lncRNA KTN1-AS1 promotes glioma cell proliferation and invasion by negatively regulating miR-505-3p

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Abstract. Glioblastoma (GBM) is one of the most prevalent and aggressive central nervous tumors with high mobility and mortality. The prognosis of patients with GBM is poor. It is therefore essential to explore the therapeutic strategies for the treatment of GBM. Previous studies have demonstrated that the long non-coding RNA (lncRNA) Kinectin 1-Antisense RNA 1 (KTN1-AS1) can participate in the development of several types of cancer. However, the underlying mechanism of KTN1-AS1 in GBM remains unknown. The present study aimed to determine the potential role of KTN1-AS1 in GBM. In this study, reverse transcription quantitative PCR analysis was conducted and the results demonstrated that KTN1-AS1 was upregulated in GBM tissues and cell lines compared with normal tissues and astrocytes (NHA). Furthermore, KTN1-AS1 knockdown decreased the viability and invasive ability of glioma cells *in vitro* and *in vivo*. In addition, high level of KTN1-AS1 was correlated with poor prognosis in TCGA GBM database. Furthermore, microRNA-505-3p (miR-505-3p) was a promising target of KTN1-AS1, and the suppressing effects of miR-505-3p on cell proliferation and invasive ability was reversed by downregulating KTN1-AS1. Taken together, the results from the present provided novel insights into the roles of KTN1-AS1 in GBM, and suggested that the KTN1-AS1/miR-505-3p axis may be considered as a novel therapeutic target for the treatment of patients with GBM.

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Introduction

Glioblastoma (GBM) is one of the most common malignant central nervous tumors. GBM is a high-grade glioma (grade IV) that accounts for ~3% of all tumors, with ~90,000 patients dying from GBM each year (1-3). In addition, the 2-year survival rate of patients with GBM is ≤35% (4). GBM originates from brain glial cells. In the 2016 World Health Organization classification, glioma is divided into four different grades and two classes as follows: Grades I and II (low-grade gliomas); and grades III and IV (high-grade gliomas) (5). Despite the progress in therapeutic and new diagnostic approaches, current treatments are not considered to be effective (6,7). Although surgical procedures are the recommend treatment strategies at present, total resection of glioma is difficult to complete (~10%) (8). However, the underlying molecular mechanisms of glioma progression remains unknown (9,10). It is therefore essential to determine novel molecular biomarkers that could be used to predict prognosis and develop new therapeutic approaches for patients with glioma.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs of >200 nucleotides in length that have limited protein coding capacity (11). lncRNAs regulate the expression level of genes on epigenetic, transcriptional and post-transcriptional levels (12,13). The modes of regulation by lncRNAs include chromosome modification and transcriptional activation or interference (14). Previously, these long non-coding transcripts were regarded as transcriptional 'noise' or cloning artifacts (15). However, increasing evidence revealed that lncRNAs serve crucial roles in various cellular processes, including transcriptional regulation, cell proliferation and nuclear import (16). These findings suggest that lncRNAs might be considered as promising therapeutic targets in cancer. Previous studies have demonstrated the biological functions of lncRNAs in various types of human cancer, including glioma (17-20).

The lncRNA Kinectin 1-Antisense RNA 1 (KTN1-AS1) is located on chromosome 14 and was demonstrated to be upregulated in non-small cell lung cancer, hepatocellular carcinoma and head and neck squamous cell carcinoma, where it was confirmed to act as an independent prognostic factor (21-24). Previous studies reported that KTN1-AS1 promotes the development of hepatocellular carcinoma and non-small cell lung cancer (21,22).

Jiang *et al* (23) also demonstrated that KTN1-AS1 can suppress the epithelial-mesenchymal transition in head and neck squamous cell carcinoma. Furthermore, analyzing RNA-sequencing data derived from the TANRIC database revealed that KTN1-AS1 might be considered as a novel biomarker for patients with head and neck squamous cell carcinoma (24). The present study aimed to determine the biological function and underlying mechanisms of KTN1-AS1 in glioma.

Materials and methods

Tissues and cell lines. The human glioma (LN229, U251, A172 and T98G) and Normal Human Astrocyte (NHA) cell lines were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were incubated in DMEM (Gibco; Thermo Fisher Scientific, Inc.; cat. no. 670087) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.; cat. no. 16140071) and maintained at 37˚C in an atmosphere containing 5% CO_2.

Human GBM specimens and adjacent normal brain tissues (located 0.5-1 cm away from the tumor) were obtained from 35 patients with histologically confirmed GBM who underwent surgery at the Second Affiliated Hospital of Harbin Medical University between May 2018 and December 2019 (Harbin, Heilongjiang, China). All GBM samples and adjacent normal brain tissues (17 women and 18 men; age range, 22-74 years; median age, 47.31 years) were confirmed by two senior pathologists. None of the patients had received any preoperative treatment. The specimens were immediately snap-frozen in liquid nitrogen for further research. Written informed consent was obtained from each patient. All procedures were performed in agreement with the Declaration of Helsinki (25). The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Harbin Medical University (No.2018HMUIRB0113, Harbin, China). The clinical characteristics of the patients are summarized in Table I.

Gene expression profiles with KTN1-AS1 expression. The high-throughput sequencing data of nine glioma tissues and three normal samples were acquired from the Gene Expression Omnibus (GEO) database (no. GSE4290 and GSE104267; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc) (26). The overall survival data were obtained from GBM patients in The Cancer Genome Atlas (TCGA) GBM database (http://cancergenome.nih.gov) (27). Gene Ontology (GO) term analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (https://david.ncifcrf.gov/). The edgeR package was conducted to explore significantly abnormally expressed lncRNAs for the normalized gene expression profile data. The DEseq2 package was used to analyze the differentially expressed lncRNA with the threshold set as log2 fold-change (FC) level >2 and false-discovery rate (FDR) <0.01. Furthermore, the Venn diagram was generated by FunRich to visualize the overlapping lncRNAs among TCGA database, GSE4290 and GSE104267 datasets.

Isolation of cytoplasmic and nuclear RNA. Glioma cell cytoplasmic and nuclear RNA were harvested and purified by using the PARIS Kit (cat. no. AM1921; Thermo Fisher Scientific, Inc.) according to the manufacturers' instructions.

Reverse transcription quantitative (RT-q) PCR. Total RNA was collected from clinical specimens and T98G and U251 cells using TRIzol Reagent (Beijing Transgen Biotech Co., Ltd.; cat. no. R1021) and reversely transcribed into cDNA using the PrimeScript™ RT Kit (Takara Biotechnology Co., Ltd.; cat. no. RR014A) according to the manufacturers' protocol. RT-qPCR was conducted three times on an ABI 7500HT Real-Time PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturers' protocols. The thermocycling conditions were as follows: Initial denaturing step (94˚C, 10 min), followed by 40 cycles of denaturing (94˚C, 5 sec), annealing (60˚C, 30 sec) and extending (72˚C, 45 sec). The sequences of the primers used were as follows: KTN1-AS1, forward 5'-ATGCACACTTCTCGGCTAAGAGTC-3', reverse, 5'-CTACAATGCCACAAGTGATTCCAG-3'; miR-505-3p, forward 5'-CGCGGATCCCAGACTCCCAGCAATCAC-3', reverse 5'-CCGGAATTCGCAGTATTCCCACCATTT-3'; MMP-9, forward 5'-AGACCTGGGCAGATTCCAAAC-3', reverse 5'-CGGCAAGTCTTCCGAGTAGT-3'; U6, forward 5'-GGATATTGTTGCCATCAATGACC-3', reverse 5'-AGC CTTCTCCATGGTGGTGAAGA-3'; and GAPDH, forward 5'-AAGAAGGTGGTGAAGCAGGC-3' and reverse 5'-GTC AAAGGTGGAGGAGTGGG-3'. U6 served as the endogenous control for miRNA, and GAPDH served as the endogenous control for lncRNA and MMP-9. To verify the expression of KTN1-AS1 and miR-505-3p, endogenous mRNA was synthesized using a SYBR Green PCR Master Mix Kit (Invitrogen; Thermo Fisher Scientific, Inc.; cat. no. 4309155). Relative gene expression levels were normalized to endogenous controls and were expressed as $2^{-\Delta\Delta Ct}$ (28).

Plasmids, oligonucleotides and cell transfection. KTN1-AS1 siRNA, pcDNA3.1-KTN1-AS1 (OE), negative control-siRNA (si-NC), miR-505-3p mimics, miR-505-3p inhibitor and negative control-miRNA (miR-NC) were obtained from Shanghai GenePharma Co., Ltd. T98G and U251 cells were seeded at the density of $5x10^5$ cells per well in 6-well plates and cultured in DMEM containing 10% FBS at 37˚C. When the cell confluence reached 70-80%, cells were transfected with 20 μM of each construct using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.; cat. no. 11668030) according to the manufacturers' protocol and maintained at 37˚C for 6 h. The culture medium was then replaced by fresh DMEM containing 10% FBS, and subsequent experiments were conducted at 24 h post-transfection. The sequences of the constructs were as follows: KTN1-AS1 siRNA, forward 5'-GACUGUGGAUAGAGAUAG AAA-3', reverse 5'-UCUAUCUCUAUCCACAGUCUG-3'; si-NC, forward 5'-GGUAAGCAGUGGCUCCUCUAA-3', reverse 5'-ACGUGACACGUUCGGAGAAUU-3'; miR-505-3p mimics, forward 5'-GGGAGCCAGGAAGUAUUGAUGU-3', reverse 5'-ACAUCAAUACUUCCUGGCUCUU-3'; miR-505-3p inhibitor, forward 5'-GGGAGCCAGGAAGUAUUGAUGU-3', reverse 5'-CAGUACUUUUGUGUAGUACAA-3'; and miR-NC, forward 5'-CAGUACUUUUGUGUAGUACAA-3' and reverse 5'-CAGUACUUUUGUGUAGUACAA-3'.

Dual-luciferase reporter assay. DIANA tools (http://carolina.imis.athena-innovation.gr/diana_tools) and StarBase V2.0 (http://starbase.sysu.edu.cn/) database were used to identify the potential target miRNAs of KTN1-AS1. Among all the statistically relevant

Table I. Clinical characteristics of patients with glioblastoma according to long non-coding RNA KTN-AS1 level in tissues (n=35).

Variables	n	KTN1-AS1 expression		P-value
		Low	High	
Age, years				
<60	14	9	5	0.129
≥60	21	8	13	
Sex				
Male	18	4	14	0.001[a]
Female	17	13	4	
Karnofsky performance scale score				
<60	18	8	10	0.615
≥60	17	9	8	
Mean tumor diameter, cm				
<5	21	6	15	0.004[a]
≥5	14	11	3	
Necrosis on MRI				
Yes	14	7	7	0.89
No	21	10	11	
Seizure				
Yes	17	9	8	0.615
No	18	8	10	

[a]P<0.05. KTN1-AS1, Kinectin 1 Antisense RNA 1; MRI, magnetic resonance imaging.

miRNAs, miR-23b-3p, miR-23c-3p and miR-505-3p were obtained from two databases, and were therefore selected for further experiments. The human KTN1-AS1 Luc-reporter was used in the ligation of the KTN1-AS1 3'-untranslated region (UTR) PCR product. The psiCHECK2 vector (GeneChem, Inc.) was conducted to construct KTN1-AS1 3'-UTR containing reporter. T98G cells were seeded in 6-well plates at the density of $1x10^5$ cells per well and the miRNA mimics were co-transfected with psiCHECK2-KTN1-AS1-wild type (WT) or mutant (MUT) (20 μl) and miR-505-3p mimics or miR-NC (20 μM) by Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.; cat. no. 11668030). The medium was changed at 4 h post-transfection. After 48 h, the intensity of Firefly luciferase was measured using a Dual-Luciferase Reporter Assay System (cat. no. BA0180; BioVision Inc.) according to the manufacturers' instructions. *Renilla* luciferase intensity was used as an internal control.

Cell viability assay. The viability of glioma cells was evaluated using Cell Counting Kit-8 (CCK-8; cat. no. CK04; Dojindo Molecular Technologies, Inc.). T98G and U251 cells were seeded in 96-well plates (100 μl containing 3,000 cells/well). Cells were cultured in DMEM at 37˚C under 5% CO_2 conditions for 24, 48 or 72 h. CCK-8 solution (10 μl) was then added to the cells for 4 h and the optical density was detected at 490 nm using a Tecan microplate reader (Infinite F50; Tecan Group, Ltd.).

Transwell assays. Transfected T98G and U251 cells ($5x10^4$) were seeded into the upper chamber of a Transwell on a Matrigel-coated membrane (cat. no. 3495; Costar; Corning, Inc.) and cultured in serum-free medium for 24 h. DMEM containing 20% FBS was placed in the lower chamber. Subsequently, the upper chamber medium was changed and cells on the upper side of the filter were removed. Then, cells that have invaded the lower side were fixed with 4% paraformaldehyde at room temperature for 10 min and stained with 0.1% crystal violet (cat. no. IC0600; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 10 min. Stained cells were counted in five randomly selected fields under a light microscope (Nikon Corporation) at x100 magnification.

Immunofluorescence staining. Cell slides were precoated with 0.1% poly-L-lysine at 4˚C for 24 h and placed into 24-well plates. Subsequently, T98G and U251 cells ($1x10^5$) were transfected with si-KTN1-AS1/si-NC in culture dish. After 24 h, cells were collected and cultured on the cell slides in 24-well plates for 24 h. Then the medium was removed and cell slides were incubated with 4% paraformaldehyde in 24-well plates at room temperature for 20 min and 0.1% Triton X-100 for 5 min. The cell slides were washed in 24-well plates and fixed with 5% BSA (cat. no. 9048-46-8; Sigma Aldrich; Merck KGaA) dissolved in PBS at room temperature for 1 h, incubated with primary antibodies targeting MMP-9 (rabbit polyclonal antibody; cat. no. 13667; 1:50; Cell Signaling Technology, Inc.) at 4˚C overnight, and with fluorescence-labeled rabbit secondary antibody [tetramethylrhodamine (TRITC)-conjugated goat anti-rabbit IgG; cat. no. SA00007-2; 1:100; ProteinTech Group, Inc.)] for 1 h at room temperature. Nuclei were stained with DAPI (1 μg/ml; cat. no. 4083s; Cell Signaling Technology, Inc.)

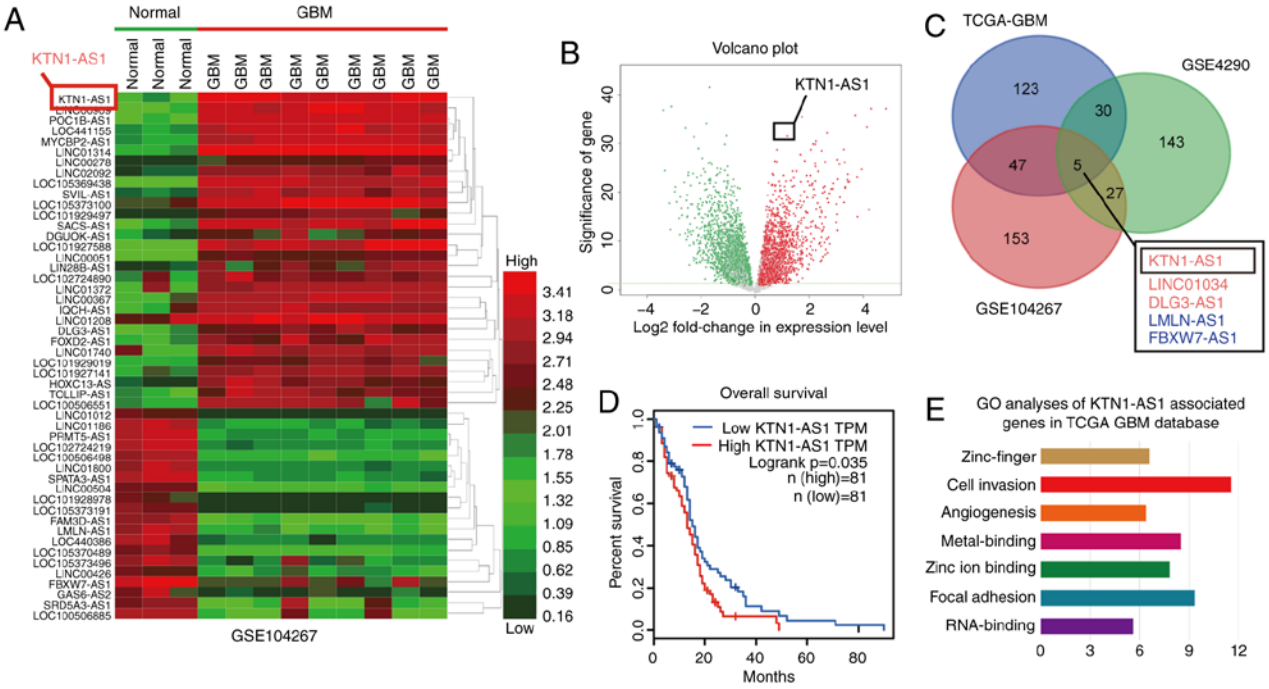

Figure 1. KTN1-AS1 was overexpressed in GBM tissues. (A) Hierarchical clustering analysis showing lncRNAs that are differentially expressed in the GEO dataset (GSE104267; glioma tissues, n=3; normal tissues, n=9; FC>2.0; P<0.01). (B) Volcano plot suggested that KTN1-AS1 was upregulated in the two groups of lncRNA from GEO database. (C) Five significantly differentially expressed lncRNAs were determined in TCGA, GSE104267 and GSE4290 datasets. (D) Kaplan-Meier survival curve of GBM patients (TCGA) based on the levels of KTN1-AS1 (log-rank test; P=0.0053). (E) Top-ranked genes in GO term analysis using DAVID, indicating that genes associated with cell invasion were enriched among those affected by KTN1-AS1. lncRNA, long non-coding RNA; KTN1-AS1, Kinectin 1 Antisense RNA 1; GBM, glioblastoma; GO, Gene Ontology; TCGA, The Cancer Genome Atlas; GEO, the Gene Expression Omnibus; TPM, Trans per kilobase of exon model per million. FC, fold change; FDR, false discovery rate.

for 10 min. Cell slides were subsequently collected and placed on glass slides. The cells were observed under a fluorescence microscope (Nikon Corporation) at x400 magnification.

In vivo xenograft tumor models. Four-week-old female BALB/c nude mice (weight, 15-20 g; n=16) were obtained from Beijing Vital River Laboratory Animal Technology, Co., Ltd. The vehicle of the luciferase lentivirus (GV260-purinomycin) and the corresponding transfection reagent (HitransG Infection enhancer fluid) were purchased from GeneChem and transfection was conducted according to the manufacturers' protocol. Then T98G cells were stable transfected with luciferase lentivirus and transfection reagent for 4 h. Then purinomycin was fixed and incubated for 15 day. The mice were assigned to two groups (si-NC and si-KTN1-AS1; n=8 per group) and placed in an anesthesia induction box. Mice were anesthetized with isoflurane (induced concentration, 3-4%) for 2-3 min and maintained anesthetized using at 1-1.5% isoflurane. Subsequently, si-KTN1-AS1 or si-NC-Luc T98G cells ($1x10^6$ cells/mouse in 5 μl) were intracranially injected using a stereotactic instrument. Tumor growth was evaluated by bioluminescence imaging (photons/s/cm^2) using a Bruker In-Vivo FX PRO Imaging System (Bruker Corporation). Mice were anesthetized using isoflurane and D-luciferin sodium salt injected into the abdomen of mice. Tumor size in the head of mice was evaluated by bioluminescence imaging (BLI). The fluorescence value is associated with the tumor size. After 40 days, the remaining mice were then sacrificed using CO_2 (28% volume displacement per min) and the overall survival time was recorded. All applicable international, national and/or institutional guidelines for the care and use of animals were followed. The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Harbin Medical University (approval no. 2018HMUIRB0113, Harbin, China).

Statistical analysis. The data were presented as the means ± standard deviation of the mean of three independent experiments. Statistical analyses were conducted using the statistical software SPSS version 19.0 (IBM Corp.). Pearson's rank correlation test was used to determine the correlation between KTN1-AS1 and miR-505-3p or MMP-9 expression. Comparisons between groups were performed using two-tailed Student's t-test or ANOVA followed by Tukey's post hoc test. The association between KTN1-AS1 level and clinicopathological characteristics of the patients was analyzed using χ^2 test or Fisher's exact test. Kaplan-Meier curve and log-rank test were conducted for survival analysis using GraphPad Prism v.5.0 (GraphPad Software, Inc.). The aberrantly expressed lncRNAs were explored according to Benjamini-Hochberg method (29). P<0.05 was considered to indicate a statistically significant difference.

Results

KTN1-AS1 expression is increased in GBM tissues. To identify whether KTN1-AS1 was involved in the development of glioma, KTN1-AS1 expression was evaluated using microarray data downloaded from GEO (GSE104267). The top 50 aberrantly expressed lncRNAs were identified with cut-off values of log2FC>2 and FDR<0.01, and it was observed that KTN1-AS1 level was overexpressed in GBM tissues compared with normal brain tissues (Fig. 1A and B). In total,

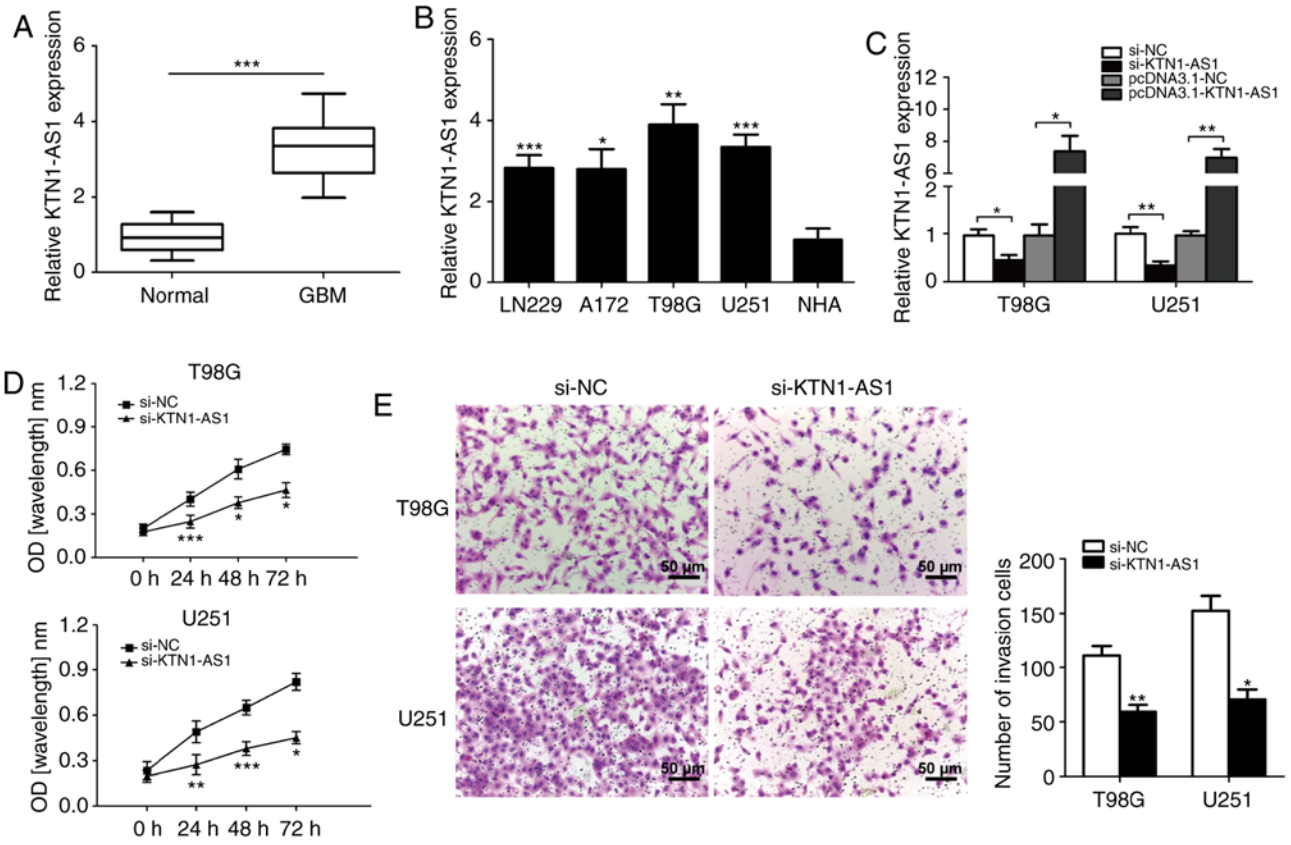

Figure 2. KTN1-AS1 knockdown attenuates the proliferation and invasive ability of glioma cells *in vitro*. (A) Upregulated level of KTN1-AS1 was measured in GBM tissues compared with adjacent normal brain tissues (n=35). GAPDH was used as a normalization control (P<0.001). The data were analyzed with Student's t-test. (B) KTN1-AS1 level in glioma cell lines was measured by RT-qPCR. (C) KTN1-AS1 level was transfected with siRNAs or pcDNA3.1 vector of KTN1-AS1 in T98G and U251 cells, and detected by RT-qPCR. (D) Glioma cell viability was analyzed using a CCK-8 assay. (E) Invasive ability was measured by Transwell assays. Magnification, x100; scale bar, 50 μm. Data were presented as the means ± standard error of the mean of at least three independent experiments. *P<0.05, **P<0.01 and ***P<0.001. KTN1-AS1, Kinectin 1 Antisense RNA 1; GBM, glioblastoma; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; siRNA, small interfering RNA.

five intersecting lncRNAs were identified and investigated according to their log2FC level, as displayed in Fig. 1C. The data from TCGA database indicated that patients with glioma and higher KTN1-AS1 expression had a shorter overall survival (Fig. 1D; n=162, P<0.01). To further elucidate the function of KTN1-AS1, the associated gene expression profiles were detected using Co-lncRNA program from the GO database. The results demonstrated that KTN1-AS1 was associated with cell invasion, focal adhesion and zinc ion binding (Fig. 1E). Since GBM is the most malignant and common primary nervous system tumor worldwide (1,2), it is crucial to identify the clinical characteristics of patients with GBM (grade IV in glioma) according to tissue expression of KTN1-AS1. The results demonstrated that expression level of KTN1-AS1 was significantly associated with sex and mean tumor diameter in the clinical GBM samples (n=35; grade IV; P=0.001 and P=0.004, respectively; Table I). Subsequently, KTN1-AS1 expression level was examined in 35 GBM tissues and adjacent normal brain tissues by RT-qPCR. The results demonstrated that KTN1-AS1 expression level was significantly increased in GBM tissues (P<0.001; Fig. 2A) compared with adjacent normal tissues. These findings suggested that KTN1-AS1 may function as an oncogene in glioma progression.

Effect of miR-505-3p on glioma cell viability and invasion. KTN1-AS1 expression was also investigated in glioma cells compared with NHA cells, and the results demonstrated that KTN1-AS1 was most highly expressed in T98G and U251 cells among the glioma cell lines (P<0.01 and P<0.001). These two cell lines were therefore chosen for subsequent experiments (Fig. 2B). To identify the biological function of KTN1-AS1 in the progression of glioma, T98G and U251 cells were transfected with si-KTN1-AS1/si-NC or pcDNA3.1-KTN1-AS1/NC, and the cell proliferation and invasive ability were determined. The transfection efficiencies were confirmed by RT-qPCR, where KTN1-AS1 expression level was significantly increased or increased compared with corresponding NC groups (Fig. 2C). The results from CCK-8 assay indicated that KTN1-AS1 knockdown significantly decreased T98G and U251 cell proliferation after 24 h (Fig. 2D; P<0.05). Furthermore, silencing KTN1-AS1 could significantly decrease glioma cell invasive ability *in vitro* (Fig. 2E; T98G cells, P<0.01; U251 cells, P<0.05).

Invasion and metastasis serve crucial roles in tumorigenesis. To further determine the underlying mechanism by which KTN1-AS1 could affect the invasive ability of glioma cells, analysis of the TCGA-GBM database was performed. The results demonstrated that KTN1-AS1 expression was positively correlated with expression of matrix metalloproteinase-9 (MMP-9; r=0.281; P=0.0043; Fig. 3A), which is a marker of cell invasion (30). Then, the expression level and

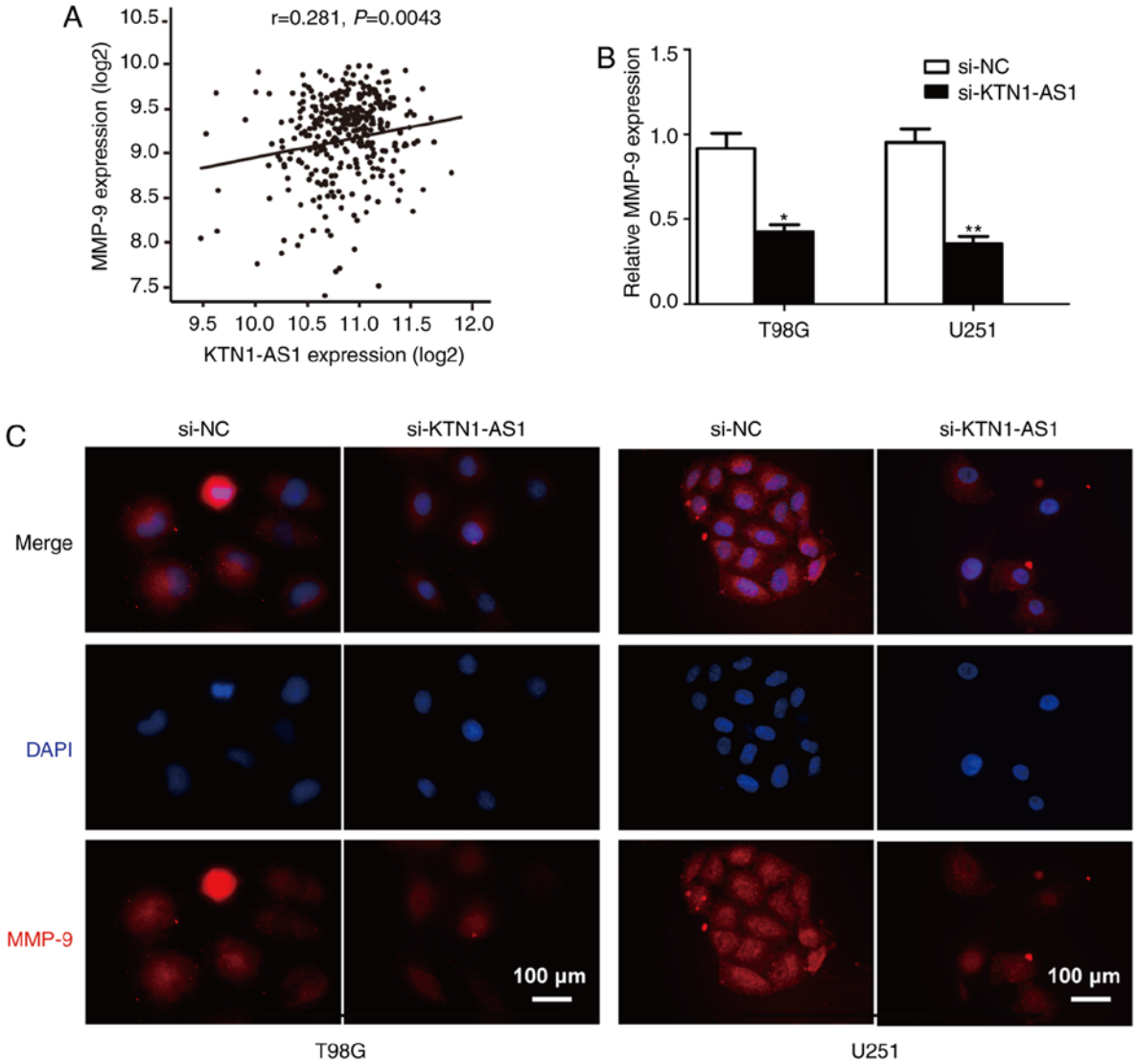

Figure 3. Expression of MMP-9 was evaluated in glioma cells. (A) Correlation between KTN1-AS1 and miR-505-3p expression in TCGA database (r=0.281; P=0.0043). (B) Reverse transcription-quantitative PCR analysis of MMP-9. (C) Immunofluorescent staining revealed downregulated expression of MMP-9 in si-KTN1-AS1 cells. Magnification, x400; scale bar, 100 μm. Data were presented as the means ± standard error of the mean of at least three independent experiments. *P<0.05 and **P<0.01. GBM, glioblastoma; KTN1-AS1, Kinectin 1 Antisense RNA 1; MMP, matrix metalloproteinase; siRNA, small interfering RNA; NC, negative control.

cellular location of MMP-9 were determined in glioma cells following KTN1-AS silencing. The results demonstrated that KTN1-AS1 knockdown significantly decreased the expression of MMP-9 in glioma cells (Fig. 3B and C). Taken together, these findings suggested that KTN1-AS1 knockdown may inhibit proliferation and invasive ability of glioma cells.

Correlation between KTN1-AS1 and miR-505-3p. To investigate the subcellular localization of KTN1-AS1, the nuclear and cytoplasmic fractions of T98G glioma cells were explored. As presented in Fig. 4A, KTN1-AS1 was predominantly localized in the cytoplasm, indicating that KTN1-AS1 may exert both transcriptional and post-transcriptional regulatory functions on glioma cell lines. By using DIANA tools and starBase V2.0 database blast prediction, it was reported that KTN1-AS1 contained a putative targeting site for miR-23b-3p, miR-23c-3p and miR-505-3p. Subsequently, following overexpression or silencing of KTN1-AS1 in T98G cells, it was observed that miR-505-3p could be downregulated or upregulated at the mRNA level (Fig. 4B and C). In addition, miR-505-3p expression level was significantly decreased in GMB tissues compared with adjacent normal tissues (P<0.001; Fig. 4D). Analysis of TCGA-GBM database indicated that low level of miR-505-3p in patients with GBM was associated with better prognosis (n=162; P<0.05; Fig. 4E). Furthermore, miR-505-3p and KTN1-AS1 expression levels in clinical GBM tissues from patients were negatively correlated (n=35; R=-0.3552; P=0.0363; Fig. 4F). Decreased miR-505-3p expression was also observed in glioma cells compared with NHA cells (P<0.05; F=35.37; Fig. 4G). Subsequently, T98G and U251 cells were transfected with miR-505-3p mimics, miR-505-3p inhibitor and miR-NC. The results demonstrated that miR-505-3p was significantly increased or decreased in transfected cells compared with NC group (Fig. 4H). The luciferase reporter assay was then conducted to confirm the putative miR-505-3p target site. In addition, luciferase reporters carrying either the

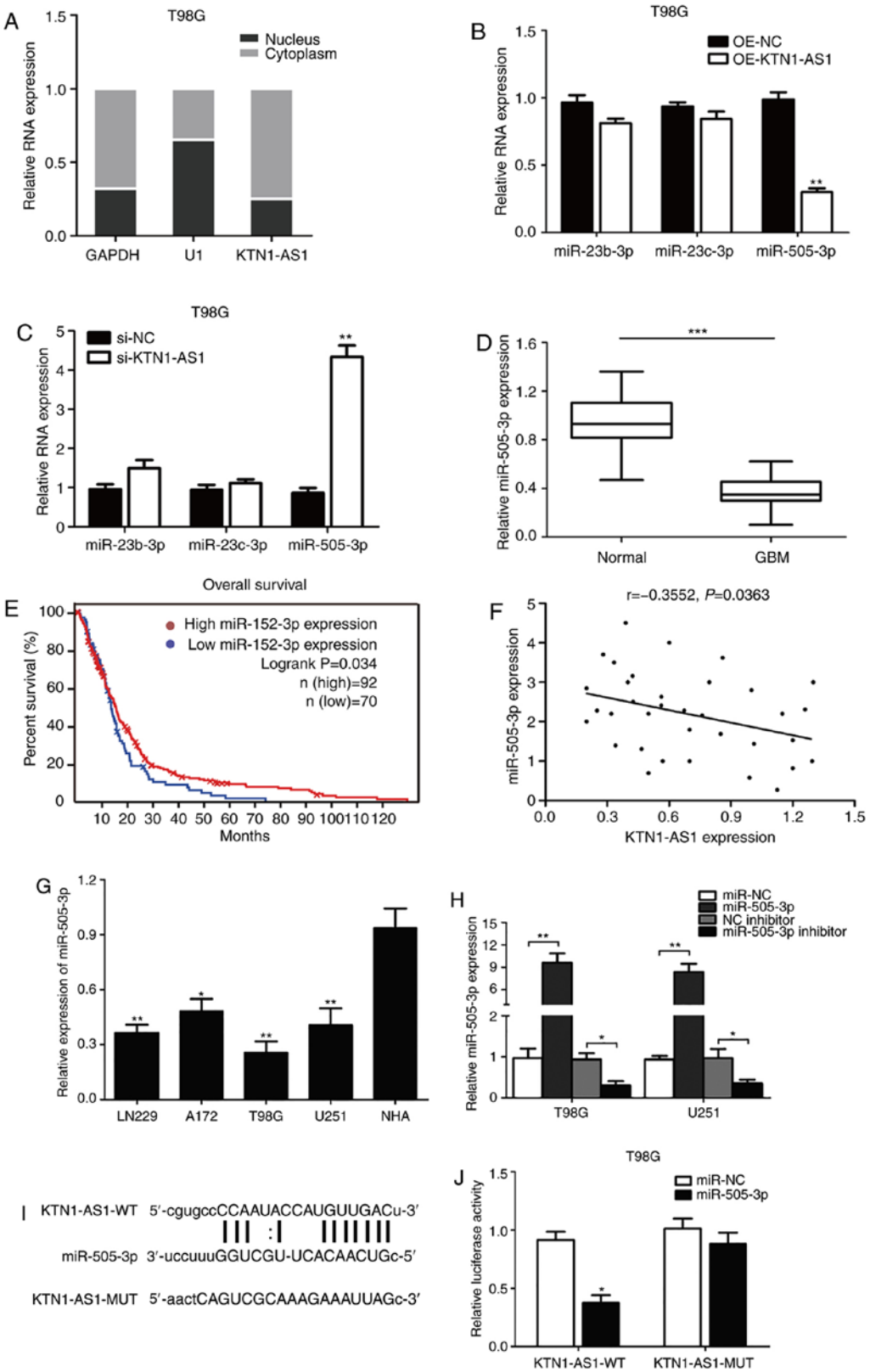

Figure 4. KTN1-AS1 was targeted by miR-505-3p at 3'-UTR. (A) Expression of KTN1-AS1 in subcellular fractions was examined by RT-qPCR in T98G cells. Black range indicates nuclear fraction and gray range indicates cytoplasmic fraction. (B and C) Expression of the predicted binding miRNAs (miR-23b-3p, miR-23c-3p and miR-505-3p) was measured by RT-qPCR following transfection with si-KTN1-AS1 or OE-KTN1-AS1. (D) Decreased expression of miR-505-3p was identified in glioma tissues compared with normal tissues (P<0.001). (E) Patients with low level of miR-505-3p had better prognosis compared with patients with high miR-505-3p level in TCGA database (log-rank test; P=0.034). (F) Correlation between KTN1-AS1 and miR-505-3p levels was detected. (G) miR-505-3p level in glioma cell lines was analyzed by RT-qPCR. (H) miR-505-3p level was determined in T98G and U251 cells transfected with miRNA mimics/miR-NC or inhibitor/NC inhibitor by RT-qPCR. (I) Predicting binding sites of miR-505-3p on KTN1-AS1. Relative luciferase activity was measured after co-transfection with miR-505-3p mimics and psiCHECK2-KTN1-AS1-WT in T98G cells. (J) Luciferase activity was determined 24 h after transfection using a dual luciferase assay. Data were presented as the means ± standard error of the mean of at least three independent experiments. *P<0.05, **P<0.01 and ***P<0.001. KTN1-AS1, Kinectin 1 Antisense RNA 1; miR, microRNA; UTR, untranslated region; GBM, glioblastoma; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; si, small interfering; WT, wild-type; MUT, mutant; OE, overexpressing.

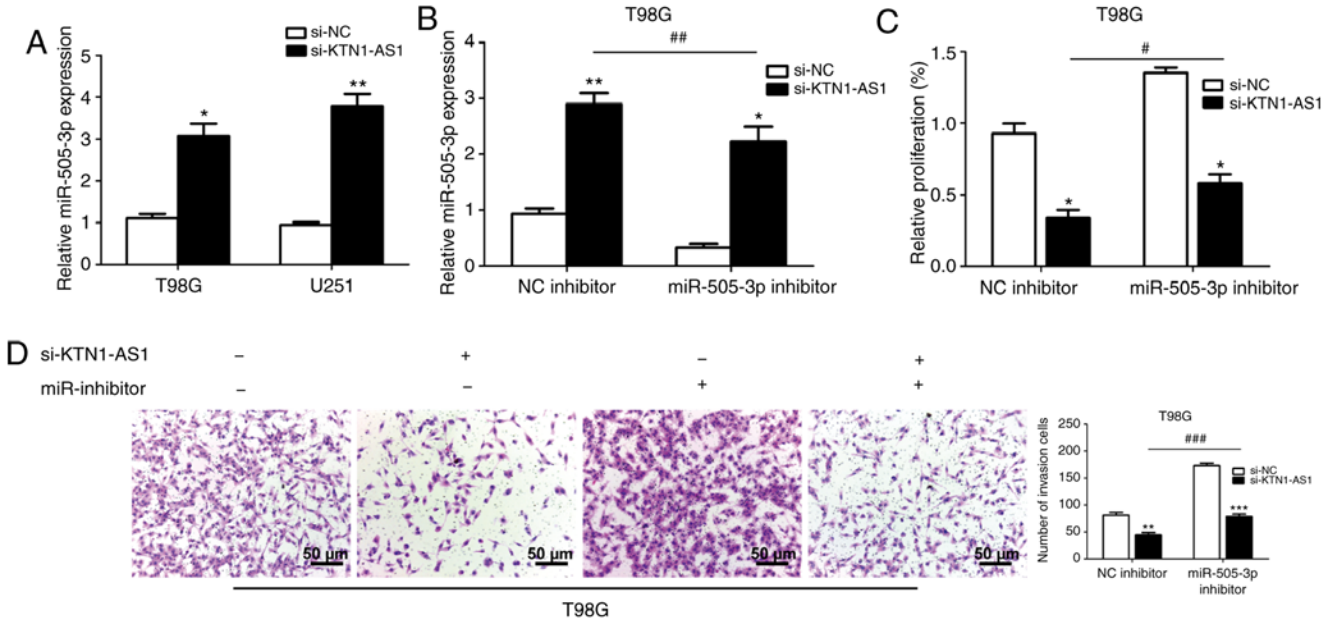

Figure 5. miR-505-3p reversed the effect of KTN1-AS1 on glioma cells. (A) miR-505-3p was upregulated following KTN1-AS1 knockdown in T98G and U251 cells. (B) Expression of miR-505-3p in T98G cells co-transfected with si-KTN1-AS1 + miR-505-3p inhibitor and with si-KTN1-AS1 + miR-inhibitor NC and in cells co-transfected with si-KTN1-AS1 + miR-505-3p inhibitor compared with si-KTN1-AS1 + miR-inhibitor NC. (C) Cell viability was conducted by CCK-8 assay. (D) Invasion ability was explored by Transwell assays. Magnification, x100; scale bar, 50 μm. Data are presented as the mean ± standard error of at least 3 independent experiments. *P<0.05, **P<0.01 and ***P<0.001; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001. *P<0.05 vs. NC. miR, microRNA; KTN1-AS1, Kinectin 1 Antisense RNA 1; GBM, glioblastoma; NC, negative control; si, small interfering.

predicted miR-505-3p WT (KTN1-AS1-WT) or its mutated fragment (KTN1-AS1-MUT) were constructed (Fig. 4I). The results indicated that miR-505-3p significantly inhibited the luciferase activity in cells transfected with KTN1-AS1-WT but not in cells transfected with KTN1-AS1-MUT 3'-UTR (Fig. 4J). These data suggested that KTN1-AS1 may interact with miR-505-3p in glioma cells.

miR-505-3p abrogates the effect of KTN1-AS1 in glioma cells. miR-505-3p was determined to be negatively associated with KTN1-AS1 in glioma tissues. The expression of miR-505-3p was significantly elevated in KTN1-AS1-silenced cells compared with si-NC cells (Fig. 5A). Subsequently, rescue experiments were performed in T98G cells. Co-transfection of miR-505-3p inhibitor and si-KTN1-AS1 led to a decreased level of miR-505-3p expression compared with miR-inhibitor NC + si-KTN1-AS1 group (Fig. 5B). In addition, the expression of miR-505-3p was increased when cells were co-transfected with si-KTN1-AS1 and miR-505-3p inhibitor compared with si-NC + miR-505-3p inhibitor group. The results from CCK-8 assay demonstrated that miR-505-3p inhibitor could decrease the proliferation induced by silencing KTN1-AS1 (Fig. 5C). Transwell assay results showed that miR-505-3p inhibitor could inhibit the invasive ability induced by silencing KTN1-AS1 (Fig. 5D). These findings suggested that miR-505-3p may be considered as an essential mediator of KTN1-AS1-regulated proliferation and invasion.

KTN1-AS1-silencing significantly suppresses tumor growth in vivo. To further determine the effect of KTN1-AS1 silencing *in vivo*, BALB/c nude mice were intracranially inoculated with T98G-luc cells with si-NC or si-KTN1-AS1. BLI of mice was conducted to identify the role of si-KTN1-AS1 on glioma tumor growth. The data demonstrated that tumor formation in KTN1-AS1 silencing group was significantly lower compared si-NC group (P<0.05; Fig. 6A and B). To further determine the inhibitory role of si-KTN1-AS1 on glioma progression in nude mice, the overall survival of mice (n=8 per group) was determined by Kaplan-Meier analysis. The si-KTN1-AS1-treated group displayed a significant improvement in survival compared with the si-NC group (P=0.0008; Fig. 6C). These findings suggested that KTN1-AS1 may promote the tumorigenicity *in vivo*.

Discussion

Glioblastoma is a highly invasive type of cancer (1,2). Despite the optimization of current treatments, the clinical outcome of patients remains poor (4). Previous studies demonstrated that aberrantly expressed molecules in tumors play an important role in tumorigenesis and predict patient survival outcomes (11-14). Cancer-specific lncRNAs have been reported to contribute to tumor progression in various types of cancer, including oesophageal cancer, pancreatic cancer, nasopharyngeal carcinoma, hepatocellular carcinoma, cervical cancer, colorectal cancer and gastric cancer (31). However, the association between lncRNAs and glioma tumorigenesis remains unclear. To the best of our knowledge, the present study was the first to investigate the expression of KTN1-AS1 in GBM. The results from this study demonstrated a higher KTN1-AS1 expression level and lower miR-505-3p expression level in GBM tissues and cell lines compared with normal tissues and cells.

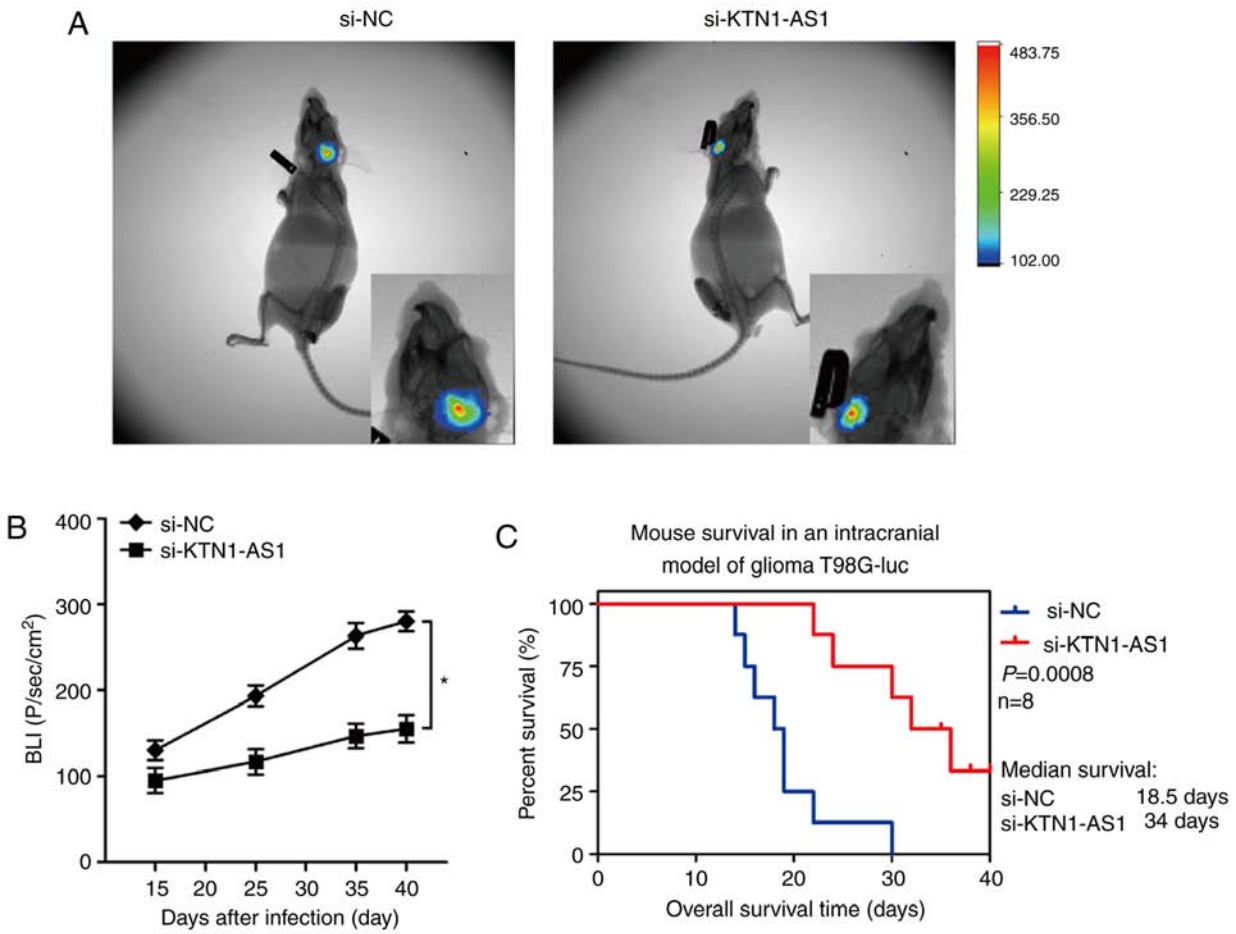

Figure 6. KTN1-AS1 knockdown inhibited tumor formation *in vivo* and prolonged mice survival. (A) Luminescence imaging showed that the BLI value of si-KTN1-AS1-treated T98G-Luc glioma cell group was suppressed compared with that of the si-NC group. (B) Line chart showed the change of tumor BLI value, which indicated the tumor size in mice. (C) Kaplan-Meier analysis indicated that mice inoculated with si-KTN1-AS1 exhibited a significantly better prognosis than those in the si-NC-treated group. *P<0.05. BLI, bioluminescence imaging; Luc, luciferase; KTN1-AS1, Kinectin 1 Antisense RNA 1; NC, negative control; si, small interfering.

The results from the present study suggested that increased KTN1-AS1 expression may predict poor outcome in patients with glioma. It was found that decreased expression of KTN1-AS1 could suppress glioma cell proliferation and invasive ability *in vitro*. The data demonstrated that KTN1-AS1 may exert its role mainly by directly targeting miR-505-3p. To support that theory, the tumor suppressor role of miR-505-3p was further investigated. The results demonstrated that transfection with miR-inhibitor could impede the si-KTN1-AS1 tumor-suppressive effect on glioma cell proliferation and invasive ability. These findings confirmed the negative correlation between KTN1-AS1 and miR-505-3p in glioma progression.

The competing endogenous RNA (ceRNA) theory is the most important theory for lncRNAs, which have been shown to act as sponges for regulating the expression and function of miRNAs (32). The lncRNA metastasis-associated lung adenocarcinoma transcript 1 was identified as an oncogene in lung cancer development (33). Furthermore, the lncRNA myocardial infarction associated transcript (MIAT) can regulate the biological functions via the MIAT/miR-29a-3p/HDAC4 axis, suggesting its vital role in the diagnosis of gastric cancer (34). Li *et al* (35) reported that the lncRNA linc00645 can promote glioma progression by modulating the miR-205-3p/ZEB1 axis.

It was concluded that KTN1-AS1 may function as a ceRNA of miR-505-3p. Numerous studies have revealed that the aberrant expression of miRNAs serves a vital functional regulator in the development and progression of certain cancers, including lung, gastric, breast and prostate cancers (36-40). Want *et al* (41) reported that downregulation of miR-505 can promote malignant biological behavior in breast cancer. Shi *et al* (42) observed that the expression of miR-505-3p is decreased in glioma and can suppress cell migration and invasion. The expression of KTN1-AS1 has been reported to be upregulated in hepatocellular carcinoma and head and neck squamous cell carcinoma (22,23), leading therefore to patients' poor survival rate. Taken together, the findings from the present results highlighted the essential role of the KTN1-AS1/miR-505-3p axis on the regulation of glioma progression.

In conclusion, this study demonstrated that KTN1-AS1 may function as an oncogene by sponging miR-505-3p or acting as its ceRNA. The present study focused on KTN1-AS1 modulated signaling via miRNAs, which was relatively novel. The data demonstrated the vital roles of KTN1-AS1 and revealed novel mechanisms underlying the proliferation

and invasive ability of glioma cells. These findings suggest that KTN1-AS1 may be considered as a potential prognostic biomarker for glioma.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YM and QT designed the study, performed experiments, analyzed the data and wrote the manuscript. YM performed the *in vitro* experiments. HF and LZ analyzed the data and drafted the manuscript. YW designed and supervised the study and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Institutional Review Board of Harbin Medical University (Harbin, China; approval no. 2018HMUIRB0113) and all procedures were performed in accordance with national (D.L.n.26, March 4th, 2014) and international laws and policies (directive 2010/63/EU). Written informed consent was provided by all patients prior to the study start.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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