Long non-coding RNA LPP-AS2 promotes glioma tumorigenesis via miR-7-5p/EGFR/PI3K/AKT/c-MYC feedback loop

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Abstract

Background: Glioma is the most common primary malignant intracranial tumor with poorly clinical prognosis in adults. Accumulating evidences indicate that long non-coding RNAs (lncRNAs) have served as important regulators in cancer progression, including glioma. Here, we identified a new lncRNA LPP antisense RNA-2 (LPP-AS2) and investigated its function and mechanism in the occurrence and development of glioma.

Methods: High-throughput RNA sequencing was performed to discriminate the differentially expression lncRNAs and mRNAs between glioma tissues and normal brain tissues. The expression of LPP-AS2, epidermal growth factor receptor (EGFR) and miR-7-5p in glioma tissues and cell lines were detected by real-time quantitative PCR (RT-qPCR). The functions of lncRNA LPP-AS2 in glioma were measured by *in vivo* and *in vitro* assays. Insights of the underlying mechanism of competitive

endogenous RNAs (ceRNAs) were originated from bioinformatic analysis, dual luciferase reporter assays, RNA pulldown assays, RNA immunoprecipitation (RIP) and rescue experiments.

Results: The results of high-throughput RNA-seq indicated that lncRNA LPP-AS2 was upregulated in glioma tissues and further confirmed by RT-qPCR. Higher LPP-AS2 expression was related to poor prognosis of glioma patients. Functional studies illustrated that LPP-AS2 depletion inhibited glioma cell proliferation, invasion and promoted apoptosis *in vitro* and restrained tumor growth *in vivo*, whereas overexpression of LPP-AS2 resulted in opposite effects. In addition, LPP-AS2 and EGFR were observed of co-expression networks, and LPP-AS2 functioned as a ceRNA to regulate EGFR expression by sponging miR-7-5p in glioma cells. Result of chromatin immunoprecipitation (ChIP) assay validated that c-MYC was directly bind with promoter region of LPP-AS2. As a downstream protein of EGFR, c-MYC was modulated by LPP-AS2 and in turn increased LPP-AS2 expression. Thus, lncRNA LPP-AS2 promoted glioma tumorigenesis via a miR-7-5p/EGFR/PI3K/AKT/c-MYC feedback loop.

Conclusions: Our study elucidated that LPP-AS2 acted as an oncogene through a novel molecular pathway in glioma and might be a potential therapeutic approach for glioma diagnosis, therapy and prognosis.

Keywords: lncRNA, LPP-AS2, EGFR, miR-7-5p, glioma, PI3K/AKT/c-MYC pathway

Introduction

As the most common primary and devastated intracranial neoplasm, glioma is characterized with high incidence and extremely poor prognosis and especially difficult to early diagnosis [1]. Gliomas are classified into numerical grades (I–IV) according to the pathological classification of World Health Organization [2]. At present, despite undergoing aggressive surgical resection and regular course of postoperative radiotherapy or chemotherapy, the overall 5-year survival rate for glioma patients remains poor [3, 4]. A number of crucial factors have been found that contributing

resistance of glioma to standard therapeutic therapy, including genetic heterogeneity, multiple genetic lesions and dysregulated pathway [5, 6]. Therefore, more in-depth research and a better understanding of molecular mechanisms underlying glioma origination that contribute to the diagnosis and treatment of glioma is urgently needed.

Non-coding RNAs are a class of RNAs formed by transcription of most genes and without protein-coding potential [7], which are divided into short non-coding RNAs and long non-coding RNAs (lncRNAs) based on the lengths of its fragments [8]. LncRNAs are a major class of ncRNAs sized greater than 200 nucleotides and mostly produced by RNA polymerase II transcription and possessing 5'-Cap and 3'-poly(A) structures [9, 10]. Increasing studies indicated that lncRNAs were not only involved in the regulation of various important biological processes, including chromatin modification, alternative splicing, epigenetic regulation, scaffolds and decoys with other molecules, transcriptional and post-transcriptional regulation [11-15], but also exerted crucial functions in cell development and diseases [16-18]. For instance, lncRNAs, such as MALAT-1, HOTAIR, PVT1 and NEAT1 may be of great significance in carcinogenesis [19-23]. LncRNA MIR155HG is upregulated in glioma and inhibits mesenchymal transition via directly targeting with miR-155 [24]. However, the clinical significance and underlying mechanism of lncRNAs, especially in glioma, need to be further explored.

LPP-AS2 (No: NR_036497.1) is a recently detected lncRNA with the length of 2897 bp. Reportedly, LPP-AS2 was considered to be associated with recurrent soft tissue sarcoma and breast cancer through bioinformatic analysis and lack of further verification by experiment [25, 26]. Nevertheless, MYC-repressed lncRNA LPP-AS2 was down-regulated in colorectal cancer and inhibited cell proliferation by regulating GADD45A [27]. However, the molecular mechanism and clinical prognosis of LPP-AS2 in glioma has not been elucidated.

MicroRNAs (miRNAs) are endogenous ~23 nucleotide RNAs that are processed from stem-loop regions of longer RNA transcripts, and regulate gene expression by base-pairing to mRNAs to induce mRNA decay and inhibit translation [28-30]. In recent years, hundreds of different miRNAs have been identified in humans, many of

which are play increasingly appreciated roles in development process of disease including cancers [31-32]. For example, miR-7-5p is downregulated and exerts tumor suppressor functions in different tumors [33-35]. Importantly, the suppressive influence of miR-7-5p in glioma has not been fully clarified.

EGFR is a tyrosine kinase receptor, playing an integral part in signaling pathways that control normal and aberrant cell growth [36]. Recent studies have suggested that EGFR was dysregulated in many different tumor types, including melanoma [37], breast cancer [38], Non-small cell lung cancer [39] and colorectal cancer [40]. Moreover, EGFR is important for glioblastoma cell growth and tumor initiation [41, 42]. However, the potential upstream regulatory mechanism of EGFR in glioma currently remains unknown.

In this present study, based on next-generation analysis, we newly identified an upregulated lncRNA LPP-AS2 in glioma, whose biological implications in tumorigenesis has not been expounded. Higher LPP-AS2 expression was associated with poor prognosis of glioma patients. Subsequent functional studies showed that LPP-AS2 affected glioma cell proliferation, apoptosis and invasion through *in vitro* and *in vivo* assays. Mechanistically, we found LPP-AS2 sponged miR-7-5p to increase EGFR expression and activate PI3K/AKT/c-MYC pathway. Moreover, LPP-AS2 was directly and transcriptionally regulated by c-MYC protein and inversely increased c-MYC protein level. Our study revealed that LPP-AS2 exerted as oncogene in a positive feedback loop and might be a novel therapeutic approach for the treatment of gliomas.

Materials and Methods

Patients and clinical samples

In the study, the cohort of tissue specimens were contained 106 glioma tissues and 23 normal brain tissues, which were obtained from department of neurosurgery of the First Affiliated Hospital of USTC between February 2014 and September 2019. All tumor samples were clinicopathologically confirmed as glioma (WHO I/II 27, WHO III/IV 79). Normal brain tissues were collected from patients undergoing brain tissue resection

from craniocerebral injury. All samples were immediately stored in liquid nitrogen with RNAhold (TransGen). Besides, written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the First Affiliated Hospital of USTC. The basic characteristics of included patients are shown in **Table 1.**

RNA extraction and transcriptome data analysis

Samples (three glioma tissues and three corresponding normal brain tissues) were used to extracted total RNA by using Trizol reagent (Invitrogen) according to the manufacturer's instructions. The RNA quality was measured by NanoDrop ND-3300 and verified by gel electrophoresis. The Ribo-minus transcriptome libraries were constructed with TruSeq Ribo Profile Library Prep Kit (Illumina) according to the manufacturer's protocols. The libraries were then subjected to 151nt paired-end sequencing generating a depth of ~ 100 million read pairs with an Illumina Nextseq 500 system (Novogene). The adapters were first trimmed with cut adapt to obtain clean reads and the left reads were then aligned to the human genome (hg19) with bowtie2 allowing one mismatch. The continuous or non-continuous mapped reads were subjected for the following mRNA and lncRNA analyses. The linear expression levels were evaluated with TopHat2 and Cufflinks followed by the annotation references of Refseq. Differentially expressed lncRNAs were identified using a strictly filtering criteria ($|\log2(\text{fold change})| \geq 1.5$ and P value < 0.01), and ($|\log2(\text{fold change})| \geq 1$ and P value < 0.01) for mRNAs.

Gene expression profile data GSE50161 and GSE33331 [43, 44] were downloaded from Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/) [45]. The two databases were based on GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. Then the two databases were merged to research expression trend of lncRNAs. Besides, GEPIA (Gene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn) [46], a web-based tool to deliver fast and customizable functionalities based on TCGA and GTEx data which was used to further verify the expression profile of lncRNAs.

Bioinformation analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways database (http://www.genome.jp/kegg/) was widely applicable to systematic analysis of gene functions [47]. The database for annotation, visualization, and integrated discovery (DAVID) is an analytical tool that used for integrative analysis of large gene lists [48]. In this study, we used DAVID (version 6.8) to perform KEGG pathway enrichment analyses for different expression genes, and the cutoff thresholds were as follows: enrichment gene number > 2 and P value < 0.05.

Cell lines and culture conditions

In this study, human glioma cell lines (U251, U87, SHG44, T98G, GOS-3, TJ905, U373) and normal cells (HEB) were maintained in our lab. All cell lines were received mycoplasma contamination test and determined to be mycoplasma-free. All Cells were cultivated in high-glucose Dulbecco's Modified Eagle medium (DMEM, Hyclone) containing 10% FBS (Clark) and stored in an incubator with constant temperature containing 5% CO2 at 37 °C.

RNA extraction and PCR reactions

The total RNA from glioma tissues and cell lines were extracted using Trizol Reagent (Invitrogen) according to the manufacturer's protocols, and 1μg of RNA quantified by NanoDrop ND-3300 (Thermo Fisher Scientific) was reverse transcribed using GoScript Reverse Transcription System (Promega), with the corresponding primes. Real-time PCR analyses were performed with TransStart Top Green qPCR SuperMix (+Dye II) (TransGen) on an ABI Q5 Sequence Detection system (Applied Biosystems), and GAPDH were used as internal controls. Bulge-Loop miRNA-specific Primer (RiboBio) was used to measure miR-7-5p expression according to the manufacturer's synopsis, and U6 was used as endogenous control. The relative mRNA and miRNA expression levels were analyzed using the $2^{-\Delta\Delta}$Ct method. All primers were synthesized by Sangon Biotech and concrete information is included in **Table S1**.

Nuclear-cytoplasmic fractionation

Nuclear/cytoplasmic fractionation was performed with a Nuclei Isolation Kit (KeyGEN BioTECH) according to the manufacturer's protocols. The nuclear and cytoplasmic RNA was analyzed by real-time quantitative PCR, and U6 was used as nuclear control while GAPDH treated as cytoplasmic control.

Plasmids, siRNAs, and transfection

For LPP-AS2 and EGFR overexpression, the full-length LPP-AS2 and EGFR cDNA was amplified and subcloned into pEGFP-C1, and empty vector was used as negative control. All plasmids were isolated using Endo-free Plasmid DNA Mini Kit I (OMEGA). SiRNAs, miRNA mimics and inhibitors were all obtained from RiboBio. All siRNAs were uploaded to BLAST searching to ensure that no more than 17-nt matches occur in the corresponding genomes [49]. SiRNAs and plasmids transfection were conducted with Lipofectamine 3000 reagent (Invitrogen) or lipo8000 reagent (Beyotime) in accordance with the manufacturer's protocol.

Lentiviral vector construction and stable transfection

The lentiviral constructs of sh-LPP-AS2 was conducted by Hanbio Biotechnology and constructed into SHG44 cell lines. Cells were transfected with lentivirus or negative control virus (NC) in order to select the stably transfected cells. Then, the cells were treated with puromycin (2μg/mL) (Solarbio) for two weeks. GFP-positive cells were selected as sh-LPP-AS2 and sh-NC stably transfected cells and validated by real-time quantitative PCR.

Tumor xenografts model

Female BALB/c nude mice (aged 4–5 weeks, 18–20 g) were purchased from Vital River Laboratory Technology, and raised in laminar airflow cabinets under specific pathogen-free conditions. Subsequently, 1×10^7 stably transfected with sh-LPP-AS2 or sh-control cells were suspended in 0.1 mL PBS and 0.1ml Matrigel substrate and injected subcutaneously into the armpit regions of the mice. Tumor volumes were measured

every 3 days and calculated using the following formula: volume (cm3) = (length × width2) / 2. Bioluminescent imaging was performed using the IVIS Lumina LT Series III Imaging System (IVIS Lumina) with administration of D-luciferin (150 mg/kg i.v.). Then, the mice were sacrificed after 18 days post-injection, and the tumors were gathered for the subsequent researches. The animal studies were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of USTC.

RNA pull-down with biotinylated antisense oligonucleotides

RNA pull-down with 5′-biotinylated AS oligos was originated from a previously described method [50]. Cells were cross-linked in a UV cross-linker (UVP) at 200-mJ strength. The cells were granulated and resuspended in RIPA buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1% SDS, 1 mM DTT, 1× protease-inhibitor cocktail (Roche), and 0.1 U/μl RNase inhibitor) for 10 min on ice, then harvested and sonicated for 10 min. Cell debris in lysate was removed by centrifugation at 13,000g for 20 minutes. Subsequently, Biotinylated AS oligos (100 pmol) or Scramble oligos (as control) were added to the supernatant at 4 °C for 2 h. M-280 Streptavidin Dynabeads (Thermo) were washed three times in RIPA buffer, then blocked with 500 ng/μl yeast total RNA and 1 mg/ml BSA for 1.5 h at room temperature. Subsequently, M-280 Streptavidin Dynabeads were washed three times again in RIPA buffer and half of washed/blocked Dynabeads were added per 100 pmol of biotin-DNA oligonucleotides. Then, the mixture was rotated for 4 h at 4 °C. Beads were captured by magnets (Life Technologies) and washed three times with RIPA buffer supplemented with 500 mM NaCl. RNAs and proteins were extracted from beads for further analysis.

RNA immunoprecipitation assay (RIP)

Briefly, the 10^7 cells that were washed with cold 1× PBS three times and irradiated in a UV cross-linker (400 mJ/cm^2, 2 min). Then, the whole cells were harvested in ice-cold lysis buffer (10 mM HEPES, pH 7.4, 200 mM NaCl, 30 mM EDTA, 0.5% Triton-X 100, 100 units/ml RNasin Plus RNase Inhibitor (Promega), 1.5 mM DTT, 1× protease-inhibitor cocktail (Roche)). Cells were sonicated for 5 min with an Ultrasonic

Disruptor (SONICS), the cell suspension was centrifuged at 13,000g for 15 min at 4 °C and the supernatant was collected. Subsequently, anti-AGO2 (Proteintech) or IgG (Sangon Biotech) antibody was added into cell suspension and incubation 2h at 4 °C. Protein G Dynabeads (Life Technology) suspension was washed by RIP buffer three times, then blocked with 500 ng/μl yeast total RNA and 1 mg/ml BSA for 1.5 h at room temperature. Protein G Dynabeads were washed three times again in RIP buffer and then added it into cell suspension for binding at least 4 h at 4 °C. The antibody–Protein G bead complexes were washed five times with lysis buffer and the complexes were digested with 30 μg of proteinase K at 65 °C for 1 h. Finally, the immunoprecipitated RNA was purified and detected by quantitative real-time PCR. Antibody validation is provided on the manufacturers' websites.

Chromatin immunoprecipitation assay (ChIP)

ChIP was carried out as previously described, with modifications [51]. Cells were cross-linked in a UV cross-linker (UVP) at 200-mJ strength. cell granules were lysed in 1 ml of SDS lysis buffer (1% (w/v) SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1, Complete protease-inhibitor cocktail (Roche)) after being washed by cold-PBS three times and then were incubated for 20 min on ice. The cell mixture was sonicated for 5 minutes with an Ultrasonic Disruptor (SONICS) to gain up to 500-bp DNA fragments. A 100-μl sample of the supernatant was saved as input. The chromatin solution was immunoprecipitated with antibody to c-MYC (Proteintech, validation provided on the manufacturer's website) or IgG, and the mixture were rotated 2h at room temperature. Then, Protein G Dynabeads (Life Technology) suspension was washed by lysis buffer three times and blocked with 500 ng/μl yeast total RNA and 1 mg/ml BSA for 1.5 h at room temperature. Protein G Dynabeads were washed three times again in lysis buffer and then added it into cell suspension for binding at least 4 h at room temperature. The beads were collected and digested with proteinase K for 1 h at 45 °C, and the DNA was extracted by Endo-free Plasmid DNA Mini Kit (OMEGA). Eluted DNA was subjected to quantitative real-time PCR using the corresponding PCR primers to detect the enriched genomic DNA region.

Dual luciferase reporter assay

Approximately 1×10^4 Human U251 cells were co-transfected with 50 nM of empty pmirGLO-NC, pmirGLO-LPP-AS2-wt (or pmirGLO-EGFR-wt) or pmirGLO-LPP-AS2-mut (or pmirGLO-EGFR- mut) (RiboBio) and 50 nM miR-7-5p mimics or miR-NC by using Lipofectamine 3000 (Invitrogen) according to the manufacturer's protocols. Firefly luciferase gene in the vector pmirGLO-control (Promega) was used as the endogenous control to detect transfection efficiency. Firefly and Renilla luciferase activity were measured by a Dual-Luciferase Reporter Assay System (Promega) after 48h transfection. Firefly luciferase activity was normalized to the corresponding Renilla luciferase. Experiments were performed in triplicate, and the data are represented as mean ± SD.

Cell proliferation assay

Cell proliferation of U251 and SHG44 glioma cells were measured by using Cell Counting Kit-8 (Biosharp) assay. 4×10^3 U251 or SHG44 glioma cells/well were seeded in 96-well plates and incubation in 5% CO_2 atmosphere at 37 °C. 10 uL of CCK-8 solution was added to each well at 0, 24, 48, 72h after cell transfection. After incubation for 3 hours, the absorbance value of per well was determined with an ultraviolet spectrophotometer at 490 nm.

Apoptosis detection by flow cytometry and TUNEL assay

U251 and SHG44 cells (2×10^5 cell/well) was seeded into 6-well plates and were harvested at 48 hours post-transfection. The cells were washed with PBS and centrifuged twice and then resuspended in Annexin-V binding buffer. Subsequently, the mixture was stained with Annexin V-FITC/PI according to the manufacturer's protocols and the apoptosis rate was analyzed by Gallios flow cytometry (BECKMAN COULTER). Besides, cell apoptosis of U251 and SHG44 were also measured by One-Step TUNEL Apoptosis Kit (RiboBio) according to the manufacturer's introductions.

Cell migration and invasion assay

Cell migration and invasion abilities were detected by performing transwell assays. For cell migration detection, the transfected U251 or SHG44 cells (1×10^4 cell) were harvested after 24 h transfection and resuspended in 100 uL serum-free medium. Then, the cells were seeded into the upper chamber of Transwell assay insert (Millipore), and 700 uL 10% FBS medium was added in the lower chamber. After incubation at 37 °C for 48h, the cells on the lower side were washed three times with PBS, fixed in 4% paraformaldehyde for 20 minutes, and stained with crystal violet solution for 15 minutes. Five random fields were chosen to count stained cells for statistics under a inverted microscope (Olympus) and photographs were taken. For the invasion assay, transwell chambers were coated with Matrigel for 1 h at 37 °C. The transfected cells (1×10^4 cell) were resuspended in 100 uL serum-free medium and seeded into upper chamber. Then, the 700 uL 10% FBS medium was added in the lower chamber. After a 48 hours incubation period, the invasive ability was evaluated as mentioned previously for the cell migration.

Wound-healing assay

The transfected U251 and SHG44 cells (5×10^4 cell) were seeded into each side of a Culture-Insert 2 Well (Ibidi), and the μ-Dish was filled with 2 ml 2% FBS medium. Images of the different stages of wound healing were photographed via microscopy at 0, 12 and 24 h. Relative wound-healing rates were calculated by using CellSense Standard software (Olympus) and each experiment was performed in triplicate.

Colony formation assay

For the colony formation assays, the transfected U251 or SHG44 cells were harvested after 24 h transfection and 300 cells were chosen to inoculate into 6-well plates. After Incubation at 37 °C for 14 days, the colonies were fixed with 4% paraformaldehyde and stained with crystal violet solution.

Immunohistochemistry (IHC)

Immunohistochemistry was originated from a previously described method [52]. The dissected tumors from mouse model were fixed overnight in formalin solution, dehydrated in ethanol, embedded in paraffin, and cut into 5 μm sections. Then, the specimens were treated with xylene and ethanol in order to remove paraffin. The slides were blocked with 5% normal goat serum and incubated with anti-ki67, anti-MMP-9 or anti-EGFR antibodies overnight at 4 °C and washed three times with PBS. After incubation with HRP-conjugated secondary antibody, the sections were counterstained with hematoxylin. The average integral optical density of each positively stained slide was measured using an ImageJ software. Three fields were chosen randomly from each section for measurement.

Western blotting

For western blots, samples were separated on 10% SDS-PAGE gels and electrophoretically transferred to PVDF membranes (Millipore). Membranes were processed according to the ECL western blotting protocol (GE Healthcare) and scanned by Amersham Imager 680 (GE Healthcare). The following primary antibodies was used in western blots: anti-EGFR (BBI, D260292); anti-PI3K (BBI, D155308); anti-AKT (Proteintech, 10176-2-AP); anti-p-PI3K Tyr458 (Cell Signaling Technology, 4228S); anti-p-AKT Ser473 (Proteintech, 66444-1-Ig); anti-c-MYC (Proteintech, 10828-1-AP) and anti-β-actin (BBI, D110001). β-actin antibody was used as endogenous control for normalization. Antibody validation is provided on the manufacturers' websites.

Statistical analysis

All experiments were performed in triplicate, data are presented as the mean ± SD, and analyzed by using SPSS (version 23.0) or GraphPad Prism software (version 7.0). Student's t tests were used to calculate P values, as indicated in the figure legends. One-way analysis of variance for multiple groups. The correlations between lncRNA LPP-AS2 and miR-7-5p as well as EGFR in human specimens were analyzed by Spearman's rank test. Survival curves were generated using the Kaplan-Meier method and log-rank tests. P value < 0.05 was considered statistically significant.

Results

LncRNA LPP-AS2 is significantly upregulated in glioma tissues and transcriptional regulated by c-MYC

To investigate the expression profiles of lncRNA and mRNA in glioma, high-throughput RNA sequencing was performed in 3 tissues from glioma patients (diagnosed by pathological biopsy) and 3 normal brain tissues from craniocerebral trauma patients. To ensure the authenticity and validity of RNA-seq data, we examined the expression levels of some previously reported mRNAs such as TGFB2, HOXB3, FOXM1 and CD44, and lncRNAs such as H19, HCP5, PART-1 and MEG3 in our data (Figure S1a) [53-60]. The same pattern of these positive controls in glioma made our analyses of RNA-seq more convincing. Hierarchical cluster analysis was constructed to reveal the differential expression of lncRNAs and mRNAs in glioma tissues and controls (Figure S1b). Volcano plots were also performed for all expressed mRNAs in glioma tissues and controls (Figure S1c). A total of 183 lncRNAs were identified as significantly dysregulated in glioma, of which 62 lncRNAs were upregulated while 121 lncRNAs were downregulated (step1 in Fig. 1a). For the further validation with RT-qPCR, SMIM30, SNORA53 and LINC01354 were significantly upregulated while EFEMP2 was markedly downregulated in glioma compared to controls (Figure S1d). In addition, 542 differentially expressed mRNAs were also identified, with 144 upregulated and 398 downregulated (step1 in Fig. 1a). To validate these results, 6 mRNAs were randomly picked for RT-qPCR analysis with 15 glioma samples and 6 normal tissues. TOP2A, COL4A1 and PXDN were significantly upregulated while ANK3, ARRB1 and ANO4 were markedly downregulated in glioma compared to controls (Figure S2a). To dig out pivotal lncRNAs that involving in glioma, a co-expression network analysis was constructed between 35 mRNAs in neuron apoptotic process (Fig. 1b) which was predicted by KEGG pathway analysis and the top 50 lncRNAs (Fig. 1c). The network revealed that LPP-AS2 might play crucial roles in

tumor apoptosis (step3 in Fig. 1a). Thus, LPP-AS2 was selected for the further investigations.

Notably, we found the expression of LPP-AS2 were markedly increased in 106 glioma tissues (27 Grade I and II, 79 Grade III and IV) compared with 23 normal brain tissues by RT-qPCR (Fig. 1d). In order to reduce false positive, GSE50161 and GSE33331, microarray profile based on GPL570 platform, were downloaded from GEO database, together with GEPIA (Gene Expression Profiling Interactive Analysis) tool to further validated the upregulated expression of LPP-AS2 (Figure S2b, c). Especially, increased level of LPP-AS2 was significantly correlated with advanced grade in glioma patients (Table 1). In addition, Kaplan-Meier analysis of GEPIA demonstrated that patients with higher LPP-AS2 expression were more likely to be poor overall survival (Fig. 1e).

It was reported that c-MYC inhibited the expression of lncRNA LPP-AS2 in colorectal cancer [27]. To explore whether c-MYC could affect the expression of LPP-AS2 in glioma, the promoter sequence analysis tool (USCS) was performed to search the 2000 bp upstream region of LPP-AS2, and predicted that the LPP-AS2 promoter regions might harbor two putative c-MYC binding sites. The chromatin immunoprecipitation (CHIP) followed by RT-PCR and RT-qPCR assays were used to verify the putative correlation between LPP-AS2 promoter regions and c-MYC. Meanwhile, we found that knockdown of c-MYC could significantly decrease the expression level of LPP-AS2 (Figure S2d, e). These results supported the claim that c-MYC directly bind to the two putative chromatin fragment of promoter regions of LPP-AS2 and then regulated the transcription of LPP-AS2 (Fig. 1f-h). Collectively, these data suggested that a c-MYC transcriptional regulated lncRNA LPP-AS2 was highly expressed in glioma tissues, and it may act as a promising indicator of glioma prognosis.

LPP-AS2 promotes glioma progression *in vitro* and *in vivo*

RT-qPCR analysis of nuclear and cytoplasmic RNAs was carried out to show that LPP-AS2 was preferentially localized in the cytoplasm (Fig. 1i). Besides, the expression of LPP-AS2 were significantly elevated in seven glioma cell lines (U251, SHG44, T98G,

U373, U87, GOS-3, TJ905) compared to normal cell line HEB, especially higher in U251 and SHG44 cell lines (Fig. 1j). Thus, we selected the U251 and SHG44 cell lines for subsequent functional analysis.

To explore the biological functions of LPP-AS2, two independent small interfering RNAs (siRNAs) against LPP-AS2 were designed, which were effective knockdown the expression of LPP-AS2 in U251 and SHG44 cells (Fig. 2a). We also constructed an overexpression plasmid and successfully overexpress LPP-AS2 in U251 and SHG44 cells (Figure S3A). Next, CCK8 and colony formation assays were demonstrated that depletion of LPP-AS2 markedly inhibited cell proliferation, whereas overexpressed LPP-AS2 expression facilitated cell viability (Fig. 2b, d and Figure S3b, d). Wound-healing and transwell assays revealed that knockdown of LPP-AS2 significantly suppressed cell invasion and migration capacities, while upregulation of LPP-AS2 exhibited the opposite effects (Fig. 2c, g and Figure S3c, g). In parallel, the ability of apoptosis was analyzed following LPP-AS2 silencing or overexpression by TUNEL assay and flow cytometry. As we speculated, knockdown of LPP-AS2 induced cell apoptosis, whereas upregulation of LPP-AS2 inhibited cell apoptosis (Fig. 2e, f and Figure S3e, f).

To evaluate the regulatory roles of LPP-AS2 on tumor formation *in vivo*, a nude mice xenograft tumor model was constructed. SHG44 stable cells were established with lentivirus to downregulate LPP-AS2. Then, a total of 1×10^7 SHG44 cells with stabled expression were subcutaneously injected into BALB/c nude mice (two groups, n = 3 each group). The procedure of animal study is as follows (Figure S3h). After injection, smaller tumor sizes and weights were observed in LV-sh-LPP-AS2 group compared with those in LV-sh-control group (Fig. 2h-j and Figure S3i-j). The effective expression of LPP-AS2 was downregulated in LV-sh-LPP-AS2 group (Figure S3k). Furthermore, H&E and immunohistochemistry staining demonstrated that ki-67, matrix metalloprotease (MMP-9) and epidermal growth factor receptor (EGFR) were inhibited upon LPP-AS2 downregulation in dissected tumors (Fig. 2k). These findings suggested that LPP-AS2 promoted cell proliferation, migration, invasion and inhibited apoptosis of glioma cells *in vitro* and *in vivo*.

LPP-AS2 facilitates glioma progression through enhancing EGFR expression

To elucidate the potential molecular mechanisms of LPP-AS2 in glioma initial and progression, we first detected expression level of the parent gene LPP. It turned out that overexpression and knockdown of LPP-AS2 had no effect on the expression of LPP (Figure S4a, b). Therefore, we constructed a co-expression analysis network of LPP-AS2 and the corresponding mRNAs according to the data of high-throughput RNA sequencing. EGFR was found to be the top predicted co-expressed mRNA and markedly upregulated in glioma tissues (Fig. 3a). We further confirmed the upregulation of EGFR in 17 glioma tissues compared to normal controls (Fig. 3b). Survival analysis of TCGA database revealed that patients with high expression of EGFR were significantly associated with poor prognosis (Fig. 3c). Moreover, correlation analysis from results of RT-qPCR showed a positively correlation between expression level of EGFR and LPP-AS2 (Fig. 3d). The expression of EGFR was significantly increased in seven glioma cell lines (U251, SHG44, T98G, U373, U87, GOS-3, TJ905) compared to normal cell line HEB (Figure S4c). The results of RT-qPCR and western blot suggested that knockdown LPP-AS2 expression significantly reduced the mRNA and protein level of EGFR, whereas EGFR mRNA and protein level was upregulated by overexpressed LPP-AS2 (Fig. 3e, f and Figure S4d, e). Taken together, EGFR was considered as a major candidate target of LPP-AS2.

Furthermore, the findings of rescue functional experiments revealed that the cell proliferation, invasion, migration and colony formation abilities were promoted/inhibited by overexpression/knockdown of EGFR and was reversed by silencing/increasing the expression of LPP-AS2 (Fig. 3g-k and Figure S5a-e), whereas restoration/downregulation of LPP-AS2 expression partially rescued the suppressive/accelerate effects of EGFR knockdown/overexpression on glioma cell apoptosis (Fig. 3l and Figure S5f). All these findings illustrated that LPP-AS2 plays an oncogenic role in glioma progression through EGFR.

LPP-AS2 functions as a ceRNA and competitively absorbs miR-7-5p in glioma

cells

Recently, quantity of cytoplasmic lncRNAs have been confirmed to function as miRNAs sponge to regulate downstream targets [61-64]. As shown in Fig. 1i, LPP-AS2 was predominantly localized in the cytoplasm. We hypothesized that LPP-AS2 exerts as a ceRNA based on these findings. Subsequently, to validate the hypothesis, we used the online bioinformatic tools (lncRNASNP2.0) to predicted miRNAs that could bind with LPP-AS2, and found LPP-AS2 contains potential complementary binding sequences to miR-7-5p, miR-200c-5p, miR-297, miR-143-5p and miR-330-5p seed regions. Results of RT-qPCR suggested that miR-7-5p was the most downregulated miRNA (Fig. 4a). MiR-7-5p showed an obviously downregulation in 17 glioma tissues compared to normal control samples (Figure S6a), and miR-7-5p expression was significantly downregulated in multiple glioma cell lines including U251 and SHG44 cells (Figure S6b). Additionally, knockdown of LPP-AS2 expression significantly increased the expression level of miR-7-5p (Fig. 4b). Overexpressed LPP-AS2 could reduce the expression level of miR-7-5p (Figure S6c), but have no effect on LPP-AS2 expression when altered the level of miR-7-5p by miR-7-5p mimics or inhibitor (Figure S6d). Correlation analysis suggested a negative correlation between the expression of LPP-AS2 and miR-7-5p (Fig. 4c). Ago2 was known as a crucial component of the RNA-induced silencing complex (RISC) [58]. Then, we performed an Ago2 immunoprecipitation assay in U251 and SHG44 cells, and determined that LPP-AS2 can serve as a platform for Ago2 and miR-7-5p (Fig. 4d). We then used the dual luciferase reporter assays to validated the direct binding of miR-7-5p with LPP-AS2. The result demonstrated that miR-7-5p overexpression distinctly suppressed the luciferase activity of LPP-AS2-WT group, but without statistical changes in LPP-AS2-MUT group (Fig. 4e). Besides, RNA pull-down assay demonstrated that biotin-coupled LPP-AS2 successfully pulled down the competitive binding of miR-7-5p, while biotin-labeled miR-7-5p also pulled down the binding of LPP-AS2 (Fig. 4f, g). Taken together, these all findings indicated that LPP-AS2 acts as a molecular sponge for miR-7-5p in glioma cells.

It is widely reported that miR-7-5p exerts important regulatory roles in a variety of

cancers [65, 66]. In glioma, as shown in Figure S7, miR-7-5p was markedly inhibited the proliferation and invasion, as well as migration and colony formation abilities of glioma cells. These results suggested that miR-7-5p function as a suppressor in glioma tumorigenesis. To further verify the cellular phenotype caused by binding of LPP-AS2 with miR-7-5p, rescued functional studies revealed that miR-7-5p mimics suppressed the viability, invasion, migration and colony formation abilities of glioma cell that stimulated by abnormal overexpression of LPP-AS2. In contrast, miR-7-5p inhibitor rescued the proliferation, invasion, migration and colony formation abilities of glioma cell with downregulated LPP-AS2 expression (Fig. 4h-k and Figure S8a-d). Moreover, miR-7-5p inhibitor rescued the cell apoptosis arose by LPP-AS2 knockdown, while transfection of miR-7-5p mimics distinctly reversed the suppression of cell apoptosis under LPP-AS2 overexpression (Fig. 4l and Figure S8e). All of these results indicated that LPP-AS2 functioned as an oncogene via miR-7-5p.

LPP-AS2 decoys miR-7-5p to upregulate the expression of EGFR

To dig out a gene sharing the regulatory role of miR-7-5p and LPP-AS2, we used four online databases (TargetScan, miRWalk, miRTarBase and miRDB) to predict potential target genes of miR-7-5p (Fig. 5a), and 62 mRNAs might be the potential targets of miR-7-5p including EGFR that previously validated. Then, dual luciferase assays were performed to confirm the relationship between miR-7-5p and EGFR. The results indicated that compared with EGFR-MUT group, the fluorescence intensity of co-transfection EGFR-WT plasmids and miR-7-5p mimics group markedly decreased. however, no significant differences observed in the co-transfection EGFR-MUT plasmids and miR-7-5p mimics group (Fig. 5b). Moreover, the influences of miR-7-5p on mRNA and protein level of EGFR was measured. The results indicated that inhibited the miR-7-5p expression significantly improved the level of EGFR, whereas the level of EGFR was reduced by transfecting miR-7-5p mimics in U251 and SHG44 cells (Fig. 5c, d and Figure S9a). Meanwhile, correlation analysis showed a negative correlation between the expression of miR-7-5p and EGFR in 21 glioma tissues (Fig. 5e).

Previous studies have demonstrated that EGFR led to the autophosphorylation of

receptor tyrosine kinase and further activated the downstream intracellular signaling cascades especially the phosphatidylinositol 3-kinase-AKT serine/threonine kinase 1 (PI3K-AKT) pathway through binding with its cognate ligands [67, 68].Then, we further investigated the regulatory ability of LPP-AS2/miR-7-5p axis to influence EGFR expression and activate the downstream PI3K/AKT/c-MYC pathway. LPP-AS2 overexpression/knockdown distinctly increased/reduced the mRNA and protein level of EGFR, phospho-PI3K (p-PI3K), phospho-AKT (p-AKT) as well as c-MYC protein, and these sequels were partially reversed by co-transfection with miR-7-5p mimics/inhibitors, whereas the total protein level of PI3K or AKT was almost unchanged (Fig. 5f-j and Figure S9c-g). Besides, knockdown LPP-AS2 remarkedly reduced the level of EGFR and its downstream effectors phospho-PI3K, phospho-AKT and c-MYC, whereas no significantly changes were observed in the total protein level of PI3K or AKT on U251 and SHG44 cells (Fig. 5k, l and Figure S9b). In contrast, overexpression of LPP-AS2 obviously elevated the protein level of EGFR, phospho-PI3K, phospho-AKT and c-MYC, and the level of PI3K or AKT was still not obviously changed (Figure S9h-j). Taken together, these findings demonstrated that LPP-AS2 decoys miR-7-5p to upregulate the expression of EGFR and stimulate the PI3K/AKT/c-MYC signaling pathway.

More importantly, we found that c-MYC could regulate the expression of LPP-AS2 in the beginning of previous study. These all findings suggested that LPP-AS2 was transcriptional regulated by c-MYC, and functions as an oncogene in glioma via miR-7-5p/EGFR/PI3K/AKT/c-MYC feedback loop (Fig. 6).

Discussion

Currently, a growing number of studies have demonstrated that lncRNAs are required for human diseases and individual development. Abnormal expression of these lncRNAs may lead to tumorigenesis and aggressive progression and making these molecules attractive therapeutic targets [61, 62]. For instance, a newly identified lncRNA called PTAR, whose expression is significantly correlated with tumor subtype,

promotes epithelial-mesenchymal transition (EMT) and metastasis in ovarian cancer via miR-101-3p/ZEB1 pathway [61]. The proliferation and tumor formation abilities of gastric cancer cells are inhibited by knockdown of LINC01234, which is a potential molecular target of tumor therapy [62]. In addition, the lncRNA upregulated in colorectal cancer liver metastasis (UICLM) is significantly increased in cases of CRC with liver metastasis and leads to poor clinical survival [69]. However, the functions and concrete molecular mechanism of LPP-AS2 in tumors have not been elucidated to date, including glioma.

Glioma, which is a devastated intracranial neoplasm with highly prevalence and poor clinical prognosis, was hardly achieved substantial progress through using current surgical resection and chemoradiotherapy. Therefore, a novel potential effective therapeutic target is urgently needed. Here, we used next-generation analysis and integrated GEO datasets to investigate the profiling of aberrantly expressed lncRNAs and mRNAs between glioma and normal brain tissues. We then identified a novel lncRNA LPP-AS2, whose expression is distinctly upregulated in glioma specimens and cell lines. The upregulated expression of LPP-AS2 was observed to be related with poorly prognosis and adverse clinical survival among patients with gliomas. Gain-of-function and loss-of-function experiments demonstrated that LPP-AS2 was promoted the occurrence and development of glioma *in vitro* and *in vivo*. These data indicate that LPP-AS2 plays a carcinogenic role and deserves to be investigated the molecular mechanism in glioma.

By comparing the differential expressed genes in the results of high-throughput sequencing and constructing a co-expression network analysis, we identified a positive relationship between LPP-AS2 and EGFR, a well-known gene involved in cell proliferation and apoptosis through activating PI3K/AKT signaling pathway. Knockdown of LPP-AS2 could decrease the expression of EGFR, while ectopic expression of LPP-AS2 elevated EGFR expression in glioma cells. Besides, the protein level of phospho-PI3K, phospho-AKT and c-MYC were also modulated by LPP-AS2. Furthermore, functional studies showed that overexpression/knockdown of EGFR could partially rescue the inhibition/promotion caused by silencing/elevating LPP-AS2

in glioma cells. These findings indicated that LPP-AS2 regulated the expression of EGFR and then activated PI3K/AKT pathway to affect the biological behaviors of glioma cells.

An increasing number of studies have demonstrated that the molecular mechanisms of lncRNAs greatly relied on their subcellular localization [70]. Besides, with the continuous understanding of tumor occurrence, a novel and extensive interaction network that involving ceRNAs was reported, in which lncRNAs could regulate the expression of miRNAs by competitively binding their target sites on protein-coding mRNA elements [71, 72]. For instance, lncRNA PDIA3P1 (protein disulfide isomerase family A member 3 pseudogene 1) affected chemotherapy sensitivity by acting as a microRNA sponge for miR-125a/b/miR-124 to elevate TRAF6 expression and augment NF-κB signaling pathway [73]. LncRNA LINC00336 suppressed ferroptosis and promoted the tumorigenesis of lung cancer through serving as a competitively endogenous sponge of miR-6852 to promote cystathionine-β-synthase expression [74]. In our research, we found that LPP-AS2 was predominantly localized in the cytoplasm of glioma cells and showed an inverse relationship between LPP-AS2 and miR-7-5p by RT-qPCR, which indicated that LPP-AS2 may function through posttranscriptional regulation. Further evidence performed by bioinformatics analysis, dual luciferase report assays, RNA immunoprecipitation and RNA pulldown assays, confirmed that LPP-AS2 interacted with miR-7-5p by directly binding to its complementary sequences. Besides, emerging evidence demonstrated that miR-7-5p was downregulated in various cancers and treated as a tumor suppressor. For example, restoring expression of miR-7-5p could suppress tumorigenesis in colorectal cancer cells and reversed by SP1-induced lncRNA TINCR [65]. MiR-7-5p was a potential biomarker in neuroendocrine neoplasms of the small intestine [66]. In this study, we found that miR-7-5p is distinctly downregulated in glioma cells and overexpression of miR-7-5p could repress biological behaviors of glioma, which was consistent with the results of a recent report [35]. Moreover, the effects of silencing LPP-AS2 on proliferation and invasion were reversed by miR-7-5p inhibitor, whereas transfecting miR-7-5p mimics into glioma cells could partially rescue the effects which was caused by overexpression of LPP-AS2. Taken

together, our findings suggest that LPP-AS2 functions as an oncogenic role in glioma by activating cell viability, which can be partially rescued by miR-7-5p mimics.

Generally, miRNAs exert function in cancer signaling pathways by depending on depression or degradation of target genes, thereby affecting cell fate and biological function [75]. In our study, we used four online databases to predict the potential target of miR-7-5p. Surprisingly, a target gene EGFR was observed in the intersection of four subsets, which was negative correlation with miR-7-5p. The result of dual luciferase report assays validated that EGFR was directly bound with miR-7-5p, and it is also in agreement with the former reports concerning EGFR in glioma and other disease [35, 76]. Moreover, overexpression/knockdown of miR-7-5p repressed/promoted EGFR mRNA and protein expression. Given that both LPP-AS2 and EGFR interact with miR-7-5p, we hypothesized that LPP-AS2 could indirectly regulate the expression of EGFR by competing bind to miR-7-5p. Subsequently, we conducted the RT-qPCR and western blot to validate the hypothesis. The mRNA and protein level of EGFR were downregulated by silencing LPP-AS2 and could be rescued by miR-7-5p inhibitor, while overexpressing LPP-AS2 also partially reversed by miR-7-5p mimics. Therefore, we demonstrated that LPP-AS2 could regulate EGFR expression and activate the PI3K/AKT/c-MYC signaling pathway by competitively sponging miR-7-5p, thereby promoting glioma cell viability.

As a transcriptional factor, c-MYC is regarded as a human proto-oncogene and leads to multiple features of cancer. In the early stage, quantity of studies suggested that c-MYC transcriptional targets are involved in various biological processes, such as cell proliferation, apoptosis and metabolism [77]. Interestingly, a recent study suggested that LPP-AS2 was transcriptionally repressed in CRC cells with high level of c-MYC, although the regulation maybe indirect [27]. However, it remains unclear whether c-MYC could transcriptionally regulate the expression of LPP-AS2 in glioma. Here, we found that knockdown of c-MYC significantly inhibited the expression of LPP-AS2. ChIP was performed to prove that c-MYC could specifically bind the promoter of LPP-AS2 and elevate LPP-AS2 expression in glioma. These results indicated that there was existed a positive feedback loop between LPP-AS2 and c-MYC, thus affecting the

biological behaviors of glioma cells.

Conclusion

In general, our study revealed that elevated LPP-AS2 expression is a common event in glioma, and aberrant expression of LPP-AS2 significantly associated with poor prognosis in glioma patients. LPP-AS2 functioned as an oncogenic lncRNA in glioma both *in vitro* and *in vivo*. In addition, LPP-AS2 was involved in the regulation of EGFR expression through competitively sponging miR-7-5p and then stimulated the PI3K/AKT/c-MYC pathway as well as conversely transcriptionally regulated by c-MYC. Overall, our study provides a new mechanistic insight into the pathogenesis of glioma and suggests a potentially novel therapeutic target for glioma patients.

Abbreviations

lncRNA: Long non-coding RNA; LPP-AS2: LPP antisense RNA 2; ceRNA: Competing endogenous RNA; EGFR: Epidermal growth factor receptor; RT-qPCR: Real-time quantitative PCR; IHC: Immunohistochemistry; RIP: RNA immunoprecipitation; GEO: Gene Expression Omnibus; ChIP: Chromatin immunoprecipitation; TCGA: The Cancer Genome Atlas

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Authors' contributions

CSN and SSH conceived of the research and accomplish the design. XMZ carried out all the cellular experiments. MLM, WXN and ZXB collected tissue specimens and information of patients. XMZ performed statistical analysis and finished draft manuscript independently. All authors read and approved the final manuscript.

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Availability of data and materials

All data in this study are available upon request.

Ethics approval and consent to participate

All tissues were obtained with informed patient consent and approval by Human Research Ethics Committee of the First Affiliated Hospital of USTC.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interests.

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References

1. Lv D, Li Y, Zhang W, Alvarez AA, Song L, Tang J, Gao WQ, Hu B, Cheng SY, Feng H. TRIM24 is an oncogenic transcriptional co-activator of STAT3 in glioblastoma. Nat Commun. 2017;8:1454.
2. Ostrom QT, Gittleman H, de Blank PM, Finlay JL, Gurney JG, McKean-Cowdin R, Stearns DS, Wolff JE, Liu M, Wolinsky Y, et al. American Brain Tumor Association adolescent and young adult primary brain and central nervous system tumors diagnosed in the United States in 2008-2012. Neuro Oncol. 2016;18(Suppl 1):i1–i50.
3. Lu M, Wang Y, Zhou S, e t a l. MicroRNA-370 suppresses the progression and proliferation of human astrocytoma and glioblastoma by negatively regulating beta-catenin and causing activation of FOXO3a. Exp Ther Med 2018;15:1093–1098.
4. Van Meir E G, Hadjipanayis C G, Norden A D, Shu HK, Wen PY, Olson JJ. Exciting new advances in neuro-oncology: the avenue to a cure for malignant glioma. Ca A Cancer Journal for Clinicians. 2010; 60(3):166-193.
5. Raguz S, Yague E. Resistance to chemotherapy: new treatments and novel insights into an old problem. Br J Cancer. 2008;99(3):387 –391.
6. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004;10(8):789 –799.
7. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10(3):155–159.
8. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013;152(6):1298–307.
9. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature. 2004;431(7011):931.
10. Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell. 2013;154(1):26-46.
11. Khalil AM, Mitchell Guttman, Maite Huarte, Manuel Garber, Arjun Raj, Dianali

Rivea Morales, Kelly Thomas, Aviva Presser, Bradley E Bernstein, Alexander van Oudenaarden, Aviv Regev, Eric S Lander, John L Rinn. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. Proc Natl Acad Sci U S A. 2009;106:11667–72.

12. Lee JT. Epigenetic regulation by long noncoding RNAs. Science. 2012;338:1435-9.
13. Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, et al. Long Noncoding RNA as Modular Scaffold of Histone Modification Complexes. Science. 2010;329(5992):689-693.
14. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem. 2012;81:145–166.
15. Hu S, X Wang and G Shan. Insertion of an Alu element in a lncRNA leads to primate-specific modulation of alternative splicing. Nat Struct Mol Biol. 2016;23(11):1011-1019.
16. Mondal T, Juvvuna PK, Kirkeby A, Mitra S, Kosalai ST, Traxler L, et al. Sense-Antisense lncRNA Pair Encoded by Locus 6p22.3 Determines Neuroblastoma Susceptibility via the USP36-CHD7-SOX9 Regulatory Axis. Cancer Cell. 2018;33(3):417-34.
17. Leucci E, Vendramin R, Spinazzi M, Laurette P, Fiers M, Wouters J, Radaelli E, Eyckerman S, Leonelli C, Vanderheyden K, et al. Melanoma addiction to the long non-coding RNA SAMMSON. Nature. 2016;531(7595):518-522.
18. Lin A, Hu Q, Li C, Xing Z, Ma G, Wang C, Li J, Ye Y, Yao J, Liang K, et al. The LINK-A lncRNA interacts with PI(3,4,5)P3 to hyperactivate AKT and confer resistance to AKT inhibitors. Nat Cell Biol. 2017;19(3):238.
19. Kim J, Piao H, Kim B, et al. Long noncoding RNA MALAT1 suppresses breast cancer metastasis. Nat Genet. 2018;50:1705-1715.
20. Fu WM, X Zhu, WM Wang, YF Lu, BG Hu, H Wang, WC Liang, SS Wang, C H Ko, M M Waye, H F Kung, G Li and JF Zhang. Hotair Mediates Hepatocarcinogenesis through Suppressing MiRNA-218 Expression and Activating P14 and P16 Signaling. J Hepatol. 2015;63(4):886-895.

21. Cho S W, J Xu, R Sun, M R Mumbach, A C Carter, Y G Chen, K E Yost, J Kim, J. He, S A Nevins, S F Chin, C Caldas, S J Liu, M A Horlbeck, D A Lim, J S Weissman, C Curtis and H Y Chang. Promoter of lncRNA Gene PVT1 Is a Tumor-Suppressor DNA Boundary Element. Cell. 2018;173(6):1398-1412 e1322.
22. Wedge D C, G Gundem, T Mitchell, D J Woodcock, I Martincorena, M Ghori, J Zamora, A Butler, H Whitaker, Z R A Eeles. Sequencing of prostate cancers identifies new cancer genes, routes of progression and drug targets. Nat Genet. 2018;50(5):682-692.
23. Wang S, K Liang, Q Hu, P Li, J Song, Y Yang, J Yao, L S Mangala, C Li, W Yang, P K Park, D H Hawke, J Zhou, Y Zhou, W Xia, M C Hung, J R Marks, G E Gallick, G Lopez-Berestein, E R Flores, A K Sood, S Huang, D Yu, L Yang and C Lin. JAK2-binding long noncoding RNA promotes breast cancer brain metastasis. J Clin Invest. 2017;127(12):4498-4515.
24. Wu X, Y Wang, T Yu, E Nie, Q Hu, W Wu, T Zhi, K Jiang, X Wang, X Lu, H Li, N Liu, J Zhang and Y You. Blocking MIR155HG/miR-155 axis inhibits mesenchymal transition in glioma. Neuro Oncol. 2017;19(9):1195-1205.
25. Huang R, T Meng, R Chen, P Yan, J Zhang, P Hu, X Zhu, H Yin, D Song and Z Huang. The construction and analysis of tumor-infiltrating immune cell and ceRNA networks in recurrent soft tissue sarcoma. Aging. 2019;11(22):10116-10143.
26. Xin Chen, Zuyuan Yang, Chao Yang, Kan Xie, Weijun Sun, and Shengli Xie. Sparse Gene Coexpression Network Analysis Reveals EIF3J-AS1 as a Prognostic Marker for Breast Cancer. Complexity. 2018;2018:1-12.
27. Ingram I. lncRNAs and MYC: An Intricate Relationship. Int. J. Mol. Sci. 2017;18(7):1497.
28. Bartel D P. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-297.
29. Bartel D P. Metazoan MicroRNAs. Cell. 2018;173(1):20-51.
30. Benjamin K, Shi C Y, Joanna S and Bartel D P. A Network of Noncoding Regulatory RNAs Acts in the Mammalian Brain. Cell. 2018;174(2):350-362 e317.

31. Li L, Li Z, Kong X, Xie D, Jia Z, Jiang W, Cui J, Du Y, Wei D, Huang S, Xie K. Downregulation of MicroRNA 494 via Loss of SMAD4 Increases FOXM1 and βcatenin Signaling in Pancreatic Ductal Adenocarcinoma Cells. Gastroenterology. 2014;147(2):485-497.e18
32. Xue J, A Zhou, Y Wu, S A Morris, K Lin, S Amin, R Verhaak, G Fuller, K Xie, A B Heimberger and S Huang. miR-182-5p Induced by STAT3 Activation Promotes Glioma Tumorigenesis. Cancer Res. 2016;76(14):4293-4304.
33. Kabir T D, C Ganda, R M Brown, D J Beveridge, K L Richardson, V Chaturvedi, P Candy, M Epis, L Wintle, F Kalinowski, C Kopp, L M Stuart, G C Yeoh, J George and P J Leedman. A microRNA-7/growth arrest specific 6/TYRO3 axis regulates the growth and invasiveness of sorafenib-resistant cells in human hepatocellular carcinoma. Hepatology. 2018;67(1):216-231.
34. Shi Y, X Luo, P Li, J Tan, X Wang, T Xiang and G Ren. miR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ. Cancer Lett. 2015;358(1):27-36.
35. Li G, M Huang, Y Cai, Y Yang, X Sun and Y Ke. Circ-U2AF1 promotes human glioma via derepressing neuro-oncological ventral antigen 2 by sponging hsa-miR-7-5p. J Cell Physiol. 2019;234(6):9144-9155.
36. Ji H, D Li, L Chen, T Shimamura, S Kobayashi, K McNamara, U Mahmood, A Mitchell, Y Sun, R Al-Hashem, L R Chirieac, R Padera, R T Bronson, W Kim, P A Janne, G I Shapiro, D Tenen, B E Johnson, R Weissleder, N E Sharpless and K K Wong. The impact of human EGFR kinase domain mutations on lung tumorigenesis and in vivo sensitivity to EGFR-targeted therapies. Cancer Cell. 2006;9(6):485-495.
37. Sun C, L Wang, S Huang, G J Heynen, A Prahallad, C Robert, J Haanen, C Blank, J Wesseling, S M Willems, D Zecchin, S Hobor, P K Bajpe, C Lieftink, C Mateus, S Vagner, W Grernrum, I Hofland, A Schlicker, L F Wessels, R L Beijersbergen, A Bardelli, F Di Nicolantonio, A M Eggermont and R Bernards. Reversible and adaptive resistance to BRAF(V600E) inhibition in melanoma. Nature. 2014;508(7494):118-122.

38. Mertins P, D R Mani, K V Ruggles, M A Gillette, K R Clauser, P Wang, X Wang, J W Qiao, S Cao, F Petralia, E Kawaler, F Mundt, K Krug, Z Tu, J T Lei, M L Gatza, M Wilkerson, C M Perou, V Yellapantula, K L Huang, C Lin, M D McLellan, P Yan, S R Davies, R R Townsend, S J Skates, J Wang, B Zhang, C R Kinsinger, M Mesri, H Rodriguez, L Ding, A G Paulovich, D Fenyo, M J Ellis, S A Carr and C Nci. Proteogenomics connects somatic mutations to signaling in breast cancer. Nature. 2016;534(7605):55-62.
39. Jamal-Hanjani M, G A Wilson, N McGranahan, et al. Tracking the Evolution of Non-Small-Cell Lung Cancer. N Engl J Med. 2017;376(22):2109-2121.
40. Srivatsa S, M C Paul, C Cardone, M Holcmann, N Amberg, P Pathria, M A Diamanti, M Linder, G Timelthaler, H P Dienes, L Kenner, F Wrba, G W Prager, S Rose-John, R Eferl, G Liguori, G Botti, E Martinelli, F R Greten, F Ciardiello and M Sibilia. EGFR in Tumor-Associated Myeloid Cells Promotes Development of Colorectal Cancer in Mice and Associates with Outcomes of Patients. Gastroenterology. 2017;153(1):178-190 e110.
41. Gimple R C, R L Kidwell, L J Y Kim, T Sun, A D Gromovsky, Q Wu, M Wolf, D Lv, S Bhargava, L Jiang, B C Prager, X Wang, Q Ye, Z Zhu, G Zhang, Z Dong, L Zhao, D Lee, J Bi, A E Sloan, P S Mischel, J M Brown, H Cang, T Huan, S C Mack, Q Xie and J N Rich. Glioma Stem Cell Specific Super Enhancer Promotes Polyunsaturated Fatty Acid Synthesis to Support EGFR Signaling. Cancer Discov. 2019; 9(9): 1248-1267.
42. Katanasaka Y, Y Kodera, Y Kitamura, T Morimoto, T Tamura and F Koizumi. Epidermal growth factor receptor variant type III markedly accelerates angiogenesis and tumor growth via inducing c-myc mediated angiopoietin-like 4 expression in malignant glioma. Mol Cancer. 2013;12(1):31.
43. Griesinger A M, D K Birks, A M Donson, V Amani, L M Hoffman, A Waziri, M Wang, M H Handler and N K Foreman. Characterization of Distinct Immunophenotypes across Pediatric Brain Tumor Types. J Immunol. 2013;191(9):4880-4888.
44. Donson A M, D K Birks, S A Schittone, B K Kleinschmidt-DeMasters, D Y Sun,

M F Hemenway, M H Handler, A E Waziri, M Wang and N K Foreman. Increased immune gene expression and immune cell infiltration in high-grade astrocytoma distinguish long-term from short-term survivors. J Immunol. 2012;189(4):1920-1927.

45. Barrett T, T O Suzek, D B Troup, S E Wilhite, W C Ngau, P Ledoux, D Rudnev, A E Lash, W Fujibuchi and R Edgar. NCBI GEO: mining millions of expression profiles—database and tools. Nucleic Acids Res. 2005;33(1):D562-566.
46. Tang Z, C Li, B Kang, G Gao, C Li and Z Zhang. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-W102.
47. Minoru K, Susumu G. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 2000;28(1):27-30.
48. Huang D W, Sherman B T, Lempicki R A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2008;4(1):44-57.
49. Shan G. RNA interference as a gene knockdown technique. Int J Biochem Cell Biol. 2010;42(8):1243-1251.
50. Chen S, V Huang, X Xu, J Livingstone, F Soares, J Jeon, Y Zeng, J T Hua, J Petricca, H Guo, M Wang, F Yousif, Y Zhang, N Donmez, M Ahmed, S Volik, A Lapuk, M L K Chua, L E Heisler, et al. Widespread and Functional RNA Circularization in Localized Prostate Cancer. Cell. 2019;176(4):831-843 e822.
51. Li Z, C Huang, C Bao, L Chen, M Lin, X Wang, G Zhong, B Yu, W Hu, L Dai, P Zhu, Z Chang, Q Wu, Y Zhao, Y Jia, P Xu, H Liu and G Shan. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2015;22(3):256-264.
52. Li L, Liang Y, Kang L, Liu Y, Gao S, Chen S, Li Y, You W, Dong Q, Hong T, Yan Z, Jin S, Wang T, Zhao W, Mai H, Huang J, Han X, Ji Q, Song Q, Yang C, Zhao S, Xu X, Ye Q. Transcriptional Regulation of the Warburg Effect in Cancer by SIX1. Cancer Cell. 2018;33(3):368-385 e367.
53. Zhang C, Zhang X, Xu R, Huang B, Chen AJ, Li C, Wang J, Li XG. TGF-β2

initiates autophagy via Smad and non-Smad pathway to promote glioma cells' invasion. J Exp Clin Cancer Res. 2017;36(1):162.

54. Xu K, Qiu C, Pei H, Mehmood MA, Wang H, Li L, Xia Q. Homeobox B3 promotes tumor cell proliferation and invasion in glioblastoma. Oncol Lett. 2018;15(3):3712-3718.
55. Zhang C, Han X, Xu X, Zhou Z, Chen X, Tang Y, Cheng J, Moazzam NF, Liu F, Xu J, Peng W, Du F, Zhang B, Song Z, Zeng J, Gong A. FoxM1 drives ADAM17/EGFR activation loop to promote mesenchymal transition in glioblastoma. Cell Death Dis. 2018;9(5):469.
56. Mooney K L, Choy W, Sidhu S, Pelargos P, Bui TT, Voth B, Barnette N, Yang I. The role of CD44 in glioblastoma multiforme. J Clin Neurosci. 2016;34:1-5.
57. Li W, Jiang P, Sun X, Xu S, Ma X, Zhan R. Suppressing H19 Modulates Tumorigenicity and Stemness in U251 and U87MG Glioma Cells. Cell Mol Neurobiol. 2016;36(8):1219-1227.
58. Teng H, Wang P, Xue Y, Liu X, Ma J, Cai H, Xi Z, Li Z, Liu Y. Role of HCP5-miR-139-RUNX1 Feedback Loop in Regulating Malignant Behavior of Glioma Cells. Mol Ther. 2016;24(10):1806-1822.
59. Zhang X Q, Sun S, Lam K F, Kiang KM, Pu JK, Ho AS, Lui WM, Fung CF, Wong TS, Leung GK. A long non-coding RNA signature in glioblastoma multiforme predicts survival. Neurobiol Dis. 2013;58:123-131.
60. Balci T, Susluer S Y, Kayabasi C, Ozmen Yelken B, Biray Avci C, Gunduz C. Analysis of dysregulated long non-coding RNA expressions in glioblastoma cells. Gene. 2016;590(1):120-122.
61. Liang H, T Yu, Y Han, Jiang H, Wang C, You T, Zhao X, Shan H, Yang R, Yang L, Shan H, Gu Y. LncRNA PTAR promotes EMT and invasion-metastasis in serous ovarian cancer by competitively binding miR-101-3p to regulate ZEB1expression. Mol Cancer. 2018;17(1):119.
62. Chen X, Chen Z, Yu S, Nie F, Yan S, Ma P, Chen Q, Wei C, Fu H, Xu T, Ren S, Sun M, Wang Z. Long noncoding RNA LINC01234 functions as a competing endogenous RNA to regulate CBFB expression by sponging miR-204-5p in

gastric cancer. Clin Cancer Res. 2018;24(8):2002-2014.

63. Yang X Z, T T Cheng, Q J He, Lei ZY, Chi J, Tang Z, Liao QX, Zhang H, Zeng LS, Cui SZ. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/beta-catenin pathway. Mol Cancer. 2018;17(1):126.
64. Zheng Z Q, Z X Li, G Q Zhou, Lin L, Zhang LL, Lv JW, Huang XD, Liu RQ, Chen F, He XJ, Kou J, Zhang J, Wen X, Li YQ, Ma J, Liu N, Sun Y. Long Noncoding RNA FAM225A Promotes Nasopharyngeal Carcinoma Tumorigenesis and Metastasis by Acting as ceRNA to Sponge miR-590-3p/miR-1275 and Upregulate ITGB3. Cancer Res. 2019;79(18):4612-4626.
65. Yu S, D Wang, Y Shao, Zhang T, Xie H, Jiang X, Deng Q, Jiao Y, Yang J, Cai C, Sun L. SP1-induced lncRNA TINCR overexpression contributes to colorectal cancer progression by sponging miR-7-5p. Aging. 2019;11(5):1389-1403.
66. Heverhagen A E, N Legrand, V Wagner, Fendrich V, Bartsch DK, Slater EP. Overexpression of MicroRNA miR-7-5p Is a Potential Biomarker in Neuroendocrine Neoplasms of the Small Intestine. Neuroendocrinology. 2018;106(4):312-317.
67. He L, X Liu, J Yang, Li W, Liu S, Liu X, Yang Z, Ren J, Wang Y, Shan L, Guan C, Pei F, Lei L, Zhang Y, Yi X, Yang X, Liang J, Liu R, Sun L, Shang Y. Imbalance of the reciprocally inhibitory loop between the ubiquitin-specific protease USP43 and EGFR/PI3K/AKT drives breast carcinogenesis. Cell Res. 2018;28(9):934-951.
68. Dong P, Z Xu, N Jia, Li D, Feng Y. Elevated expression of p53 gain-of-function mutation R175H in endometrial cancer cells can increase the invasive phenotypes by activation of the EGFR/PI3K/AKT pathway. Mol Cancer. 2009;8(1):103.
69. Chen D L, Y X Lu, J X Zhang, Wei XL, Wang F, Zeng ZL, Pan ZZ, Yuan YF, Wang FH, Pelicano H, Chiao PJ, Huang P, Xie D, Li YH, Ju HQ, Xu RH. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a ceRNA for microRNA-215 to regulate ZEB2 expression. Theranostics. 2017;7(19):4836-4849.

70. Chen L L. Linking Long Noncoding RNA Localization and Function. Trends Biochem Sci. 2016;41(9):761-772.
71. Thomson D W, Dinger M E. Endogenous microRNA sponges: evidence and controversy. Nat Rev Genet. 2016;17(5):272-283.
72. Salmena L, L Poliseno, Y Tay, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011;146(3):353-358.
73. Xie C, L Z Zhang, Z L Chen, Zhong WJ, Fang JH, Zhu Y, Xiao MH, Guo ZW, Zhao N, He X, Zhuang SM. A hMTR4-PDIA3P1-miR-125/124-TRAF6 Regulatory Axis and Its Function in NF kappa B Signaling and Chemoresistance. Hepatology. 2019.
74. Wang M, C Mao, L Ouyang, Liu Y, Lai W, Liu N, Shi Y, Chen L, Xiao D, Yu F, Wang X, Zhou H, Cao Y, Liu S, Yan Q, Tao Y, Zhang B. Long noncoding RNA LINC00336 inhibits ferroptosis in lung cancer by functioning as a competing endogenous RNA. Cell Death Differ. 2019;26(11):2329-2343.
75. Bracken C P, Scott H S, Goodall G J. A network-biology perspective of microRNA function and dysfunction in cancer. Nat Rev Genet. 2016;17(12):719-732.
76. Cao Y, Wen J, Li Y, Chen W, Wu Y, Li J, Huang G. Uric acid and sphingomyelin enhance autophagy in iPS cell-originated cardiomyocytes through lncRNA MEG3/miR-7-5p/EGFR axis. Artif Cells Nanomed Biotechnol. 2019;47(1):3774-3785.
77. Hua Q, M Jin, B Mi, Xu F, Li T, Zhao L, Liu J, Huang G. LINC01123, a c-Myc-activated long non-coding RNA, promotes proliferation and aerobic glycolysis of non-small cell lung cancer through miR-199a-5p/c-Myc axis. J Hematol Oncol. 2019;12(1):91.

Fig. 1 RNA-seq analysis reveals that LPP-AS2 is significantly upregulated in glioma tissues and transcriptional regulated by c-MYC. **a** Flow diagram describes the steps for identifying and validating lncRNAs in glioma. **b** The KEGG pathway analysis of differentially expressed lncRNAs. **c** Heatmaps of top 50 lncRNAs that were

differentially expressed between glioma and normal brain tissues. Upregulated lncRNAs are shown in red and downregulated lncRNAs are shown in blue. **d** Relative expression of LPP-AS2 was significantly elevated in (27 grade I + II and 79 Grade III + IV) glioma tissues compared with 23 normal brain tissues. **e** Kaplan-Meier analysis of overall survival in glioma patients with low (n=167) and high (n=168) level of LPP-AS2. **f** Pulldown of LPP-AS2 in Chromatin immunoprecipitation assay with c-MYC. Results of western blots suggesting c-MYC could effective pulldown of LPP-AS2, and β-actin as the negative control. **g** Illustration of c-MYC bound to the promoter regions of LPP-AS2. Transcription start site (TSS) was designated as +1. The putative binding sites were listed. **h** C-MYC significantly enhanced the fold enrichment of LPP-AS2 probe compared with IgG in U251 and SHG44 cells. **i** Relative level of LPP-AS2 in the nuclear and cytoplasmic fractions of U251 and SHG44 cells. **j** Relative expression of LPP-AS2 in seven glioma cell lines and HEBs by RT-qPCR. Error bars, s.e.m. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (Student's t test).

Fig. 2 LPP-AS2 suppresses glioma progression *in vitro* and *in vivo*. **a** Relative expression of LPP-AS2 in U251 and SHG44 cells transfected with siRNAs. **b** CCK-8 assays in U251 and SHG44 cells with silencing of LPP-AS2. **c** Transwell assays in U251 and SHG44 cells with silencing of LPP-AS2. Representative staining images are presented (bar=50 μm). **d** Colony formation assays in U251 and SHG44 with silencing of LPP-AS2. **e** TUNEL assays in U251 and SHG44 cells with silencing of LPP-AS2 (bar=200 μm). **f** Flow cytometry analysis in U251 and SHG44 cells with silencing of LPP-AS2. **g** Wound healing assays of U251 and SHG44 cells with silencing of LPP-AS2. **h, i** Representative images of tumor formation of the lv-sh-control group and lv-sh-LPP-AS2 group. The dissected tumors from two groups were photographed. **j** Tumor volume was measured every 3 days. **k** IHC staining revealed that transfection of lv-sh-LPP-AS2 contributed to decreased Ki-67, MMP9 and EGFR expression in the subcutaneous tumors. Data represent the mean ± s.e.m. of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (Student's t test).

Fig. 3 LPP-AS2 facilitates tumor progression by regulating expression of EGFR. **a** Corresponding mRNAs in co-expression analysis network of LPP-AS2. Upregulated mRNAs are shown in red and downregulated mRNAs are shown in blue. **b** Relative expression of EGFR in 17 glioma tissues and 17 normal brain tissues by RT-qPCR. **c** Kaplan-Meier analysis of overall survival in glioma patients with low (n=203) and high (n=203) level of EGFR. **d** A positive relationship was existed in glioma tissues between LPP-AS2 and EGFR expression. **e** Knockdown of LPP-AS2 significantly inhibited EGFR expression in U251 and SHG44 cells. **f** Protein level of EGFR in U251 and SHG44 cells after treated with siRNAs of LPP-AS2. **g** Relative expression of EGFR in U251 and SHG44 cells transfected with pEGFP-C1/EGFR alone or co-transfection with siRNAs of LPP-AS2. **h** CCK8 assays in U251 and SHG44 cell treated with pEGFP-C1/EGFR alone or co-transfection of si-LPP-AS2-1. **i, j** Histogram of invasion and migration assay in U251 and SHG44 cells treated with pEGFP-C1/EGFR alone or co-transfection of si-LPP-AS2-1. **k** Colony formation assays in U251 and SHG44 cells treated with pEGFP-C1/EGFR alone or co-transfection of si-LPP-AS2-1. **l** Histogram of cell apoptosis rate in U251 and SHG44 cells treated with pEGFP-C1/EGFR alone or co-transfection of si-LPP-AS2-1. Error bars, s.e.m. from three independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$; $^{\#\#\#\#}P < 0.0001$ (Student's t test).

Fig. 4 LPP-AS2 regulates glioma progression by directly binding with miR-7-5p. **a** Relative expression of miRNAs align to LPP-AS2. **b** Knockdown of LPP-AS2 significantly upregulated the expression of miR-7-5p in U251 and SHG44 cells. **c** A negative relationship between the expression level of LPP-AS2 and miR-7-5p. **d** Ago2 protein immunoprecipitation was used to detect the fold enrichment of LPP-AS2 in U251 and SHG44 cells. **e** Schematic representation of the potential binding sites between LPP-AS2 and miR-7-5p. Luciferase report activity of constructed plasmids (LPP-AS2-WT or LPP-AS2-MUT) in 293T cells co-transfected with miR-7-5p mimics or miR-NC mimics. **f** Lnc_LPP-AS2 pulldown was used to measure the fold enrichment of LPP-AS2 and miR-7-5p in U251 and SHG44 cells. **g** Enrichment of LPP-AS2 in

U251 and SHG44 cells after treated with biotinylated miR-7-5p. **h** RT-qPCR analysis of miR-7-5p expression in U251 and SHG44 cells transfected with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **i** CCK8 assays in U251 and SHG44 cell treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **j** Histogram of invasion assays in U251 and SHG44 cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **k** Histogram of colony formation assay in glioma cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **l** Histogram of flow cytometry assays in U251 and SHG44 cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. Data represent the mean ± s.e.m. of three independent experiments. ns (no significance); *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001; $^{\#\#\#\#}$P < 0.0001 (Student's t test).

Fig. 5 LPP-AS2 decoys miR-7-5p to regulate the expression of EGFR and its downstream PI3K/AKT/c-MYC signaling pathway. **a** A total of 62 targeted mRNAs of miR-7-5p from four online databases (miRDB, miRTarBase, miRWalk, TargetScan). **b** Schematic representation of the potential binding sites between miR-7-5p and EGFR. Luciferase reporter activity of constructed plasmids (EGFR-WT or EGFR-MUT) in 293T cells co-transfected with miR-7-5p mimics or miR-NC mimics. **c** Relative expression of EGFR in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. **d** Protein level of EGFR in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. **e** Correlation analysis between miR-7-5p and EGFR in 21 paired glioma tissues. **f** Western blotting analysis of EGFR, PI3K, AKT, p-PI3K, p-AKT, c-MYC in U251 and SHG44 cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **g** Histogram of EGFR protein levels in U251 and SHG44 cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **h** EGFR mRNA expression in U251 and SHG44 cells treated with miR-7-5p inhibitor alone or co-transfection with siRNAs of LPP-AS2. **i, j** Ratio of p-PI3K/PI3K and p-AKT/AKT in U251 and SHG44 cells. **k** Western blot analysis of EGFR, PI3K, p-PI3K, AKT, p-AKT and c-MYC in U251 and SHG44 cells treated

with pEGFP-C1/EGFR plasmids alone or co-transfection with si-LPP-AS2-1. **l** Changes of p-PI3K/PI3K and p-AKT/AKT in U251 and SHG44 cells treated with pEGFP-C1/EGFR plasmids alone or co-transfection of si-LPP-AS2-1. Data represent the mean ± s.e.m. of three independent experiments. ns (no significance); *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001; $^{\#\#\#\#}$P < 0.0001 (Student's t test).

Fig. 6 Schematic illustration of the proposed mechanism of LPP-AS2 in glioma.

Figure S1. Identification of differentially expressed lncRNAs and mRNAs in high-throughput RNA sequencing. **a** Heatmap of reported lncRNAs and mRNAs in high-throughput RNA sequencing. **b** Hierarchical cluster analysis of differential expressed mRNAs in three glioma and normal samples. **c** Volcano plot of differential expressed mRNAs in three glioma and normal samples. **d** Four dysregulated lncRNAs obtained from RNA-seq were validated in 6 normal brain tissues and 15 GBM tissues by RT-qPCR. *P < 0.05; **P < 0.01; ***P < 0.001 (Student's t test).

Figure S2. LPP-AS2 was upregulated in glioma and regulated by c-MYC. **a** Six dysregulated mRNAs obtained from RNA-seq were validated in 6 normal brain tissues and 15 GBM tissues by RT-qPCR. **b** Relative expression level of LPP-AS2 in TCGA (207 normal brain tissues and 163 glioma tissues). **c** Relative LPP-AS2 expression in glioma tissues (n=75) and normal brain tissues (n=13) of the GEO databases (GSE50161 and GSE33331). **d, e** Relative expression of LPP-AS2 in U251 and SHG44 cells treated with siRNAs of c-MYC. Error bars, s.e.m. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (Student's t test).

Figure S3. LPP-AS2 promotes glioma progression *in vitro* and *in vivo*. **a** Relative expression of LPP-AS2 in U251 and SHG44 cells transfected with pEGFP-C1/LPP-AS2. **b** CCK-8 assays in U251 and SHG44 cells with overexpression of LPP-AS2. **c** Transwell assays in U251 and SHG44 cells with overexpression of LPP-AS2.

Representative staining images are presented. **d** Colony formation assays in U251 and SHG44 cells with overexpression of LPP-AS2. **e** TUNEL assays in U251 and SHG44 cells with overexpression of LPP-AS2. **f** Flow cytometry analysis in U251 and SHG44 cells with overexpression of LPP-AS2. **g** Wound healing assays in U251 and SHG44 cells with overexpression or silencing of LPP-AS2. **h** Schematic diagram of the entire experimental process. The divergent arrows suggest the different stages (first, inject tumor cells; second, tumor formation; third, measure tumor size; fourth, tissue harvesting and treatment). **i** Volumes of xenograft tumors in lv-sh-LPP-AS2 group and lv-sh-control group. **j** Weights of xenograft tumors in lv-sh-LPP-AS2 group and lv-sh-control group. **k** Relative expression of LPP-AS2 in Xenograft tissues were measured by RT-qPCR. Representative images are presented. Data represent the mean ± s.e.m. of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (Student's t test).

Figure S4. Regulation relationship between LPP-AS2 and EGFR. **a** Relative expression of LPP in U251 and SHG44 cells treated with siRNAs of LPP-AS2. **b** Relative expression of LPP in U251 and SHG44 cells with overexpression of LPP-AS2. **c** Relative expression of EGFR in seven glioma cell lines and HEBs by RT-qPCR. **d** Overexpression of LPP-AS2 significantly increased EGFR expression in U251 and SHG44 cells. **e** Protein level of EGFR in U251 and SHG44 cells with overexpression of LPP-AS2. Error bars, s.e.m. from three independent experiments. ns (no significance); *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; (Student's t test).

Figure S5. LPP-AS2 requires EGFR to facilitate tumor progression. **a** Relative expression of EGFR in U251 and SHG44 cells transfected with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. **b** CCK8 assays in U251 and SHG44 cell treated with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. **c** Transwell assays in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. **d** Migration assays in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. **e**

Colony formation assays in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. **f** Flow cytometry assays in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection of pEGFP-C1/LPP-AS2. Error bars, s.e.m. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001 (Student's t test).

Figure S6. Characterization of miR-7-5p in glioma. **a** Relative expression of miR-7-5p in 17 normal brain tissues and 17 glioma tissues. **b** Relative expression of miR-7-5p in seven glioma cell lines and HEBs by RT-qPCR. **c** Relative expression of miR-7-5p in U251 and SHG44 cells treated with Overexpression of LPP-AS2. **d** Knockdown or overexpression of miR-7-5p in U251 and SHG44 cells have no significantly effect on LPP-AS2 expression. Data represent the mean ± s.e.m. of three independent experiments. ns (no significance); **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001; $^{\#\#\#\#}$P < 0.0001 (Student's t test).

Figure S7. Functions of miR-7-5p in glioma. **a** CCK8 assays in U251 and SHG44 cell transfected with miR-7-5p mimics or miR-7-5p inhibitor. **b** Colony formation assays in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. **c** Transwell assays in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. **d** Migration assays in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. **e** Flow cytometry assays in U251 and SHG44 cells transfected with miR-7-5p mimics or miR-7-5p inhibitor. Error bars, s.e.m. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (Student's t test).

Figure S8. LPP-AS2 decoys miR-7-5p to regulate glioma progression. **a** CCK8 assays in U251 and SHG44 cell transfected with miR-7-5p mimics alone or co-transfection of pEGFP-C1/LPP-AS2. **b** Migration assays in U251 and SHG44 cells transfected with miR-7-5p mimics alone or co-transfection of pEGFP-C1/LPP-AS2. Migration assays in U251 and SHG44 cells transfected with miR-7-5p inhibitor alone or co-transfection

of siRNAs of LPP-AS2. **c** Transwell assays in U251 and SHG44 cells transfected with miR-7-5p mimics alone or co-transfection of pEGFP-C1/LPP-AS2. Representative staining images are presented. **d** Colony formation assays in U251 and SHG44 cells transfected with miR-7-5p mimics alone or co-transfection of pEGFP-C1/LPP-AS2. **e** Flow cytometry assays in U251 and SHG44 cells transfected with miR-7-5p mimics alone or co-transfection of pEGFP-C1/LPP-AS2. Error bars, s.e.m. from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001 (Student's t test).

Figure S9. LPP-AS2 functions as an oncogene via miR-7-5p/EGFR/PI3K/AKT/c-MYC axis. **a** Relative protein levels of EGFR in U251 and SHG44 cells treated with miR-7-5p mimics or miR-7-5p inhibitor. **b** Relative protein levels of EGFR in U251 and SHG44 cells treated with pEGFP-C1/EGFR alone or co-transfection of si-LPP-AS2-1. **c** Western blotting analysis of EGFR, PI3K, AKT, p-PI3K, p-AKT, c-MYC in U251 and SHG44 cells treated with miR-7-5p mimics alone or co-transfection with pEGFP-C1/LPP-AS2. **d** Histogram of EGFR protein levels in U251 and SHG44 cells treated with miR-7-5p mimics alone or co-transfection with pEGFP-C1/LPP-AS2. **e** Relative expression of EGFR in U251 and SHG44 cells treated with miR-7-5p mimics alone or co-transfection with pEGFP-C1/LPP-AS2 were validated by RT-qPCR. **f, g** Ratio of p-PI3K/PI3K and p-AKT/AKT in U251 and SHG44 cells. **h** Western blot analysis of EGFR, PI3K, p-PI3K, AKT, p-AKT and c-MYC in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection with pEGFP-C1/LPP-AS2. **i** Histogram of EGFR protein levels in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection with pEGFP-C1/LPP-AS2. **j** Ratio of p-PI3K/PI3K and p-AKT/AKT in U251 and SHG44 cells treated with siRNAs of EGFR alone or co-transfection with pEGFP-C1/LPP-AS2. Data represent the mean ± s.e.m. of three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01; $^{\#\#\#}$P < 0.001; $^{\#\#\#\#}$P < 0.0001 (Student's t test).