

MYC-activated lncRNA *HNF1A-AS1* overexpression facilitates glioma progression via cooperating with *miR-32-5p/SOX4* axis

Jianheng Wu¹ | Rong Li² | Linfan Li¹ | Yimian Gu¹ | Hui Zhan¹ | Changbao Zhou¹ | Chuanhong Zhong³ 

¹Department of Neurosurgery, Gaozhou People's Hospital, Gaozhou, Guangdong, China

²Department of Radiation Oncology, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, China

³Department of Neurosurgery, The Affiliated Hospital of Southwest Medical University, Neurosurgical Clinical Research Center of Sichuan Province, Luzhou, Sichuan, China

Correspondence

Chuanhong Zhong, Department of Neurosurgery, the Affiliated Hospital of Southwest Medical University, Neurosurgical Clinical Research Center of Sichuan Province, Luzhou 646000, Sichuan, China.

Email: neurosurgery@swmu.edu.cn

Funding information

Project Program of Neurosurgical Clinical Research Center of Sichuan Province; Science and Technology Foundation of Southwest Medical University (2017-ZRQN-180, 2017-ZRQN-110).

Abstract

Mounting literatures have revealed the crucial effects of long noncoding RNA (lncRNA) in various cancers, including glioma. *HNF1A-AS1*, a novel lncRNA, is reported to modulate tumorigenesis and development of multiple cancers. However, the tumorigenic function of lncRNA *HNF1A-AS1* in glioma remains largely unknown. quantitative reverse transcription and polymerase chain reaction and western blot assays were applied to evaluate the expression of relevant mRNAs and proteins. 5-Ethynyl-2'-deoxyuridine, terminal deoxynucleotidyl transferase dUTP nick-end labeling, flow cytometry, and transwell assays were conducted for examining the influence of *HNF1A-AS1* on glioma cell functions. The relationship among RNAs was investigated by mechanical experiments. The results demonstrated that *HNF1A-AS1* was predominantly highly expressed in glioma cell lines compared with nontumorigenic epithelial cell, which was associated with the stimulation of transcription factor myelocytomatosis oncogene. Knockdown of *HNF1A-AS1* remarkably inhibited glioma cells proliferation, migration, and invasion, while accelerating cell apoptosis in vitro. Mechanically, *HNF1A-AS1* served as a *miR-32-5p* sponge. Moreover, *SOX4* was discovered as a target of *miR-32-5p*. Inhibited *miR-32-5p* or upregulated *SOX4* could markedly counteract the inhibitory effects of silencing *HNF1A-AS1* on glioma malignant biological behaviors. *HNF1A-AS1* exerted oncogenic property in glioma progression via upregulating *miR-32-5p*-mediated *SOX4* expression, suggesting potential novel therapeutic target for future glioma treatment.

KEYWORDS

glioma, *HNF1A-AS1*, *miR-32-5p*, *SOX4*

1 | INTRODUCTION

Glioma is the most common intracranial malignant tumor and is one of the leading causes of cancer deaths.¹ The occurrence of brain tumors is 21/100 000, which is much lower

than other type of cancers, occupying only approximately 2% of all human cancers. However, the morbidity of glioma accounts for almost 60% among all kinds of brain tumors.²

Accumulating evidence highlighted the importance of abundantly transcribed noncoding transcripts, among

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Cancer Medicine* published by John Wiley & Sons Ltd.

which long noncoding RNAs (lncRNAs) emerged as critical mediator in many processes of cell biology. Recently, *HNF1A-AS1*, as a novel identified lncRNA, gained much attention. *HNF1A-AS1* is correlated with tumorigenic functions including cancer cell proliferation, migration, and invasion. For instance, *HNF1A-AS1* was revealed to facilitate colon cancer metastatic progression via regulating miR-34a/SIRT1/p53 feedback loop.³ Besides, overexpressed *HNF1A-AS1* activates the Wnt/ β -catenin signaling pathway to promote colorectal cancer carcinogenesis.⁴

Substantial studies manifested that lncRNAs can function as competing endogenous RNAs (ceRNAs) in regulating the protein production of mRNA via binding to shared miRNAs post-transcriptionally.⁵ This discovery deepened the understanding of human genome and highlighted the important gene regulatory role of lncRNAs, which were previously thought as “transcription noise”.⁶ Long noncoding RNAs could mediate various cellular biological functions including cell proliferation, apoptosis, migration, and invasion in pathology evolution.^{7,8} Increasing studies revealed the implication of lncRNAs in progression of brain tumors.^{9,10} To date, the molecular relationship between lncRNA and glioma onset and progression has been unveiled. For instance, LINC00909 serves as a ceRNA to positively mediate the expression of MUC1-C via binding to miR-194, and consequently promotes glioma cell migration and invasion.¹¹ Long noncoding RNA UBE2R2-AS1 serves as a ceRNA against miR-877-3p to upregulate TLR4, and inhibits the malignant phenotype of glioblastoma cells.¹² These studies indicated that lncRNAs are essential for glioma progression. Nevertheless, as a novel lncRNA, the biological role and molecular mechanisms of *HNF1A-AS1* in glioma has not been explored yet. This study aimed to reveal the underlying molecular mechanism of *HNF1A-AS1* in glioma progression.

2 | MATERIALS AND METHODS

2.1 | Tissue samples

In total, 35 glioma tissues and 10 normal tissues (excised from patients with other brain diseases) were obtained from Gaozhou People's Hospital with the approval from the Ethics Committee of Gaozhou People's Hospital. Each participant has signed informed consent. No patients have received chemo- or radiotherapy before surgery. After surgical resection, fresh tissues were instantly frozen in liquid nitrogen and stored at -80°C .

2.2 | Cell lines

Human glioma cell lines (LN229, A172, SHG-44, and U87) and normal brain glial cell line (HEB) were both procured from American Type Culture Collection (ATCC;

Manassas, VA) and cultured under 95% air, 5% CO_2 at 37°C . Dulbecco's Modified Eagle's medium (Invitrogen) adding 10% fetal bovine serum were applied for cell culture.

2.3 | Quantitative reverse transcription and polymerase chain reaction (qRT-PCR)

Total RNA from A172 and U87 cells were extracted by TRIzol reagent from Invitrogen in line with the guidebook. A Reverse Transcription Kit (Toyobo) was applied for synthesizing cDNA for conducting qRT-PCR with SYBR Green Super Mix (Bio-Rad). All RNA levels were measured via $2^{-\Delta\Delta\text{CT}}$ method, with U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference.

2.4 | Cell transfection

Cells of A172 and U87 were seeded in the 24-well plates for 48 hours of transfection with Lipofectamine 2000 (Invitrogen). The duplex sh-RNAs of *HNF1A-AS1* (sh-*HNF1A-AS1*#1/2) and negative control (NC; termed short hairpin-negative control [sh-NC]), pcDNA3.1/myelocytomatosis oncogene (MYC), pcDNA3.1/*SOX4*, and NC (pcDNA3.1), as well as the *miR-32-5p* mimics and NC mimics, *miR-32-5p* inhibitor and NC inhibitor, all these were designed and synthesized by RiboBio.

2.5 | 5-Ethynyl-2'-deoxyuridine (EdU) staining

A172 and U87 cells on sterile coverslips were transfected and plated in the 24-well plates for treatment with the EdU incorporation assay kit (RiboBio). Cell nucleus was counterstained with EdU and DAPI (Beyotime), and visualized with laser confocal microscopy (Olympus).

2.6 | Terminal deoxynucleotidyl transferase dUTP nick-end labeling assay

Glioma cells after transfection were cultured with the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) Apoptosis Assay Kit (Beyotime) in light of the user guide. After treating with DAPI, apoptotic cells were analyzed under confocal microscopy.

2.7 | Flow cytometry of apoptosis

70% cold ethanol on ice was used to fix transfected glioma cells for 1 hour. Then, cells were treated with Annexin

V-APC and 7-AAD staining in the dark. A flow cytometer (BD Biosciences) was used to assess cell apoptosis.

2.8 | Transwell assay

Transfected cells were reaped and planted into the upper chamber of transwell inserts (Corning Incorporated) coating with or without Matrigel membrane (BD Biosciences) for invasion or migration analysis. After 48 hours, the invaded and migrated cells were stained in crystal violet. Then, a microscope (magnification, $\times 200$) was used to count the cells.

2.9 | Chromatin immunoprecipitation assay

Cells of A172 and U87 were fixed for 10 minutes to generate DNA-protein cross-links. After shearing into 200- to 1000-bp chromatin fragments, cell lysates were immunoprecipitated with anti-MYC or normal control anti-immunoglobulin G (IgG) antibody (Millipore). The retrieved chromatin DNA by beads was subjected to qRT-PCR analysis.

2.10 | Dual-luciferase reporter assays

HEK-293T cells (ATCC) in the 24-well plates were co-transfected with the pGL3 vector containing *HNFI A-ASI* promoter, pRL-TK-Renilla plasmid (Promega Corporation), and pcDNA3.1/MYC or pcDNA3.1. Besides, the wild-type (WT) or mutant (MUT) reporter constructs of *HNFI A-ASI* and *SOX4* were generated by pmirGLO vectors and co-transfected into HEK-293T cells with *miR-32-5p* mimics and NC mimics. The Dual-Luciferase Reporter Assay System (Promega) was employed to evaluate luciferase intensity after 48 hours of transfection.

2.11 | Subcellular fractionation

A PARIS™ Kit (Invitrogen) was applied for isolating nuclear and cytoplasmic RNAs in 1×10^6 glioma cells, which were previously rinsed in precooled PBS. The isolated *HNFI A-ASI* was analyzed via qRT-PCR. U6 and GAPDH, respectively, served as the nuclear and cytoplasmic controls.

2.12 | Fluorescence in situ hybridization assay

After fixation, the glioma cells were collected for incubation with RNA Fluorescence in situ hybridization (FISH) probe

for *HNFI A-ASI* (RiboBio) in the hybridization buffer. After slides were cultured with DAPI solution, cells were observed under a Olympus microscope.

2.13 | RNA pull down

The protein extracts from glioma cells were obtained for mixing with biotin-labeled *HNFI A-ASI* (Biotin *HNFI A-ASI* WT/MUT) and Biotin NC, as well as the beads for 1 hour. qRT-PCR was followed for analyzing the mixture of pull-down.

2.14 | RNA immunoprecipitation assay

1×10^7 glioma cells in RNA immunoprecipitation lysis buffer were collected and immunoprecipitated with anti-Ago2 or normal mouse control anti-IgG antibody (Millipore). Finally, the purified RNA was analyzed by qRT-PCR.

2.15 | Western blot

The cellular protein samples from glioma cells were prepared for separation with SDS-PAGE (10%) and electrotransferred to PVDF membranes. After culturing with 5% skimmed milk, membranes were incubated with primary antibodies (Abcam) against *SOX4* and control GAPDH all night, then with horseradish peroxidase-tagged secondary antibody for 2 hours. The band intensity was determined by enhanced chemiluminescence reagent (Santa Cruz Biotechnology).

2.16 | Animal study

Six-week-old male nude mice was maintained in the SPF-grade animal laboratory, acquired from the National Laboratory Animal Center (Beijing, China). All procedures were approved by the Animal Research Ethics Committee of Southwest Medical University. Animal study was achieved through subcutaneous inoculation of 5×10^6 A172 cells to mice for 4 weeks. Tumor volume was recorded every 4 days. After killing mice, tumors were excised and weighed for analysis.

2.17 | Statistical analysis

All data were analyzed statistically via a *t* test or one-way ANOVA by using PRISM 6 (GraphPad), with $P < .05$ as significant level. Results were given as the mean \pm SD of three or more repetitive assays.

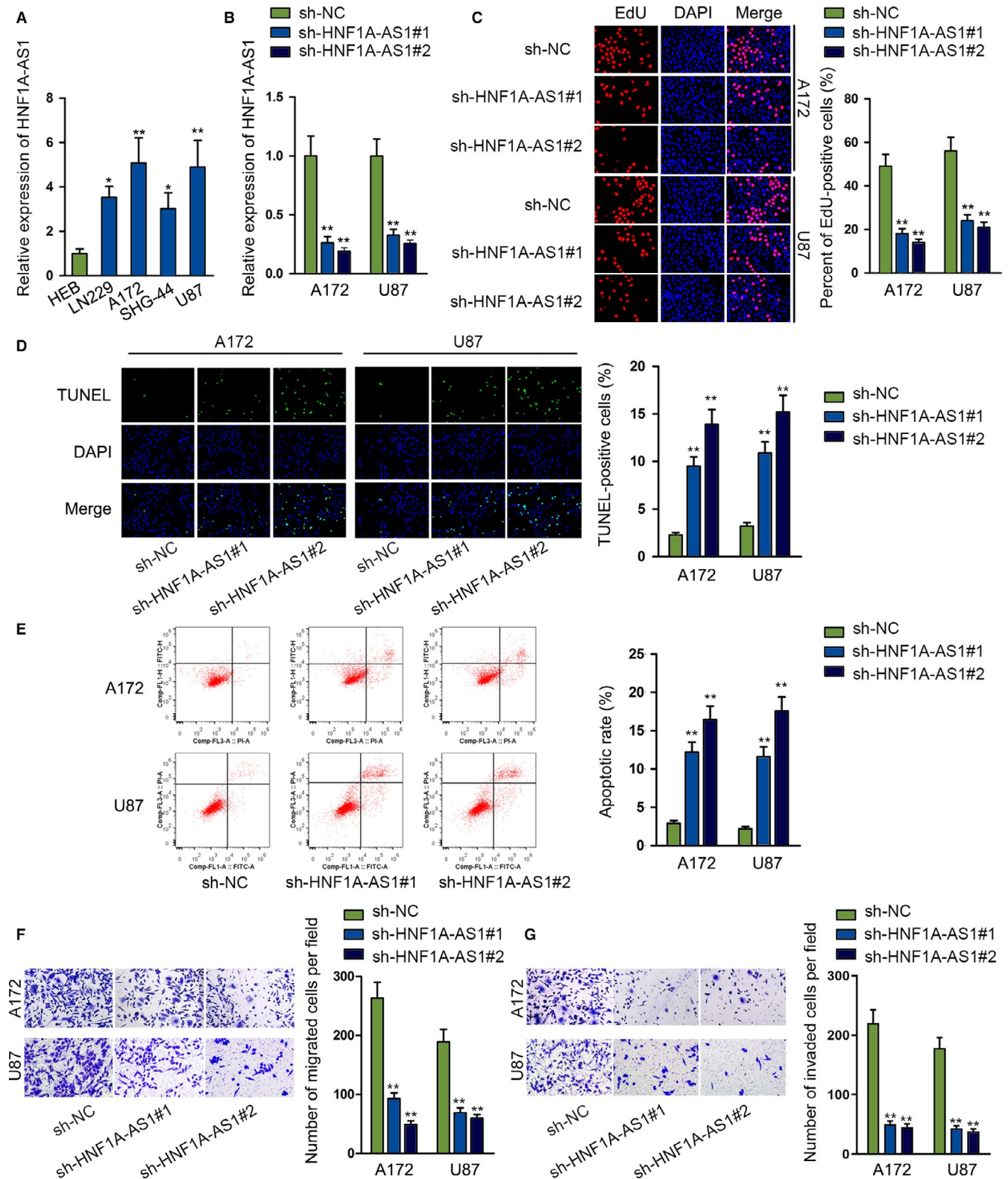


FIGURE 1 Silencing *HNF1A-AS1* dampens glioma cells proliferation, migration, and invasion, while facilitating apoptosis. A, qRT-PCR analysis was used to determine the expression profile of *HNF1A-AS1* in glioma cell lines and normal brain glial cell. B, The transfection efficiency of sh-*HNF1A-AS1*#1 and sh-*HNF1A-AS1*#2 were detected by qRT-PCR analysis. C, EdU assay was performed to assess glioma cells proliferation ability. D and E, TUNEL and flow cytometry assays were performed to determine cell apoptosis. F and G, Transwell migration and invasion assays were conducted to assess glioma cells migration and invasion ability. TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling. * $P < .05$, ** $P < .01$

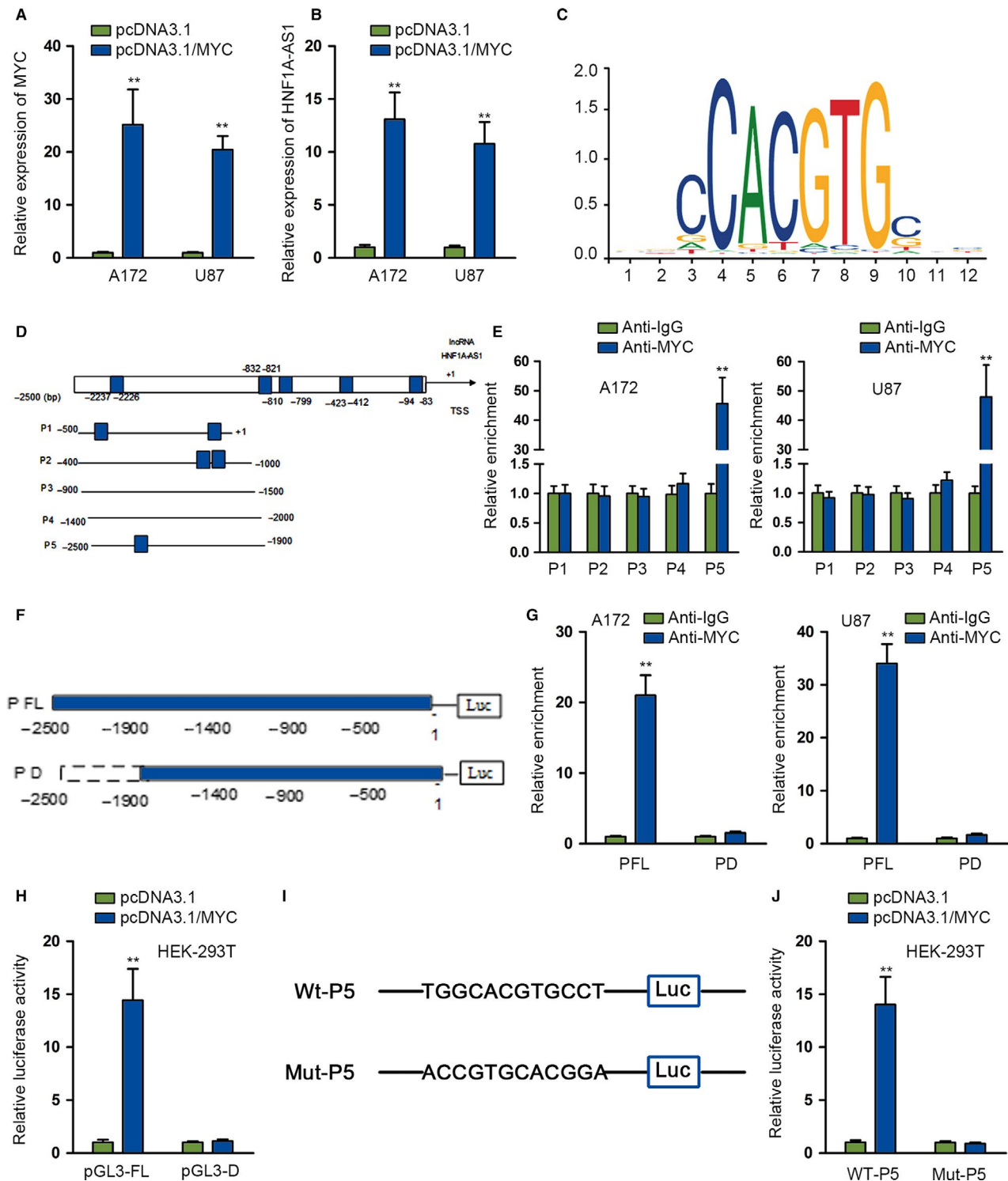


FIGURE 2 MYC induces overexpression of *HNF1A-AS1* via transcription activation. The expression of MYC was determined by quantitative RT-PCR (qRT-PCR) analysis after transfecting with pcDNA3.1 and pcDNA3.1/MYC plasmids. B, qRT-PCR was performed to study the effects of overexpressing MYC on *HNF1A-AS1* expression. C, The putative MYC binding motif in human *HNF1A-AS1* promoter. D, *HNF1A-AS1* promoter was divided into four sections according to potential MYC binding sites. E, Chromatin immunoprecipitation (ChIP) assay using antibody targeting MYC and IgG was conducted to detect the affinity of MYC with *HNF1A-AS1* promoter. F, The full *HNF1A-AS1* promoter (P FL) and *HNF1A-AS1* P5 deleted (P D) were subcloned into pGL3 luciferase reporter vector. G, ChIP assay was used to investigate the relative enrichment of *HNF1A-AS1* promoter. H, Luciferase reporter assay was performed in HEK-293T. I, pGL3-WT-P5, pGL3-MUT-P5 luciferase vectors were constructed accordingly. J, HEK-293T was co-transfected with pcDNA3.1 and pcDNA3.1/MYC and a luciferase reporter was performed between WT-P5 and MUT-P5. ** $P < .01$

3 | RESULTS

3.1 | Silencing *HNFI A-AS1* dampens glioma cell proliferation, migration, and invasion, while facilitating apoptosis

To understand the expression status of *HNFI A-AS1* in glioma, we performed qRT-PCR. It was revealed that *HNFI A-AS1* was significantly upregulated in glioma tissues than in normal tissues (Figure S1A). We also noticed that *HNFI A-AS1* was significantly overexpressed in glioma cells (LN229, A172, SHG-44, and U87) in contrast to normal brain glial cell line (HEB) (Figure 1A). We also noticed that A172 and U87 cells contained the most significant upregulation of *HNFI A-AS1*; thus they were used for following assays. For loss-of-function assays, we utilized two sh-RNAs directly targeting *HNFI A-AS1* and verified the inhibitory efficiency through qRT-PCR (Figure 1B). The EdU assay showed that silencing *HNFI A-AS1* significantly inhibited cell proliferation, as illustrated in Figure 1C. The TUNEL assay indicated that *HNFI A-AS1* knockdown could markedly promote glioma cell apoptosis (Figure 1D). Flow cytometry data showed notably elevated cell apoptosis after silencing *HNFI A-AS1*, which further confirmed the proapoptosis effects of *HNFI A-AS1* knockdown (Figure 1E). Besides, silencing *HNFI A-AS1* could significantly restrain cell migration and invasion, as shown in transwell assays (Figure 1F,G). Together, *HNFI A-AS1* played an oncogenic role in glioma progression.

3.2 | MYC induces overexpression of *HNFI A-AS1* via transcription activation

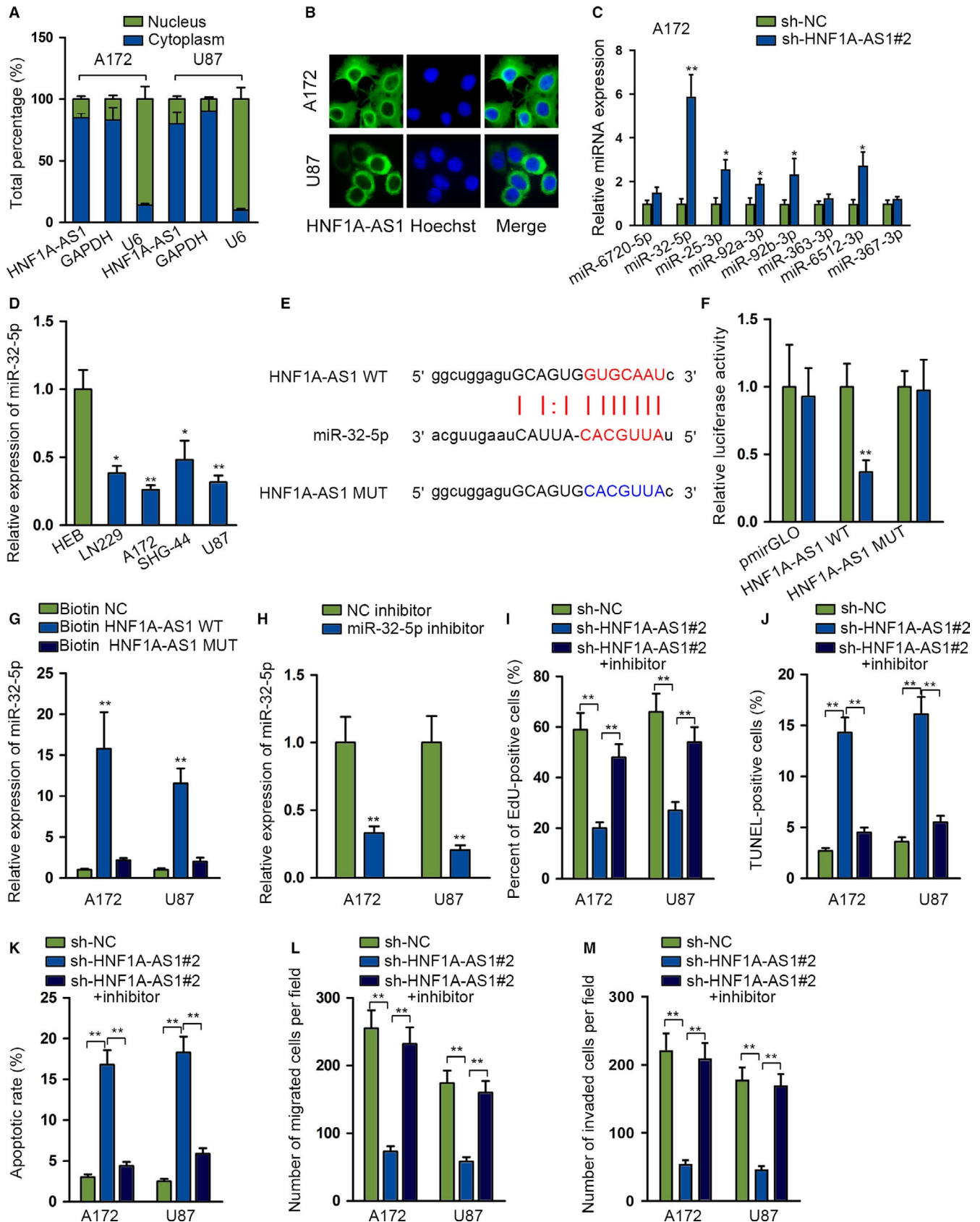
We hypothesized that transcription factor might be associated with *HNFI A-AS1* overexpression in glioma cells. MYC might bind to *HNFI A-AS1* promoter region by prediction of UCSC (<http://genome.ucsc.edu/>) and Jaspar (<http://jaspar.genereg.net/>) database jointly. The influence of MYC on *HNFI A-AS1* expression was subsequently detected. We observed that pcDNA3.1/MYC could force the overexpression of MYC efficiently (Figure 2A). We noticed that *HNFI A-AS1* expression was elevated after overexpressing MYC (Figure 2B). With the aid of Jaspar, we detected the

putative MYC motif in human *HNFI A-AS1* promoter region, as shown on Figure 2C. Then, we found five concrete putative MYC binding sequences in *HNFI A-AS1* promoter by Jaspar. We divided the promoter region into five sectional area from -2500 bp according to putative binding sites predicted by Jaspar (Figure 2D). Then, the binding capacity of MYC to *HNFI A-AS1* promoter was investigated by chromatin immunoprecipitation (ChIP) assay. Its findings depicted that MYC bound to P5 section (Figure 2E). Moreover, we constructed pGL3 luciferase vector containing *HNFI A-AS1* full promoter region (P FL) and *HNFI A-AS1* promoter P5 deleted region (P D) (Figure 2F). Deletion of P5 abrogated the increased promoter enrichment in anti-MYC, indicating that P5 fragment in *HNFI A-AS1* promoter region was indispensable for the interaction of *HNFI A-AS1* promoter with MYC (Figure 2G). This phenomenon was further elucidated by luciferase reporter assay on HEK-293T cell. It indicated that pcDNA3.1/MYC elevated the promoter activity of pGL3-FL, not that of pGL3-D (Figure 2H). These data represented that MYC interacted with *HNFI A-AS1* promoter at approximately -2500 to -1900 bp upstream transcription start site (TSS). We found that there was only one specific binding site (-2237 to -2226) predicted by Jaspar in P5 fragment. Therefore, we intended to verify whether MYC bound to these sequences in P5 segment. We constructed P5-WT and P5-MUT (which mutated -2237 to -2226 sequence) accordingly (Figure 2I). Promoter activity of P5-WT was strengthened after overexpressing MYC, while P5-MUT, which lacked the sequence form -2237 to -2226 , showed no alternation in luciferase activity after co-transfection with pcDNA3.1/MYC (Figure 2J). Collectively, these data provided substantial proof for the activation of *HNFI A-AS1* transcription activity by MYC in glioma cells.

3.3 | *HNFI A-AS1* directly binds to *miR-32-5p* and *miR-32-5p* inhibition could reverse the restraining effects of *HNFI A-AS1* knockdown on glioma

Many cytoplasmic lncRNAs have been found as ceRNAs in the carcinogenesis of cancers. *HNFI A-AS1* was found mainly situated in cytoplasm through subcellular fraction and FISH assays, as illustrated in Figure 3A,B. By

FIGURE 3 *HNFI A-AS1* directly binds to *miR-32-5p* and *miR-32-5p* inhibition could reverse the restraining effects of *HNFI A-AS1* knockdown on glioma. A and B, Subcellular fractionation and FISH assays were performed to determine the location of *HNFI A-AS1*. C, Quantitative RT-PCR (qRT-PCR) analysis was performed to determine miRNAs expression after *HNFI A-AS1* depletion. D, The expression status of *miR-32-5p* was detected in glioma cell lines and normal brain glial cell lines. E, Putative wild and mutant *miR-32-5p* binding sites with *HNFI A-AS1*. F, Luciferase reporters were performed in HEK-293T. MiR-32-5p mimics could impair the luciferase activity of *HNFI A-AS1*-WT. G, RNA pull down showed enriched *miR-32-5p* by biotin-labeled *HNFI A-AS1*-WT. H, qRT-PCR was used to examine the transfection efficiency of *miR-32-5p* inhibitor. I-M, Functional experiments were conducted in A172 and U87 cell lines to examine the rescue effects of silenced *miR-32-5p* on silenced *HNFI A-AS1* in cell proliferation, apoptosis, migration, and invasion. * $P < .05$, ** $P < .01$



browsing starBase tool,¹³ eight miRNAs were found to interact with *HNF1A-AS1* (clip data: low stringency ≥ 1). Among them, *miR-32-5p* was upregulated significantly in

A172 after silencing *HNF1A-AS1* compared with other miRNA candidates from qRT-PCR results (Figure 3C). Subsequently, we detected an abnormal low expression

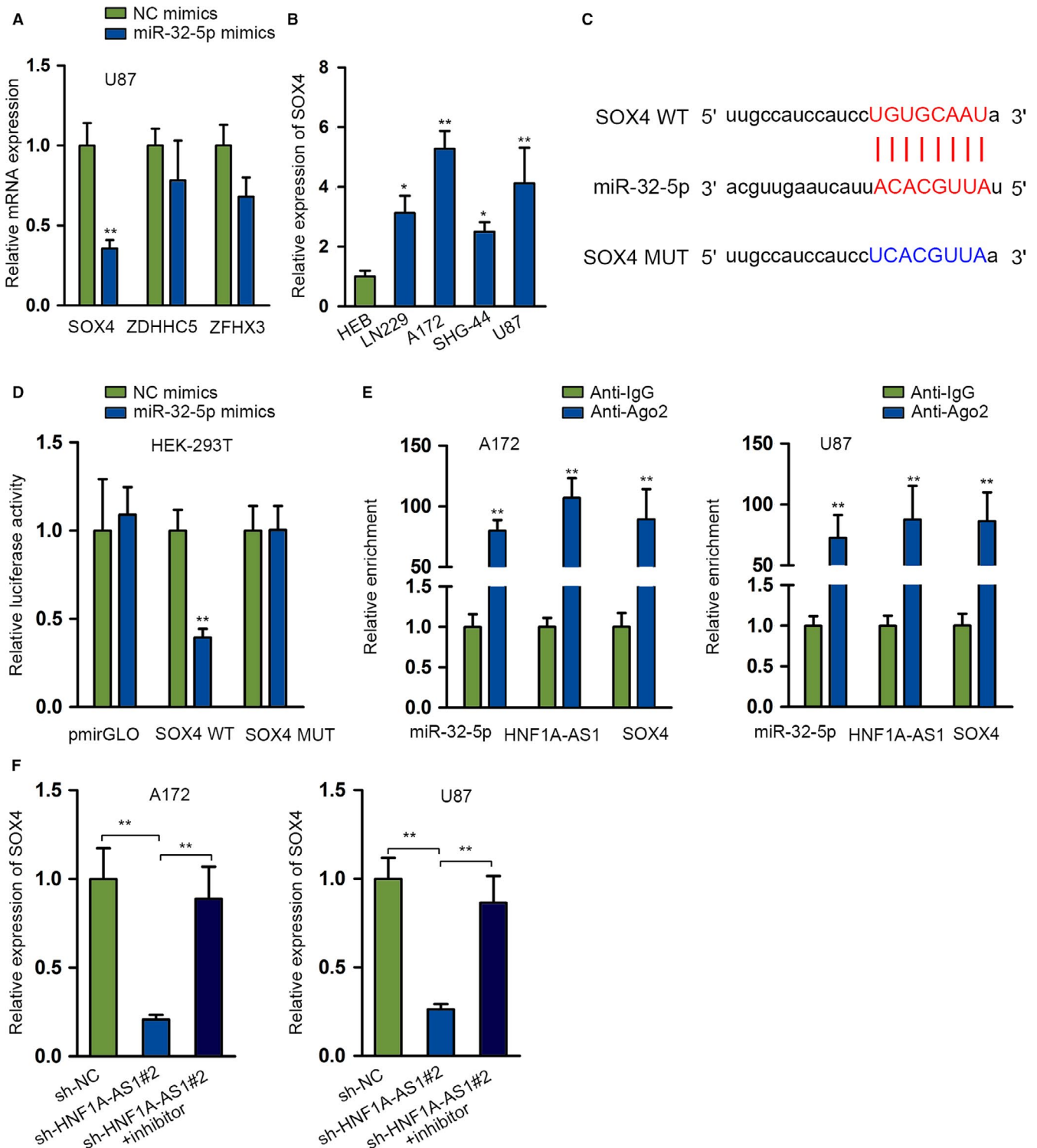


FIGURE 4 *SOX4* is a direct target gene of *miR-32-5p*. A, The expression of potential target genes was detected by quantitative RT-PCR (qRT-PCR) after transfecting *miR-32-5p* mimics. B, The expression profile of *SOX4* in cell lines was detected by qRT-PCR. C, Putative wild and mutant *miR-32-5p* binding sites in 3'UTR sequence of *SOX4* based on starBase data. D, Luciferase reporter assays were performed to investigate the association between *miR-32-5p* and *SOX4*. E, RIP was used performed to verify the mechanical relationship among *miR-32-5p*, *HNF1A-AS1* and *SOX4*. F, The effects of silenced *miR-32-5p* on silenced *HNF1A-AS1* in *SOX4* expression were studied by qRT-PCR. * $P < .05$, ** $P < .01$

status of *miR-32-5p* in glioma cells (Figure 3D). By browsing starBase, putative *miR-32-5p* binding site with *HNF1A-AS1* was detected. We mutated the putative binding

site sequence correspondingly, as shown in Figure 3E. Both *HNF1A-AS1*-WT and *HNF1A-AS1*-MUT were subcloned into pmirGLO luciferase reporter vector. After

co-transfecting *miR-32-5p* mimics with pmirGLO vector containing *HNF1A-AS1-WT* or *HNF1A-AS1-MUT*, the luciferase activity of *HNF1A-AS1-WT* was markedly attenuated, instead of *HNF1A-AS1-MUT* in HEK-293T, as illustrated in Figure 3F. *MiR-32-5p* was only significantly pulled down by Bio-*HNF1A-AS1-WT* group, which verified the interaction between *HNF1A-AS1* and *miR-32-5p* (Figure 3G). *HNF1A-AS1* bound with *miR-32-5p* and negatively modulated the expression of *miR-32-5p* in glioma cells.

After that, we carried out rescue assays to determine whether *HNF1A-AS1* contributed to glioma progression via inhibiting *miR-32-5p*. *MiR-32-5p* expression was silenced via transfection of plasmids containing *miR-32-5p* inhibitor into A172 and U87 cell lines (Figure 3H). *MiR-32-5p* inhibition obviously restored the oncogenic effects of *HNF1A-AS1* in glioma cell proliferation (Figure 3I), apoptosis (Figure 3J,K; Figure S1B), migration (Figure 3L; Figure S1C), and invasion (Figure 3M; Figure S1D). These results elucidated that *HNF1A-AS1* mediated glioma development via downregulating *miR-32-5p*.

3.4 | *SOX4* is a direct target gene of *miR-32-5p*

We aimed to find mRNA targets of *miR-32-5p* to better understand its role in glioma. Aided by starBase, we screened three target genes (*SOX4*, *ZDHHC5*, *ZFHX3*) according to below circumstance (clip data: strict stringency ≥ 5 ; degradome data: high stringency ≥ 3 ; program number: five; predicted program: microT, miRanda, miRmap, PITA, PicTar). We found that *SOX4* was significantly downregulated in the presence of *miR-32-5p* mimics compared with the other two mRNA candidates (Figure 4A). From qRT-PCR results, we found a significant elevation of *SOX4* in four glioma cells (Figure 4B). This expression status is contrary to that of *miR-32-5p* in glioma cells, thus we chose *SOX4* to study. The putative *miR-32-5p* binding site in the 3'-untranslated region (3'UTR) sequence of *SOX4* (Figure 4C) was verified by the results of dual luciferase reporter assays, which showed significant decreased luciferase activity only in *SOX4-WT* by *miR-32-5p* upregulation (Figure 4D). RNA immunoprecipitation assay data showed significant enriched *miR-32-5p*, *HNF1A-AS1*, *SOX4* in the anti-Ago2 group instead of the IgG group (Figure 4E). The expression of *SOX4* was examined after silencing *HNF1A-AS1* and then inhibiting *miR-32-5p* in A172 and U87. It was manifested that *SOX4* was downregulated after silencing *HNF1A-AS1*, yet increased again in the presence of *miR-32-5p* inhibition (Figure 4F). Together, these results confirmed the ceRNA role of *HNF1A-AS1* in glioma via regulating *miR-32-5p/SOX4* axis.

3.5 | *SOX4* enrichment restores the carcinogenesis role of *HNF1A-AS1*

To further determine whether *HNF1A-AS1* facilitated glioma via acting as a ceRNA in regulating *miR-32-5p/SOX4* network, we performed rescue experiments functionally. First, we validated the overexpression efficiency of *SOX4* (Figure 5A). Next, we co-transfected pcDNA3.1/*SOX4* into *HNF1A-AS1*-depleted A172 and U87 cell lines. The mRNA and protein levels of *SOX4* were inhibited after silencing *HNF1A-AS1*, while recovered again after co-transfecting with pcDNA3.1/*SOX4* form qRT-PCR and western blot assays (Figure 5B,C). Based on the findings of functional rescue experiments, *SOX4* upregulation could reverse *HNF1A-AS1* silencing produced biological effects on cell proliferation (Figure 5D), apoptosis (Figure 5E,F; Figure S1E), migration (Figure 5G; Figure S1F), and invasion (Figure 5H; Figure S1G). Furthermore, we constructed xenograft mice models by inoculating A172 cell lines transfected with sh-NC, sh-*HNF1A-AS1*#2, and sh-*HNF1A-AS1*#2 + pcDNA3.1/*SOX4*. We found that silencing *HNF1A-AS1* could suppress tumor growth, but this effect was reversed by overexpressing *SOX4* (Figure 5I; Figure S1H). Tumor volume and weight were reduced by silenced *HNF1A-AS1* while co-transfection of *SOX4* rescued the suppressive influence of silenced *HNF1A-AS1* on tumor volume and weight (Figure 5J,K). To sum up, *HNF1A-AS1* contributed to glioma progression by targeting *miR-32-5p/SOX4* axis.

4 | DISCUSSION

Glioma is one of the most common types of intracranial tumors which initiate from the central nervous system. The prognosis of patients with high-grade glioma is much worse than those with low-grade glioma.¹⁴ Substantial studies supported the crucial regulator roles of lncRNAs in the initiation, development, and progression of various diseases, including tumors.¹⁵⁻¹⁷ *HNF1A-AS1* has been reported to exhibit carcinogenesis property in gastric cancer¹⁸ and non-small cell lung cancer.¹⁹ In the present study, we found the aberrant overexpression of *HNF1A-AS1* in glioma cell lines, which was in accordance with *HNF1A-AS1* overexpression in urothelial carcinoma of the bladder.²⁰ We noticed that the overexpression of *HNF1A-AS1* was activated by transcription factor MYC. Previously, MYC amplification has been discovered to promote homologous recombination via targeting CDK18 in glioblastoma.²¹ We found that *HNF1A-AS1* knock-down could suppress glioma cells proliferation, migration, and invasion abilities, while markedly enhance apoptosis capacity, which revealed the crucial oncogenic role of *HNF1A-AS1* in glioma progression. These findings accord with the function of *HNF1A-AS1* in oral squamous cell carcinoma.²²

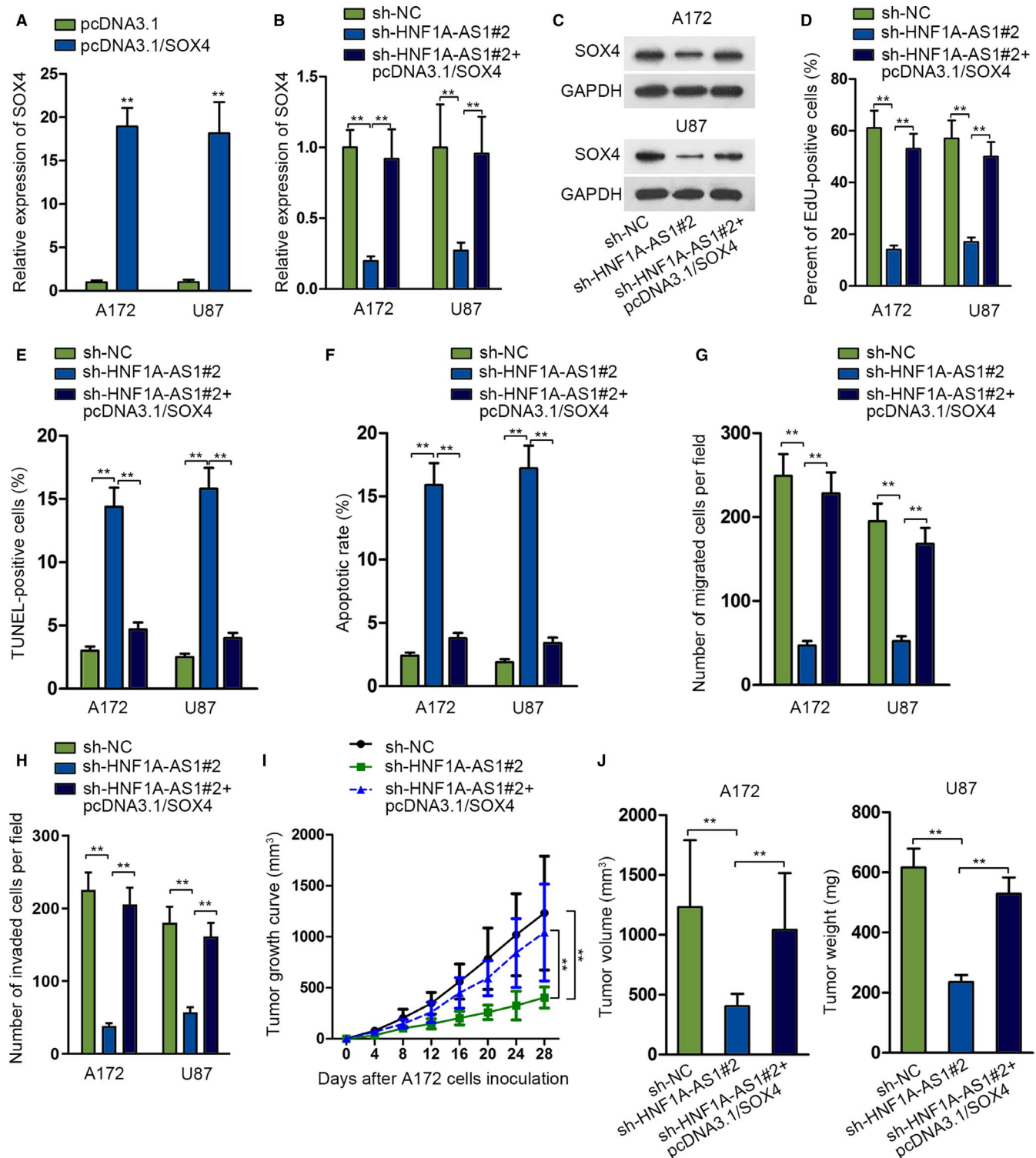


FIGURE 5 *SOX4* enrichment restores the carcinogenesis role of *HNF1A-AS1*. A, The expression of *SOX4* after transfecting with pcDNA3.1/*SOX4* was studied through quantitative RT-PCR (qRT-PCR) assay. B-C, qRT-PCR and western blot assays were used to detect the expression of *SOX4* mRNA and protein expression level. D-H, Rescue functional experiments were conducted among sh-NC, sh-*HNF1A-AS1*#2, and sh-*HNF1A-AS1*#2 + pcDNA3.1/*SOX4* groups in A172 and U87 cells. I-K, Xenograft tumor growth curve, volume, and weight were compared among sh-NC, sh-*HNF1A-AS1*#2, and sh-*HNF1A-AS1*#2 + pcDNA3.1/*SOX4* groups. ** $P < .01$

Over the last decade, the ceRNA crosstalk is prevalent. Numerous reports have found that a large number of lncRNAs were involved in the ceRNA network.²³ lncRNAs could

mediate the protein production of genes via acting as ceRNA according to substantial documents. Here, we detected the cytoplasmic localization of *HNF1A-AS1* through subcellular

fractionation and FISH assays. We screened downstream combinable miRNAs with *HNF1A-AS1* and selected *miR-32-5p* through multiple channel. Inhibited *miR-32-5p* counteracted the curbing influence of inhibited *HNF1A-AS1* in glioma progression. We initially uncovered that *HNF1A-AS1* bound with *miR-32-5p* and decreased its expression in glioma cells. MiR-32-5p also presents low expression and involve in the ceRNA axis of *SNHG14/miR-32-5p/SKIL* in colorectal cancer.²⁴ MiR-32-5p targets *HOXB8* to repress the cellular malignant behavior in cervical cancer cells.²⁵ LncRNA *GAS5* modulates *miR-32-5p/PTEN* axis to suppress pancreatic cancer metastasis.²⁶

Subsequently, we screened *SOX4* as the most potential target gene of *miR-32-5p* with putative binding site in its 3'UTR sequences. *SOX4* has been reported to be an oncogene supported by multiple lines of evidence. *SOX4* gene has been reported to be frequently amplified and upregulated in over 20 types of malignant tumors.^{27,28} In the field of glioma, *SOX4* has been found to be significantly elevated in glioblastoma multiforme.²⁹ Herein, we also detected the abnormal upregulation of *SOX4* in glioma cells in comparison to normal control cell, which was the same expression profile previously revealed in medulloblastoma.³⁰ We clarified the oncogenic role of *SXO2* in glioma via a collection of functional experiments. These data showed that *SOX4* overexpression could dampen cells proliferation, migration, and invasion, while stimulating apoptosis in glioma cells. Last but not least, *SOX4* overexpression could significantly reduce the biological effects induced by silencing *HNF1A-AS1* in glioma cells, which further confirmed the ceRNA role of *SOX4*.

All in all, we initially found that *MYC* induced *HNF1A-AS1* overexpression promoted glioma progression via modulating *miR-32-5p/SOX4* axis, providing potent reference value for targeting *HNF1A-AS1* in future therapeutic strategies of glioma.

ACKNOWLEDGMENT

We are very grateful to all individuals and groups involved in this study.

CONFLICTS OF INTERESTS

None.

AUTHORS' CONTRIBUTIONS

Jianheng Wu designed this study. Rong Li interpreted data. Linfan Li recorded data. Yimian Gu and Hui Zhan were responsible for preparation and investigation. Jianheng Wu, Rong Li and Changbao Zhou devoted to data curation and methods. Chuanhong Zhong wrote the manuscript. All authors reviewed the manuscript.

ETHICAL APPROVAL

All procedures were approved by the Ethics Committee of Gaozhou People's Hospital.

DATA AVAILABILITY STATEMENT

Research data and material are not shared.

ORCID

Chuanhong Zhong  <https://orcid.org/0000-0003-3283-9057>

REFERENCES

- Fouladseresht H, Ziaee SM, Erfani N, Doroudchi M. Serum levels of APRIL increase in patients with glioma, meningioma and schwannoma. *Asian Pac J Cancer Prev*. 2019;20(3):751-756.
- Ahammed Muneer KV, Rajendran VR, Paul Joseph K. Glioma tumor grade identification using artificial intelligent techniques. *J Med Syst*. 2019;43(5):113.
- Fang C, Qiu S, Sun F, et al. Long non-coding RNA HNF1A-AS1 mediated repression of miR-34a/SIRT1/p53 feedback loop promotes the metastatic progression of colon cancer by functioning as a competing endogenous RNA. *Cancer Lett*. 2017;410:50-62.
- Zhang X, Xiong Y, Tang F, Bian Y, Chen Y, Zhang F. Long noncoding RNA HNF1A-AS1 indicates a poor prognosis of colorectal cancer and promotes carcinogenesis via activation of the Wnt/beta-catenin signaling pathway. *Biomed Pharmacother*. 2017;96:877-883.
- Denzler R, Agarwal V, Stefano J, Bartel DP, Stoffel M. Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol Cell*. 2014;54(5):766-776.
- Hu X, Hong Y, Shang C. Knockdown of long non-coding RNA SNHG5 inhibits malignant cellular phenotypes of glioma via Wnt/CTNNB1 signaling pathway. *J Cancer*. 2019;10(5):1333-1340.
- Zhang C, Qu Y, Xiao H, et al. LncRNA SNHG3 promotes clear cell renal cell carcinoma proliferation and migration by upregulating TOP2A. *Exp Cell Res*. 2019;384(1):111595.
- Zhang C, Liu J, Zhang Y, et al. LINC01210 accelerates proliferation, invasion and migration in ovarian cancer through epigenetically downregulating KLF4. *Biomed Pharmacother*. 2019;119:109431.
- Zhang Y, Yang G, Luo Y. Long non-coding RNA PVT1 promotes glioma cell proliferation and invasion by targeting miR-200a. *Exp Ther Med*. 2019;17(2):1337-1345.
- Dong H, Cao W, Xue J. Long noncoding FOXD2-AS1 is activated by CREB1 and promotes cell proliferation and metastasis in glioma by sponging miR-185 through targeting AKT1. *Biochem Biophys Res Comm*. 2019;508(4):1074-1081.
- Liu Z, Lu C, Hu H, et al. LINC00909 promotes tumor progression in human glioma through regulation of miR-194/MUC1-C axis. *Biomed Pharmacother*. 2019;116:108965.
- Xu W, Hu GQ, Da Costa C, et al. Long noncoding RNA UBE2R2-AS1 promotes glioma cell apoptosis via targeting the miR-877-3p/TLR4 axis. *Onco Targets Ther*. 2019;12:3467-3480.
- Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. 2014;42(D1):D92-D97.

14. Ordys BB, Launay S, Deighton RF, McCulloch J, Whittle IR. The role of mitochondria in glioma pathophysiology. *Mol Neurobiol*. 2010;42(1):64-75.
15. Liu Y, Yin L, Chen C, Zhang X, Wang S. Long non-coding RNA GAS5 inhibits migration and invasion in gastric cancer via interacting with p53 protein. *Dig Liver Dis*. 2020;52(3):331-338.
16. Vafadar A, Shabaninejad Z, Movahedpour A, et al. Long non-coding RNAs: epigenetic regulators in cancer. *Curr Pharm Des*. 2019;25(33):3563-3577.
17. Xu YH, Deng JL, Wang G, Zhu YS. Long non-coding RNAs in prostate cancer: functional roles and clinical implications. *Cancer Lett*. 2019;464:37-55.
18. Liu HT, Liu S, Liu L, Ma RR, Gao P. EGR1-mediated transcription of lncRNA-HNF1A-AS1 promotes cell-cycle progression in gastric cancer. *Can Res*. 2018;78(20):5877-5890.
19. Liu L, Chen Y, Li Q, Duan P. lncRNA HNF1A-AS1 modulates non-small cell lung cancer progression by targeting miR-149-5p/Cdk6. *J Cell Biochem*. 2019;120(11):18736-18750.
20. Wang YH, Liu YH, Ji YJ, Wei Q, Gao TB. Upregulation of long non-coding RNA HNF1A-AS1 is associated with poor prognosis in urothelial carcinoma of the bladder. *Eur Rev Med Pharmacol Sci*. 2018;22(8):2261-2265.
21. Ning J-F, Stanciu M, Humphrey MR, et al. Myc targeted CDK18 promotes ATR and homologous recombination to mediate PARP inhibitor resistance in glioblastoma. *Nat Commun*. 2019;10(1):2910.
22. Liu Z, Li H, Fan S, Lin H, Lian W. STAT3-induced upregulation of long noncoding RNA HNF1A-AS1 promotes the progression of oral squamous cell carcinoma via activating Notch signaling pathway. *Cancer Biol Ther*. 2019;20(4):444-453.
23. Gou L, Zou H, Li B. Long noncoding RNA MALAT1 knock-down inhibits progression of anaplastic thyroid carcinoma by regulating miR-200a-3p/FOXA1. *Cancer Biol Ther*. 2019;20(11):1355-1365.
24. Ye T, Zhang N, Wu W, et al. SNHG14 promotes the tumorigenesis and metastasis of colorectal cancer through miR-32-5p/SKIL axis. *In Vitro Cell Dev Biol Anim*. 2019;55(10):812-820.
25. Liu YJ, Zhou HG, Chen LH, et al. MiR-32-5p regulates the proliferation and metastasis of cervical cancer cells by targeting HOXB8. *Eur Rev Med Pharmacol Sci*. 2019;23(1):87-95.
26. Gao Z-Q, Wang J-F, Chen D-H, et al. Long non-coding RNA GAS5 suppresses pancreatic cancer metastasis through modulating miR-32-5p/PTEN axis. *Cell Biosci*. 2017;7:66.
27. Moreno CS. SOX4: the unappreciated oncogene. *Semin Cancer Biol*. 2019;S1044-579X(18):30145-30147.
28. Ha Thi HT, Kim HY, Kim YM, Hong S. MicroRNA-130a modulates a radiosensitivity of rectal cancer by targeting SOX4. *Neoplasia (New York, NY)*. 2019;21(9):882-892.
29. Lin B, Madan A, Yoon J-G, et al. Massively parallel signature sequencing and bioinformatics analysis identifies upregulation of TGFBI and SOX4 in human glioblastoma. *PLoS One*. 2010;5(4):e10210.
30. de Bont, JM Kros JM, Passier MMCJ, et al. Differential expression and prognostic significance of SOX genes in pediatric medulloblastoma and ependymoma identified by microarray analysis. *Neuro-oncology*. 2008;10(5):648-660.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Wu J, Li R, Li L, et al. MYC-activated lncRNA *HNF1A-AS1* overexpression facilitates glioma progression via cooperating with *miR-32-5p/SOX4* axis. *Cancer Med*. 2020;9:6387–6398. <https://doi.org/10.1002/cam4.3186>