LncRNA SBF2-AS1 Promotes Malignant Phenotypes and Radioresistance of Glioblastoma Cells by Impairing miR-338-3p-targeted MAP4K3 Inhibition

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Research

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Abstract

Background: Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumors in adults. LncRNA SBF2-AS1 has been reported to promote malignant progression in multiple human cancers. However, the roles and underlying mechanisms of SBF2-AS1 in GBM are still unknown.

Methods: The levels of SBF2-AS1 were identified in GBM tissues and normal brain tissues, and in GBM cell lines and normal human astrocytes. The effect of SBF2-AS1 on proliferation, invasion and radiosensitivity was evaluated by CCK-8 assay, transwell assay, γ -H2AX foci assay and clonogenic survival assay in vitro and in vivo. The interactions among SBF2-AS1, miR-338-3p and MAP4K3 were validated through luciferase report, RNA immunoprecipitation and western blot assays.

Results: In the present study, we confirmed that SBF2-AS1 was highly upregulated in GBM tissues and cell lines, and its levels were markedly higher in WHO stage III-IV gliomas compared with WHO stage I-II gliomas. Kaplan-Meier survival analysis revealed that glioma patients with high SBF2-AS1 expression had shorter overall survival than those with low SBF2-AS1 expression. SBF2-AS1 inhibition suppressed the proliferation of GBM cells by down-regulating of Ki-67 and CyclinD1. Moreover, silencing SBF2-AS1 increased the proportion of cells in G1 phase and decreased the proportion of cells in S phase. In addition, SBF2-AS1 inhibition suppressed the invasion of GBM cells by down-regulating of MMP2 and MMP9. Xenograft tumor models revealed that the silencing of SBF2-AS1 inhibited tumor growth in vivo. Moreover, the silencing of SBF2-AS1 significantly sensitized GBM cells to radiation in vitro and in vivo. Indeed, we observed a persistence of γ -H2AX staining after ionizing radiation, leading to enhanced DNA repair and radioresistance. Mechanistically, SBF2-AS1 inhibition reduced the expression of MAP4K3 by directly binding with miR-338-3p in GBM cells.

Conclusions: Our study demonstrated that SBF2-AS1 enhanced cell proliferation, invasion and radioresistance via modulating miR-338-3p/MAP4K3 axis, and it is an attractive therapeutic target to overcome radioresistance of GBM.

Background

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumors in adults. Currently, the standard therapeutic treatment consists of maximum surgical resection, followed by radiotherapy plus concomitant and adjuvant temozolomide chemotherapy ^[1]. However, the prognosis of GBM patients remains poor with a median survival around 12–14 months ^[2]. Several different factors attenuate the development of successful treatments against GBM, including its intrinsic aggressive and infiltrative nature, that lead to therapeutic resistance and recurrence ^[3]. Therefore, it is imperative to uncover novel mechanisms of tumorigenesis and radioresistance, and that might provide new approaches for GBM treatment.

Long non-coding RNAs (LncRNAs) are a class of remarkable RNAs with a size of more than 200 nucleotides, which can control gene expression at the transcriptional and post-transcriptional levels,

thereby inactivating or stabilizing proteins [4]. It is a key player in cell differentiation, tumorigenesis, immune response and other biological processes [5]. To date, lncRNAs can be used as a competitive endogenous RNA (ceRNA) to inhibit the level of miRNA targeted mRNA through miRNA sponge [6]. Based on this hypothesis, the "lncRNA-miRNA-mRNA" axis has been explored and validated in a variety of tumors including GBM [7, 8].

lncRNA SBF2 antisense RNA1 (SBF2-AS1) was initially identified in non-small cell lung cancer, which plays the role of oncogene and promotes the occurrence and development of lung cancer, and is closely related to poor prognosis [9]. SBF2-AS1 can adsorb miRNAs (including miR-188-5p, miR-361-5p and miR-140-5p) to promote the evolution and deterioration of acute myeloid leukemia, cervical cancer and liver cancer, respectively [10–12]. It was reported that SBF2-AS1 was closely related to the occurrence of autophagy in GBM, and which is a tolerance mechanism of cells against tumor therapy [9]. It was also found that SBF2-AS1 was significantly up-regulated in temozolomide (TMZ)-resistant GBM cell lines and tissues, and overexpression of SBF2-AS1 enhanced TMZ resistance [10]. However, the function and underlying mechanism of SBF2-AS1 in GBM remain unclear.

Materials And Methods

Patients and tissue samples, cell culture, and irradiation

All primary GBM tissues and adjacent normal tissues were collected from patients who had undergone surgery at the Department of Neurological Surgery, Provincial Hospital Affiliated to Shandong First Medical University between March 2014 and March 2016. Experienced pathologist confirmed all tumors and paired adjacent tissues. This study was approved by the Ethics Committee of Provincial Hospital Affiliated to Shandong First Medical University. All methods were performed in accordance with the relevant guidelines and regulations. U87MG, LN229, T98G, LN18 and U251 were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. All the cells were cultured in DMEM medium supplemented with 10% fetal bovine serum in humidified air at 37 with 5% CO₂. IR was carried out with 6 MV X-ray produced by linear accelerator (Varian 23EX). The dose rates were about 2 Gy/min.

SiRNA and mimic transfection

The control siRNA and siRNA pool against SBF2-AS1 or miR-338-3p were purchased from Invitrogen. U87MG and LN18 cells were grown to 50% confluence, and the transfection was conducted with Lipofectamine 2000 according to the manufacturer's instructions.

Quantitative real-time PCR

Total RNAs in GBM tumor tissues and cells were isolated using Qiazol kit (Qiagen) and TRIzol reagent (Invitrogen). The synthesis of cDNA was performed using GlodScript one-step qRT-PCR Kit (Applied Biosystems). Real-time fluorescence quantitative PCR reaction was carried out on ABI 7500 Fast Instrument with SYBR Green Kit (Takara). Primers were designed with Primer Express 3.0 software. Relative quantities were normalized to U6 or GAPDH, and the data were explicated by $2^{-\Delta\Delta Ct}$ method.

Western blot analysis

Whole-cell lysates were prepared as described previously. The antibodies against Ki-67, CyclinD1, matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 9 (MMP9), Mitogen-activated protein kinase kinase kinase 3 (MAP4K3) and β -actin were purchased from Abcam and incubated overnight at 4°C. The membrane was scanned and analyzed using Odyssey Infrared Imaging System (LI-COR Biosciences).

Cell proliferation assay

Cell proliferation was determined by using Cell Counting Kit (CCK)-8 Assay, according to the manufacturer's instructions. Briefly, 1×10^4 cells were incubated into 96-well plate for 24 hours. Then, 10 μ l CCK-8 solution was added and incubated for 2 hours. The optical density (OD) values of each well were measured at 450 nm by microplate reader (Bio-Rad).

Cell cycle assay

Cell cycle assay was performed using U87MG and LN18 cells transfected with si-NC and si-SBF2-AS1. After transfecting for 48 h, the cells were harvested and then stained with propidium iodide. Flow cytometric analysis was performed using BD FACS Calibur (BD Biosciences). Experiments were performed in triplicate.

Transwell assay

Transwell assay was used to assess cell invasion ability using a BioCoat™ Matrigel™ Invasion. The plate was warmed up to room temperature for 10 min. Then, 750 μ l DMEM containing 10% FBS was added to the lower chambers and incubated for 2 hours at 37°C. Cells (3×10^4 cells, 500 μ l) were added to the upper chambers and incubated for 22 hours, and then proceeded to fix cells. The cells were fixed by 4% paraformaldehyde for 5 min, stained by 0.4% crystal violet for 20 min, and photographed under a microscope.

γ -H2AX foci assay

Briefly, cells were fixed in 4% paraformaldehyde for 15 minutes and washed 2 times with PBS. Cells were permeabilized in 0.2% Triton X-100 for 5 minutes on ice, and blocked in 10% normal goat serum. Then, cells were stained with γ -H2AX primary antibody (Millipore) for 1 hour and incubated with secondary antibody for 1 hour at room temperature. Cells were washed 3 times with PBS and mounted using Vectashield-mounting medium with DAPI. Fluorescent images were acquired using Carl Zeiss Fluorescence microscope (Germany) equipped with Axiovision software (version 4.8) for camera control, image acquisition, processing, and a module for multichannel display.

Clonogenic survival assay

Cell radio-sensitivity was evaluated by loss of colony-forming ability. Briefly, 2×10^5 cells were plated, per 60 mm dish with 3 ml of medium. The cells were irradiated and plated to obtain densities of 20 to 200 colonies per dish. Duplicate dishes were prepared for each radiation dose. The cells were incubated for

12–14 days to allow colonies to develop. Colonies were stained with crystal violet in 100% methanol solution.

Tumor xenograft in nude mice and radio-sensitivity studies

We carefully followed a protocol approved by the Ethics Committee of Provincial Hospital Affiliated to Shandong First Medical University to perform this animal work. Briefly, 2×10^6 cells lines were subcutaneously injected into both hind legs of each mouse as described in our previous report [15, 16]. Tumor volumes were measured at different time points. When the xenografts formed at 12 days after cell inoculation, the mice carrying similar size tumors were chose at both hind legs for the following experiments (each type of cells formed 6 tumors in 3 mice, 3 tumors in 3 mice were exposed to x-ray and 3 tumors in 3 mice were exposed to iron exposure). After the mice were anesthetized, the left hind leg of each mouse was exposed to radiation and the right leg was used as a mock-irradiation control. The same legs were irradiated with 3 Gy x 3 again at 48 hours intervals. The mice were sacrificed about two weeks after the final irradiation, and the tumors were removed and weighed.

Luciferase assay

The binding sites of SBF2-AS1 and miR-338-3p were divided and interpreted using RNA 22 website. The wild-type (WT) and mutant (MUT) sequences of SBF2-AS1 3' untranslated region (3'UTR) gene fragment were inserted into pMIR-REPORTER™ Luciferase plasmid (Premega) to generate the SBF2-AS1-WT and SBF2-AS1-MUT, respectively. 293T cells were transfected with the plasmids in 48-well plates. The cells were harvested 48 hours after transfection. The cells were then lysed with Luciferase Assay Kit according to the manufacturer's protocol and were measured using Glomax 20/20 luminometer (Premega).

RNA immunoprecipitation (RIP) assay

RIP assay was performed using EZMagna RIP RNA-binding protein immunoprecipitation Kit (Millipore) according to the manufacturer's instructions. The cells were lysed and incubated with RIP buffer containing magnetic beads coated with Ago2 antibodies (Millipore), while IgG acted as a negative control. After incubation for 2 hours at 4°C, co-precipitated RNAs were isolated by TRIzol reagent and measured by PCR analysis.

Results

SBF2-AS1 was up-regulated in GBM tissues and cell lines and was correlated with tumor progression and poor survival in GBM patients.

The online system GEPIA (<http://gepia.cancer-pku.cn/>) suggested that SBF2-AS1 is highly expressed in the TCGA-GBM DataBase (Fig. 1A). Kaplan-Meier survival analysis revealed that glioma patients with high SBF2-AS1 expression had shorter overall survival than those with low SBF2-AS1 expression (Fig. 1B). To explore the role of SBF2-AS1 in GBM progression, we further detected the expression SBF2-AS1 in GBM tissues and adjacent normal tissues by qRT-PCR. The results showed that the mRNA level of SBF2-AS1 was clearly elevated, and miR-338-3p was attenuated in GBM tissues compared to the

adjacent normal tissues (Fig. 1C). SBF2-AS1 was also significantly overexpressed in the high-grade gliomas (HGGs; grade III or IV) compared with that in the low-grade gliomas (LGGs; all grade II) (Fig. 1D). The mRNA level of SBF2-AS1 was further determined in GBM cell lines, including U87MG, LN18, U251 and T98G cells, and the normal astrocyte cells. Our results showed that the expression of SBF2-AS1 was much higher in all five GBM cell lines compared with normal astrocyte cells (Fig. 1E). U87 and LN18 cells were selected for further studies. These results suggested that SBF2-AS1 served as an independent factor for advanced tumor progression and poor prognosis in patients with GBM.

SBF2-AS1 promotes proliferation and invasion of GBM cells

To identify the function of SBF2-AS1 on GBM progression, si-SBF2-AS1 was transfected into U87MG and LN18 cells. CCK-8 assay showed that the proliferation ability of U87MG and LN18 cells was remarkably decreased after silencing of SBF2-AS1 (Fig. 2A-B). The protein expression of Ki-67 and CyclinD1 in U87MG and LN18 cells were further measured by Western blot analysis, and the data showed that the protein level of Ki-67 and CyclinD1 was significantly declined in the si-SBF2-AS1 group, contrast to the blank group and the si-NC group (all $P < 0.05$) (Fig. 2C-D). Next, transwell assay was conducted to evaluate the migration and invasion of U87MG and LN18 cells. The results indicated that the migration and invasion ability of GBM cells was inhibited in the si-SBF2-AS1 group, contrast to the blank group and the si-negative control (NC) group (all $P < 0.05$) (Fig. 2E). Moreover, the protein level of MMP2 and MMP9 was clearly reduced in the si-SBF2-AS1 group, contrast to the blank group and the si-NC group (all $P < 0.05$) (Fig. 2F).

SBF2-AS1 inhibition promotes proliferation of GBM cells by repressing CyclinD1

To identify the function of SBF2-AS1 on GBM proliferation, si-SBF2-AS1 was transfected into U87MG and LN18 cells. CCK-8 assay showed that the proliferation ability of U87MG and LN18 cells was remarkably decreased after silencing of SBF2-AS1 (Fig. 2A-B). Flow cytometry showed that silencing SBF2-AS1 increased that proportion of cells in G1 phase and decreased the proportion of cells in S phase (Fig. 2C-D). The protein expression of Ki-67 and CyclinD1 in U87MG and LN18 cells were further measured by Western blot analysis, and the data showed that the protein level of Ki-67 and CyclinD1 was significantly declined in the si-SBF2-AS1 group, contrast to the blank group and the si-NC group (all $P < 0.05$) (Fig. 2E-F).

SBF2-AS1 inhibition promotes invasion of GBM cells by repressing MMP2 and MMP9

Next, transwell assay was conducted to evaluate the migration and invasion of U87MG and LN18 cells. The results indicated that the migration and invasion ability of GBM cells was inhibited in the si-SBF2-AS1 group, contrast to the blank group and the si-NC group (all $P < 0.05$) (Fig. 3A-B). Moreover, the protein level of MMP2 and MMP9 was clearly reduced in the si-SBF2-AS1 group, contrast to the blank group and

the si-NC group (all $P < 0.05$) (Fig. 3C-E). These results indicated that SBF2-AS1 inhibition attenuated the invasion of GBM cells.

SBF2-AS1 inhibition increases GBM cells sensitivity to ionizing radiation in vitro

To investigate whether the specific inhibition of SBF2-AS1 may modify the cellular radiosensitivity, γ -H2AX assay was used to analyze the DNA double strand breaks (DSBs) repair efficiency because the signals of γ -H2AX reflect the amounts of DNA DSBs in irradiated cells and indirectly describe the efficiency of DNA DSBs repair. The results showed that there was no apparent difference in the γ -H2AX-positive ratios among the examined cells immediately after IR (30 minutes), suggesting that there was not much difference in the induction of DNA DSBs in these irradiated cells, however, the γ -H2AX-positive ratios clearly increased in the irradiated cells in siRNA-SBF2-AS1 group compared with that in si-NC group (Fig. 4A-C). Next, the impact of silencing SBF2-AS1 on GBM cell survival after irradiation was examined by a clonogenic assay to analyze the reproductive death in U87 and LN18 cell. The results showed SBF2-AS1 inhibition made U87 and LN18 cells more sensitive to radiation after a 2 Gy irradiation (Fig. 4D-E). These results indicated that SBF2-AS1 inhibition increases GBM cells radio-sensitivity and radiation-induced cell death.

SBF2-AS1 inhibition increases GBM cells sensitivity to ionizing radiation in vivo

To evaluate the effects of SBF2-AS1 inhibition on GBM cells growth and radiosensitivity in xenograft mouse model, we subcutaneously injected the cell lines (U87MG and LN18 cells, or expressing si-NC and si-SBF2-AS1) to both hind legs of each nude mouse. After tumor formed, we exposed the left hind leg of each mouse including the tumor area to X-ray radiation (3 Gy, 3 times at 48 h intervals) and the right hind leg including the tumor area was used as the mock-irradiation control. Twelve days after final irradiation, the mice were killed and tumors were removed. The results showed that the volume and weight of the tumors was clearly reduced in si-SBF2-AS1 group, contrast to the blank and si-NC group, indicating that si-SBF2-AS1 inhibition could suppress the tumor growth in GBM (Fig. 5A-B). Similar to the *in vitro* data, the size and weight of the tumors in si-SBF2-AS1 group were much more smaller than that of the tumors in the blank and si-NC group (Fig. 5C-F), indicating that SBF2-AS1 plays an important role in the resistance of irradiation.

SBF2-AS1 directly binds with miR-338-3p in GBM

As increasing studies revealed that lncRNAs could function as ceRNAs for miRNA in tumor progression, we explored the interaction between SBF2-AS1 and miRNA in GBM cells. RNA 22 website revealed that SBF2-AS1 could specifically bind to miR-338-3p (Fig. 6A). Dual-luciferase assay results showed that the luciferase activity was remarkably attenuated in co-transfected with miR-338-3p mimics group (Fig. 6B).

RIP assay results showed that SBF2-AS1 and miR-338-3p expression were significantly enriched in Ago2 pellet compared to IgG control (Fig. 6C-D).

SBF2-AS1 regulates MAP4K3 expression via miR-338-3p in GBM

Our previous study revealed that MAP4K3 was a target of miR-338-3p in glioma. In the present study, MAP4K3 was significantly increased in GBM tumor tissues and cell lines (Fig. 7A-B). Then, we explored whether SBF2-AS1 could regulate MAP4K3 via miR-338-3p. The data showed that SBF2-AS1 inhibition clearly reduced MAP4K3 expression in U87MG and LN18 cells, while miR-338-3p mimics could reverse this effect (Fig. 7D). Furthermore, MTT assay showed that miR-338-3p mimics significantly rescued the proliferation of SBF2-AS1 silenced U87MG and LN18 cells. These results indicated that SBF2-AS1 promotes GBM progression through regulating miR-338-3p/MAP4K3 axis.

Discussion

Increasing studies have demonstrated that lncRNAs might act as tumor oncogenes in the initiation and progression of human cancers, and several lncRNAs may even become novel biomarkers in cancer. For example, lncRNA ZFAS1 might be a poor prognostic factor and promote tumor progression, including non-small cell lung cancer and esophageal squamous cell carcinoma [17, 18]. However, the roles and underlying mechanisms of lncRNAs in GBM remain unknown. In the present study, we identified that SBF2-AS1 was up-regulated in GBM and its upregulation is clinically associated with advanced pathologic stages and poor prognosis in GBM patients. Silencing SBF2-AS1 inhibited the proliferation, invasion and radioresistance of GBM *in vitro* and *in vivo*. Mechanistically, SBF2-AS1 served as a microRNA sponge for miR-338-3p and consequently modulates the overexpression of MAP4K3, suggesting a promising therapeutic and diagnostic direction for GBM.

In our study, there presented high expression of SBF2-AS1 was correlated with tumor progression and poor survival in GBM patients. Consistent with our study, a study reported that SBF2-AS1 was found to be independent prognostic factors for glioma patients, and it was an autophagy-related lncRNA with unfavorable factor [13]. SBF2-AS1 may serve as a diagnostic marker for GBM chemoresistance [14]. Moreover, silencing SBF2-AS1 inhibited cell proliferation and invasion of GBM cells by repressing CyclinD1, MMP2 and MMP9, indicating that SBF2-AS1 might play important role in GBM progression. Recently, increasing studies showed that SBF-AS1 promoted tumorigenesis and progression by sponging miRNAs. For example, Tian et al. reported that SBF2-AS1 acted as a ceRNA to modulate cell proliferation via binding with miR-188-5p in acute myeloid leukemia [10]. Gao et al. showed that SBF2-AS1 promoted the progression of cervical cancer by regulating miR-361-5p [11]. Li et al. found that SBF2-AS1 promotes hepatocellular carcinoma progression through regulation of miR-140-5p-TGFBR1 pathway [19]. In the present study, we showed that miR-338-3p was clearly decreased and negatively correlated with SBF2-AS1 expression in GBM. In addition, luciferase reporter and RIP assay showed that SBF2-AS1 acted as a ceRNA to sponge miR-338-3p in GBM progression.

MAP4K3 (also known as serine/threonine protein kinase GLK) is a member of MAPK kinase that regulates a variety of cellular activities, including cell proliferation, cell migration, and cell differentiation [20, 21]. Upregulation of MAP4K3 is a major factor in the development and progression of various tumors [22–24]. MAP4K3 was firstly identified to play an oncogenic role in paediatric high grade glioma [25]. However, the underlying mechanisms remain unclear. Our previous study found that miR-338-3p could inhibit the malignant biological behavior of glioma cells via targeting MAP4K3 [26]. Our study firstly showed that SBF2-AS1 suppression significantly reduced MAP4K3 expression, while miR-338-3p mimics significantly reversed the effects and further rescued the proliferation of SBF2-AS1 silenced GBM cells. Silencing MAP4K3 could suppress the proliferation of GBM cells. These results indicated that MAP4K3 played an important role in the progression of GBM.

Postoperative radiotherapy is one of the main modalities of GBM treatment, but it often fails to achieve the expected results mainly due to tumor radioresistance. Increasing studies showed that lncRNAs have been found to modulate radiosensitivity by regulating various mechanisms [27]. Wang et al. reported that lncRNA PVT1 served an oncogenic role and played an important role in radiosensitivity in malignant nasopharyngeal carcinoma via activating the KAT2A acetyltransferase and stabilizing HIF-1 α [28]. Another study suggested that lncRNA TP73-AS1 was highly expressed in hepatocellular carcinoma and participated in radioresistance via PTEN/AKT signaling pathway [29]. In the present study, silencing of SBF2-AS1 enhanced radiosensitivity of GBM cells by down-regulating MAP4K3 via miR-338-3p. However, the mechanisms by which MAP4K3 regulates cell radiosensitivity remained unclear.

Conclusions

Over all, the results of this study demonstrated that SBF2-AS1 served as a sponge of miR-338-3p to elevate MAP4K3. SBF2-AS1 facilitates cell proliferation, cell invasion, tumor growth and radiosensitivity in GBM via miR-338-3p/MAP4K3 axis, which provides a novel therapeutic target for GBM treatment.

Abbreviations

GBM
glioblastoma multiforme; lnc RNA:long non-coding RNA; ceRNA:competitive endogenous RNA; SBF2-AS1:lncRNA SBF2 antisense RNA1; TMZ:temozolomide; DMEM:Dulbecco's modified eagle's medium; qRT-PCR:quantitative real-time PCR; MMP:matrix metalloproteinase; MAP4K3:mitogen-activated protein kinase kinase kinase kinase 3; OD:optical density; WT:wild-type; MUT:mutant; 3' UTR:3' untranslated region; RIP:RNA immunoprecipitation; HGG:high-grade glioma; LGG:low-grade glioma; NC:negative control; DSBs:double strand breaks

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Provincial Hospital Affiliated to Shandong First Medical University.

Consent for publication

Authors confirmed that this work can be published. The content of this manuscript is original and it has not yet been accepted or published elsewhere.

Availability of data and materials

Materials are available upon request.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Hong pan conducted all experiments with the assistance of Jiajun Xu and Zheng Wang. Guodong Wang and Zhigang Yao made statistical analysis. Zhiming Zheng designed the study and wrote this paper. All authors read and approved the final manuscript.

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Figures

Figure 1

SBF2-AS1 was up-regulated in GBM tissues and cell lines, and was correlated with tumor progression and poor survival in GBM patients. (A) SBF2-AS1 was highly expressed in the TCGA-GBM DataBase (GEPIA). (B) Kaplan-Meier survival analysis revealed that glioma patients with high SBF2-AS1 expression had shorter overall survival than those with low SBF2-AS1 expression. (C-D) SBF2-AS1 expression in normal brain tissues and GBM tissues was analyzed by qRT-PCR. (F) SBF2-AS1 expression in astrocytes and GBM cell lines was analyzed by qRT-PCR. *P<0.05.

Figure 2

SBF2-AS1 promoted proliferation of GBM cells by regulating Ki-67 and CyclinD1. (A-B) SBF2-AS1 inhibition reduced the growth of U87MG and LN18 cells. (C-D) Cell cycle assay was performed using U87MG and LN18 cells transfected with si-NC and si-SBF2-AS1. (E-F) The protein levels of Ki-67 and CyclinD1 were determined by Western blot. *P<0.05.

Figure 3

SBF2-AS1 promoted invasion of GBM cells by regulating MMP2 and MMP9. (A-B) Cell invasion was evaluated by Transwell assay. (C-E) The protein levels of MMP2 and MMP9 were determined by Western blot. * $P < 0.05$.

Figure 4

Silencing of SBF2-AS1 sensitized GBM cells to IR-induced killing. (A) Incidence of γ -H2AX foci in irradiated U87MG and LN18 cells. Cells were irradiated with 2Gy, incubated at 37 at different times and then processed for γ -H2AX foci counting). (B-C) The effect of SBF2-AS1 inhibition on γ -H2AX foci in GBM cells at different times after 2Gy exposure. (D-E) The effect of SBF2-AS1 inhibition on cell radiosensitivity was conducted by clonogenic assay. * $P < 0.05$.

Figure 5

Silencing of SBF2-AS1 inhibited cell growth and enhanced radiosensitivity of GBM in vivo. (A) The effects of SBF2-AS1 on U87MG tumor growth, the volume of tumor=length×width×height. (B) The effects of SBF2-AS1 on LN18 tumor growth, the volume of tumor=length×width×height.(C) U87MG tumor in nude mice treated with radiotherapy after injected with si-NC and si-SBF2-AS1. The left hand leg that born the developed tumor was exposed to IR(3Gy 3, at 48h intervals). (D) LN18 tumor in nude mice treated with radiotherapy after injected with si-NC and si-SBF2-AS1. The left leg borning the tumor was exposed to IR (3Gy × 3, at 48h intervals). The data shown are the mean and SE.*P<0.05.

Figure 6

miR-338-3p was sponged by SBF2-AS1. (A) Luciferase reporter vectors that carry cDNA sequences of SBF2-AS1 containing the wild-type or mutant miR-338-3p binding site and the seed region of miR-338-3p. (B) Relative luciferase activity in HEK293T cells which were co-transfected pMIR-SBF2-AS1-Wt or Mut and miR-338-3p mimics were determined. (C, D) RNA immunoprecipitation assay was performed with normal mouse IgG or anti-Ago2 in U87 cells. Relative expression of SBF2-AS1 was determined by qRT-PCR. *, # P<0.05; **P<0.01.

Figure 7

MAP4K3 was upregulated and was involved in SBF2-AS1-mediated GBM cell-driven proliferation. (A) Expression of MAP4K3 was detected in GBM tissues by qRT-PCR. (B) Expression of MAP4K3 was detected by Western blot in normal human astrocytes, U87MG, LN18, T98G and U251 cell lines. (C) Association of MAP4K3 expression levels with WHO grade. (D) SBF2-AS1 regulated MAP4K3 expression via miR-338-3p in GBM cells. (E, F) Effects of MAPK4K3 inhibition on GBM cell growth were measured. $*P < 0.05$; #, $**P < 0.01$.