RESEARCH PAPER

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lncRNA MAFG-AS1 enhances radioresistance of glioblastoma cells *via* miR-642a-5p/Notch1 axis

Xi Zhang[1], Rui Li[2]*

[1] Department of Oncology, Ya'an People's Hospital, Ya'an, P.R. China,
[2] Department of Neurosurgery, Wenjiang District People's Hospital, Chengdu, P.R. China,
** Email: lirui197606@163.com*

This study was designed to explore the function of lncRNA MAFG-AS1/miR-642a-5p/Notch1 in glioblastoma (GBM) cells under radiation. GBM cells (M059K and M059J) were transfected or/and irradiated. Western blotting was used to detect Notch1 protein and its downstream Hes1 protein. Cell Counting Kit-8 assay and flow cytometry were applied for viability and apoptosis tests, respectively. Luciferase reporter plasmids and Ago2 antibody were used to verify the predicted binding of miR-642a-5p to MAFG-AS1 or Notch1 mRNA. Notch1 and MAFG-AS1 were highly expressed and miR-642a-5p was lowly expressed in radioresistant M059K cells. Knockdown of Notch1 inhibited radioresistance and promoted apoptosis in M059K cells. MAFG-AS1 competed with Notch1 mRNA for binding of miR-642a-5p and therefore promoted the expression of Notch1. Overexpression of miR-642a-5p or silencing of MAFG-AS1 inhibited the radioresistance of M059K cells. Knockdown of Notch1 or overexpression of miR-642a-5p reduced viability and increased apoptosis in irradiated M059J cells overexpressing MAFG-AS1. MAFG-AS1 reduces the radiosensitivity of GBM cells *via* miR-642a-5p/Notch1 axis.

Key words: lncRNA MAFG-AS1, miR-642a-5p, Notch1, glioblastoma, radioresistance

INTRODUCTION

Glioblastoma (GBM) is the most common and malignant brain tumor, with an average survival of only 15 months after diagnosis (Lah et al., 2020). The current standard treatment for most newly diagnosed GBMs is composed of surgical resection followed by radiotherapy in combination with temozolomide chemotherapy (Le Rhun et al., 2019). However, instances of GBM with resistance to standard treatment often display high levels of both intra- and inter-tumoral heterogeneity that resists anti-tumor therapies (Chen and Hambardzumyan, 2018). Although the precise pathogenic mechanisms of GBM remain unknown, increasing evidence has supported a strong connection of GBM with different types of epigenetic modifications (Uddin et al., 2020). Molecules that control these alterations have emerged as promising targets for improving the outcomes of GBM.

Long noncoding RNAs (lncRNAs) are a heterogeneous class of transcripts (> 200 nucleotides) that have a critical role in transcription regulation (Kopp and Mendell, 2018). Over the past decade, an increasing number of lncRNAs have been demonstrated to have associations with therapy resistance and malignant potential of tumor cells in GBM, such as SNHG12, MALAT1, HOXA-AS3 and AHIF (Chen et al., 2020; Liao et al., 2019; Lu et al., 2020). A recent study found that lncRNA MAFG-AS1 was highly expressed in GBM and promoted the viability of GBM cells by suppressing the maturation of microRNA-34 (miR-34a) (Zhao et al., 2021). However, no report has indicated whether MAFG-AS1 is implicated in the radioresistance of GBM.

Notch signaling is an important player in both cancer cell and tumor microenvironment, in structures including vascular vessels, immune infiltrates, fibroblasts and extracellular matrix (Meurette and Mehlen, 2018). Notch1 signaling is activated in GBM and pro-

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motes the self-renewal, invasion and growth of glioma-initiating cells by stimulating the CXCR4/AKT pathway (Yi et al., 2019). Vasorin, induced by hypoxia, maintains the stemness and tumorigenic potential of glioma stem-like cells by stabilizing Notch1 protein in the cell membrane (Man et al., 2018). Knockdown of Notch1 sensitizes glioma stem cells to radiation by reducing AKT activity and Mcl-1 levels (Wang et al., 2010), but how Notch1 is regulated in the radiosensitivity of glioma remains unknown.

Since MAFG-AS1 and Notch1 are both demonstrated to be oncogenic regulators in GBM, we speculated that MAFG-AS1 might cooperate with Notch1 in promoting GBM. lncRNAs often act as microRNA (miRNA) sponges to suppress the regulatory effects of miRNAs (Akhade et al., 2017). MiRNAs are another class of noncoding RNAs that control cellular activities through complementary binding with target mRNAs and subsequent translation inhibition or mRNA degradation (Rupaimoole and Slack, 2017). Based on bioinformatics analysis, we found miR-642a-5p was a target of MAFG-AS1 and could also bind Notch1 mRNA. In this context, this original research explored the function of MAFG-AS1/miR-642a-5p/Notch1 axis in the radioresistance of GBM cells.

METHODS

Cell culture

GBM cells (M059K and M059J) and HEK-293T cells (ATCC, Manassas, VA, USA) were cultured in DMEM/F12 (PM150312B, Procell Life Science & Technology Co., Ltd., Wuhan, China) and DMEM, respectively (37°C, 5% CO_2). All media contained 10% fetal bovine serum, 1% penicillin and 1% streptomycin, and media for GBM cells was additionally supplemented with 1% nonessential amino acids and 1 mM sodium pyruvate (S104174, Aladdin, Shanghai, China). Adherent cells were passaged using 0.25% trypsin (HyClone, Logan, UT, USA). Experiments were performed on cells in the logarithmic growth phase.

Cell transfection and radiation treatment

miR-642a-5p mimic and the negative control of miR-642a-5p mimic (mimic NC), were transfected into cells at a concentration of 50 nM; from RiboBio, Guangzhou, China. Short hairpin Notch1 RNA (sh-Notch1), the negative control of sh-Notch1 (sh-NC_1), Notch1 overexpression pcDNA3.1 vector (oe-Notch1), the negative control of oe-Notch1 (oe-NC_1, empty pcDNA3.1 vector), MAFG-AS1 suppression vector (sh-AS1), the negative control of sh-AS1 (sh-NC_2), MAFG-AS1 overexpression pcDNA3.1 vector (oe-AS1), the negative control of oe-AS1 (oe-NC_2, empty pcDNA3.1 vector), were transfected into cells at a concentration of 2 μg; from GenePharma, Shanghai, China. The above transfection reagents were introduced into GBM cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). GBM cells were accordingly named: blank group (untreated cells), sh-Notch1 group, sh-NC_1 group, miR-642a-5p mimic group, mimic NC group, sh-AS1 group, sh-NC_2 group, oe-AS1 + miR-642a-5p mimic group, and oe-AS1 + sh-Notch1 group. Successfully transfected cells were cultivated in serum-free medium for 8 h and then in 10% FBS-containing medium (5% CO_2, 37°C, 95% humidity) for 48 h prior to experiments.

GBM cells were exposed to radiation (1 to 10 Gy) from VARIAN 2300 EX linear accelerator 6MVX for 48 h.

MAFG-AS1 location

M059K cells were washed in 500 μL phosphate buffered saline (PBS) and collected *via* centrifugation at 4°C for 5 min. PARIS™ kit (Invitrogen, Carlsbad, CA, USA) was used to isolate MAFG-AS1 from the nucleus and cytoplasm of M059K cells. Quantitative reverse transcription PCR (qRT-PCR) was performed to analyze the level of MAFG-AS1 (U6 was used as the internal control for nuclear MAFG-AS1, and GAPDH for cytoplasmic MAFG-AS1).

Cell counting kit (CCK)-8 assay

GBM cells were pre-cultured for 24 h and transfected in a 96-well plate (1×10^4 cells/well). Transfected or/and irradiated GBM cells were incubated with CCK-8 reagent (10 μL per well; CK04, Dojindo, Kumamoto, Japan) at 37°C. Optical density (OD) was measured by a microplate reader at 450 nm to determine cell proliferation 3 h after the cells were incubated with CCK-8 reagent.

Flow cytometry

GBM cells were digested with 0.25% EDTA-depleted trypsin (YB15050057, Shanghai Yu Bo Biotech Co., Ltd., Shanghai, China) and centrifuged twice at 2000 rpm for 5 min, with the supernatant discarded after each centrifugation. Cells (1×10^6) were incubated with 100 μL of staining solution (K201-100, BioVision, Milpitas, CA, USA) at room temperature for 15 min and mixed with 1 mL of HEPES buffer (PB180325, Procell, Wuhan,

China). Then the cells were counted by flow cytometry to determine cell apoptosis.

qRT-PCR

PrimeScript RT kit (RR037A, Takara, Kyoto, Japan) and miRcute Plus miRNA first-strand cDNA synthesis kit (TIANGEN Biotech Co., Ltd., Beijing, China) were used for synthesis of cDNA from total RNA extracted by TRIzol method (Invitrogen, Carlsbad, CA, USA). The ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) and SYBR® Premix Ex Taq™ II kit (RR820A, Takara) were used for PCR analysis. GAPDH or U6 acted as the internal reference. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of each target gene (Livak and Schmittgen 2001).

$$\Delta\Delta Ct = \Delta Ct_{\text{experimental group}} - \Delta Ct_{\text{control group}}$$

$$\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{reference gene}}$$

Primers used in PCR experiments were synthesized by Sangon Biotech (Shanghai, China) (Table 1).

Western blotting

GBM cells were lysed with enhanced RIPA buffer (Boster, Wuhan, China) containing protease inhibitors, followed by measurement of protein concentration using a BCA kit (Boster). Proteins separated by 10% SDS-PAGE were electroblotted onto a PVDF membrane which was then placed in 5% BSA at room temperature for 2 h before incubation with diluted antibodies against: Notch1 (1:1000; ab52627, Abcam, Cambridge, UK), Hes1 (1:1000; ab108937, Abcam) or GAPDH (1:2500; ab9485, Abcam) at 4°C overnight. The membrane was then washed and incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibodies (1:2000; ab6721, Abcam) at room temperature for 1 h. The membrane was treated with ECL working reagent (EMD Millipore, Burlington, MA, USA) at room temperature for 1 min, after which excess ECL solution was removed. The membrane was sealed with plastic wrap and exposed to an X-ray film in a dark box for 5 to 10 min. Protein expression was quantified by ImageJ software (NIH, USA) with GAPDH as the reference.

Table 1. Primer sequences.

Name of primer	Sequences
lncRNA MAFG-AS1-F	CGGGAGGAAGATAAACGGGG
lncRNA MAFG-AS1-R	TGACCACGGAACACCTTCAG
miR-642a-5p-F	GCGGTCCCTCTCCAAATGT
miR-642a-5p-R	AGTGCAGGGTCCGAGGTATT
Notch1-F	ACTTGTACTCCGTCAGCGTG
Notch1-R	GTGGGAACTAACTGCCCTGG
GAPDH-F	GCAAGGATGCTGGCGTAATG
GAPDH-R	TACGCGTAGGGGTTTGACAC
U6-F	CTCGCTTCGGCAGCACA
U6-R	ACGCTTCACGAATTTGCGT

F, forward; R, reverse

Dual-luciferase reporter assay

miR-642a-5p binding site in MAFG-AS1 was predicted by starBase (http://starbase.sysu.edu.cn/). miR-642a-5p binding site in Notch1 was predicted by Jefferson (https://cm.jefferson.edu/rna22/Precomputed/). Artificially synthesized wild and mutant MAFG-AS1 3'UTR and Notch1 3'UTR sequences (AS1-WT, AS1-MUT, Notch1-WT and Notch1-MUT; Genscript, Shanghai, China) complementary to the seed sequence of miR-642a-5p were digested by restriction endonucleases SpeI and HindIII and introduced by T4 DNA ligase into pMIR-reporters (Promega, Madison, WI, USA). Luciferase reporter plasmids with correct sequences were co-transfected with miR-642a-5p mimic or mimic NC into HEK-293T cells. Luciferase activity was detected by the Dual-Luciferase Reporter Assay System (Promega) and the luminescence value was measured by a luminescence detector (Promega). Relative luciferase activity=target fluorescence/reference fluorescence. The assay was independently repeated 3 times.

RNA immunoprecipitation (RIP)

Magna RIP™ RNA-Binding Protein Immunoprecipitation Kit (17-700, Millipore, Burlington, MA, USA) was used to detect Ago2-binding complex. Cells were washed with precooled PBS and lysed with an equal volume of RIPA buffer (P0013B, Beyotime, Shanghai, China) in an ice bath for 5 min. The lysate was centrifuged at 14,000 rpm, 4°C, for 10 min and supernatant was taken as input or incubated with antibody. Briefly, every 50 µL of magnetic beads were washed, resuspended in 100 µL of RIP Wash Buffer, and incubated with 5 µg of rabbit Ago2 antibody (1:50; ab186733, Abcam, Cambridge, UK) or rabbit anti-human IgG antibody (1:100; ab172730, Abcam) at room temperature for 30 min. The

bead-antibody complex was washed, resuspended in 900 µL of RIP Wash Buffer, and incubated with 100 µL of cell lysate at 4°C overnight. The bead-protein-RNA complex was washed 3 times with RIP Wash Buffer and collected on a magnetic grate. The bead-protein-RNA complex and input were digested with proteinase K and isolated RNA was detected by qRT-PCR.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7. All data were expressed as mean ± standard deviation. Student's t-test was used for comparisons between two groups, and one-way analysis of variance for pairwise comparisons among multiple groups. Tukey's test was used for post-hoc multiple comparisons. The normality of the data was confirmed before they were analyzed with the t-test. Significant differences were represented by $P<0.05$.

RESULTS

Upregulation of Notch1 and radioresistance in GBM cells

Gene Expression Profiling Interactive Analysis (GEPIA) of the expression of Notch1 in the TCGA database showed higher expression of Notch1 in GBM than in normal tissues (Fig. 1A). Radiation-resistant GBM cells M059K and radiation-sensitive GBM cells M059J were exposed to different doses of radiation and subjected to CCK-8 test for viability. The viability of M059J cells decreased significantly with the increase of radiation dose and vanished when the radiation dose reached 10 Gy (Fig. 1B). The viability of M059K cells decreased slightly with the increase of radiation dose but significantly exceeded that of M059J cells (Fig. 1B, $P<0.05$), which demonstrated the resistance of M059K cells to radiation. A radiation dose of 5 Gy caused a significant but not excessive decrease in the viability of M059J cells, so this dose was applied in the following experiments. Flow cytometry was used to detect the apoptosis of M059K and M059J cells after treatment with 5 Gy radiation. M059K cells had a lower apoptosis rate than M059J cells (Fig. 1C, $P<0.05$). Moreover, the results of qRT-PCR and Western blotting showed that Notch1 and Hes1 were more highly expressed in M059K cells than in M059J cells (Fig. 1D-E, $P<0.05$).

To explore the effect of Notch1 on the radioresistance of GBM cells, we knocked down the expression of Notch1 in M059K cells before radiation treatment (Fig. 1F, $P<0.05$). Knockdown of Notch1 reduced viability (Fig. 1G, $P<0.05$) and increased apoptosis (Fig. 1H, $P<0.05$) in M059K cells under radiation.

MAFG-AS1 promotes the expression of Notch1 in GBM by binding miR-642a-5p

The starBase database showed that miR-642a-5p had binding sites with Notch1 mRNA (Fig. 2A) and MAFG-AS1 (Fig. 2B). MAFG-AS1 was predominantly located in the cytoplasm of M059K cells (Fig. 2C). In the dual-luciferase reporter assay, decreases in luciferase activity were observed in cells transfected with miR-642a-5p mimic and Notch1-WT (Fig. 2D, $P<0.05$) and in cells transfected with miR-642a-5p mimic and MAFG-AS1-WT (Fig. 2E, $P<0.05$). Transfection of Notch1-MUT or MAFG-AS1-MUT had no significant influence on luciferase activity. Compared with IgG control antibody, Ago2 antibody significantly pulled down Notch1 mRNA, miR-642a-5p and MAFG-AS1 in a common eluate from 5 Gy-irradiated M059K cells (Fig. 2F, $P<0.05$). The above two experiments both verified the binding of miR-642a-5p to MAFG-AS1 and Notch1 mRNA. In addition, MAFG-AS1 was highly expressed and miR-642a-5p was lowly expressed in M059K cells compared with M059J cells (Fig. 2G, $P<0.05$).

Overexpression of miR-642a-5p sensitizes GBM cells to radiation

To explore the effect of miR-642a-5p on the response of GBM cells to radiation, we used miR-642a-5p mimic to induce overexpression of miR-642a-5p in M059K cells (Fig. 3A, $P<0.05$). Overexpression of miR-642a-5p reduced the expression of MAFG-AS1, Notch1 and Hes1 (Fig. 3A, 3D, $P<0.05$). After exposure to radiation (5 Gy), M059K cells overexpressing miR-642a-5p showed lower viability (Fig. 3B, $P<0.05$) and higher apoptosis (Fig. 3C, $P<0.05$).

Knockdown of MAFG-AS1 sensitizes GBM cells to radiation

To explore the effect of MAFG-AS1 on the response of GBM cells to radiation, we knocked down the expression of MAFG-AS1 in M059K cells (Fig. 4A, $P<0.05$). Knockdown of MAFG-AS1 reduced the levels of Notch1 and Hes1 and increased that of miR-642a-5p (Fig. 4A, 4D, $P<0.05$). Moreover, knockdown of MAFG-AS1 impaired the viability (Fig. 4B, $P<0.05$) and enhanced the apoptosis (Fig. 4C, $P<0.05$) of M059K cells under radiation (5 Gy).

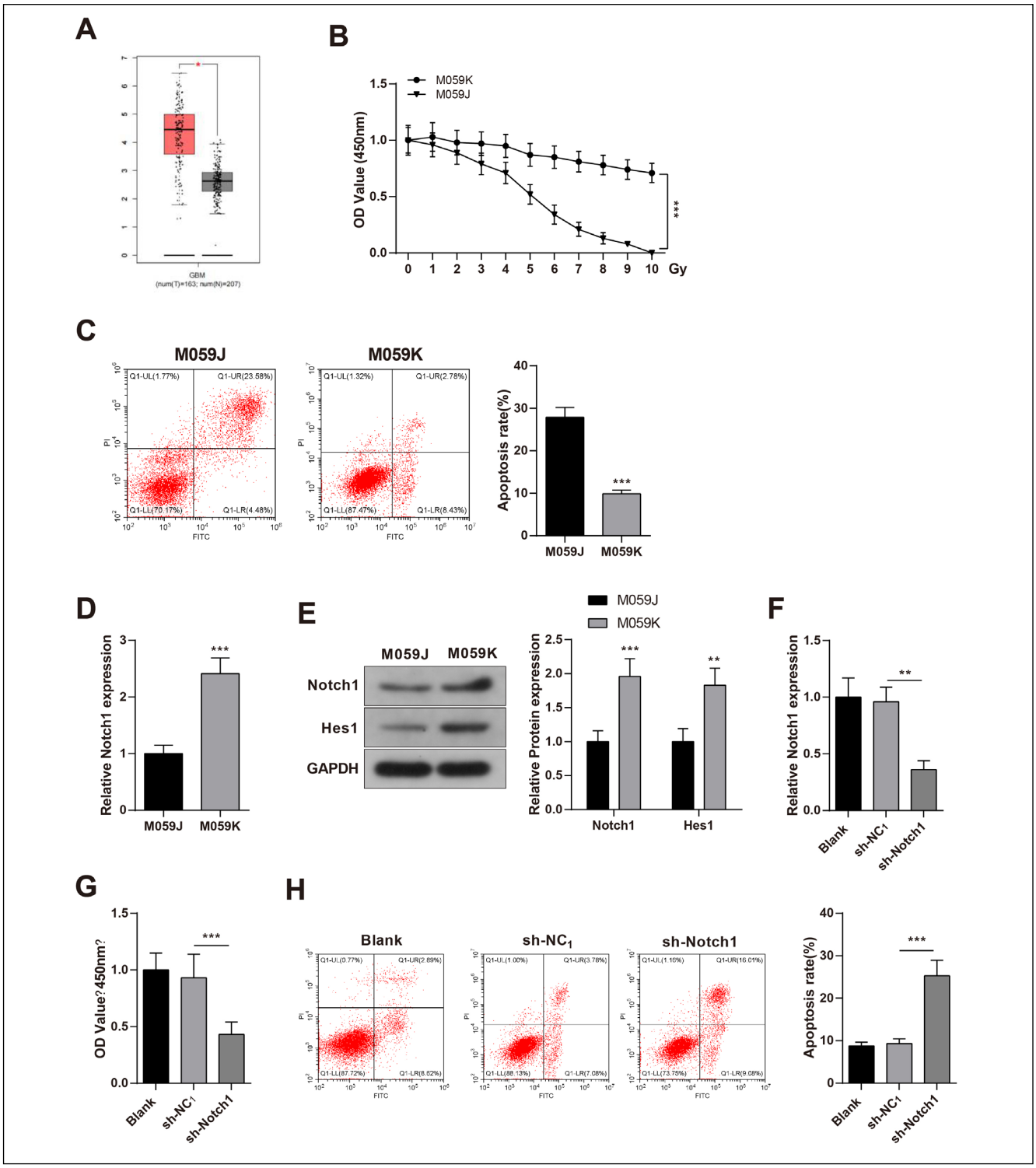

Fig. 1. Notch1 expression is positively correlated with glioma radioresistance. (A) The expression of Notch1 in GBM and normal tissues in the TCGA database. (B) CCK-8 was used to detect the viability of M059K and M059J cells after exposure to different doses of radiation (1 ~ 10 Gy). (C) Flow cytometry was used to detect apoptosis of M059K and M059J cells after exposure to 5 Gy radiation. qRT-PCR and Western blotting were used to detect the expression of Notch1 (D-E) and Hes1 (E) in M059K and M059J cells. M059K cells were transfected with sh-Notch1 or sh-NC_1 before treatment with 5 Gy radiation: (F) qRT-PCR was used to detect the expression of Notch1 in M059K cells. (G) CCK-8 was used to detect the viability of M059K cells. (H) Flow cytometry was used to detect the apoptosis of M059K cells. * P<0.05, ** P<0.01, *** P<0.001. Independent-samples t-test was used for comparisons between two groups. Each experiment was repeated 3 times. The above numerical results are all measurement data and expressed as mean ± standard deviation. GBM, glioblastoma; CCK-8, cell counting kit-8; qRT-PCR, quantitative reverse transcription polymerase chain reaction; NC, negative control.

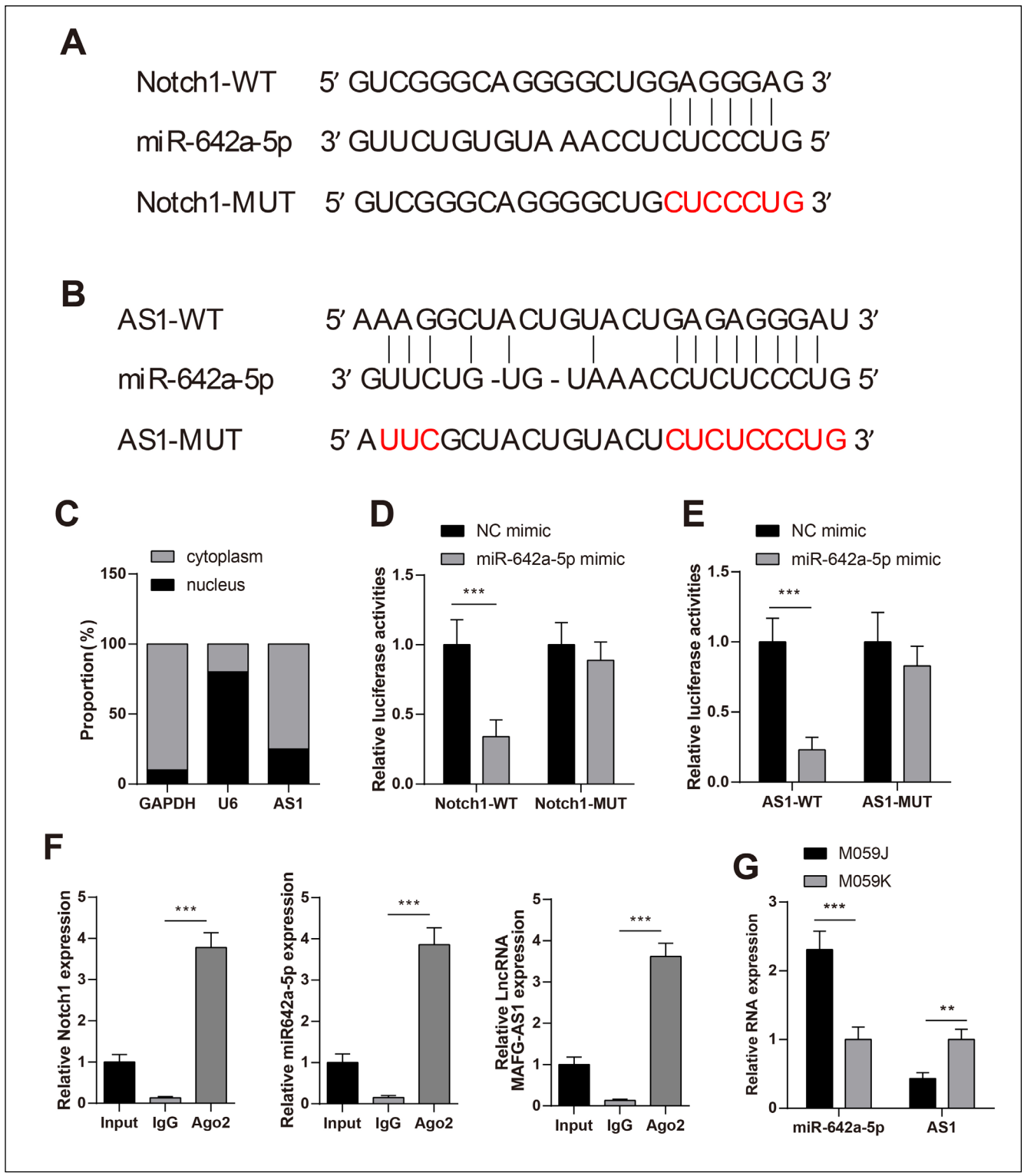

Fig. 2. MAFG-AS1 and Notch1 are miR-642a-5p targets. (A) The binding sites between Notch1 mRNA and miR-642a-5p in the starBase database. (B) The binding sites between miR-642a-5p and MAFG-AS1 in the starBase database. (C) Localization of MAFG-AS1 in M059K cells. Dual-luciferase reporter assay was performed to verify the binding of miR-642a-5p to Notch1 mRNA (D) or MAFG-AS1 (E). (F) qRT-PCR was used to detect the expression of Notch1 mRNA, miR-642a-5p and MAFG-AS1 pulled down by Ago2 antibody. (G) qRT-PCR was used to detect the expression of MAFG-AS1 and miR-642a-5p in M059J and M059K cells. * P<0.05, ** P<0.01, *** P<0.001. Independent-samples t-test was used for comparisons between two groups. Each experiment was repeated 3 times. The above numerical results are all measurement data and expressed as mean ± standard deviation. GBM, glioblastoma; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

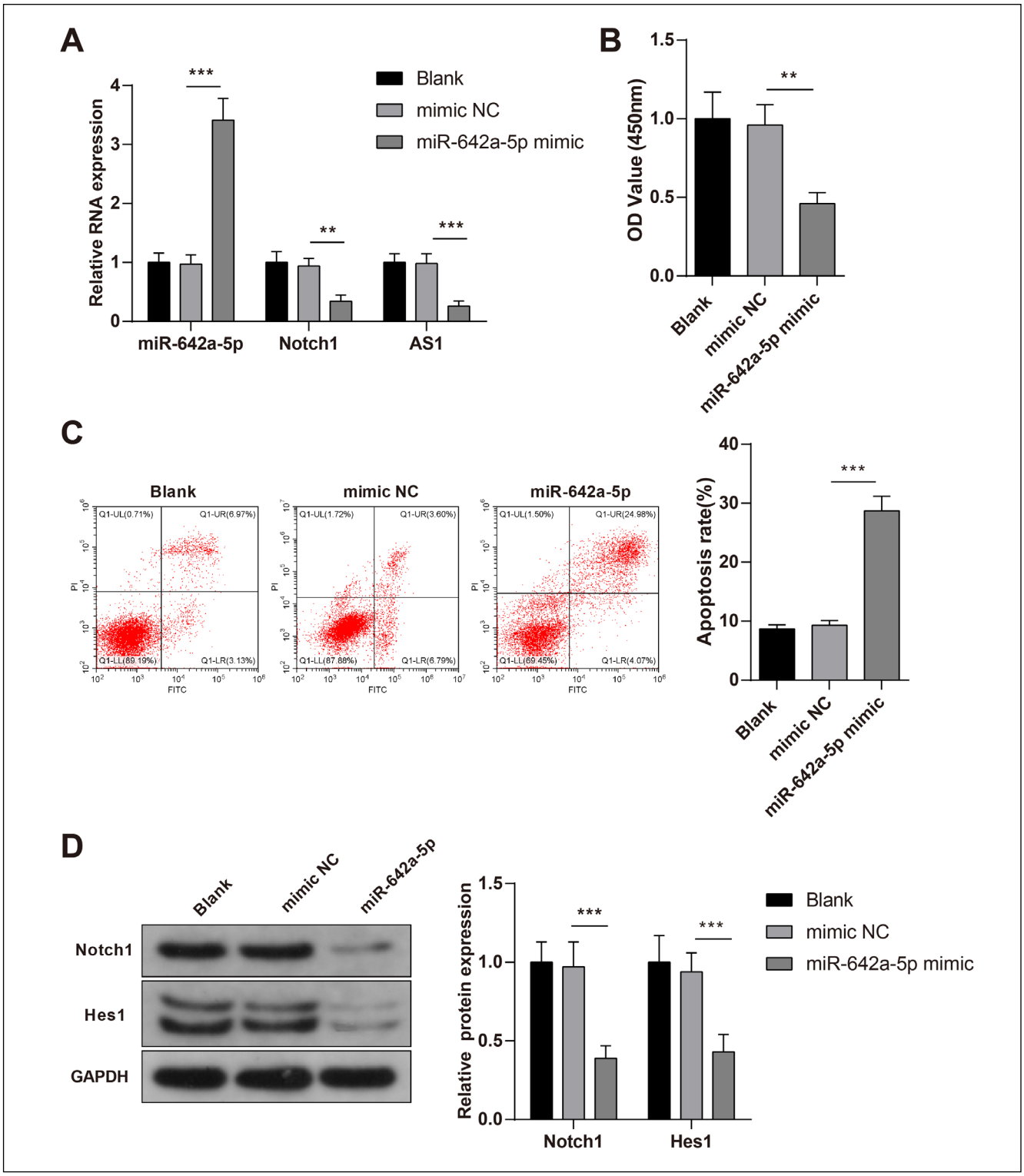

Fig. 3. Overexpression of miR-642a-5p sensitizes GBM cells to radiation. M059K cells were transfected with miR-642a-5p mimic or mimic NC. Forty-eight h after the transfection, (A) qRT-PCR was used to detect the expression of miR-642a-5p, Notch1 mRNA and MAFG-AS1. The transfected M059K cells were incubated for another 48 h after exposure to 5 Gy radiation: (B) CCK-8 was used to detect the viability of M059K cells. (C) Flow cytometry was used to detect the apoptosis of M059K cells. (D) Western blotting was used to detect Notch1 and Hes1 proteins. * P<0.05, ** P<0.01, *** P<0.001. Independent-samples t-test was used for comparisons between two groups. Each experiment was repeated 3 times. The above numerical results are all measurement data and expressed as mean ± standard deviation. GBM, glioblastoma; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction; CCK-8, cell counting kit-8.

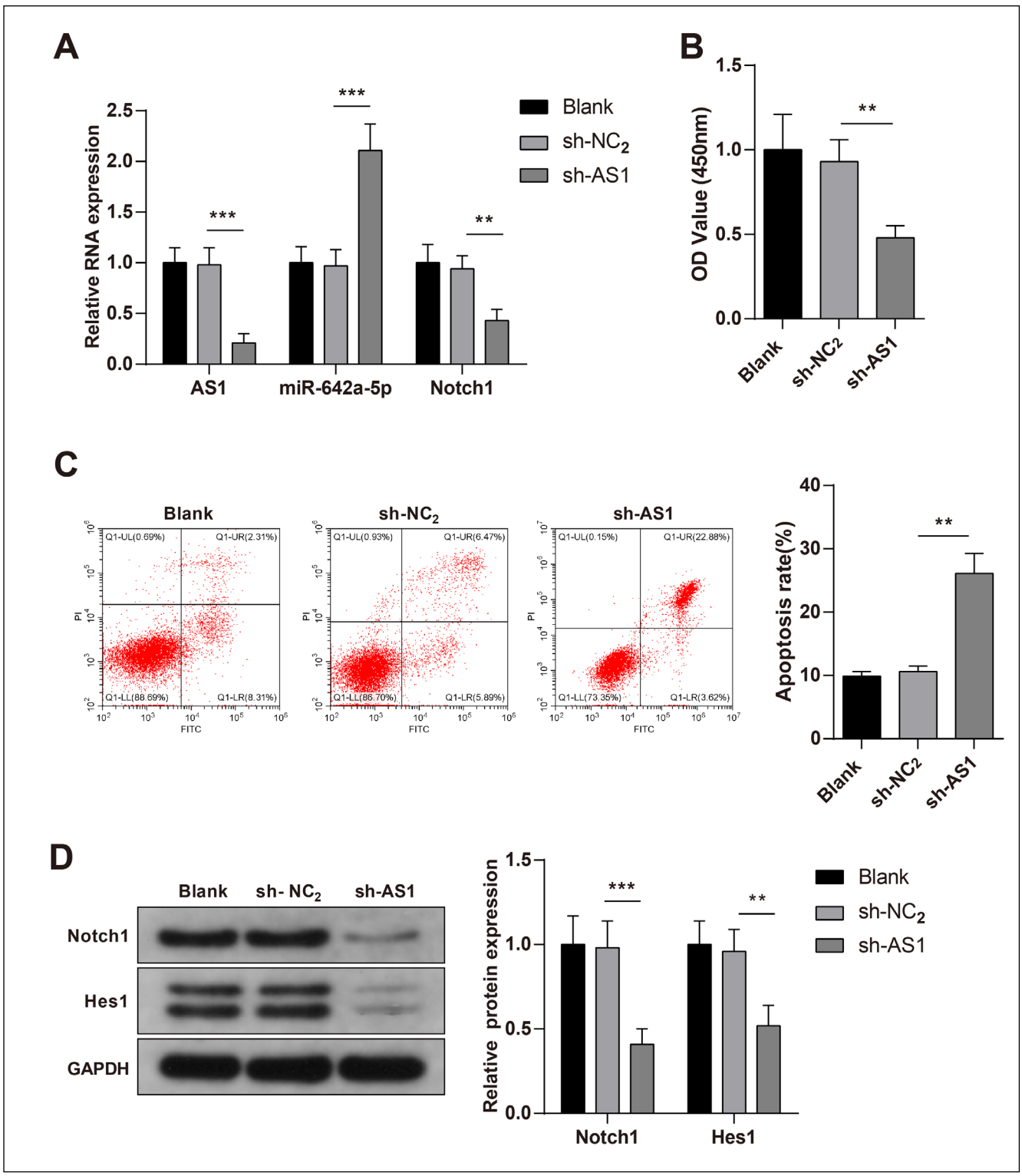

Fig. 4. Knockdown of MAFG-AS1 sensitizes GBM cells to radiation. M059K cells were transfected with sh-AS1 or sh-NC_2. Forty-eight h after the transfection, (A) qRT-PCR was used to detect the expression of miR-642a-5p, Notch1 mRNA and MAFG-AS1. The transfected M059K cells were incubated for another 48 h after exposure to 5 Gy radiation: (B) CCK-8 was used to detect the viability of M059K cells. (C) Flow cytometry was used to detect apoptosis of M059K cells. (D) Western blotting was used to detect Notch1 and Hes1 proteins. * P<0.05, ** P<0.01, *** P<0.001. Independent-samples t-test was used for comparisons between two groups. Each experiment was repeated 3 times. The above numerical results are all measurement data and expressed as mean ± standard deviation. GBM, glioblastoma; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction; CCK-8, cell counting kit-8.

Function of MAFG-AS1/miR-642a-5p/Notch1 axis in irradiated M059J cells

To confirm the involvement of MAFG-AS1/miR-642a-5p/Notch1 axis in the radioresistance of GBM cells, we transfected M059J cells with either: oe-NC, oe-AS1, mimic NC, miR-642a-5p mimic, oe-Notch1, oe-AS1 + miR-642a-5p mimic, oe-AS1 + sh-NC or oe-AS1 + sh-Notch1, and exposed them to 5 Gy radiation. qRT-PCR was used to detect the expression of MAFG-AS1, miR-642a-5p and Notch1 mRNA. Compared with the control group, the oe-AS1 group showed higher expression of MAFG-AS1 and Notch1 mRNA and lower expression of miR-642a-5p; and the oe-Notch1 group showed higher expression of Notch1 mRNA (Fig. 5A-C, $P<0.05$). Overexpression of miR-642a-5p reduced the expression levels of MAFG-AS1 and Notch1 mRNA (Fig. 5A-C, $P<0.05$). miR-642a-5p expression increased and Notch1 mRNA expression decreased in the oe-AS1 + sh-Notch1 group compared with the oe-AS1 + sh-NC group (Fig. 5B-C, $P<0.05$). There were no significant differences in the expression levels of MAFG-AS1, miR-642a-5p, and Notch1 among the control, oe-NC, and mimic NC groups.

Overexpression of MAFG-AS1 or Notch1 enhanced viability (Fig. 5D, $P<0.05$), reduced apoptosis (Fig. 5E, $P<0.05$), and increased protein levels of Notch1 and Hes1 (Fig. 5F, $P<0.05$) in irradiated M059J cells. Knockdown of Notch1 reduced the viability of M059J cells overexpressing MAFG-AS1 (Fig. 5D, $P<0.05$). Compared with the oe-AS1 group, the oe-AS1 + miR-642a-5p mimic and oe-AS1 + sh-Notch1 groups showed higher apoptosis (Fig. 5E, $P<0.05$) and lower expression of Notch1 and Hes1 proteins (Fig. 5F, $P<0.05$).

DISCUSSION

GBM is one of the most lethal human cancers and approaches for improving survival are challenged by therapy resistance, intrinsic and adaptive heterogeneity, and highly evolved metabolic machinery for proliferation (Noch et al., 2018). Genetic findings of the molecular mechanisms of GBM have greatly stimulated the development of targeted therapeutics (Aldape et al., 2015). This study found Notch1 was highly expressed in GBM and affected the response of GBM cells to radiation. MAFG-AS1 promoted the expression of Notch1 by binding miR-642a-5p and therefore strengthened the radioresistance of GBM cells.

Notch1 and its downstream Hes1 exhibited higher expression in radioresistant M059K cells than in nonresistant M059J cells. Knockdown of Notch1 reduced viability and increased apoptosis in M059K cells exposed to radiation. Notch1 is a well-characterized oncogenic molecule regulating tumorigenesis, immune infiltration, metastasis and angiogenesis in various malignancies, such as gastric cancer, tongue cancer, triple-negative breast cancer, colorectal cancer and bladder cancer (Gan et al., 2018; Gu et al., 2019; Hu et al., 2020; Jackstadt et al., 2019; Miao et al., 2020; Zang et al., 2017). SMARCD1 protein suppressed the proliferation, invasion and chemoresistance of GBM cells through downregulation of Notch1, which in turn stimulated the expression of SMARCD1 *via* inhibition of Hes1 (Zhu et al., 2021). M2 muscarinic receptors induced cell cycle arrest and apoptosis in GBM stem cells by interfering with Notch1/EGFR pathway (Cristofaro et al., 2020). Melatonin inhibited the malignant properties of GBM stem-like cells by downregulating EZH2 protein that controlled the transcriptional activity of Notch1 (Zheng et al., 2017). Fassl et al. (2012) found that Notch1 signaling increased the resistance of GBM cells to radiation-induced apoptosis by regulating anti-apoptotic Mcl-1 protein. By far, little attention has been paid to the mediators of Notch1 signaling in the radioresistance of GBM cells.

In this study, miR-642a-5p inhibited the expression of Notch1 protein by targeting Notch1 mRNA. A lower level of miR-642a-5p was observed in radioresistant M059K cells compared with nonresistant M059J cells. miR-642a-5p is a newly characterized regulator in cancers. Aberrant expression of miR-642a-5p was found in osteosarcoma, classical Hodgkin lymphoma, and renal cell carcinoma with Xp11 translocation (Marchionni et al., 2017; Monterde-Cruz et al., 2018; Paydas et al., 2016). lncRNA PCGEM1 enhanced the malignancy of cervical carcinoma cells by elevating the level of legumain *via* miR-642a-5p (Liu et al., 2020). lncRNA LINC01234 promoted the proliferative potential of colon cancer cells by stimulating SHMT2-dependent serine/glycine metabolism through inhibition of miR-642a-5p (Lin et al., 2019). Underexpression of miR-642a-5p was associated with poor prognosis in colon cancer and promoted collagen type I α1-mediated mobility of colon cancer cells (Wang et al., 2021). Current evidence indicates that miR-642a-5p primarily acts as a tumor suppressor. Consistently, overexpression of miR-642a-5p inhibited viability and increased apoptosis in M059K cells exposed to radiation. We further searched for the regulator of miR-642a-5p in GBM cells.

MAFG-AS1 was highly expressed in M059K cells and inhibited the expression of miR-642a-5p. In addition, there was mutual regulation of MAFG-AS1 and miR-642a-5p expression. Overexpression of miR-642a-5p reduced the expression of MAFG-AS1. An lncRNA is composed of fragments such as introns

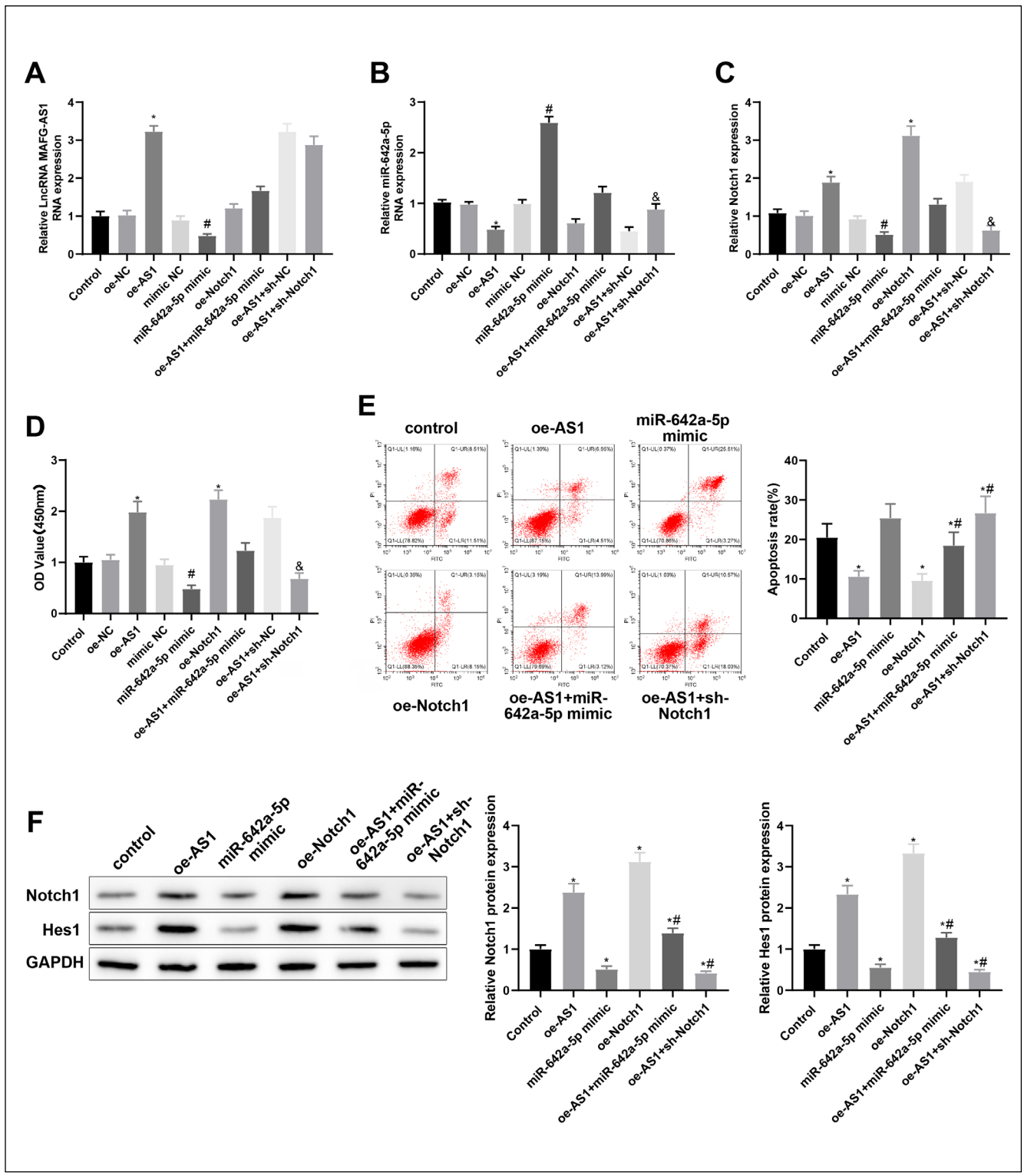

Fig. 5. Function of MAFG-AS1/miR-642a-5p/Notch1 axis in irradiated GBM cells. Forty-eight h after cell transfection, (A-C) qRT-PCR was used to detect the expression of MAFG-AS1 (A), miR-642a-5p (B), and Notch1 mRNA (C) in M059J cells; * P<0.05 *vs.* the control group, # P<0.05 *vs.* the mimic NC group, & P<0.05 *vs.* the oe-AS1 + sh-NC group. The transfected M059J cells were incubated for another 48 h after exposure to 5 Gy radiation: (D) CCK-8 was used to detect the viability of M059J cells. (E) Flow cytometry was used to detect the apoptosis of M059J cells. (F) Western blotting was used to detect Notch1 and Hes1 proteins. * P<0.05 *vs.* the control group, *# P<0.05 *vs.* the oe-AS1 group. Independent-samples t-test was used for comparisons between two groups. Each experiment was repeated 3 times. The above numerical results are all measurement data and expressed as mean ± standard deviation. GBM, glioblastoma; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction; CCK-8, cell counting kit-8.

and can reach thousands of nucleotides in length; which provides a good basis for binding a large number of miRNAs. lncRNAs occupy miRNAs in cells like sponges and reduce miRNA-induced suppression of target mRNAs. The relationship between such an lncRNA and mRNA is called "competing endogenous RNA (ceRNA)" (Salmena et al., 2011). MAFG-AS1, miR-642a-5p and Notch1 formed a ceRNA network in regulating radioresistance of GBM cells. Knockdown of MAFG-AS1 reduced the competitive binding of MAFG-AS1 to miR-642a-5p and increased free miR-642a-5p through the release of miR-642a-5p from MAFG-AS1.Therefore, the qRT-PCR analysis showed a higher relative expression level of miR-642a-5p after MAFG-AS1 was knocked down. This finding showed that this process only increased the relative expression of miR-642a-5p but did not affect the expression and maturation of miR-642a-5p itself. More importantly, knockdown of MAFG-AS1 reduced viability and enhanced apoptosis in irradiated M059K cells. Overexpression of MAFG-AS1 increased the resistance of M059J cells to radiation and this effect was reversed in the presence of miR-642a-5p mimic. Overexpression of Notch1 increased viability and inhibited apoptosis in irradiated M059J cells overexpressing both MAFG-AS1 and miR-642a-5p. Upregulation of MAFG-AS1 has been demonstrated to have association with the progression of diverse cancers. For example, MAFG-AS1 promoted the proliferative and invasive capability of bladder cancer cells by increasing the expression of COX-2 *via* miR-143-3p (Li et al., 2020b). Silencing of MAFG-AS1 suppressed the malignancy of pancreatic cancer cells *via* downregulation of nuclear factor I X (Ye et al., 2020). High expression of MAFG-AS1 was associated with advanced disease states and predicted poor survival in patients with gastric cancer (Li et al., 2020a).

Although the Notch pathway does not alter the DNA damage response of glioma stem cells, Notch1 protein expression is associated with aldehyde dehydrogenase activity that stimulates DNA double-strand break repair in radioresistant tumor cells (Dehghan Harati et al., 2019). This suggests that the Notch1 pathway may stimulate DNA damage repair to mediate radioresistance through other mechanisms other than by changing phosphorylation of DNA damage checkpoint kinases Chk1 and Chk2. Previous findings have shown that p53 plays a role in the effects of radiation on cells (Fu et al., 2020; Nakajima et al., 2017; Park et al., 2019). The bioinformatics analysis in our preliminary experiments has shown that miR-642a-5p may bind the 3'UTR of p53 to regulate p53 translation. Moreover, p53 can regulate ERK and JNK signaling pathways to control cellular radioresistance. Therefore, the difference in radioresistance of the cells does not solely rely on Notch1 expression but may also rely on p53, which needs further validation.

This study shows for the first time the role of MAFG-AS1 in the radioresistance of GBM cells *via* upregulation of Notch1 and a negative feedback loop with miR-642a-5p. These findings may assist the efficacy of radiotherapy for GBM and also provide novel targets for precision medicines.

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