor prognosis in patients with glioma. Furthermore, the downregulation of MFI2-AS1 suppresses the growth, aggressiveness, and induces apoptosis of glioma cells. Also, MMP14 is the target gene of miR-485-5p and its level is positively modulated by MFI2-AS1. The rescue experiments reveal that miR-485-5p inhibition reverses the suppressive impacts of MFI2-AS1 silencing on the growth, migration, and invasive abilities of glioma cells. Finally, we elaborate that MFI2-AS1 silencing represses glioma cell growth *in vivo* and suppress the expression of MMP14.

CONCLUSIONS: In summary, the downregulation of MFI2-AS1 restrains the aggressive phenotypes of glioma cells *via* downregulating the expression of MMP14.

Key Words:
MFI2-AS1, Glioma, MMP14, MiR-485-5p.

Introduction

Glioma, which is one of the common types of primary tumors in the human brain, accounts for almost 80% of all malignant human brain tumors. Despite the enormous improvement in the treatment options in glioma, the overall survival of patients with glioma remains unsatisfactory. Hence, it is urgent to investigate the molecular mechanisms behind the development of glioma and explore the potential therapeutic target. Long noncoding RNAs (lncRNAs) have been proved to have crucial roles in tumorigenesis. Dysregulation of lncRNAs regulates various cancer-associated biological processes, including cell growth, invasion, metastasis, and epithelial-mesenchymal transition (EMT) process. For example, lncRNA MFI2-AS1 has been proved to be overexpressed in sporadic localized clear cell RCC (ccRCC) and pancreatic cancer cells[1,2]. In ccRCC, MFI2-AS1 promotes the growth and metastatic abilities of colorectal carcinoma cell by regulating the miR-574-5p/MYCBP axis[2]. LncRNA MFI2-AS1 expression is closely related to the poor disease-free survival (DFS) and the recurrence of patients with sporadic localized ccRCC[1]. Recently, increasing evidence has demonstrated that the dysregulation of lncRNAs participates into the development of glioma. LncRNA LINC00515 induces the growth and suppresses apoptosis of glioma cell by sponging miR-16 and increasing the expression of protein arginine methyltransferase 5 (PRMT5)[3]. LncRNA LINC01503 is closely associated with the poor prognosis in patients with glioma and facilitates the development of glioma by regulating the Wnt/β-catenin[4]. However, the potential biological activities of MFI2-AS1 in glioma still are not elucidated.

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Theoretically, lncRNA functions as a miRNA sponge to regulate the miRNA/mRNA axis. For instance, lncRNA UCA1 impacts cell proliferation, invasion, and migration of pancreatic cancer by regulating miR-96/FOXO3[5]. Emerging evidence has indicated that non-coding RNAs, especially miRNAs, have important functions in the pathogenesis of cancer metastasis by modulating the expression of cancer-suppressors or oncogenes[6-8]. MiR-485-5p has been reported to suppress the metastasis of breast cancer cell by inhibiting the expression of PGC-1α[9]. A previous investigation[10] has indicated that miRNA-485-5p suppresses the proliferation of glioma cells *via* directly targeting paired box 3 (PAX3). In addition, lncRNA DSCR8 serves as a molecular sponge for miR-485-5p to activate Wnt/β-catenin in human hepatocellular carcinoma (HCC)[11]. Nevertheless, the relations between MFI2-AS1 and miR-485-5p in glioma has not yet been definitively explored.

In the present research, we observe that MFI2-AS1 is significantly overregulated in glioma cells and tissues. In addition, the downregulation of MFI2-AS1 inhibits growth, metastatic traits, and induces the apoptosis of glioma cells. Furthermore, MFI2-AS1 regulates the expression of MMP14 by binding with miR-485-5p and then exerts its important functions in glioma. All these results provide the theoretical basis for a deeper understanding of MFI2-AS1/miR-485-5p/MMP14 in glioma.

Patients and Methods

Glioma Tissues and Cell Culture

36 cases of clinical glioma tissues and normal tissues were obtained from the Affiliated Hospital of Weifang Medical University. Written informed consent was obtained from all patients with glioma. This research was approved by the Ethics Committee of the Affiliated Hospital of Weifang Medical University and was complied with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985). The clinicopathological features of all the patients are summarized in Table I. Glioma cell lines (U251, SHG-44, TJ905, and U87) and normal astrocyte cell NHA were purchased from Nanjing Cobioer Biological Technology co., LTD (Nanjing, Jiangsu, China) and cultured using Roswell Park Memorial Institute-1640 (RPMI-1640; Thermo Fisher Scientific, Waltham, MA, USA) + 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) at 37°C with 5% CO_2.

Table I. Association of lncRNA MFI2-AS1 expression with clinicopathologic features in patients with glioma.

Clinical parameter	MFI2-AS1		*p*-value
	High	Low	
Age (years)			0.41
≤55	7	10	
>55	11	8	
Gender			0.063
Female	6	9	
Male	13	8	
Tumor size (cm)			0.024
≥5	13	3	
<5	15	5	
WHO grade			0.0013
I-II	14	6	
III-IV	13	3	
KPS score			0.09
≥90	8	9	
<90	12	7	

Abbreviations: KPS = Karnofsky Performance Scale; WHO = World Health Organisation.

Quantitative Real Time PCR (qPCR) Assay

Total RNAs were extracted using the TRIzol kit (Thermo Fisher Scientific, Waltham, MA, USA). The miRNAs were extracted using the Mir-VanaTM MiRNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA). The level of MMP4 or MFI2-AS1 was detected using the SYBR® Premix Ex Taq™ II (Takara, Dalian, China). The primers for miR-485-5p or miR-491-5p were obtained from RiboBio (Guangzhou, Guangdong, China). The level of miR-485-5p or miR-491-5p was determined using a miDETECT A Track™ miRNA qPCR Starter Kit (RiboBio, Guangzhou, Guangdong, China). The comparative cycle threshold (Ct) method was applied to quantify the expression levels by calculating the $2^{(-\Delta\Delta Ct)}$ method. The primers were used for PCR reaction as follows: MFI2-AS1 forward, 5'-TACATACAGTGACCCAAAGAGCA-3', MFI2-AS1 reverse, 5'-CAGTGCTTCTGAACGCCTCTT-3'; MMP14 forward, 5'-ACCCACACACAACGCTCAC-3', MMP14 reverse, 5'-GCCTGTCACTTGTAAACCATAGA-3'. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5'-AATGGATTTGGACGCATTGGT-3', GAPDH reverse, 5'-TTTGCACTGGTACGTGTTGAT-3'. miR-485-5p forward, 5'-GGAGAGGCTGGCCGTGAT-3'; miR-485-5p reverse, 5'-CAGTGCGTGTCGTGGAGT-3'; miR-491-5p forward, 5'- ATCCAGTGCGTGTCGTG-3'; miR-491-5p reverse, 5'-TGCTAGTGGGGAACCCTTC-3'; U6 snRNA

forward, 5'-GCTTCGGCAGCACATATACTA-AAAT-3'; U6 snRNA reverse, 5'-CAGTGCGT-GTCGTGGAGT-3'. GAPDH or small nuclear RNA (snRNA) U6 were used as an internal reference.

Cell Transfections

MiR-485-5p mimics (miR-485-5p), miR-491-5p mimics (miR-485-5p), scrambled miRNA control (miR-con), miRAN inhibitor control (anti-miR-con), miR-485-5p inhibitor (anti-miR-485-5p), and miR-491-5p inhibitor (anti-miR-485-5p) were purchased by GenePharma (Shanghai, China). Negative siRNA control (si-con) and small interfering RNA (siRNA) against MFI2-AS1 (si-MFI2-AS1), empty vector pcDNA3.1 (Vector), and MFI2-AS1 overexpressing vector pcDNA-MFI2-AS1 (MFI2-AS1) were purchased from RiboBio (Guangzhou, Guangdong, China). Cell transfection was conducted using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA).

MTT Assay

U87 or U251 cell was cultured into 96 well plates and grown for 24 h, 48 h, 72 h or 96 h. Then, 20 µl of MTT (5 mg/ml; Sigma-Aldrich, St. Louis, MO, USA) was plated into plates and the cell was continuously incubated for another 4 h. After that, the OD value was detected at 490 nm.

Migration and Invasion Assay

The cell was cultured into six-well plates overnight. Next, a straight line was made using a100 µl pipette tip on the monolayer. After washing with phosphate-buffered saline (PBS), the cell was cultured with FBS-free medium. The percentage of migration was counted as the following formula: (width of 0 h - width of 48 h)/ width of 0 h × 100%. For the invasion assay, the 8 µm membrane in 24-well transwell chamber (Corning, Corning, NY, USA) was firstly coated with 1:4 diluted Matrigel (BD Biosciences, Bedford, MA, USA). Total 200 µl cell suspension was added into the upper chamber and 600 µl medium containing 20% FBS was plated into the lower chamber contained. After 24 h, the invaded cells were fixed using 4% paraformaldehyde and were stained by crystal violet (0.1%). The invaded cell was counted from five random fields.

Apoptosis Analysis

U87 or U251 cell was stained according to the protocol of Annexin V-FITC/PI kit (Beyotime Biotechnology, Nanjing, Jiangsu, China). The apoptosis of the glioma cell was analyzed by flow cytometry (BD Biosciences, San Jose, CA, USA).

Luciferase Reporter Gene Assay

U87 or U251 cell was cotransfected with the luciferase reporter vector containing mutant type or wild type MFI2-AS1 fragment (MFI2-AS1-MUT or MFI2-AS1-WT) and miR-485-5p. After 48 h, the luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). To analyze the relation among MFI2-AS1, MMP14, and miR-485-5p, U87 or U251 cell were cotransfected with the luciferase reporter vector (containing MMP14-WT or MMP14-MUT) and miR-485-5p, or luciferase reporter vector and MFI2-AS1+miR-485-5p. After 48 h post-transfection, the luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Xenograft Model

100 µl U87 cells (2×10^7) were stably transfected with sh-MFI2-AS1 and were subcutaneously inoculated into BALB/c-nude mice (n=6 for each group, Shanghai Slake Experimental Animal Co., Ltd, Shanghai, China). The tumor volume was measured each week and was calculated using the formula: Tumor volume = $0.5 \times (\text{length} \times \text{width}^2)$. After five weeks, mice were sacrificed and the tumor tissues were weighted. All animal procedures were approved by the Animal Research Committee of the Ethics Committee of the Affiliated Hospital of Weifang Medical University.

Immunoblotting Analysis

Total proteins were extracted from tumor tissues using radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology, Nanjing, Jiangsu, China) containing protease and phosphatase inhibitors. 30 µg proteins were separated using 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were blocked using Tris-Buffered Saline and Tween-20 (TBST) buffer containing 5% low-fat milk for 90 min. Next, PVDF membrane was incubated with MMP14 (1:1000, Abcam, Shanghai, China) or GAPDH antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight followed by incubation with horseradish peroxidase (HRP)-conjugated IgGs (1:10000, Bioworld Biotechnology, Wuhan, Hubei, China). Target proteins were detected by the enhanced chemiluminescence (ECL) system (Millipore, Braunschweig, Germany) and visualized with the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All results were shown as Means±SD. Results were analyzed using the SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). Differences in the results of the two groups were evaluated using either two-tailed Student's *t*-test or one-way ANOVA followed by post-hoc Dunnett's test. The result was statistically significant if $p<0.05$.

Results

MFI2-AS1 is Overexpressed in Glioma

To explore the level of miR-485-5p in glioma, the levels of MFI2-AS1 in 36 cases of glioma tissues and adjacent normal tissues were detected using qPCR. As shown in Figure 1A, MFI2-AS1 was remarkably overexpressed in glioma tissues when compared to that in adjacent normal tissues. Consistently, the levels of MFI2-AS1 in glioma cell lines (U251, SHG-44, TJ905, and U87) and normal astrocyte cell NHA were also measured. As shown in Figure 1B, the level of MFI2-AS1 was significantly increased in glioma cell, especially in U87 and U251 cell which were used for further investigations when compared with NHA. Furthermore, MFI2-AS1 was significantly overregulated in glioma tissues with high-grade (III-IV) when compared to that in tissue with low-grade (I-II) (Figure 1C). Finally, the Kaplan-Meier survival analysis suggested that glioma patients with a high level of MFI2-AS1 had a poor prognosis (Figure 1D). All these results implied that a higher level of MFI2-AS1 may be connected to the progression of glioma.

Downregulation of MFI2-AS1 Inhibits Glioma Cell Growth and Invasion

To further elaborate the action of MFI2-AS1 in glioma, U251 or U87 cell were transfected with si-MFI2-AS1 to reduce the level of MFI2-AS1. As shown in Figure 2A, the qPCR assay suggested that si-MFI2-AS1 transfection strikingly reduced MFI2-AS1 level in both U87 and U251 cell.

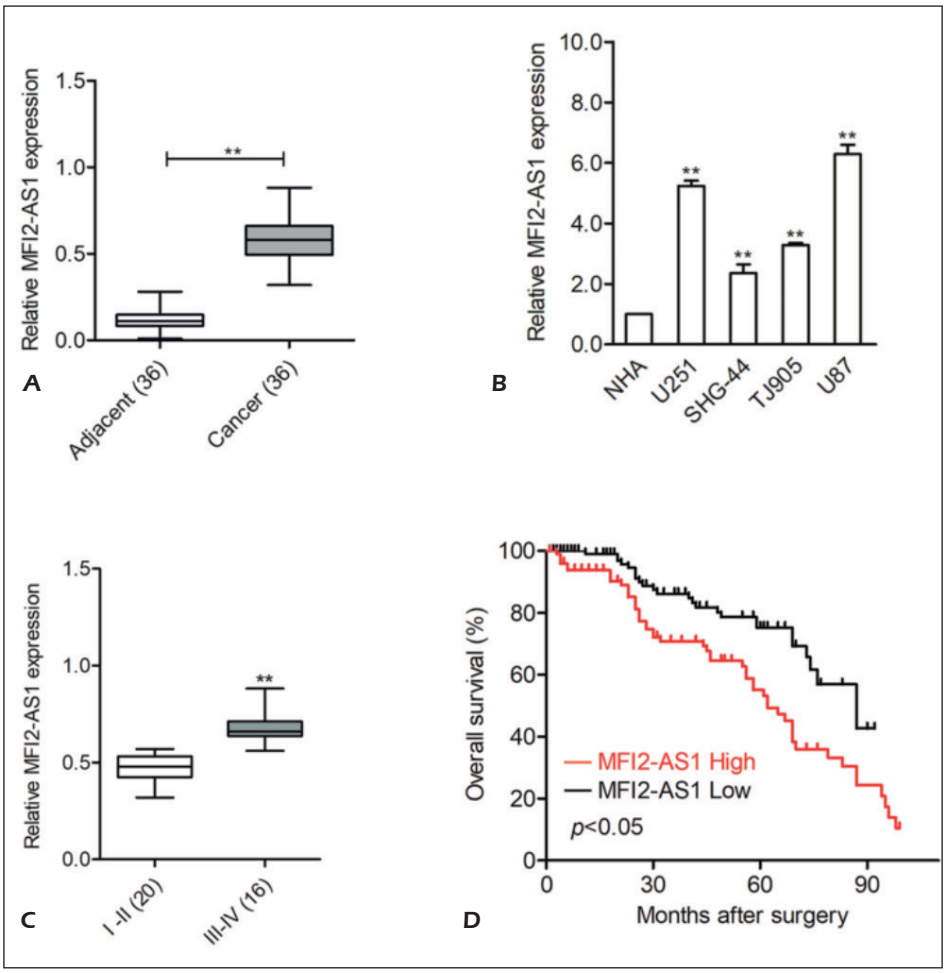

Figure 1. The expression of MFI2-AS1 is increased in glioma tissues and cell lines. **A**, Levels of MFI2-AS1 in glioma or normal tissues were detected by qPCR. $^{**}p<0.01$ compared to the adjacent. **B**, Levels of MFI2-AS1 in glioma cell lines (U251, SHG-44, TJ905, and U87) and normal astrocyte cell and NHA were detected by qPCR. $^{**}p<0.01$ compared to NHA. **C**, Relative level of MFI2-AS1 in I-II and III-IV stage. $^{**}p<0.01$ compared to I-II. **D**, Overall survival was estimated by Kaplan-Meier curve between high and low MFI2-AS1 expression groups.

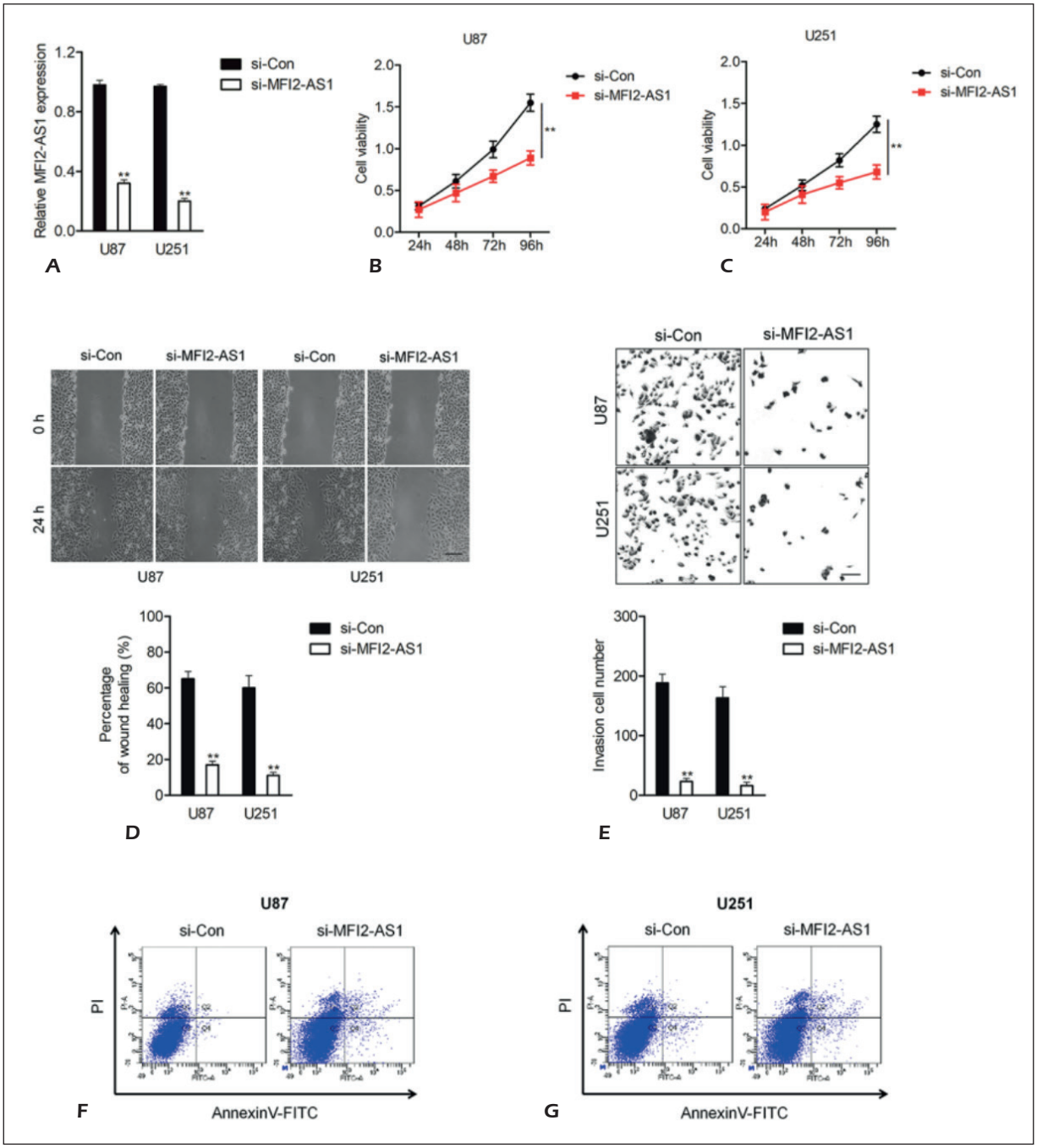

Figure 2. MFI2-AS1 silencing inhibits proliferation, migration, and invasion, and induces apoptosis of glioma cells. A, U87 and U251 cell were transfected with si-con or si-MFI2-AS1. qPCR analysis detected MFI2-AS1 expression in U87 and U251 cell. **B**-**C**, Proliferation activity of U87 and U251 cell was evaluated by MTT assay. **D**, Migration of U87 and U251 cell were determined by wound healing assay. **E**, Invasion of U87 and U251 cells were determined by transwell assays. **F**-**G**, Cell apoptosis was determined by flow cytometry analysis in U87 and U251 cell. $^{**}p<0.01$ compared to si-con.

To analyze the role of MFI2-AS1 on the growth of glioma cell *in vitro*, the MTT test was carried out using U87 or U251 cell. As showed in Figures 2B and 2C, the downregulation of MFI2-AS1 markedly suppressed the proliferation of glioma cells *in vitro*. To reveal the function of MFI2-AS1 on the migration and invasive ability of glioma cell, wound closure, and transwell invasion assays were conducted using U87 or U251 cell. As showed in Figures 2D-2E, si-MFI2-AS1 reduced

the migration and invasion abilities of U87 and U251 cell when compared to the control cell. To study the impact of MFI2-AS1 on the apoptosis of glioma cells, flow cytometry analysis was conducted using U251 and U87 cells. As shown in Figures 2F-2G, MFI2-AS1 silencing significantly increased the apoptosis of U251 and U87 cells. All these data suggest that the downregulation of MFI2-AS1 inhibits the growth and aggressiveness of glioma cells and induces apoptosis of glioma cells.

MFI2-AS1 Regulates the Level of MiR-485-5p

To study the precise mechanism of MFI2-AS1 in regulating the aggressiveness of glioma, starBase bioinformatics analysis was selected to find the relation between miRNAs and MFI2-AS1. The prediction data suggested that MFI2-AS1 bound with two miRNAs, miR-485-5p, and miR-491-5p (Figure 3A). To verify this speculation, the luciferase reporter gene analysis was conducted. As showed in Figures 3B-3C, miR-485-5p transfection significantly decreased the luciferase activity in U251 and U87 that was transfected with the wild type reporter vector (MFI2-AS1-WT). Nevertheless, no significant inhibition was observed in the cell that was cotransfected with miR-491-5p and MFI2-AS1-WT. The effect of MFI2-AS1 on the level of miR-485-5p in U87 and U251 cell were further analyzed using the qPCR analysis. As shown in Figures 3D-3E, the downregulation of MFI2-AS1 significantly elevated the miR-485-5p level, while the MFI2-AS1 transfection inhibited miR-485-5p level in U251 and U87. Besides, miR-485-5p was significantly downregulated in glioma tissues (Figure 3F). The correlation analysis indicated that MFI2-AS1 was inversely associated with miR-485-5p in glioma tissues (Figure 3G). All findings suggest that MFI2-AS1 binds to miR-485-5p in glioma cells.

MiR-485-5p Inhibits the Aggressive Phenotypes of Glioma Cell

To analyze the impacts of miR-485-5p on glioma cells, U87 or U251 were transfected with miR-485-5p to increase the level of miR-485-5p (Figure 4A). Next, the role of miR-485-5p on the viability of glioma cells was measured by the MTT assay. As showed in Figures 4B-4C, transfection of miR-485-5p distinctly suppressed the viability in U87 and U251 cells. In addition, wound healing and transwell invasion assays indicated that miR-485-5p weakened the migration and invasion capacities of U87 and U251 cells (Figures 4D-4E). Furthermore, miR-485-5p transfection increased U87 and U251 cell apoptosis when compared to control group (Figure 4F). All findings imply that miR-485-5p suppresses the aggressiveness and induces apoptosis of glioma cells.

Downregulation of MiR-485-5p Reverses the Impacts of MFI2-AS1 on Glioma Cell

To ascertain whether MFI2-AS1 exerted its biological activities *via* modulating miR-485-5p, U251 or U87 cells were transfected with si-MFI2-AS1 alone or si-MFI2-AS1+anti-miR-485-5p. The result of the qPCR assay suggested that the anti-miR-485-5p treatment repressed si-MFI2-AS1-mediated increasing on the level of miR-485-5p (Figure 5A). Further assays implied that the suppressive functions of MFI2-AS1 silencing on the aggressive phenotypes and apoptosis in U87 and U251 cells were neutralized by anti-miR-485-5p (Figures 5B-5F). These findings indicate that MFI2-AS1 contributes to the progression of glioma by sponging miR-485-5p.

MFI2-AS1 Increases the Level of MMP14 in Glioma Cell

Previous investigations have proved that lncRNAs exert vital functions *via* acting as a ceRNA to modulate the expression of miRNAs' targets. Hence, the potential target of miR-485-5p was then studied. Among the known targets of miR-485-5p, MMP14 was reported to play a critical role in glioma metastasis (Figure 6A). To prove whether MFI2-AS1 bound to miR-485-5p and further reduced the expression of MMP14, the luciferase reporter gene assay was conducted using U87 and U251 cell. As showed in Figures 6B-6C, the transfection of miR-485-5p distinctly reduced the luciferase activity in the cell transfected with wild-type MMP14 (MMP14-WT), while the inhibitory effect of miR-485 was abolished by the overexpression of MFI2-AS1. The qPCR analysis also suggested that miR-485-5p transfection or knockdown MFI2-AS1 strikingly reduced the level of MMP14. Nevertheless, the downregulation of miR-485-5p eliminated the suppressive impacts of si-MFI2-AS1 on MMP14 in U87 and U251 cells (Figures 6D-6E). Furthermore, MMP14 was significantly overexpressed in glioma tissue (Figure 6F). Finally, the MMP14 expression was positively associated with the level of MFI2-AS1 in glioma tissues as demonstrated by the correlation analysis (Figure 6G). All these results prove that MFI2-AS1 functions as a miR-485-5p sponge to inhibit MMP14 in glioma.

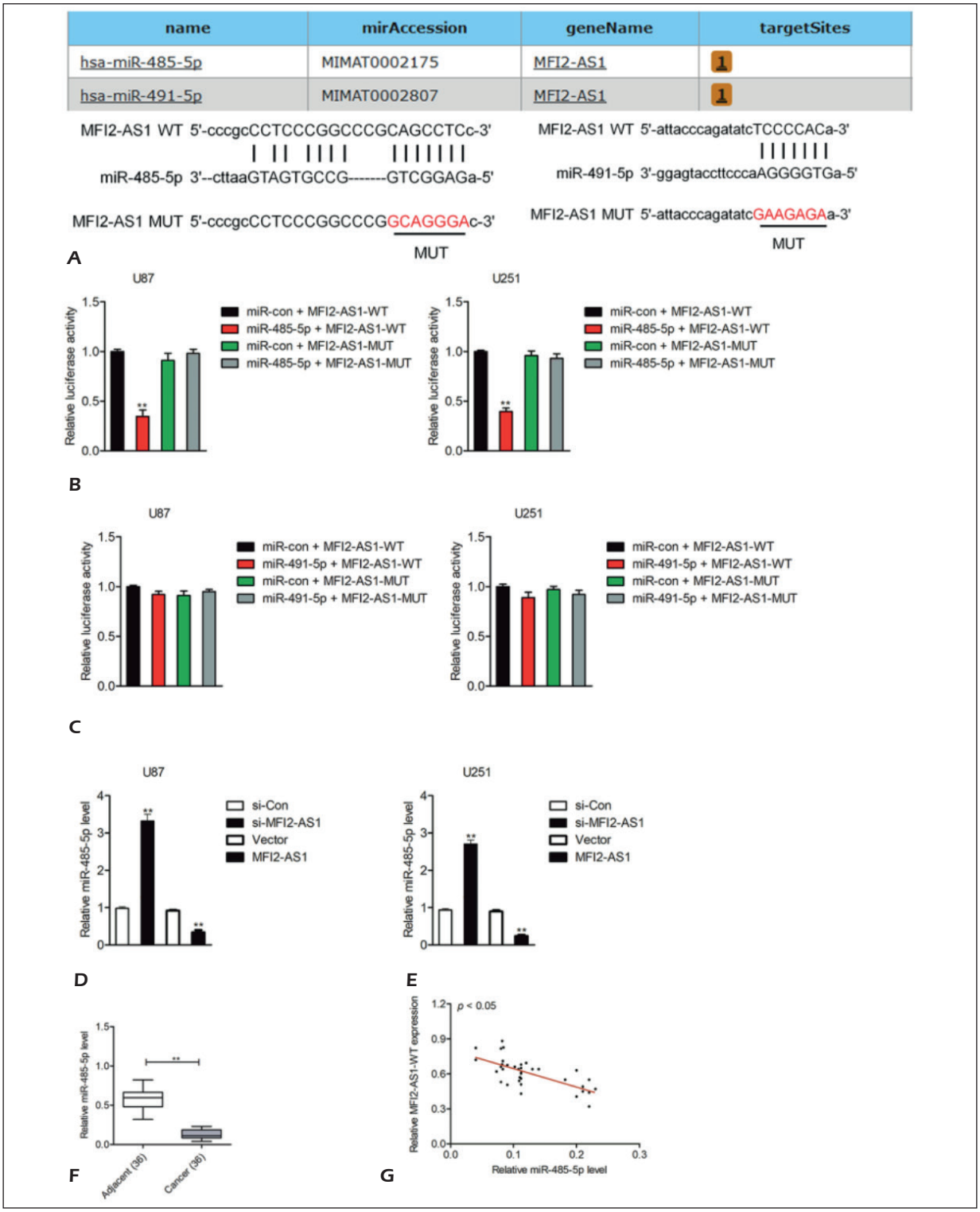

Figure 3. MFI2-AS1 binds to miR-485-5p. **A**, Predicted binding sites of miR-485-5p or miR-491-5p on MFI2-AS1. **B-C**, Luciferase reporter assays were performed to detect luciferase activity in U87 and U251 cell transfected with wild type (MFI2-AS1-WT) or mutant type (MFI2-AS1-MUT) MFI2-AS1 reporter vector and miR-491-5p or miR-485-5p. $^{**}p<0.01$ compared to miR-con + MFI2-AS1-WT. **D-E**, QPCR assays examined miR-485-5p expression in U87 and U251 cells transfected with or si-MFI2-AS1 or MFI2-AS1. $^{**}p<0.01$ compared to si-con. **F**, MiR-485-5p expression was measured in glioma tissues (n=36) or adjacent normal (n=36) tissues. $^{**}p<0.01$ compared to the adjacent. **G**, Spearman's correlation analysis of MFI2-AS1 and miR-485-5p expression in glioma tissues.

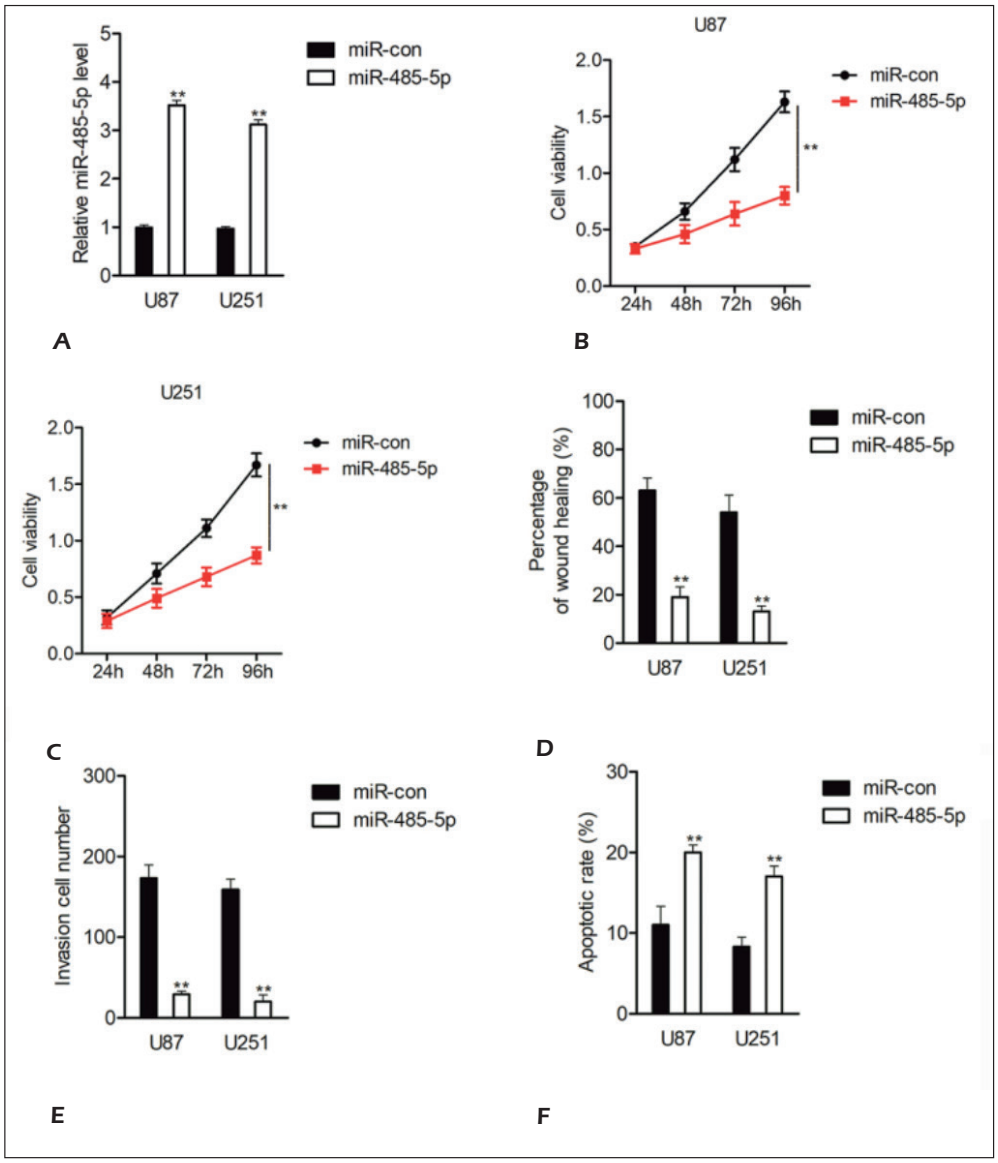

Figure 4. MiR-485-5p suppresses the proliferation, migration and invasion and enhances apoptosis of glioma cell. **A**, U87 and U251 cell was transfected with miR-NC or miR-485-5p and the level of miR-485-5p was detected using qPCR analysis. **B-C**, Cell viability of U87 and U251 cell was measured by MTT assay. **D-E**, Migration and invasion of U87 and U251 cell were determined by wound healing and transwell invasion assays. **F**, Cell apoptosis by flow cytometry analysis. **$p<0.01$ compared to miR-con.

Downregulation of MFI2-AS1 Suppresses the Growth of Glioma In Vivo

To study the actions of MFI2-AS1 on glioma cells *in vivo*, U87 cell that was stably transfected with sh-MFI2-AS1 was also subcutaneously inoculated into nude mice. As shown in Figures 7A-7B, the downregulation of MFI2-AS1 significantly inhibited the tumor growth and tumor weight. The downregulation of MFI2-AS1 evidently inhibited the mRNA level of MMP14 but elevated the miR-485-5p level in the tumor tissues (Figures 7C-7D). Moreover, MFI2-AS1 knockdown markedly decreased the protein expression of MMP14 in tumor tissues which were formed by sh-MFI2-AS1 transfected U87 cell (Figure 7E). Collectively, these data demonstrate that MFI2-AS1 silencing suppresses the tumor growth of glioma cell *in vivo* by regulating miR-485-5p/MMP14.

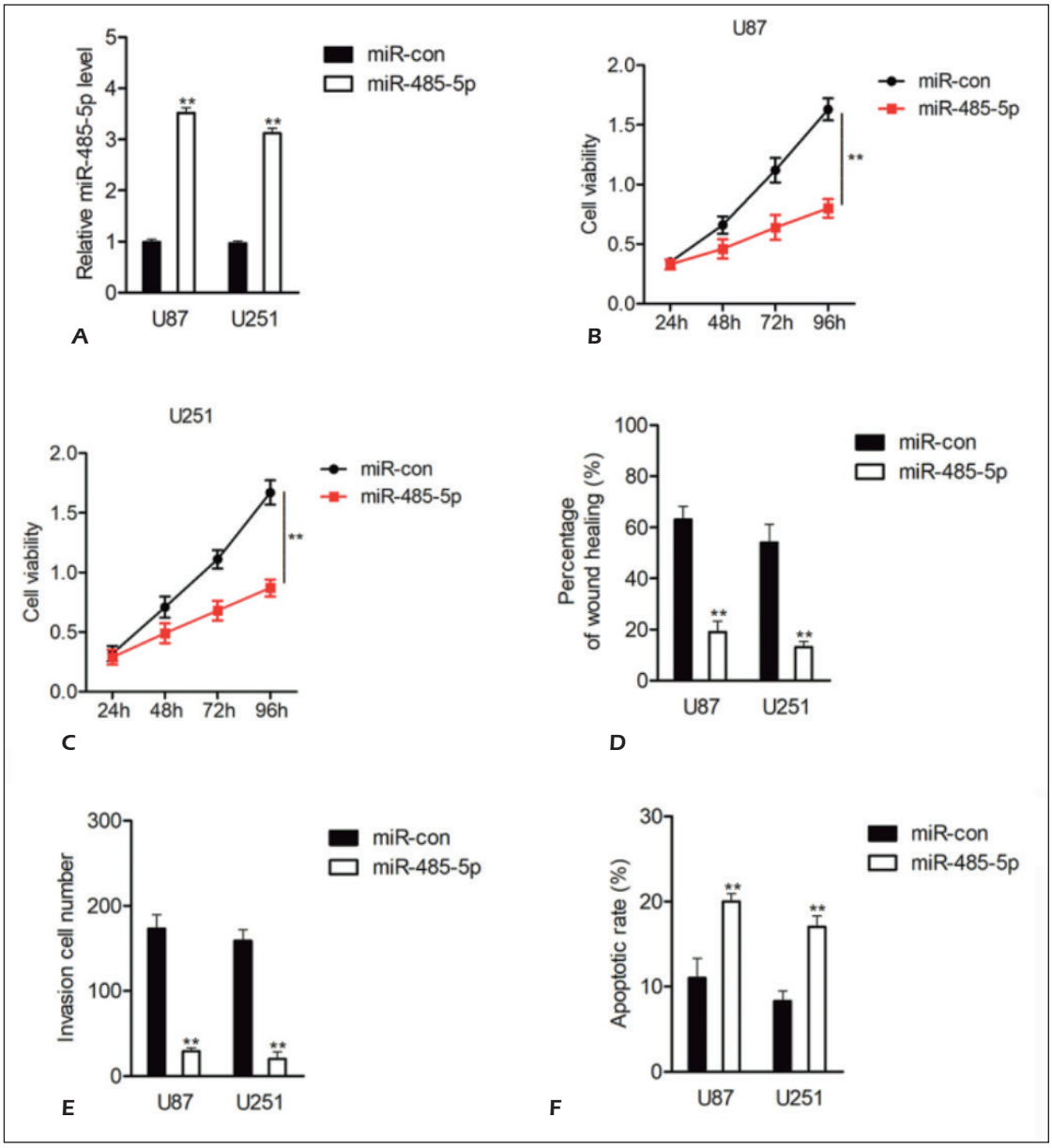

Figure 5. MFI2-AS1 promotes the proliferation, migration, and invasion and promotes apoptosis in glioma cell through binding to miR-485-5p. **A**, U87 and U251 cells were transfected with si-con, si-MFI2-AS1 or si-MFI2-AS1+anti-miR-485-5p. The level of miR-485-5p was determined by qPCR analysis. **B-C**, Cell viability was detected by MTT assay. **D-E**, Migration and invasion assays. **F**, Cell apoptosis was analyzed by flow cytometry assay. $^{**}p<0.01$ compared to si-Con, $^{\#\#}p<0.01$ compared to si-MFI2-AS1.

Discussion

Glioma remains one of the most common causes of malignancies related death worldwide[12-14]. In spite of the great advancement in the treatment of glioma, the overall survival of patients with glioma remains unsatisfactory. Hence, to explore more effective treatments target for glioma is urgent. Increasing evidence[15-17] has indicated that lncRNAs have crucial functions in carcinogens. In the current research, we observed that MFI2-AS1 was remarkably upregulated in glioma cells and tissues. Furthermore, the downregulation of MFI2-AS1 reduced glioma cell growth, aggressive phenotypes, and resulted in apoptosis by modulating MMP14 *via* a positive feedback loop *in vitro* and *in vivo*.

Accumulating evidence[18-20] has proved that lncRNAs function as suppressors or oncogenes in various kinds of cancers. Furthermore, the dysregulation of lncRNAs contributes to the development

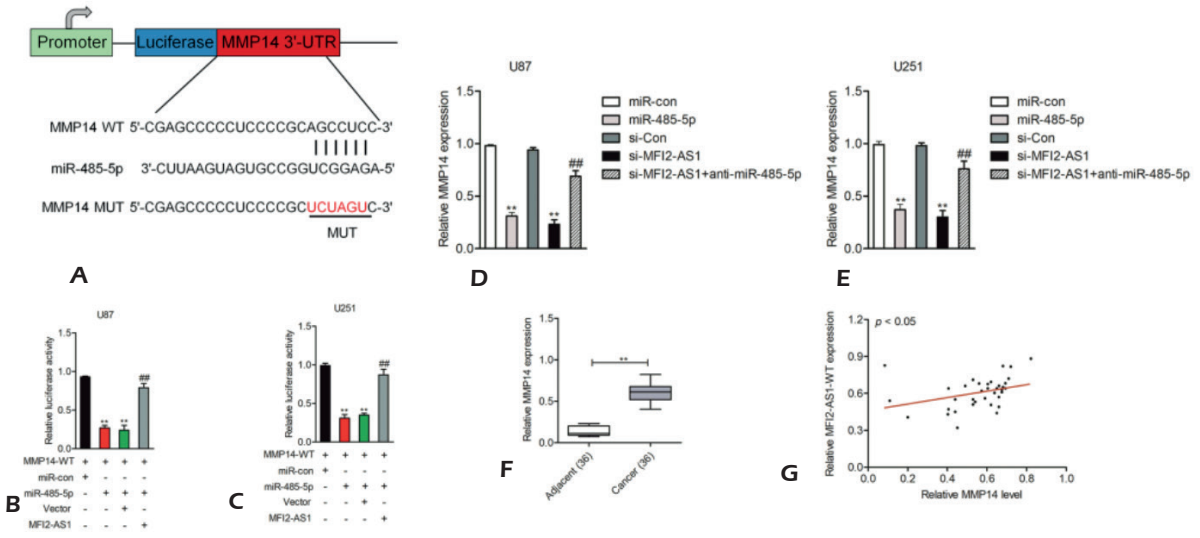

Figure 6. MFI2-AS1 positively regulates the expression of MMP14. **A-C**, Luciferase reporter assays were performed to detect luciferase activity in U87 and U251 cells transfected with MMP14 3′-UTR reporter vector and miR-485-5p or MFI2-AS1+miR-485-5p. $^{**}p<0.01$ compared to MMP14 WT+miR-con, $^{\#\#}p<0.01$ compared to MMP14 WT+miR-485-5p. **D-E**, Level of MMP14 in U87 and U251 cells transfected with miR-485-5p, si-MFI2-AS1 or si-MFI2-AS1+anti-miR-485-5p was examined by qPCR assay. $^{**}p<0.01$ compared to miR-con, $^{\#\#}p<0.01$ compared to si-MFI2-AS1. **F**, Expression of MMP14 was measured in glioma tissues (n=36) or adjacent normal (n=36) tissues. $^{**}p<0.01$ compared to the adjacent. **G**, Correlation of MMP14 levels with MFI2-AS1 expression was analyzed in glioma tissues.

of human malignancies, including glioma[12,14,21]. Recently, few evidence[1,2] indicates that MFI2-AS1 is abnormally upregulated and exerts its oncogenic function in clear-cell renal cell carcinoma (ccRCC) and colorectal carcinoma. In the current work, we demonstrated that MFI2-AS1 was overregulated in glioma cells and tissues. In addition, the higher level of MFI2-AS1 was positively related to the ad-

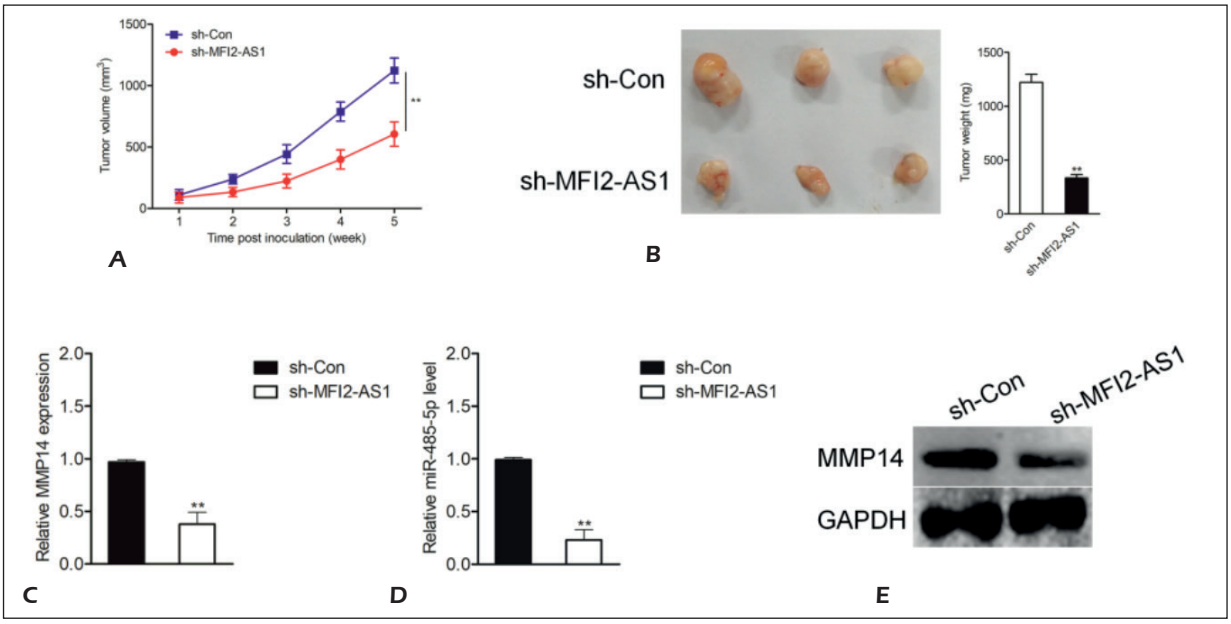

Figure 7. MFI2-AS1 knockdown suppresses tumor growth of glioma cell in vivo. **A**, U87 cells stably transfected with sh-con or sh-MFI2-AS1 were subcutaneously injected into nude mice. Tumor volumes were measured every 7-day using calipers. **B**, Tumor tissues were excised and weighted. **C-D**, Levels of miR-485-5p and MMP14 in the resected tumor tissues were determined by qPCR. **E**, Expression of MMP14 was detected using western blotting assay. $^{**}p<0.01$ compared to sh-Con.

vanced stage and poor clinical outcomes in patients with glioma. The downexpression of MFI2-AS1 restrained the growth, migration, and invasive capacities, and induced the apoptosis of glioma cells, which suggests that MFI2-AS1 was a functional oncogene. Consistent with our findings, a recent investigation indicates that the level of MFI2-AS1 is closely related to lymph metastasis, tumor histological grade, and TNM stage of colorectal cancer. MFI2-AS1 downregulation also represses the growth and aggressiveness of colon cancer cell[2]. All these data proved that MFI2-AS1 could function as a vital oncogene to induce the malignant phenotypes of glioma cell.

Increasing evidence has suggested that lncRNAs are closely related to tumor progression by serving as a ceRNA to regulate the expression of miRNAs' targets. For instance, lncRNA CASC9 modulates the invasion and migration of hemangioma endothelial cells by regulating miR-125a-3p/Nrg1[22]. In the colon cancer, lncRNA B3GALT5-AS1 suppresses the liver metastasis of cancer cell by repressing miRNA-203[23]. In the current study, online bioinformatics analysis combination with luciferase reporter gene assay indicated that MFI2-AS1 bound with miR-485-5p in glioma cells. Increasing evidence has proved that miR-485-5p exerts its inhibitory functions in various malignancies by suppressing its target proteins. MiRNA-485-5p inhibits the invasion and growth of HCC by regulating stanniocalcin 2 (Stc2)[24]. Additionally, the overexpression of miR-485-5p suppresses the growth of HCC cell *in vitro* and *in vivo* by the down-regulation of EMMPRIN[25]. Our data indicated that miR-485-5p was downregulated in glioma and the overexpression of miR-485-5p suppressed the growth, migration, and invasion in glioma cells. In addition, the downregulation of MFI2-AS1 mediated the aggressive phenotypes inhibition and was reversed by the downregulation of miR-485-5p.

Matrix metalloproteinases (MMPs) is comprised of 24 zinc-containing enzymes, which share several functional domains[26]. MMPs are involved in various aspects of cancer progression, including cancer cell diffusion, metastasis, and tumor angiogenesis[27,28]. Yan et al[29] have reported that MMP14 modulates the migration, and invasion of cancer cell by regulating EMT in nasopharyngeal carcinoma. MMP14 is also overexpressed in colorectal cancer and has prognostic value in patients with colorectal carcinoma[30]. In gliomas, the inhibition of MMP14 potentiates the therapeutic effect of temozolomide and radiation[31]. Nevertheless, whether MMP14 could be modulated by MFI2-AS1 in glioma was uncertain. Herein, we observed that MMP14 competitively bound to miR-485-5p with MFI2-AS1. In addition, the downregulation of MFI2-AS1 reduced the level of MMP14 *via* binding to miR-485-5p in glioma. All results indicated that MFI2-AS1 facilitated the development of glioma by regulating the MMP14 level.

Conclusions

We revealed that MFI2-AS1 was overexpressed in glioma and its level was associated with the progression of glioma. Downregulation of MFI2-AS1 inhibited the growth, metastatic traits, and induced apoptosis of glioma cell *via* binding with miR-485-5p and further decreasing the level of MMP14.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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