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Research article

LINC01138 expresses two novel isoforms and functions as a repressive factor in glioma cells

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ABSTRACT

Objective: The objective of this study is to investigate the aggressive infiltration of glioblastoma into adjacent brain tissue, considering its challenging prognosis. Initially classified as an intergenic non-coding RNA, we aim to elucidate the functional implications of LINC01138 in glioblastoma.

Method: Glioma grading was performed utilizing H&E staining, which unveiled distinct nuclear morphology in high-grade gliomas. The downregulation of LINC01138 in glioma tissues was corroborated through qRT-PCR and gel electrophoresis, concurrently identifying two previously unrecognized LINC01138 isoforms. Expression profiling of all four LINC01138 isoforms was executed in glioma cell lines (A172, SHG-44, U251, U87-MG). The impact of LINC01138 overexpression in U87-MG and U251 cells was evaluated for cell proliferation, migration, and invasion through cell counting, CCK-8 analysis, and Transwell assays. Furthermore, the suppression of LINC01138 in SHG-44 cells substantiated its involvement in fostering tumor malignancy. Transcriptome sequencing revealed the inhibitory influence of LINC01138 on IGF1 expression. These findings contribute to an enriched comprehension of glioma biology by exploring the engagement of LINC01138 through diverse methodologies, thereby elucidating its potential therapeutic significance.

Results: Our investigation elucidates the intricate involvement of LINC01138 in gliomas. High-grade gliomas are characterized by elevated cell density and distinctive nuclear features. LINC01138 demonstrates a substantial downregulation in glioma tissues, with the identification of two novel isoforms. The expression of all four LINC01138 isoforms is notably diminished in both glioma tissues and cell lines. Elevated expression of LINC01138 demonstrates inhibitory effects on tumor cell proliferation, migration, and invasion, while its downregulation exacerbates

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malignancy. The regulatory function of LINC01138 as a repressor of IGF1 expression was elucidated through transcriptome sequencing.
Conclusion: The LINC01138 isoforms display notable tumor-suppressive effects, suggesting a promising potential for impeding glioma progression.

1. Introduction

Malignant gliomas represent the most prevalent subtype of primary brain tumors, and a majority of patients receive a diagnosis of advanced grade 4 tumors, specifically glioblastoma multiforme (GBM). GBM, characterized by its aggressive nature, manifests as a formidable class of adult cancers exhibiting hallmark features such as invasiveness, prolific proliferation, necrosis, genetic instability, and resistance to chemotherapy [1]. Individuals afflicted with GBM experience a median survival period of 15–20 months, even when undergoing the contemporary tumor-treating fields (TTF) in conjunction with a standard care regimen encompassing surgery, chemotherapy (temozolomide, TMZ), and radiotherapy [2–4]. Despite substantial progress, the invasive characteristics of GBM pose challenges to surgical resection, limit the accessibility of chemotherapeutic agents, and contribute to unfavorable prognoses.

The primary cause of mortality in GBM stems from tumor cell invasion, leading to occurrences of both local and distal recurrences [5]. Furthermore, studies indicate that radiotherapy and chemotherapy contribute to an augmentation in the invasive potential of GBM in vivo [6,7]. It is posited that the most promising strategy for mitigating the invasion cascade involves the suppression of migratory and invasive capabilities [8]. The modification of cell phenotype is posited to represent a pivotal mechanism in the context of this invasive progression [9]. There likely exist intracellular factors governing this intricate process that warrant further exploration.

Extensive research efforts have been dedicated to unraveling the pathogenesis of GBM, resulting in significant advancements. Nevertheless, the molecular mechanisms that underlie the onset of GBM remain unclear [10,11]. In the present study, we sought to elucidate the expression levels of long non-coding RNA (lncRNA) in GBM, comparing them to levels observed in cell lines derived from malignant gliomas. Our investigation revealed a reduction in LINC01138 expression levels in cancer tissue when compared to adjacent normal tissue. Specifically, the expression levels of lncRNA in GBM and glioma cell lines were lower than those observed in adjacent normal tissue.

LINC01138 was initially recognized as an androgen-responsive lncRNA in prostate cancer through bioinformatics analysis [12]. While it has demonstrated certain capabilities to stimulate malignancies in hepatocellular carcinoma [13], clear cell renal cell carcinoma [14], and gastric cancer [15], its role in the context of brain and GBM has not been previously documented, and its fundamental biological function remains elusive.

In contrast to several other tumor types, our investigation revealed a diminished expression of LINC01138 in GBM in comparison to its levels in adjacent normal tissues. Notably, LINC01138 exhibited a primary inhibitory effect on malignancies in cell lines derived from malignant gliomas. Moreover, we identified two previously unreported isoform variants of LINC01138, denoted as LINC01138-S1

Table 1
The sequence of primers.

names	sequences
LINC01138	F:AAGCTTAGCTGGGCGGTCACATCTGGAAATGG
LINC01138-L	R:GGATCCGAGAGAAAGCAACTTTTATTGAACTG
LINC01138-S	F1: CTTTCATGCTCATCCACAGCATCAT
	R1: ATGATGCTGTGGATGAGCATG
	F:ATGATGCTGTGGATGAGCATG
	R: TAGCACTGGAGAGGTAAAATC
	F: CCTGTATTTCCTCCTCTTCAG
	R: TAGCACTGGAGAGGTAAAATC
LINC01138-S1	F: ATGGGGATGTGTTCAAGGCA
	R: GTGGTAGAGGAGGAAGAAAA
LINC01138-S2	F: CCAGAGCCTACTTGTGAAGA
	R:TGAGACTCCATCTGTAATCC
GAPDH	F:ATGACAACTTTGGTATCGTGG
	R:AGGGATGATGTTCTGGAGAG
ASNS	F:GAGATAGAAACTGTGAAGAA
	R:GGATACTGTACTTGGGCTTC
CBSL	F:ATTCTCACATCCTAGACCAG
	R:ACACTTCTCCTTCAGCTTCC
HSPA6	F:AAGCCACTGCTGGAGATACC
	R:GTGTAGAAGTCCACGCCCTC
IGF2	F:CATCGTTGAGGAGTGCTGTT
	R:TGCTTCCAGGTGTCATATTG
PARP8	F:ATTTGTCAGCAGTTAGAGAG
	R:GTAAGTGAACAGTGTAGTCG
Si–S	GTGGCTCTAAATACAACTA
Si-L	CGATGCTGTGACCTATATT

Random RNA provided by Genechem, China.

and LINC01138-S2 (Genebank submission MT674560 and MT674561, respectively). Both oligonucleotides displayed a similar expression pattern to that of the known LINC01138 isoform (NR_104014) in GBM and adjacent normal tissue, suggesting that LINC01138 may function as a suppressor in malignant gliomas.

2. Methods and materials

2.1. Patient samples, cell culture, and transfection

A total of twenty-one pairs of human glioma tissues and their corresponding normal tissues were procured from individuals diagnosed with GBM at the Chinese PLA General Hospital between 2018 and 2019. Written consents were acquired from all participants in the study, and ethical approval for the research protocol was granted by the Ethics Committee of the General Hospital of the People's Liberation Army and the Medical School of Henan University.

The U87, U251, SHG44, and A172 cell lines were purchased from Shanghai Cell Line and Bank (Shanghai, China) and later cultured in Dulbecco's modified Eagle's medium (DMEM, Corning) supplemented with 10 % fetal bovine serum (FBS, ATCC). All cell lines were incubated at 37 °C in a humidified incubator in an atmosphere of 5 % CO_2.

Plasmid transfection was performed with lipofectamine 3000 according to the manufacturer's instructions. siRNA transfection was performed with siRNA (Riborio) according to the manufacturer's instructions. The siRNA target sequences for LINC01138 are shown in Table 1.

2.2. Reverse transcription PCR and quantitative real-time PCR

Clinical tissue specimens and cultured cells utilized in this study underwent RNA extraction using TRIzol reagent (Solaibio) to obtain total RNA. First-strand cDNA synthesis was accomplished using the PrimeScript™ Reverse Transcriptase kit (Vazyme, China). Additionally, some total RNAs were reverse transcribed into cDNA utilizing the Bestar™ qPCR RT kit (Vazyme, China). The qRT-PCR assay was conducted with SYBR Green (Vazyme, China) on the Thermo Fisher PIKOREAL96 Real-Time PCR System. The primer sequences employed in this study are detailed in Table 1 mRNA expression levels were normalized to GAPDH, and the relative expression levels of RNA were calculated using the comparative CT ($2^{-\Delta\Delta CT}$) method.

2.3. CCK8 assay

The cell proliferation capacity was assessed using the CCK8 assay. Initially, glioma cells were seeded at a density of 5000 cells per well in 96-well plates and incubated at 37 °C for 6 h. Subsequently, 10 μl of CCK8 was added to 100 μl of medium in each well and maintained at 37 °C for 2 h. The absorbance value at 450 nm was measured using INFINITE F50 (Tecan Austria). Each cell experiment with three replicates was repeated three times, and the measurements were taken continuously over a period of 5 days. The data were analyzed using PRISM.

2.4. Cell counting assay

Cell proliferation in the cultured cells was assessed through cell counting. Initially, treated cells (10,000 cells/well) were seeded into 24-well plates and maintained under regular culture conditions for 24 h. Following digestion with trypsin/EDTA and centrifugation, the collected cells were suspended for counting. The cell count was continuously monitored using Countstar (Bio Tech) over a period of 4 days.

2.5. Migration and invasion analysis

Migration and invasion analyses of cells were conducted using the Transwell assay with 8-μm pore chambers (Corning USA), with or without Matrigel matrix (BD Biosciences USA). Treated cells were harvested and re-suspended in the culture medium, resulting in a final concentration of 5 × 10^4 cells/ml. Subsequently, 0.2 ml of the cell suspension solution was added to the upper chamber, and 0.7 ml of culture medium containing 10 % FBS was added to the lower chamber. After 48 h of incubation at 37 °C, the invaded and migrated cells were stained with 0.1 % crystal violet (Solarbio, China) and enumerated under a microscope.

2.6. Hematoxylin and eosin (H&E) staining

Tumor tissue and adjacent normal samples underwent dehydration in gradient alcohol, fixation in neutral-buffered formalin, and embedding in paraffin. Subsequently, they were sectioned into 3 μm-thick tissue sections and dewaxed. For H&E staining, hematoxylin and eosin were applied, followed by rinsing with water, dehydration, and sealing with neutral gum, in accordance with the manufacturer's instructions (Solarbio, Beijing, China). The prepared samples were then observed under a microscope.

2.7. RNA (transcriptome) sequencing

We transiently transfected 8 × 10^6 U87MG cells with the specified plasmid for 48 h, after which total RNA samples were collected

using TRIzol reagents and processed on a bgiseq-500 sequencing platform (BGI China) with a 150-bp paired-end run. The raw sequencing reads were aligned to the human reference genome (hg19) using the splice-aware aligner HISAT246. Read counts for each gene were normalized to FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values [47]. The cutoff for differential gene expression was set at FDR <0.05, normalized against the respective empty vector control.

2.8. Western blot analysis

Cultured cells were transfected with the LINC01138 overexpression vector. After 48 h of transfection, cells were harvested with RIPA buffer, as previously indicated [16]. Subsequently, cell lysates were separated by SDS-PAGE, and proteins were transferred to a PVDF membrane. Primary antibodies, including anti-Akt, -pAkt, -mTOR, -pmTOR (purchased from Cell Signaling Technology), and anti-IGF2 and IGF1R antibodies (procured from Proteintech), were utilized. The anti-GAPDH antibody was obtained from Bio-Rad. Primary antibodies were appropriately diluted, ranging from 500 to 2000 times, depending on individual titers. Secondary

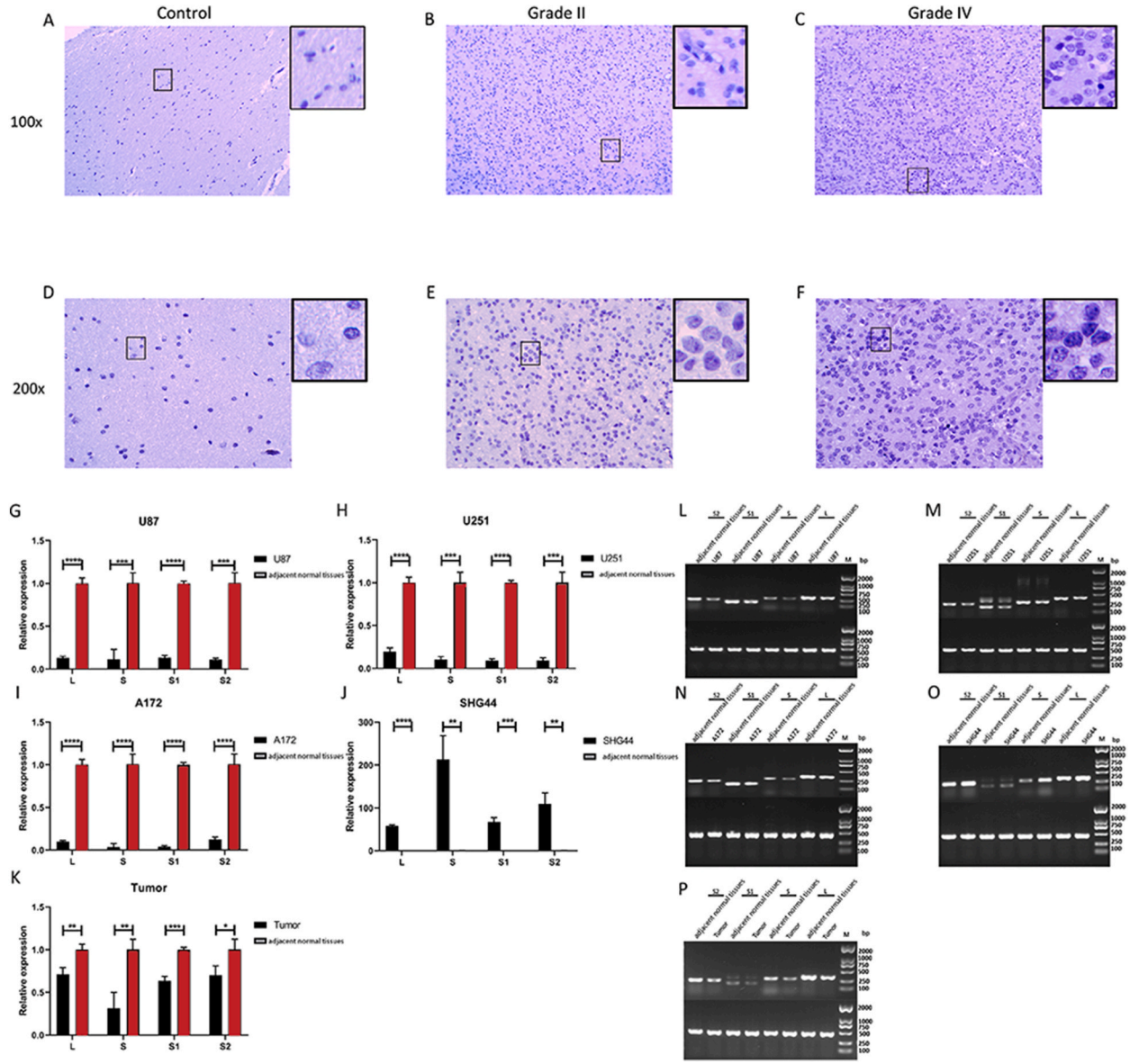

Fig. 1. Glioma and adjacent normal tissues were analyzed by H&E staining. (**C**, **F**) High grade tissues were characterized with high cell density with segmented-like nuclei compared to (A, D) normal tissues or (B, E) tissues of grade II patients. The expression of 4 LINC01138 isoforms in U87MG (G), U251 (H), A172 (I) and SHG44 (J) cells were quantified by qRT-PCR and compared to the expression level in adjacent normal tissues. The expression levels of all isoforms in tumor were reduced compared to normal tissues (**K**). These results were typically represented in agarose gel in a corresponding order from (**L**) to (**P**).

antibodies were diluted 5000 times. The final membranes were visualized using ECL reagents.

2.9. Statistical analysis

Data are presented as mean ± SD. Statistical comparisons were performed using the unpaired, two-tailed Student's *t*-tests or a one-way ANOVA. $P < 0.05$ is considered statistically significant.

3. Results

3.1. High grade GBM indicating high cell density with segmented-like nuclei

The classification of GBM was validated through H&E staining. The findings indicated that normal adjacent tissue exhibited relatively low cell density and round bland nuclei (Fig. 1A–D). In contrast, grade II tumor tissue displayed a relatively high cell density, though nuclei remained relatively round and bland (Fig. 1B–E). Notably, Grade IV tumor tissue was characterized by high cell density with a distinct protoplasmic area, where nuclei exhibited clear segmentation or appeared as multiple black dots within the nuclei (Fig. 1C–F).

3.2. LINC01138 was downregulated in GBM tissues

The qRT-PCR results revealed that the expression level of LINC01138 RNA in adjacent normal tissues was 2.8-fold (±0.26) higher than the expression level in GBM tissues (Fig. 2A). Additionally, gel electrophoresis demonstrated the downregulation of LINC01138 expression compared to adjacent normal tissues in conventional RT-PCR (Fig. 2B).

3.3. Novel isoforms of LINC01138 expressed in human glioma tissues

Upon amplification of LINC01138 with specific primers designed to match LINC01138(NR_027468) at both 5′ and 3′ primes, two novel isoforms were identified. These novel isoforms appeared to be products of alternative splicing from the same gene or transcript. Two versions of LINC01138 were accessible on NCBI/GenBank through NR_027468 and NR_104014, respectively, with NR_027468 being the longer variant. Our data suggested that the two newly discovered variants of LINC01138 differed from both NR_027468 and NR_104014 (Fig. 2C).

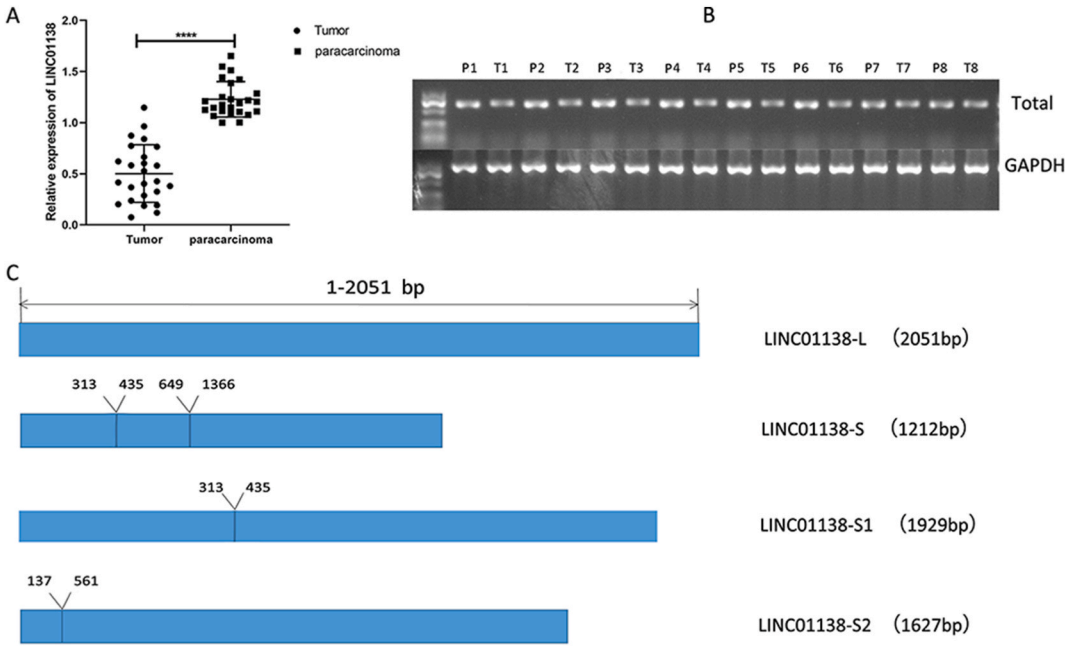

Fig. 2. (**A**) RNA expression level of LINC01138-L was quantified in 25 pairs of glioma and adjacent normal tissues using qRT-PCR. The values were normalized to GAPDH content in parallel samples. (**B**) Typical 8 pairs of samples were collected and visualized in agarose gel after PCR. (**C**) Schematic representation of all isoforms were derived from LINC01138 gene. LINC01138-S1 (MT674560) and LINC01138-S2 (MT674561) were novel isoforms identified by PCR and sequencing in the present study.

3.4. All isoforms were downregulated in GBM tissues and 3 glioma cell lines

Four human glioma cell lines, namely A172, SHG-44, U251, and U87-MG, were employed to assess the expression levels of three isoforms of LINC01138 through qRT-PCR using specific primers. The findings indicated that A172, U251-MG, and U87-MG exhibited lower expression levels of LINC01138 variants compared to the levels in adjacent normal tissue (Fig. 1G, H, I). In contrast, SHG-44 cells demonstrated a notably elevated expression level compared to adjacent normal tissues and other cell lines (Fig. 1J). The expression of all four isoforms was found to be downregulated in GBM tissues (Fig. 1K). Consistent findings were observed in glioma cell lines through regular RT-PCR as evidenced by agarose gel electrophoresis (Fig. 1L-P).

3.5. LINC01138 inhibited tumor cell proliferation, migration, and invasion

To elucidate the role of LINC01138 in glioma cells, we overexpressed all four isoforms in U87-MG and U251 cells (Fig. 3A and B). Cell proliferation was assessed through cell counting and CCK-8 assays. The results indicated that the groups transfected with any isoform displayed reduced cell numbers and a lower growth rate compared to the control group transfected with a blank vector, signifying that all four isoforms significantly inhibited tumor cell proliferation (Fig. 3C–F), including in U87MG (Fig. 3D, E) and U251 (Fig. 3G and H) as observed in the CCK-8 assay.

All four isoforms were transfected into U87MG and U251 cells. The cells were then seeded into Transwell chambers with or without Matrigel, and the capacity for cell migration and invasion was measured as described previously. The data demonstrated that both the migration and invasion cell numbers in the four experimental groups were smaller than those in the control group. These results suggest that elevating the expression levels of all four isoforms could downregulate U87MG cell migration (Fig. 4A and B) and invasion (Fig. 4E and F) in vitro. Similarly, overexpression of these isoforms reduced migration (Fig. 4C and D) and invasion (Fig. 4G and H) of U251 cells as well.

3.6. Knock-down of LINC01138 in intrinsically highly expressed SHG44 cells promoted cell malignancy

SHG44 cells inherently exhibit high expression levels of LINC01138 isoforms (Fig. 1 J, O). To investigate the impact, the expression of the three shorter isoforms, LINC01138 and LINC01138-L were knocked down with siRNA (Fig. 5A and B), and cell malignancy was assessed. Cell growth was monitored from day 2 to day 4 following siRNA transfection in the experimental and control groups. The transfection of both short and long isoform siRNA led to an increased growth rate in the counting assay (Fig. 5C) and CCK-8 assay (Fig. 5D). The data indicated a significant promotion of cell proliferation.

To assess the impact of LINC01138 expression on cell migration and invasion, a Transwell migration assay was conducted using chambers with or without Matrigel matrix to simulate the migration and invasion environment. The number of cells that migrated onto the Transwell chamber membranes was counted. Consistent with the results from the proliferation analysis, the number of cells counted in the downregulated LINC01138 expression group was higher than the cell numbers in the group where LINC01138

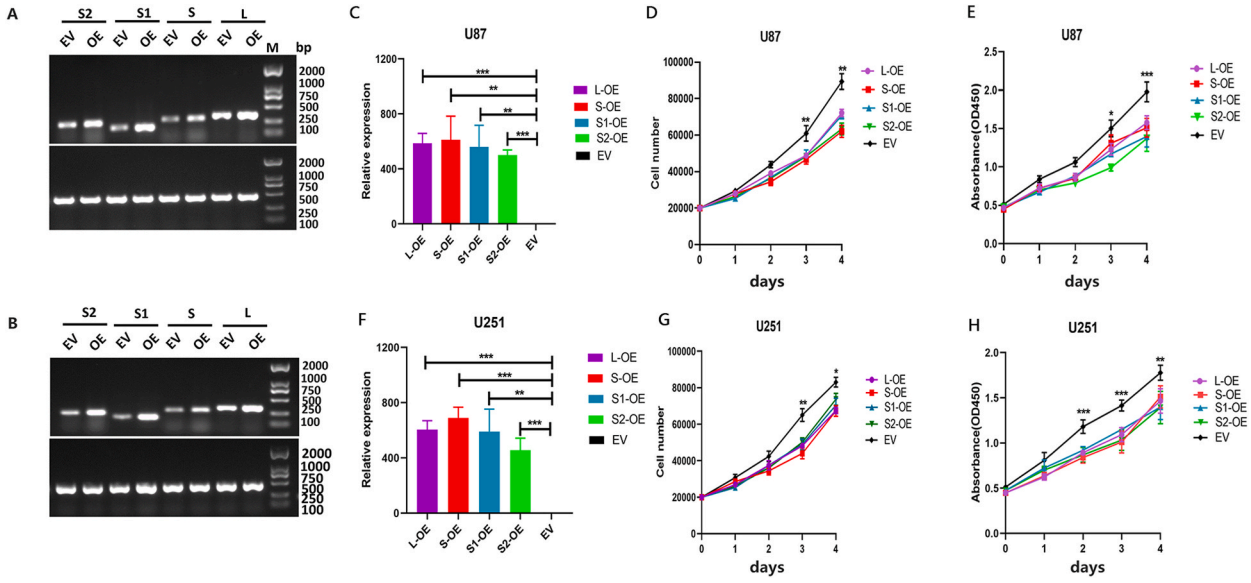

Fig. 3. Four isoforms were overexpressed and represented in U87MG (**A**) and U251 (**B**) cells measured by RT-PCR. Four isoforms were overexpressed and measured with qRT-PCR in U87MG (**C**) and in U251 (**F**) cells. Cell proliferation was examined by cell counting in U87MG (**D**) and U251 (**G**) cells, as well as by CCK8 assay in U87MG (**E**) and U251 (**H**) cells. Results showed that the groups transfected with isoforms exhibited smaller cell numbers and lower growth rate when compared to the transfected control group with the blank vector.

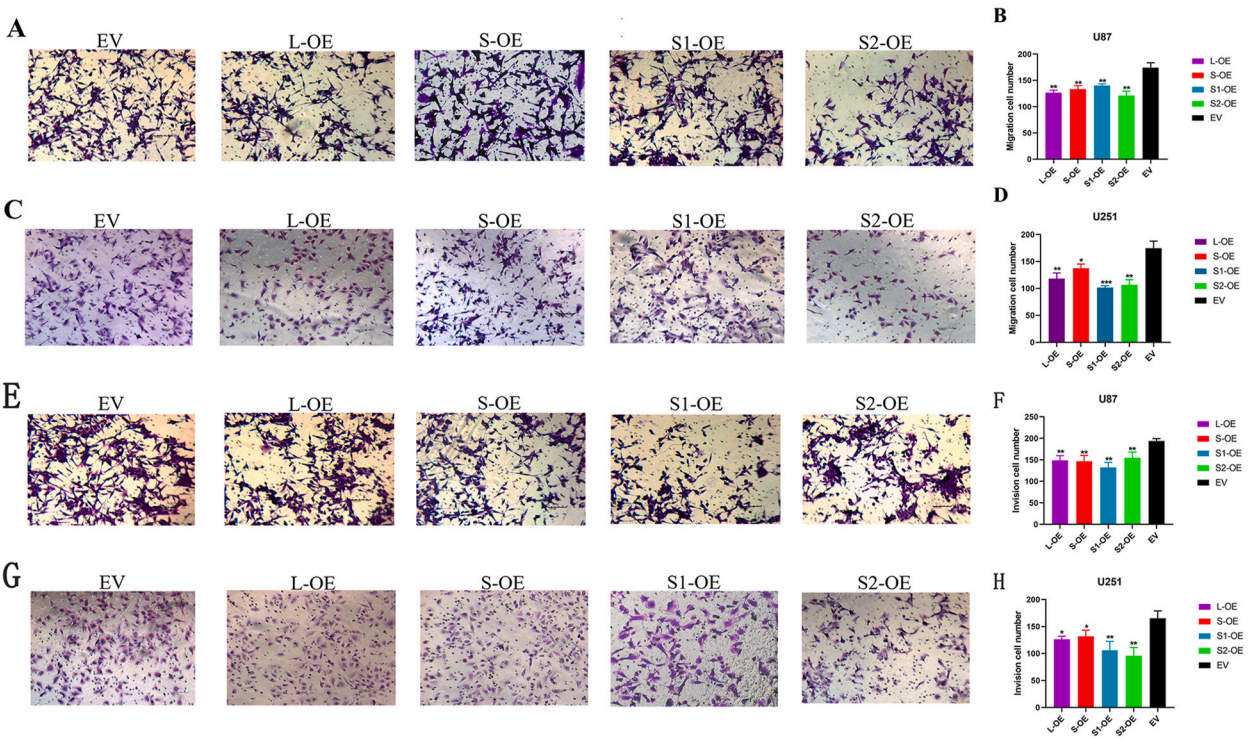

Fig. 4. Effects of overexpression of the four isoforms on cell migration were examined using Transwell (without Matrigel) in U87MG (**A, B**) cells and U251 (**C, D**) cells. At the same time, the effects of their overexpression on cell invasion were measured using Transwell (with Matrigel) in U87MG (**E, F**) cells and U251(**G, H**) cells. Data indicated that both the migration and invasion cell numbers of the four experimental groups were smaller than the ones in the control group.

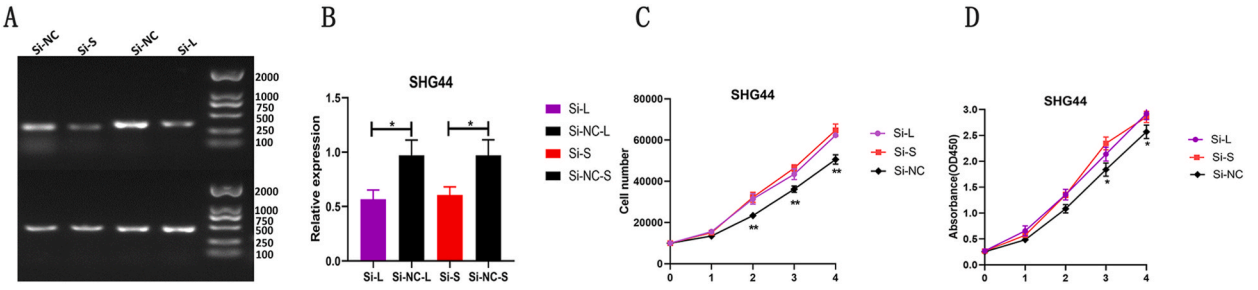

Fig. 5. LINC01138-L and LINC01138-S were knocked down in SHG44 cells with siRNA and the effects of LINC01138 on cell malignancy were measured. LINC01138 expression level was examined with RT-PCR (**A**) and qRT-PCR (**B**). Cell proliferation was measured by cell counting (**C**) and CCK8 (**D**) assay. Transfection of both the short and long isoform siRNA resulted in an increased growth rate.

expression had not been downregulated. These findings suggest that LINC01138 inhibits both migration (Fig. 6A and B) and invasion of tumor cells (Fig. 6C and D).

3.7. A screening assay of transcriptome sequencing indicated LINC01138 repressed IGF1 expression in glioma cells

To investigate gene expression potentially regulated by LINC01138, it was overexpressed in U87MG cells, which is characterized by low LINC01138 expression. Among the downregulated and upregulated genes, 20 genes were selected for measurement of their expression levels using RT-PCR. However, the expression levels of most of the genes were not found to be significantly altered (data not shown). Changes were observed in the expression levels of 5 genes, including poly(ADP-Ribose) polymerase family member 8 (PARP8), asparagine synthetase (ASNS), cystathionine beta-synthase like (CBSL), heat shock protein family A (Hsp70) member 6 (HSPA6), and insulin-like growth factor 2 (IGF2) as shown in RT-PCR (Fig. 7A). Among these, four genes displayed changes in qRT-PCR (Fig. 7B). Western blot results demonstrated that the expression of IGF2 and its receptor was significantly downregulated by LINC01138 overexpression (Fig. 7C). The Western blot analyses were independently conducted at least three times to ensure statistical

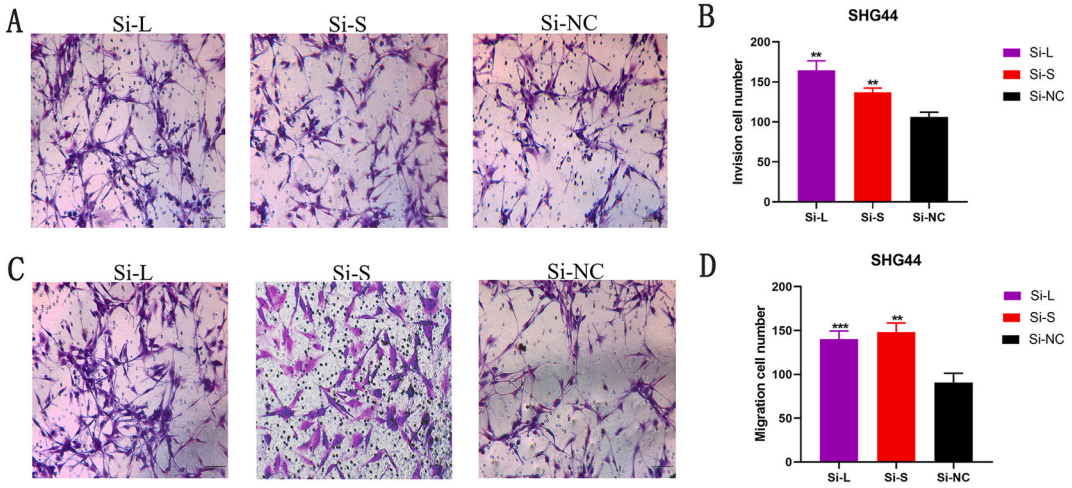

Fig. 6. Cell migration in Transwell without Matrigel (**A, B**) and cell invasion with Matrigel (**C, D**) were measured after LINC01138 was knocked down. The number counted in downregulated LINC01138 expression cells were more than the cell number of LINC01138 expression cells that had not been downregulated.

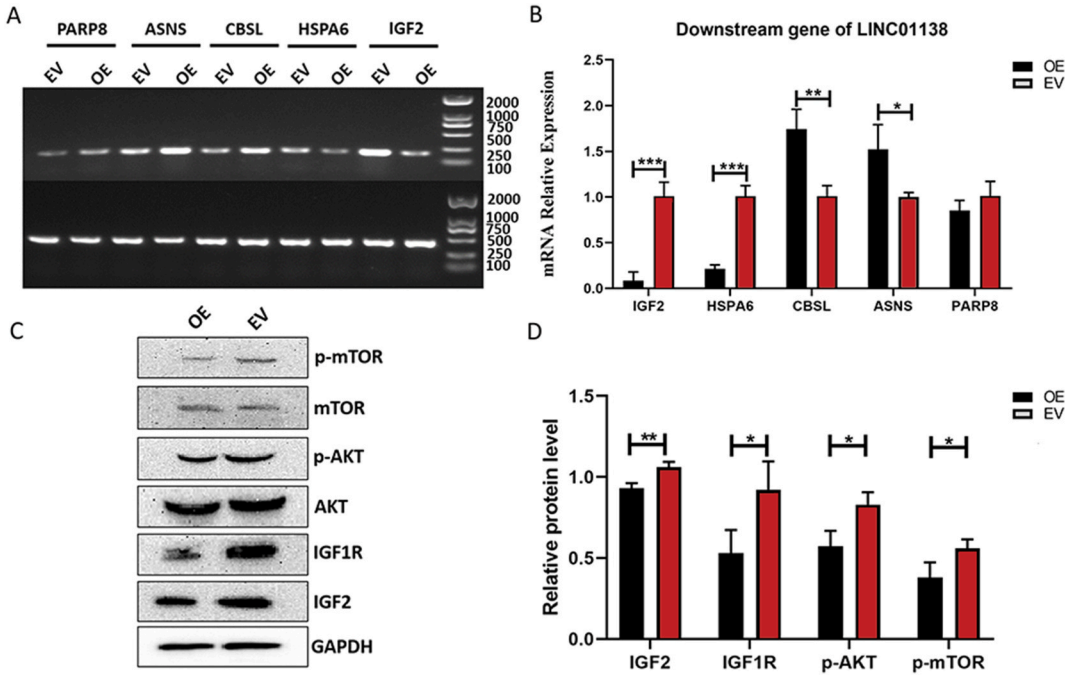

Fig. 7. PARP8, ASNS, CBSL, HSPA6, and IGF2 expression were detected with RT-PCR **(A)** or qRT-PCR (**B**). Changes were noted in the expression levels of 5 genes including PARP8, ASNS, CBSL, HSPA6, and IGF2. (**C**) The expression levels or activity of IGF2 signaling pathway were measured using Western blot analysis. The expression of IGF2 and its receptor was significantly downregulated by LINC01138 overexpression (**D**) Western blot analysis was repeated at least three times, and bands were scanned into a numerical value that was statistically analyzed.

reliability (Fig. 7D).

4. Discussions

Numerous researchers have extensively investigated the biological functions of lncRNA, primarily employing next-generation sequencing and computational approaches. It is widely recognized that lncRNA plays a role in various cellular processes, including chromatin remodeling [17], RNA transcription [18], splicing [19], RNA stability and degradation [20,21], regulation of protein translation [22], and formation of complexes with protein(s) [23,24]. The specific functions of lncRNA have been extensively reviewed

[25,26], and there have also been comprehensive reviews on the involvement of lncRNA in cancer [27]. Nevertheless, the intricate mechanism by which lncRNA contributes to the initiation and progression of glioma remains unclear.

LINC01138 was initially identified as an androgen-responsive lncRNA, with androgens acting as transcription factors capable of directly upregulating LINC01138 expression [12]. It is important to note that these initial studies were primarily conducted on cell lines with supplementary bioinformatic analyses, and many lacked comprehensive clinical investigation.

In the current study, we assessed LINC01138 expression levels in 25 pairs of clinical glioma tissues and their corresponding peritumor control tissues. Overall, LINC01138 expression was observed to be downregulated in tumors compared to peritumor control tissues (Fig. 1A and B). Determining whether the decrease in expression contributes to tumor development or if tumorigenesis leads to a decrease in expression remains challenging. Further in vivo studies are necessary to elucidate the role of LINC01138 in tumorigenesis.

Two isoforms of LINC01138 have been documented in GenBank (NR_027468 and NR_104014). NR_104014 is shorter than NR_027468, representing a full-length splicing. In the present study, two novel isoforms were identified from glioma tissues (Fig. 1C), and both were shorter than the full-length LINC01138 (NR_027468). Moreover, these two novel isoforms were detected in four glioma cell line-derived cell lines, albeit at low levels (Fig. 2G–J). However, it remains unknown whether these two novel isoforms are commonly expressed in all glioma tissues or other cell lines.

The expression of LINC01138 isoforms in four cell lines was examined through real-time RT-PCR. Expressions of all LINC01138 isoforms were downregulated in U87MG, U251, and A172 cells, but upregulated in SHG44 cells (Fig. 2I), indicating that cell lines with different genetic backgrounds have distinct regulatory mechanisms in gene expression, particularly in SHG44 cells. In the subsequent in vitro experiments, all the isoforms exhibited repressive effects in cell proliferation and migration/invasion experiments. Paradoxically, Li et al. reported that LINC01138 promoted the growth of hepatocellular cancer cells by activating arginine methyltransferase 5 (PRMT5) [13]. Other in vitro studies have indicated that LINC01138 plays a malignant role by promoting the proliferation of clear cell renal cell carcinoma [14] and gastric cancer cells [15]. It is plausible that the same molecule may play opposite or varying regulatory roles in different cell contexts, as observed with Caveolin-1 and BRD7 [28,29].

To investigate the effects of different isoforms of LINC01138 on glioma cell lines, we overexpressed them (Fig. 3A and B) and knocked them down in the cell lines (Fig. 4A). The results revealed the function of LINC01138 as a malignancy repressor (Fig. 3C–P), based on its expression levels in the tumor cells (Fig. 2P). LINC01138 functions as a repressor in cell malignancy, contrary to the findings of Li et al. [13], who reported that insulin-like growth factor 2 mRNA-binding protein 1/insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP1/IGF2BP3) could bind to and stabilize LINC01138. Moreover, LINC01138 binds to and stabilizes PRMT5 by blocking its ubiquitination and degradation, thereby contributing to cell malignancy.

Both IGF2BP1 and IGF2BP3 have been identified as oncogenes or oncogenic repressors in various studies [30,31]. These RNA-binding proteins interact with insulin-like growth factor 2 mRNA, influencing mRNA lifespan, controlling nuclear export, and regulating translation and mRNA stability. Similar RNA-binding proteins have also been identified in plants [32]. It is well-documented that IGF/IGFR signaling plays a crucial role in modulating cell growth and proliferation, and aberrant signaling can contribute to cancer [33]. Moreover, in a transcriptome sequencing screening assay, we observed that LINC01138 overexpression downregulated the expression level of IGFR1, a finding subsequently confirmed by Western blot analysis (Fig. 5C). Notably, we did not observe regulation of IGF2BP1 or IGF2BP3 expression by LINC01138 in the transcriptome sequencing assay. It is conceivable that LINC01138 may not directly reverse regulate the expression of IGF2BP1/IGF2BP3, but the downregulation of IGF1 may mediate the repressive function of LINC01138 in malignant glioma cells.

In conclusion, the mechanism through which LINC01138 mediates the repression of IGF1 expression, resulting in the suppression of cell malignancy in glioma, remains unclear. Further investigations are warranted to elucidate the regulatory mechanisms underlying the impact of LINC01138 on cell malignancy.

5. Conclusion

In this study, we have identified two novel isoforms of LINC01138, providing valuable insights into the intricate role of lncRNAs in glioma. Our findings uncover a previously unrecognized facet of tumor biology, as these isoforms demonstrate tumor-suppressive effects. This revelation deepens the comprehension of glioma pathogenesis and introduces new possibilities for targeted therapeutic interventions. Future investigations can build upon our discoveries to unravel the precise mechanisms by which LINC01138 functions, potentially paving the way for its application as a therapeutic target or a prognostic biomarker in glioma. Consequently, our study not only contributes to the advancement of knowledge on glioma but also holds promise for the development of innovative treatment strategies for this challenging condition.

Ethics approval and consent to participate

This study was conducted with approval from the Ethics Committee of the General Hospital of the People's Liberation Army and Medical School of Henan University(Approval number:S2018-268-02). This study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants.

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Availability of data and materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Chao Zhang: Writing – original draft, Funding acquisition, Conceptualization. **Ao Xu:** Writing – review & editing, Writing – original draft. **Ruoyu Liu:** Formal analysis, Data curation. **Minghang Liu:** Formal analysis, Data curation. **Wei Zhao:** Writing – review & editing, Formal analysis, Data curation. **Anhui Yao:** Writing – original draft, Formal analysis. **Guochen Sun:** Project administration, Funding acquisition, Conceptualization. **Shaoping Ji:** Project administration, Funding acquisition, Conceptualization. **Kai Zhao:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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We would like to acknowledge the hard and dedicated work of all the staff that implemented the intervention and evaluation components of the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32245.

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