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LncRNA SAMD12-AS1 down-regulates P53 to promote malignant progression of glioma

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Abstract. – OBJECTIVE: To study the expression level of long non-coding RNA (LncRNA) SAMD12-AS1 in glioma and its influence on the invasive ability of glioma cells; meanwhile, the regulation mechanism of LncRNA SAMD12-AS1 promoting the development of glioma was further explored.

PATIENTS AND METHODS: The expression level of SAMD12-AS1 in 40 pairs of tumor tissue specimens and paracancerous ones collected from glioma patients were examined through Real Time quantitative-Polymerase Chain Reaction (qRT-PCR) method, and the interplay between SAMD12-AS1 expression and clinical indicators of glioma patients was also analyzed. Meanwhile, the expression of SAMD12-AS1 in glioma cell lines was further verified by qRT-PCR. In addition, SAMD12-AS1 knockdown model was constructed using glioma cell lines (T98-G and U87). Cell Counting Kit-8 (CCK-8), cell wound healing test, and transwell assays were conducted to examine the impact of SAMD12-AS on glioma cell functions. Additionally, whether it exerted its biological characteristics through P53 was finally explored.

RESULTS: qPCR results in the study revealed that SAMD12-AS1 expression in tumor tissue specimens of glioma patients was remarkably higher than that in the adjacent ones, and the difference was statistically significant. Compared with patients with low expression of SAMD12-AS1, patients with high expression of SAMD1-AS1 had a higher incidence of lymph node or distant metastasis. In addition, compared to the NC group, knocking down SAMD12-AS1 markedly attenuated the proliferation rate, as well as the invasiveness and migration ability of glioma cells. Subsequently, in glioma tissues, it was verified that P53 expression was remarkably decreased and negatively correlated with SAMD12-AS1. Finally, cell recovery experiment also demonstrated that there may exist a mutual regulation between SAMD12-AS1 and P53, which then together affected the malignant progression of glioma.

CONCLUSIONS: LncRNA SAMD12-AS1 may accelerate the invasion and migratory capacities of glioma cells by modulating P53, and its expression was confirmed to be significantly relevant to the incidence of lymph node or distant metastasis.

Key Words:
LncRNA SAMD12-AS1, P53, Glioma, Metastasis.

Introduction

Glioma is the most common malignant tumor of adult central nervous system. According to the statistics of the American Brain Tumor Registry (CBTRUS), glioma accounts for 28% of all central nervous system tumors and 80% of intracranial malignant tumors[1,2]. Among them, the median survival time of glioblastoma is less than 1.5 years, while the 5-year survival rate is only 0.05%-4.7%[3,4]. The pathogenesis of glioma is not very clear, and it is generally believed to be related to genetic, immune dysfunction, living environment, etc. Currently, the exposure to high dose of ionizing radiation and mutation of high penetrability gene related to rare syndrome have been identified as two risk factors for glioma[5,6]. At present, the treatment of glioma mainly includes surgical resection combined with postoperative radiotherapy and chemotherapy[7]. Surgery can safely remove tumor lesions in the largest range, thus delaying clinical symptoms, improving patients' quality of life, extending survival time, and obtaining specimens for pathological diagnosis and molecular biology research[8,9]. However, due to the invasive growth of glioma, surgical treatment is difficult to achieve complete resection, which often leads to the relapse. The postoperative adjuvant therapies, such as chemotherapy and radiotherapy, cannot kill glioma cells in a highly specific way, and may also produce toxic and side

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effects of the central nervous system[10]. Therefore, it is of great importance to elucidate the underlying mechanism of glioma and find more potential therapeutic targets to improve the current diagnosis and treatment[11].

Long non-coding RNA (LncRNA), located in the nucleus or cytoplasm, is a type of RNA with a transcription length of more than 200 nucleotides and no protein coding function[12,13]. According to the positions of lncRNA and coding genes, they can be divided into five types, namely, lncRNA, antisense lncRNA, bi-directional lncRNA, intra-gene lncRNA, and extracellular lncRNA[13]. LncRNA can mediate signaling pathway, function as molecular bait, provide molecular guidance for ribonucleoprotein complex to specific chromatin sites, and function as scaffold of complex formation[14,15]. LncRNA can regulate the expression of protein-coding genes at epigenetic, transcriptional, and post-transcriptional levels[16]. Current studies[14,17] have shown that the expression levels of some lncRNA are remarkably different in normal tissues and tumor tissues, and the abnormally expressed lncRNA is closely related to the occurrence and development of tumor, suggesting that lncRNA will play a pivotal role in the diagnosis, treatment, and prognosis of tumor. Liu et al[18] have found that the newly discovered LncRNA SAMD12-AS1 may play an important role in tumor progression. However, few studies have mentioned the function of SAMD12-AS1 in glioma. Thus, exploring the specific function of LncRNA SAMD12-AS1 in glioma might provide a new understanding of the pathogenesis and provide a potential therapeutic target for glioma treatment.

Human P53 gene is located at 17p13.1, which is composed of 11 exons and 10 introns[19,20]. The wild-type P53 protein encoded by P53 transcription translation is composed of 393 amino acid residues and contains multiple functional domains[21,22]. Activated P53 protein induces a variety of biological effects by regulating the expression of its target genes and downstream signal transmission[22]. Some studies[23,24] have shown that excessive expression and activation of P53 can lead to malignant transformation of cells and the occurrence and development of tumors, so the signal transduction pathway involved in P53 has also become a research hotspot for the targeted therapy of malignant glioma. Currently, the regulation mechanism of LncRNA SAMD12-AS1 in P53 in glioma cells has rarely been explored. Therefore, in this study, the possible roles of LncRNA SAMD12-AS1 and P53 in the occurrence and development of glioma and their molecular regulatory mechanism were expounded respectively, which may bring new ideas for the diagnosis and treatment of glioma.

Patients and Methods

Glioma Samples

Tumor tissue samples and paracancerous ones of 40 glioma patients undergoing glioma radical resection were collected. All patients did not receive any radiotherapy or chemotherapy before surgery. The pathological classification and staging criteria of glioma were performed according to the international collateral cancer staging criteria (international union against cancer, UICC). Patients and their families in this study had been fully informed, and our research was approved by the Ethics Oversight Committee. This study was also approved by the Animal Ethics Committee of Soochow University Animal Center.

Cell Lines and Reagents

The human glioma cell lines (U251, U87, T98-G, A172) and Human brain normal glial cell line (HEB) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). High glucose Dulbecco's Modified Eagle's Medium (DMEM) medium and fetal bovine serum (FBS) were purchased from Life Technologies (Gaithersburg, MD, USA). All cell lines were cultured with DMEM high glucose medium containing 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) in a 37°C, 5% CO_2 incubator.

Transfection

T98-G and U87 glioma cells were seeded into 6-well plates and cultured to a cell density of about 50%-70%. sh-NC and sh-SAMD12-AS1 sequence were transfected into glioma cells. Subsequently, after simultaneously transfected with sh-SAMD12-AS1 and sh-P53, glioma cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments 48 hours later.

Cell Proliferation Assay

The transfected cells were collected and plated into 96-well plates at 2000 cells per well. After cultured for 24 h, 48 h, 72 h, and 96 h respectively, 10 μL of Cell Counting Kit-8 (CCK-8) solution (Dojindo, Kumamoto, Japan) was added per well for incubation for 1 hour, and then, the optical density

(OD) value of each well was measured in the microplate reader at 450 nm absorption wavelength.

Cell Wound Healing assay

The cells after transfection for 48 hours were digested, centrifuged, and resuspended in the medium without FBS to adjust the density to 5 x 10^5 cells/mL. The density of the plated cells was determined according to the size of the cells (the majority of the number of cells plated was set to 50000 cells/well), and the confluence of the cells reached 90% or more the next day. After the stroke, the cells were rinsed gently with phosphate-buffered saline (PBS) for 2-3 times and observed again after 24 hours incubation with low-concentration serum medium.

Transwell Assay

When the cell density reached to 3×10^5/well, they were seeded in a 6-well plate. Liposomal transfection experiments can be performed when cell confluence reached 80%. Positive clones were selected for expanded culture for subsequent transwell experiments, and the transwell chamber containing Matrigel and no Matrigel was placed in a 24-well plate. 200 μL of the cell suspension was added in the upper chamber, and 500 μL of a medium containing 10% FBS was added to the lower chamber. After incubated in a 37°C incubator for 48 hours, the chamber was removed, fixed with 4% paraformaldehyde for 30 minutes, and stained with crystal violet for 15 minutes. Subsequently, the cells were washed with PBS, and the inner surface of the basement membrane of the chamber was carefully cleaned to remove the inner layer cells. The perforated cells stained in the outer layer of the basement membrane of the chamber were observed under the microscope, and 10 fields of view were randomly selected.

QRT-PCR

The total RNA was extracted in one step by TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and reverse transcribed into the first strand of complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent reverse transcription kit. The qPCR reaction was performed using SYBR® Premix Ex Taq™ and StepOne Plus Real Time-PCR system. The following primers were used for qPCR reaction: SAMD12-AS1: forward: 5'-CGTCTCTCCAAAGCAACTGAA-3', reverse: 5'-CTTGAACTCCAGCAACTCTAGTC-3'; P53: forward: 5'-ACTGCCTTCCGGGTCACTGC-3', reverse: 5'-GTCAGTGGGGAACAAGAAGTGGAG-3'; β-actin: forward: 5'-CCTGGCACCCAGCACAAT-3', reverse: 5'-GCTGATCCACATCTGCTGGAA-3'. Three replicate wells were repeated for each sample and the assay was repeated twice. The Bio-Rad (Hercules, CA, USA) PCR instrument was used to analyze and process the data. The β-actin and U6 genes were used as internal parameters, and the gene expression was calculated by 2-ΔΔCt method.

Western Blot

The total proteins of tissues or cells were extracted by radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China). After the protein concentration was determined by the Bradford method, the protein sample was denatured at 100°C for 5 min, and an appropriate amount of the loading buffer was added. The denatured protein sample was pipetted to the sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and blocked with 5% skim milk for 1 h at room temperature. The primary antibody against P53 (1:1000) was added for incubation overnight at 4°C shaker. In the next day, the membrane was rinsed 3 times with Tris-Buffered Saline-Tween 20 (TBST) and incubated with second antibody for 1 h at room temperature. After that, the protein samples on the membrane were finally semi-quantitatively analyzed by alpha SP image analysis software.

Statistical Analysis

The statistical analysis was performed using the Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM Corp., Armonk, NY, USA). The *t*-test was used to compare the measurement data, and the categorical variables were analyzed by χ^2-test or Fisher's exact probability method. Survival analysis was performed using the Kaplan-Meier method and survival curves were plotted. Data were expressed as mean ± standard deviation, and $p<0.05$ was considered to be statistically significant.

Results

SAMD12-AS1 Was Highly Expressed in Glioma Tissues and Cell Lines

To investigate the expression pattern of SAMD12-AS1 in glioma patients, we analyzed the expression of SAMD12-AS1 by qRT-PCR and

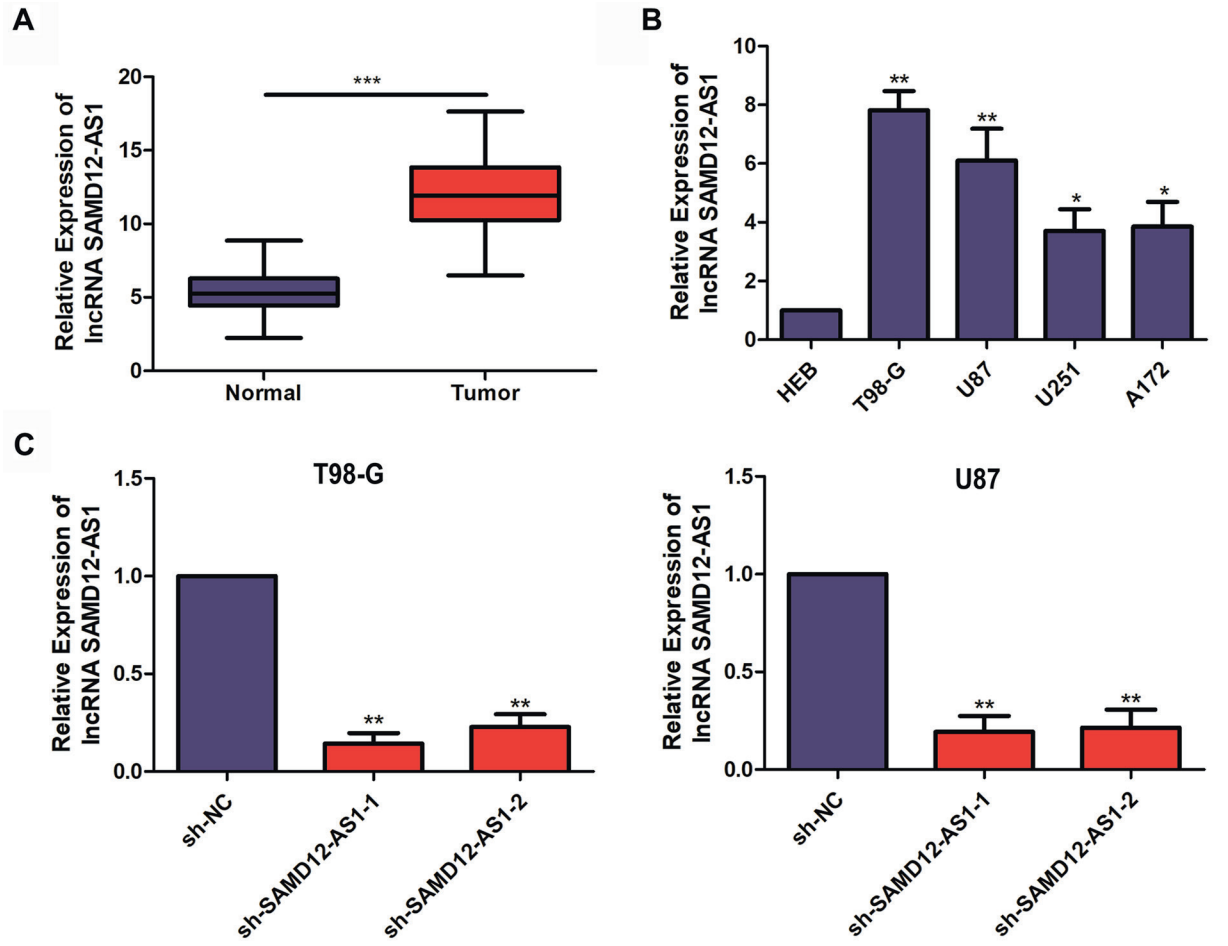

Figure 1. High expression of SAMD12-AS1 in glioma tissues and cell lines. **A,** qRT-PCR was used to detect the difference in expression of SAMD12-AS1 in glioma tumor tissues and adjacent tissues. **B,** qRT-PCR was used to detect the expression level of SAMD12-AS1 in glioma cell lines. **C,** qRT-PCR verified the interference efficiency of SAMD12-AS1 after transfection of the SAMD12-AS1 knockdown vector in the T98-G and U87 cell lines. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

found that SAMD12-AS1 was highly expressed in glioma tissues relative to paracancerous tissue samples (Figure 1A). Additionally, SAMD12-AS1 showed a markedly higher expression level in glioma cell lines than in HEB, especially in T98-G and U87 cell lines, which were therefore selected for subsequent experiments (Figure 1B). These results suggested that SAMD12-AS1 might play a crucial role in glioma.

SAMD12-AS1 Expression Was Correlated with Lymph Node and Distance Metastasis in Glioma Patients

The interplay between SAMD12-AS1 level and some clinical indicators of glioma patients including the age, sex, and pathological stage, along with the situation of lymph node or distant metastasis were analyzed according to the tissue specimens obtained from the 40 patients with glioma. As shown in Table I, SAMD12-AS1 low expression was positively correlated with the incidence of glioma lymph node or distant metastasis, but not with other indicators.

Knockout of SAMD12-AS1 Inhibited Cell Proliferation, Migration, and Invasion in Glioma

To figure out the impact of SAMD12-AS1 on the function of glioma cells, we first successfully constructed SAMD12-AS1 knockdown cell model and verified it by qPCR (Figure 1C). Subsequently, the examination of glioma cell proliferation, invasion, and migration abilities were performed in the T98-G and U87 cell lines, respectively. As a result, CCK-8 and transwell assays demonstrated that compared

with the NC group, the proliferation, as well as the invasiveness and migration abilities of glioma cells, were all remarkably attenuated after knockdown of SAMD12-AS1 (Figure 2A, 2B). Meanwhile, the cell wound healing test showed that SAMD12-AS1 downregulation caused a decrease in the crawling ability of glioma cells (Figure 2C). In sum, knockout of SAMD12-AS1 inhibited cell proliferation, migration, and invasion in glioma.

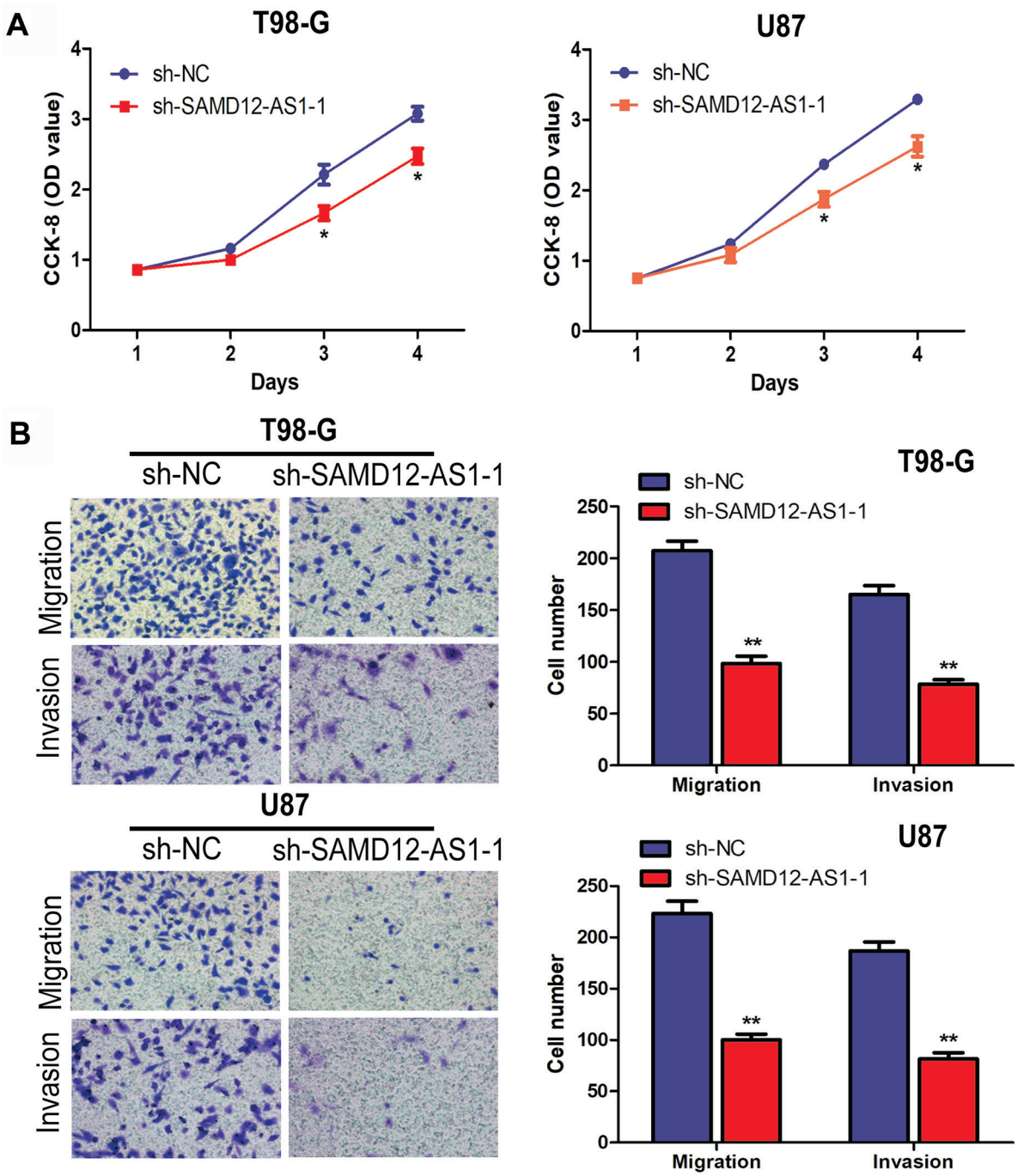

Figure 2. Knockdown of SAMD12-AS1 inhibits glioma cell proliferation and invasion and migration. **A,** The CCK-8 assay was performed to detect the effect of transfection of the SAMD12-AS1 knockdown vector on the proliferation of glioma cells in the T98-G and U87 cell lines. **B,** The transwell migration invasion assay was performed to detect the invasion and migration ability of glioma cells after transfecting the SAMD12-AS1 knockdown vector in the T98-G and U87 cell lines (magnification: 40×).

Figure continued

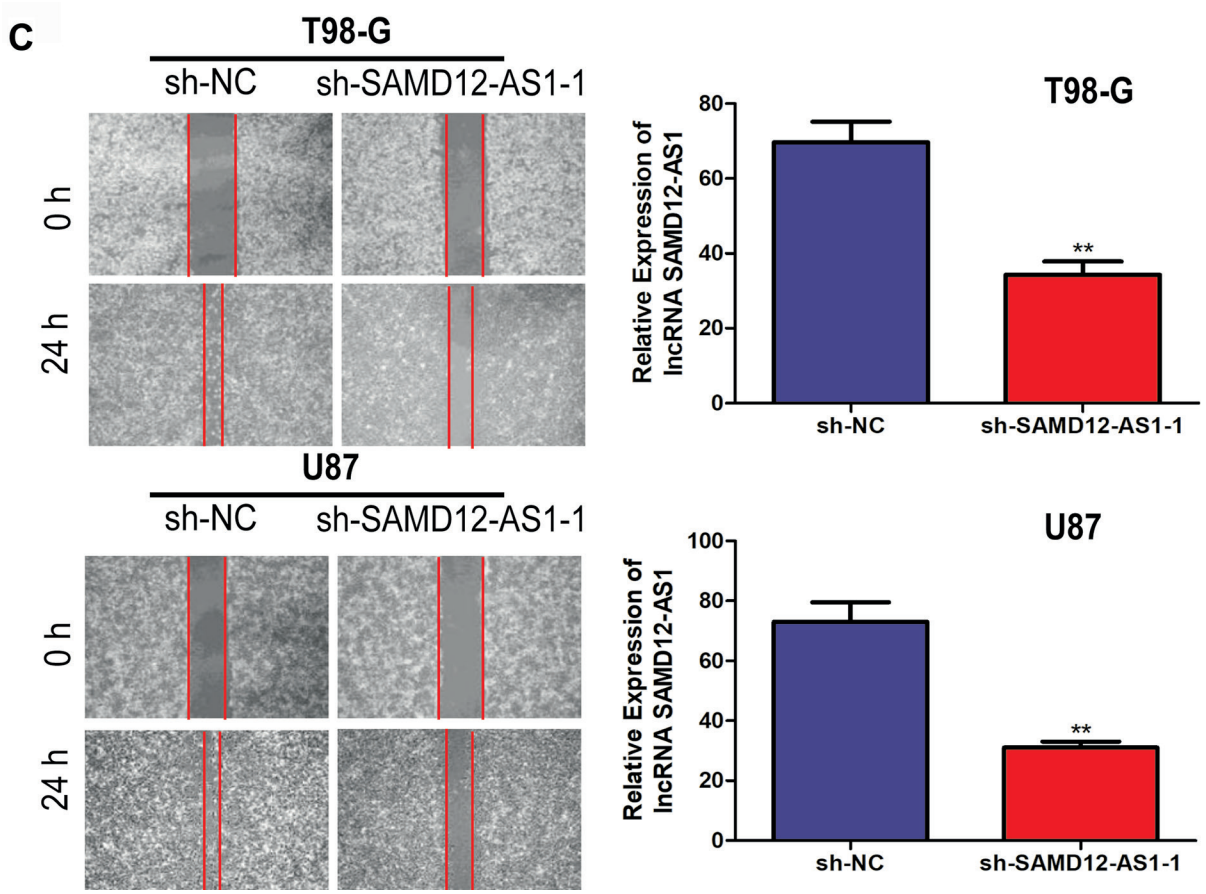

Figure 2. *(Continued).* **C,** Cell wound healing assay was performed to detect the crawling ability of glioma cells after transfecting the SAMD12-AS1 knockdown vector in the T98-G and U87 cell lines (magnification: 40×). Data are mean ± SD, *p<0.05, **p<0.01.

Table I. Association of lncRNA SAMD12-AS1 expression with clinicopathologic characteristics of glioma.

Parameters	Number of cases	SAMD12-AS1 expression		p-value
		Low (%)	High (%)	
Age (years)				0.492
<60	21	12	9	
≥60	19	9	10	
Gender				0.492
Male	19	9	10	
Female	21	12	9	
Tumor size				0.707
≤2 cm	20	8	10	
>2 cm	20	11	9	
T stage				0.907
T1-T2	25	13	12	
T3-T4	15	8	7	
Lymph node metastasis				0.028
No	27	17	10	
Yes	13	4	9	
Distance metastasis				
No	26	17	9	0.011
Yes	14	4	10	

P53 Was Lowly Expressed in Glioma Tissues and Cell Lines

Bioinformatics studies have revealed that there may be some association between SAMD12-AS1 and P53 in gliomas. It was found by Western blot that the knockdown of SAMD12-AS1 in glioma cell lines remarkably enhanced the expression of P53 protein, indicating a close interaction between SAMD12-AS1 and P53 (Figure 3A); similarly, qPCR reflected the same trend (Figure 3B). In addition, in glioma tissue samples, P53 expression was also found reduced compared to the paracancerous ones (Figure 3C), which indicated a negative correlation between SAMD12-AS1 and P53 in glioma tissues (Figure 3D).

SAMD12-AS1 Modulated P53 Expression in Human Glioma Cells

To further explore the ways in which SAMD12-AS1 and P53 promote malignant progression of gliomas, we simultaneously knocked down P53, as well as SAMD12-AS1, in glioma cell lines and verified the transfection efficiency through Western blot (Figure 3E). Subsequently, the transwell migration assay demonstrated that the co-transfection of P53 and SAMD12-AS1 remarkably elevated the number of transmembrane glioma cells in the transwell chamber, which counteracted the influence of SAMD12-AS1 knockdown on glioma cell metastasis ability (Figure 3F).

Knockout of SAMD12-AS1 Inhibited Glioma Progression In Vivo

To better explore the role of SAMD12-AS1 *in vivo*, the transfected T98-G cell line was inoculated *in situ* into each nude mouse and injected in the left axilla. As shown in Figure 4A and 4B, the tumor volume of nude mice inoculated with the SAMD12-AS1 knockdown vector was reduced. As expected, the tumor weight knockdown of SAMD12-AS1 was reduced compared to the sh-NC group (Figure 4C). Subsequently, the RNA of the tumor-forming tissues of nude mice was extracted, and qPCR results showed that the expression of SAMD12-AS1 in the tumor-forming tissues of nude mice was remarkably decreased after knocking down SAMD12-AS1 (Figure 4D), while P53 expression showed the opposite trend (Figure 4E), suggesting that the knockdown of SAMD12-AS1 could suppress tumor formation *in vivo*. These results suggested that the knockout of SAMD12-AS1 inhibited glioma progression *in vivo*.

Discussion

Fundamentally, tumor is a genetic disease that changes the flow of cell information, which not only affects cell homeostasis but also produces growth-promoting effects[25]. The discovery of the universal genetic code of protein-coding genes has completed a series of breakthroughs in the understanding of the effect of mutations on tumors, and established a scientific theoretical basis for targeted therapy of malignant tumors[25,26]. The most unexpected discovery in the age of genomics was the widespread transcription of RNA in non-coding regions of the genome[27].

LncRNA plays an indispensable role in the initiation of tumor genesis and progression through lncRNA-mediated biological mechanisms[12-14]. Abnormal lncRNA expression in tumors indicates that lncRNA can also serve as an independent predictor of tumor prognosis[15]. Functionally, many lncRNAs can regulate the expression of some key genes through epigenetic level and the effect on gene enhancers. LncRNA can also regulate the activity of tumor suppressor genes. Besides, lncRNA also plays a regulatory role in mRNA processing and translation[14,16,17]. Therefore, finding the lncRNA that is abnormally expressed in tumor tissues, determining its target mRNA, and defining the function of the expression product of target mRNA are the key steps to elucidate the mechanism of tumor occurrence and development, as well as the necessary theoretical basis for the targeted treatment of tumor with miRNA[14,17].

In this study, qPCR revealed a higher expression of SAMD12-AS1 in glioma tissue samples than in the adjacent normal ones; meanwhile, its expression was found positively correlated with the incidence of lymph node and distant metastasis, suggesting that SAMD12-AS1 may act as cancer-promoting gene in glioma. Tumor metastasis refers to the process in which tumor cells shed and spread from the *in situ* to the remote target organ and adapt to the new tissue microenvironment. The two necessary conditions for the smooth implementation of the process are movement and survival[14,18]. In this study, to further figure out the impact of SAMD12-AS1 on the biological function of glioma, lentivirus was used to construct SAMD12-AS1 knockdown model, and the results of cell scratching and transwell assay revealed that SAMD12-AS1 could promote the metastasis of glioma and play a pivotal role, but the specific molecular mechanism still remains elusive.

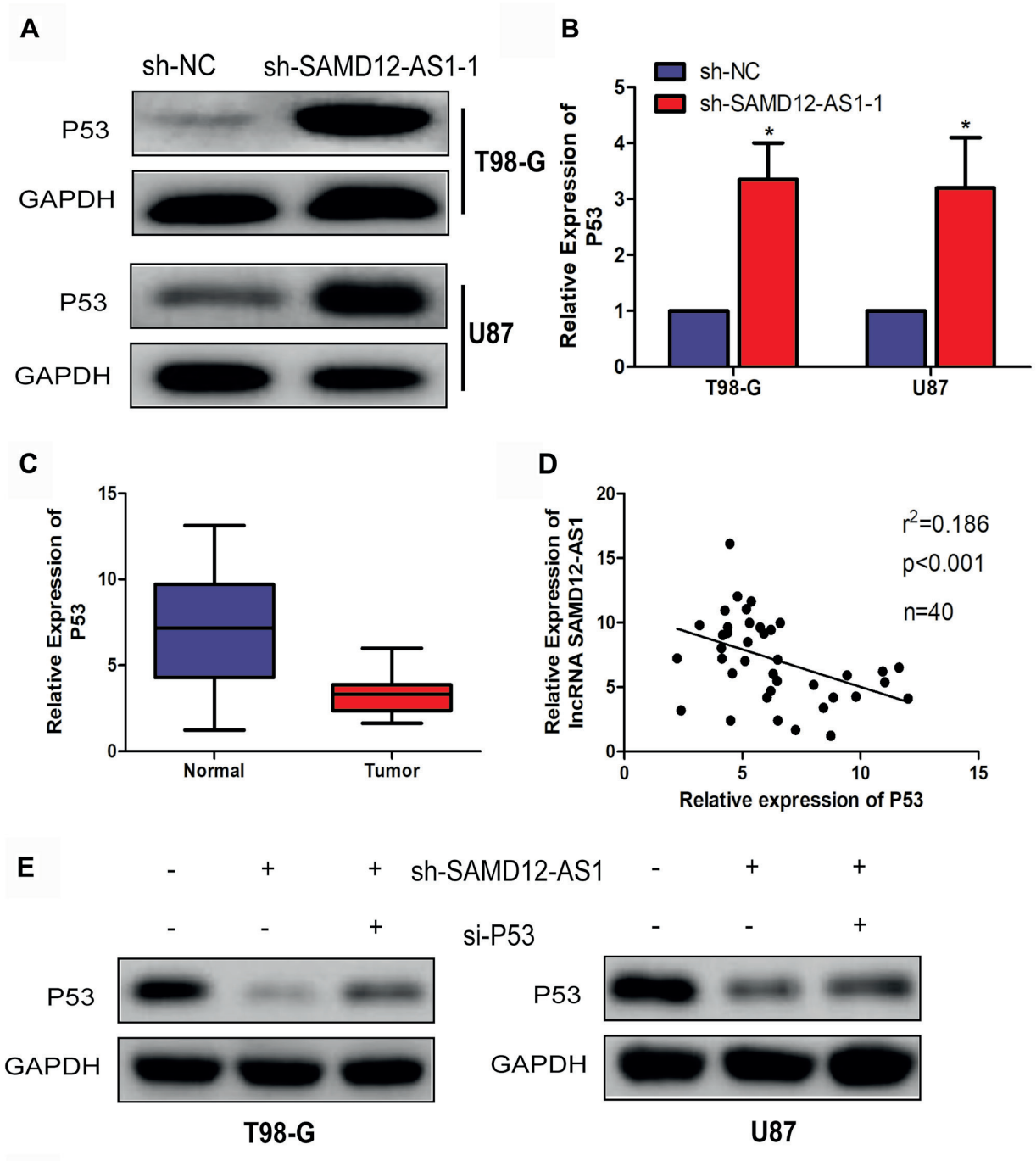

Figure 3. Low expression of P53 in glioma tissues and cell lines. **A,** Western blot verified the expression level of P53 after transfection of the SAMD12-AS1 knockdown vector in the T98-G and U87 cell lines. **B,** qRT-PCR verified the expression level of P53 after transfection of the SAMD12-AS1 knockdown vector in the T98-G and U87 cell lines. **C,** qRT-PCR was used to detect the difference in expression of P53 in glioma tumor tissues and adjacent tissues. **D,** There was a significant negative correlation between the expression levels of SAMD12-AS1 and P53 in glioma tissues. **E,** Western blot was used to detect the expression of P53 in glioma cell lines co-transfected with SAMD12-AS1 and P53.

Figure continued

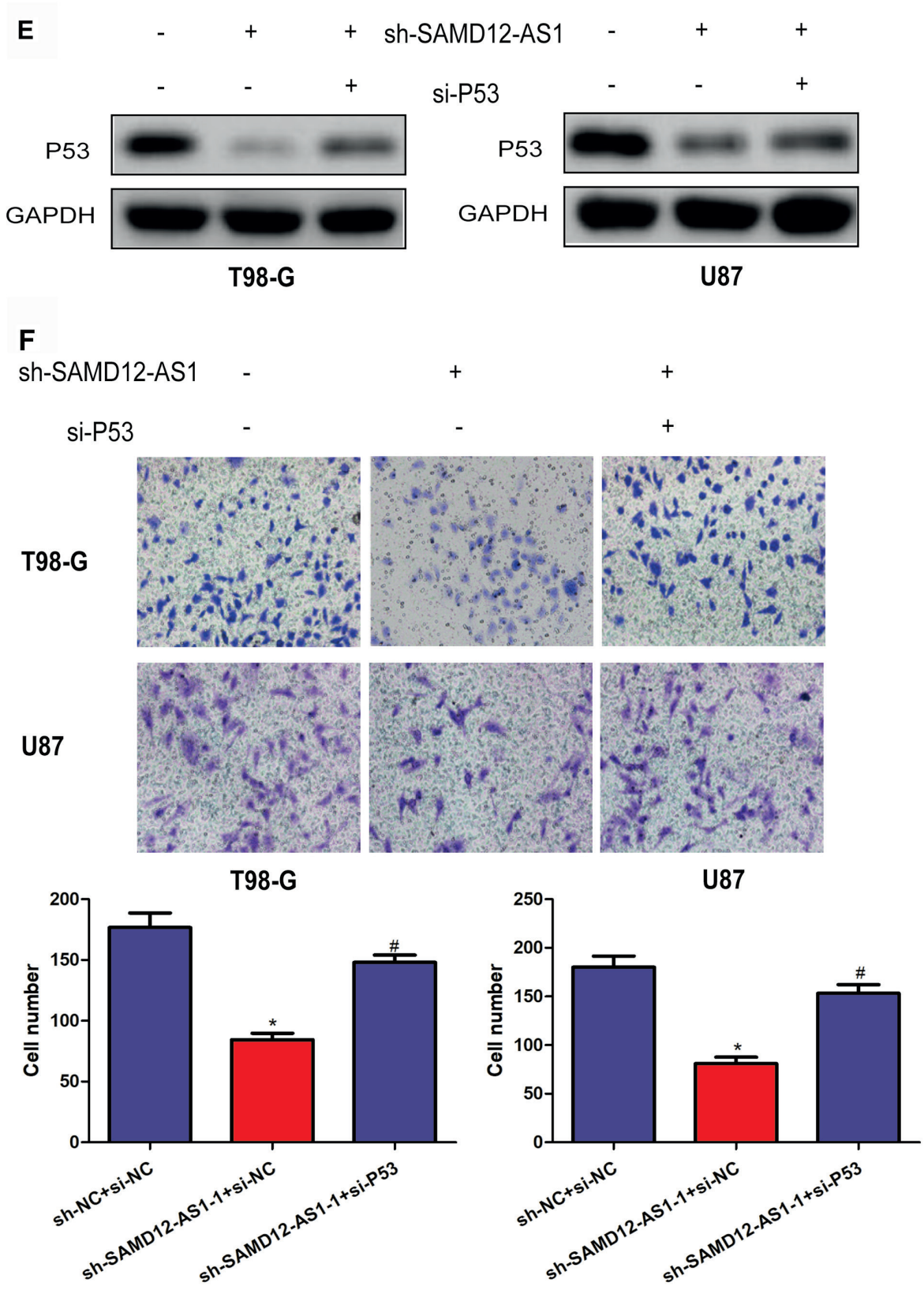

Figure 3. *(Continued).* **E,** Western blot was used to detect the expression of P53 in glioma cell lines co-transfected with SAMD12-AS1 and P53. **F,** The transwell invasion assay was performed to detect the invasion and migration ability of glioma cells after co-transfection of SAMD12-AS1 and P53 in glioma cell lines (magnification: 40×). Data are mean ± SD, *#p<0.05.

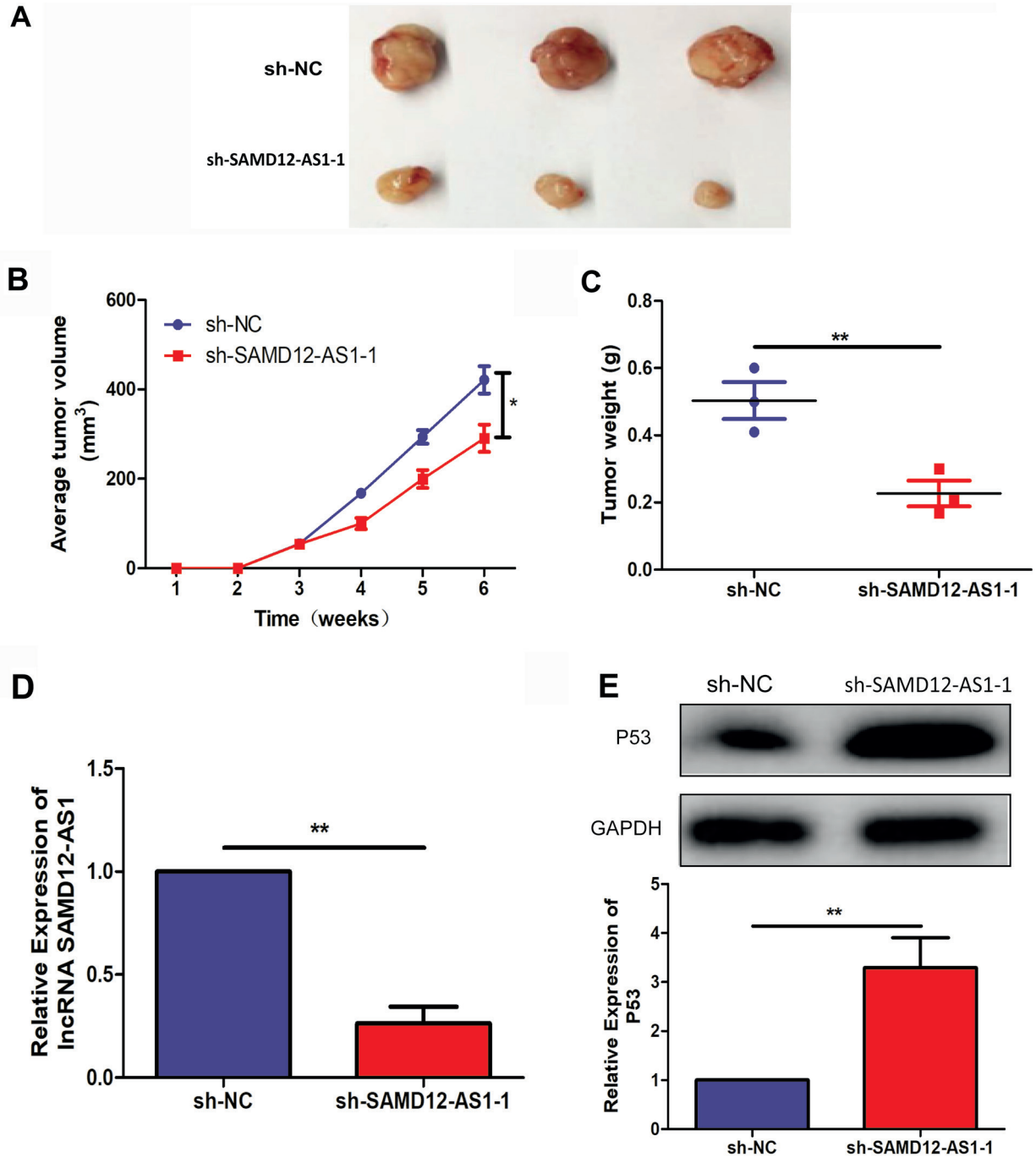

Figure 4. Knockdown of SAMD12-AS1 inhibits tumorigenicity in nude mice. **A,** Gross images of different nude mice after injection of sh-NC and sh-SAMD12-AS1 were shown, respectively. **B,** Tumor volume growth curves were calculated after injection of sh-NC and sh-SAMD12-AS1, respectively. **C,** Tumor weight growth curves were calculated for different nude mice after injection of sh-NC and sh-SAMD12-AS1, respectively. **D,** qRT-PCR was used to detect the level of SAMD12-AS1 in the tumor-forming tissues of glioma in nude mice. **E,** qPT-PCR and Western blotting were used to detect the level of P53 in glioma tumor tissue of the nude mice. Data are mean ± SD, *p<0.05, **p<0.01.

To clarify the biological function of lncRNA, we need to further search for its target genes and explore its interaction with lncRNA on the process of tumor genesis and development[19-21]. Subsequently, the expression of P53 was detected by RT-PCR, and the results showed that the expression of P53 in brain glioma tumor tissues was remarkably downregulated. In addition, it was further verified that the knockdown of SAMD12-AS1 remarkably upregulated mRNA and protein expression of P53. Subsequently, the recovery experiment verified that the knocking down of P53 could remarkably improve the number of transmembrane penetrating glioma cells in the transwell chamber and their crawling ability after silencing SAMD12-AS1, thereby offsetting the role of SAMD12-AS1 silencing in invasion and metastasis of glioma cells.

Conclusions

SAMD12-AS1 expression was remarkably correlated with lymph node and distant metastasis in glioma and it may enhance the invasiveness and migration ability of glioma cells *via* regulating P53.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Gusyatiner O, Hegi ME. Glioma epigenetics: from subclassification to novel treatment options. Semin Cancer Biol 2018; 51: 50-58.
2) Niu H, Li X, Yang A, Jin Z, Wang X, Wang Q, Yu C, Wei Z, Dou C. Cycloartenol exerts anti-proliferative effects on glioma U87 cells via induction of cell cycle arrest and p38 MAPK-mediated apoptosis. J BUON 2018; 23: 1840-1845.
3) Pierscianek D, Ahmadipour Y, Oppong MD, Rauschenbach L, Kebir S, Glas M, Sure U, Jabbarli R. Blood-based biomarkers in high grade gliomas: a systematic review. Mol Neurobiol 2019; 56: 6071-6079.
4) Yan C, Wang J, Yang Y, Ma W, Chen X. Molecular biomarker-guided anti-angiogenic targeted therapy for malignant glioma. J Cell Mol Med 2019; 23: 4876-4882.
5) Ren D, Yang C, Liu N, Wang Z, Wang X, Johnson M, Wang W, Zhang P, Yang H, Liu H, Cheng Y, Wang L, Tu Y. Gene expression profile analysis of U251 glioma cells with shRNA-mediated SOX9 knockdown. J BUON 2018; 23: 1136-1148.
6) Yuan X, Liu D, Wang Y, Li X. Significance of nuclear magnetic resonance combined with Ki-67 and VEGF detection in the diagnosis and prognosis evaluation of brain glioma. J BUON 2018; 23: 410-415.
7) Woolf EC, Scheck AC. The ketogenic diet for the treatment of malignant glioma. J Lipid Res 2015; 56: 5-10.
8) Garzon-Muvdi T, Kut C, Li X, Chaichana KL. Intraoperative imaging techniques for glioma surgery. Future Oncol 2017; 13: 1731-1745.
9) Krivosheya D, Prabhu SS, Weinberg JS, Sawaya R. Technical principles in glioma surgery and preoperative considerations. J Neurooncol 2016; 130: 243-252.
10) Molinaro AM, Taylor JW, Wiencke JK, Wrensch MR. Genetic and molecular epidemiology of adult diffuse glioma. Nat Rev Neurol 2019; 15: 405-417.
11) Ludwig K, Kornblum HI. Molecular markers in glioma. J Neurooncol 2017; 134: 505-512.
12) Ferre F, Colantoni A, Helmer-Citterich M. Revealing protein-lncRNA interaction. Brief Bioinform 2016; 17: 106-116.
13) Wang SJ, Wang H, Zhao CD, Li R. Long noncoding RNA LINC01426 promotes glioma progression through PI3K/AKT signaling pathway and serves as a prognostic biomarker. Eur Rev Med Pharmacol Sci 2018; 22: 6358-6368.
14) Pardini B, Sabo AA, Birolo G, Calin GA. Noncoding RNAs in extracellular fluids as cancer biomarkers: the new frontier of liquid biopsies. Cancers (Basel) 2019; 11. pii: E1170.
15) Jiang N, Pan J, Fang S, Zhou C, Han Y, Chen J, Meng X, Jin X, Gong Z. Liquid biopsy: circulating exosomal long noncoding RNAs in cancer. Clin Chim Acta 2019; 495: 331-337.
16) Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing miRNA-lncRNA interactions. Methods Mol Biol 2016; 1402: 271-286.
17) Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. Cancer Res 2017; 77: 3965-3981.
18) Liu Q, Liu N, Shangguan Q, Zhang F, Chai W, Tong X, Zhao X, Li Z, Qi D, Ye X. LncRNA SAMD12-AS1 promotes cell proliferation and inhibits apoptosis by interacting with NPM1. Sci Rep 2019; 9: 11593.
19) Cordeiro Y, Vieira T, Kovachev PS, Sanyal S, Silva JL. Modulation of p53 and prion protein aggregation by RNA. Biochim Biophys Acta Proteins Proteom 2019; 1867: 933-940.
20) Barbosa K, Li S, Adams PD, Deshpande AJ. The role of TP53 in acute myeloid leukemia: challenges and opportunities. Genes Chromosomes Cancer 2019. doi: 10.1002/gcc.22796. [Epub ahead of print].
21) Parrales A, Iwakuma T. Targeting oncogenic mutant p53 for cancer therapy. Front Oncol 2015; 5: 288.
22) Goeman F, Strano S, Blandino G. MicroRNAs as key effectors in the p53 network. Int Rev Cell Mol Biol 2017; 333: 51-90.
23) Yi Y, Zhang W, Yi J, Xiao ZX. Role of p53 family proteins in metformin anti-cancer activities. J Cancer 2019; 10: 2434-2442.

24) Mrakovcic M, Kleinheinz J, Frohlich LF. P53 at the crossroads between different types of HDAC inhibitor-mediated cancer cell death. Int J Mol Sci 2019; 20. pii: E2415.

25) Pahle J, Walther W. Vectors and strategies for nonviral cancer gene therapy. Expert Opin Biol Ther 2016; 16: 443-461.

26) Yu X, Yu G, Wang J. Clustering cancer gene expression data by projective clustering ensemble. PLoS One 2017; 12: e171429.

27) Shanthikumar S, Neeland MN, Saffery R, Ranganathan S. Gene modifiers of cystic fibrosis lung disease: a systematic review. Pediatr Pulmonol 2019; 54: 1356-1366.