INTRODUCTION

Glioblastoma multiforme (GBM) is a fast-growing type of aggressive and lethal primary brain tumour. Because the pathologic mechanisms of GBM are not fully understood, more research is needed to identify effective predictive biomarkers and therapeutic targets for GBM. Emerging studies, supported by whole-genome sequencing technology and microarray assays, have shown long non-coding RNAs (lncRNAs) to be key regulatory transcripts. LncRNAs are classified
as RNAs if they are longer than 200 nucleotides. Most IncRNAs are poorly annotated coding genes.5 The expression of some IncRNAs is correlated with poor cancer prognosis.6-7 LncRNAs regulate tumorigenesis and invasion by activating or silencing oncogenes via various mechanisms, such as epigenetic regulation, RNA decay and post-translational modification.8 Many IncRNAs have high levels of expression in brain tumours such as GBM and astrocytoma, and these IncRNAs have been shown to rely on various molecular mechanisms.9

In the current study, we identified a novel IncRNA, HOXA cluster antisense RNA 3 (HOXA-AS3). It was found to be one of the most up-regulated IncRNAs in GBM tissues.10,11 HOXA-AS3 is a HOX cluster, and it is a set of transcription factor genes that regulate hematopoietic lineage differentiation and embryological development.10,12 Members of the HOX cluster family, such as HOXA-AS2, have been shown to regulate cell proliferation and the formation of tumour-related vasculogenic mimicry in various tumours.13,14 In contrast, there have been very few studies on the biological mechanism underlying the action of IncRNA HOXA-AS3 in tumours.

We here identified IncRNA HOXA-AS3 and observed characteristic up-regulation of its expression in malignant glioma tissue relative to normal brain tissues, which is consistent with the information in the TCGA database. Down-regulation of IncRNA HOXA-AS3 has been shown to significantly suppress glioblastoma cell (LN229 and U251) proliferation, invasion and migration. We also found that IncRNA HOXA-AS3 could act as a miRNA sponge to reduce miR-455-5p concentration, up-regulate USP3 expression and drive the epithelial-mesenchymal transition (EMT) process of GBM cells. This study is the first to provide evidence of a positive correlation between IncRNA HOXA-AS3 and USP3. This study improved our understanding of the HOXA-AS3/miR-455-5p/USP3 signalling pathway and may facilitate future development of new treatments for glioblastoma.

2 | MATERIALS AND METHODS

2.1 | TCGA data analysis

The processed IncRNA expression data of 502 cases of GBM and five cases of normal brain tissues were downloaded from the TCGA database (http://cancergenome.nih.gov/).

2.2 | Tumour tissues samples

Malignant glioma specimens (n = 25) and normal brain tissues (NBT; n = 10) were purchased from January 2009 to December 2019 at the People’s Hospital affiliated with Jiangsu University. This study protocol was approved by the ethics committee of Jiangsu University Affiliated People’s Hospital, and the procedures were performed in accordance with the approved guidelines. All tumours were characterized by two pathologists.

2.3 | Cell culture

Glioblastoma multiforme cell lines (U251, LN229, U87 and U138) were obtained from the American Type Culture Collection. T98G and SNB19 were purchased from the BeNa culture collection (BNCC), and normal human astrocytes (NHAs) were obtained from ScienCell Research Laboratories. All cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies) supplemented with 10% foetal bovine serum (FBS; Life Technologies) and maintained in a humidified atmosphere containing 5% CO2 at 37°C. Lentivirus transfection was purchased from GenePharma (Shanghai) and used to establish LN229 cell line labelled with green fluorescent protein (GFP).

2.4 | RNA extraction and IncRNA microarray analysis

Total RNA was isolated using TRIzol reagent (Invitrogen). Qualified total RNA samples were amplified by cDNA according to the Low Input Quick Amp WT Labeling Kit (Agilent) and standard operating procedures (SOPs). The expression profile of IncRNA in five malignant glioma and five NBT were screened by using IncRNA microarray (Shanghai Biotechnology Corporation). Tissue sample preparation and microarray hybridization were separately performed according to manufacturer’s recommendations. qRT-PCR was used to further determine the nuclear/cytoplasmic level of IncRNA HOXA-AS3 in glioma cells. GAPDH and U6 were used as a nuclear and cytoplasmic control. The primer sequences for the amplification of the IncRNA HOXA-AS3 and GAPDH used were listed in Table S1.

2.5 | Quantitative RT-PCR (qRT-PCR)

SYBR™ Green PCR Master Mixs (ABI, USA) were designed for quantitative RT-PCR. A LightCycler 490 Probes Master kit was also used to detect the relative expression of gene, and GAPDH was applied as the internal control for normalization. qRT-PCR results were shown using the 2-ΔΔCt method.

2.6 | Oligonucleotide transfection

The small interfering RNA (siRNA) and vector were purchased from Shanghai Gene Pharmaceutical Company. These plasmids were transfected into LN229 and U251 cells with Lipofectamine 3000 (Invitrogen) to obtain lentivirus soups complying with the manufacturer’s instructions. siRNA sequence was shown in Table S1.

2.7 | Inducible shRNA knock-down

Short hairpin RNA (shRNA) targeting IncRNA HOXA-AS3 (sh-HOXA-AS3) is used to silence IncRNA HOXA-AS3 in vivo. Lentiviral
particles were packaged into LN229 cells by co-transfection with plasmids. All shRNA sequences were shown in Table S1.

2.8 | MTT assays

Cell proliferation was evaluated with an MTT kit (Promega) complying with manufacturer’s instructions. Thereafter, 3 x 10^3 cells were seeded in each well of a 24-well plate and maintained in medium containing 10% FBS for 2 weeks, during which time the medium was replaced with MTT reagent every 4 days. Cell proliferation and viability were measured with absorbance at 490 nm.

2.9 | Cell invasion assays

Cell invasion was analysed using Transwell chambers (Corning). In total, 1 x 10^5 cells were incubated with 0.3 mL serum-free growth medium into the upper chamber of a fibronectin-coated polycarbonate membrane embedded in a Transwell device and then incubated at 37°C for 4 hours. The medium supplemented with 10% FBS was applied to the lower chamber as a chemoattractant. After 48 hours, cells adhering to the bottom surface were stained with 0.2% crystal violet and manually calculated to determine the invasion index.

2.10 | Cell migration assays

The scratch assay was used for the study of cell motility. After 24 hours, the cells were seeded on 6-well plates at an initial density of 5 x 10^5 in DMEM supplemented with 10% FBS. After the cells reached confluence, a scuffed wound was introduced with a sterile pipette tip into each well. The injuries were observed using phase contrast microscopy on an inverted microscope.

2.11 | Dual-Luciferase reporter assays

For luciferase reporter generation, the target genes of IncRNA HOXA-AS3 were predicted by using bioinformatics analysis. USP3 3’UTR was cloned into pmirGLO-dual-luciferase reporter as described previously.\(^{15}\) Mutant and wild-type plasmids were acquired to prevent their binding with miR-455-5p from GenePharma (Shanghai). The sequence of wild-type IncRNA HOXA-AS3, mutant IncRNA HOXA-AS3, wild-type USP3 (USP3-Wt) and mutant USP3 (USP3-Mut) was inserted into pmirGLO reporter vector. IncRNA HOXA-AS3-WT and IncRNA HOXA-AS3-MUT were co-transfected into LN229 cells with negative control (miR-NC), miR-455-5p screen and miR-455-5p inhibitors. After two days, the luminescent signals of Renilla firefly were calculated with the double luciferase assay system (Promega).

2.12 | Western blotting

The proteins of the cultured GBM cells were extracted, separated by electrophoresis and transferred to PVDF membranes (Millipore). Protein concentrations were blocked with bovine serum albumin and then incubated with primary antibodies at 4°C overnight. The membranes were then incubated with secondary antibody at room temperature for one hour. Immunoreactivity was detected using the ECL chemiluminescent detection system (Thermo Fisher Scientific). USP3 and GAPDH antibodies (1:5000) were purchased from Thermo Fisher Scientific. Primary antibodies against E-cadherin, N-cadherin and vimentin (1:1000) were purchased from Abcam Biotechnology.

2.13 | RNA pull-down assay

A pull-down test was used to examine the potential association of IncRNA HOXA-AS3 with miRNA. The cells were treated and quantified with 1 mL of cellular nuclear lysate buffer for 3 days. Next, the cells were incubated with magnetic beads coated with streptavidin (Sigma) at 25°C for 2 hours, and the withdrawal test was carried out in an RNA complex coupled to biotin. After removal, the beads were washed in lysis buffer and RNA complexes bound to the beads were collected by centrifugation. The RNA in the beads was eluted and detected by qRT-PCR.

2.14 | Tumour xenograft model

Nude mice (4-5 weeks old) were purchased from the Shanghai Center for Experimental Animals, Chinese Academy of Sciences. In the subcutaneous model, LN229 cells (1 x 10^6) were injected subcutaneously into individual nude mice (n = 4/group). Tumour volume was measured once four day after injection. 32 days later, the mice were killed, and tumour weight was measured. Growth curves were displayed by plotting tumour volume against time. The tumour was removed and weighed, and then, RNA was extracted for qRT-PCR. All of the animal experiments followed the experimental animal use guidelines of the National Institutes of Health. Then, LN229 cells were transfected with lentivirus sh-HOXA-AS3 in vitro for two days. Stereotactic implantation of sh-HOXA-AS3/LN229 cells was used to establish an intracranial xenotransplantation model. Mice were monitored by using IVIS Lumina II imaging system (Waltham, MA, USA) on 7 days and 21 days.

2.15 | Statistical analysis

All statistical analyses were performed by SPSS 21.0 (IBM). Each experiment was repeated three or more times, as mentioned in each of the figure legends. Count data were expressed as a percentage or as a ratio, and the chi-square test was applied for comparison. The results were analysed using Student’s t test or unidirectional analysis of variance (ANOVA). Data represent mean ± SD. *P < .05, **P < .01 and ***P < .001 were considered statistically significant.
3 | RESULTS

3.1 | LncRNA HOXA-AS3 expression was up-regulated in GBM tissue and cell lines

LncRNA microarray assay was used to detect abnormal expression of thousands of lncRNAs in malignant glioma tissues (Figure 1A). LncRNA HOXA-AS3 showed the most up-regulation of 11.3-fold change, which was selected as a candidate for further examination. In addition, the lncRNAs expression level of GBM patients from TCGA database showed that lncRNA HOXA-AS3 was a significant up-regulated lncRNA in GBM tissue. (Figure 1B). The expression of LncRNA HOXA-AS3 in malignant glioma patients’ tissues and normal brain tissues was validated by RT-PCR (Figure 1C). Then, Malignant glioma clinical samples were divided into two subsets with low expression versus high expression level. Kaplan-Meier analysis showed that patients with higher levels of lncRNA HOXA-AS3 had significantly poorer overall survival rates than those with lower expression of this lncRNA in GBM.
patients (Figure 1D). Furthermore, qRT-PCR was used to assess the expression of lncRNA HOXA-AS3 in various glioma cell lines such as U251, LN229, U87, T98G, SNB19 and U138. The expression of lncRNA HOXA-AS3 was up-regulated in GBM cell lines (U251, LN229, SNB19 and U138) compared with normal cell lines (NHA) (Figure 1E). The LN229 and U251 cell lines were selected for the other experiments.
because lncRNA HOXA-AS3 had higher expression relative to the other GBM cell lines. LncRNA HOXA-AS3 was found in the cytoplasm of LN229 and U251 cells, suggesting that lncRNA HOXA-AS3 may regulate the process of GBM in the nucleus (Figure 1F).

3.2 | Knock-down of lncRNA HOXA-AS3 reduces GBM cell tumorigenicity and EMT process

To evaluate the functionality of lncRNA HOXA-AS3 in vitro and in vivo, we reduced lncRNA HOXA-AS3 expression in LN229 and U251 cells by using siRNA. The efficiency of lncRNA HOXA-AS3 down-regulation was tested by qRT-PCR (Figure 2A). Functionally, colony formation and MTT assay showed that lncRNA HOXA-AS3 lncRNA degradation significantly reduced cell proliferation for LN229 and U251 cells after si-HOXA-AS3 transfection (Figure 2D). Using transwell assays, we demonstrated that silencing lncRNA HOXA-AS3 could decrease invasion and migration functions of GBM cells (Figure 2E). We also found that knock-down of lncRNA HOXA-AS3 expression strongly inhibited flattening and spreading in a cell-wounding assay, suggesting that lncRNA HOXA-AS3 expression dramatically affects key tumorigenesis gene signatures (Figure 2F).
Western blotting analysis was performed to verify EMT-related biomarkers (E-cadherin, N-cadherin and vimentin) expression. These results showed knock-down of lncRNA HOXA-AS3 can increase E-cadherin protein expression and reduce inhibit N-cadherin and vimentin protein expression (Figure 2G).

3.3 Overexpression of IncRNA HOXA-AS3 promotes glioblastoma cell proliferation and invasion

To assess whether IncRNA HOXA-AS3 can also promote glioblastoma cell proliferation, we used a simulator to regulate IncRNA
HOXA-AS3 expression in LN229 cells and U251 cells. By transfecting cells with a vector carrying lncRNA HOXA-AS3, we constructed a stable lncRNA HOXA-AS3 overexpressing cell line. The efficiency of lncRNA HOXA-AS3 overexpression was detected by qRT-PCR (Figure S1A). Next, MTT and transwell examinations were performed to determine the effect on cell viability and invasion (Figure S1B-D). Cells transfected with lncRNA HOXA-AS3 showed significant cell proliferation enhancement compared with cells transfected with blank control. In addition, wound healing experiments showed that lncRNA HOXA-AS3 overexpression significantly promoted the migration capacity in two GBM cell lines (Figure S1E-F). These data suggest that lncRNA HOXA-AS3 overexpression may enhance glioblastoma cell proliferation and invasion in vitro.

3.4 | Deregulation of IncRNA HOXA-AS3 suppresses GBM cell proliferation and invasion in GBM orthotopic xenografts

To further evaluate the potential therapeutic value of IncRNA HOXA-AS3 inhibition in vivo, we transfected shRNA IncRNA HOXA-AS3 plasmid vectors into LN229 cells (sh-HOXA-AS3/LN229) for orthotopic GBM xenografts. Then, we injected nude mice subcutaneously with sh-HOXA-AS3/LN229 cells and measured the tumour volume every 4 days thereafter for 32 days. The quantification of the tumour volume showed that tumour growth was slowed down in the mice injected with sh-HOXA-AS3 cells compared to the sh-NC cells (P < .01) (Figure 3A,B). As shown in Figure 3C, treatment with sh-HOXA-AS3 also resulted in a significant reduction in tumour weight. We further extracted RNA from tumour tissues for qRT-PCR analysis, and the results showed that the expression of IncRNA HOXA-AS3 in sh-HOXA-AS3 tissues was significantly lower relative to control tissues (Figure 3D). These results suggest that suppression of IncRNA HOXA-AS3 may have therapeutic potential for established tumours.

3.5 | LncRNA HOXA-AS3 acts as a miRNA sponge and negatively regulates miR-455-5p expression

Accumulating evidence indicates that IncRNAs serve as a sponge to negatively regulate certain miRNAs. To investigate whether lncRNA HOXA-AS3 utilizes a similar mechanism, bioinformatics software (StarBase v2.0) was used to identify lncRNA HOXA-AS3 binding sites (Figure 4A,B). Luciferase activity analysis showed that compared with the blank vector control, the IncRNA HOXA-AS3 level of miR-455-5p overexpression group was significantly reduced by 51% (Figure 4C). In addition, qRT-PCR showed that knock-down of IncRNA HOXA-AS3 significantly up-regulated the expression of miR-455-5p in LN229 and U251 cells (Figure 4D). Meanwhile, qRT-PCR showed that ectopic expression of miR-455-5p reduced the expression level of IncRNA HOXA-AS3 in LN229 and U251 cells (Figure 4E). We used qRT-PCR to reveal a negative correlation between IncRNA HOXA-AS3 and miR-455-5p expression in malignant glioma tissue (Figure 4F). To further identify miR-455-5p sponge IncRNA HOXA-AS3, RNA pull-down analysis was performed. The specific biotin-labelled probe of IncRNA HOXA-AS3 and control probe was designed for pull-down assay and was confirmed the effect of the probe by qRT-PCR (Figure 4G). We also used inverse pull-down assay to test whether miR-455-5p could pull-down lncRNA HOXA-AS3 (Figure 4H). Luciferase reporter assay was also used to further prove the direct binding between IncRNA HOXA-AS3 and miR-455-5p. Next, we constructed luciferase reporters containing IncRNA HOXA-AS3 wild-type binding sites (HOXA-AS3 WT) or IncRNA HOXA-AS3-mutant binding sites (HOXA-AS3 Mut). As expected, the luciferase activity of IncRNA HOXA-AS3 WT was significantly inhibited by miR-455-5p in LN229 and U251 cells. In contrast, we did not observe any statistical change in the luciferase activity of IncRNA HOXA-AS3 Mut (Figure 4I). These findings indicate that there is a direct interaction between miR-455-5p and IncRNA HOXA-AS3, and miR-455-5p may be a promising miRNA for the IncRNA HOXA-AS3 sponge in GBM.

3.6 | USP3 is the target gene of miR-455-5p in GBM

To identify miR-455-5p-mediated downstream regulators of cell growth and invasion in glioma, we applied three miRNA target prediction algorithms (Targetscan, miRDB and miRanda). We found 34 genes clustered in the aggressive and proliferation process (Figure 5A). Among the many miR-455-5p targets, we focused on ubiquitin specific protease 3 (USP3) for further investigation due to miR-455-5p could bind to the 3′-UTR region of USP3 (Figure 5B). Therefore, we constructed USP3 3′UTR-WT and USP3 3′UTR-MUT. Both of plasmids were performed for luciferase reporter assay. These results showed that miR-455-5p mimic significantly inhibited the luciferase activity of USP3 3′UTR-WT, while USP3 3′UTR-MUT was not affected, suggesting that USP3 is a direct target of miR-455-5p (Figure 5C). We further found that USP3 expression was positively correlated with IncRNA HOXA-AS3 expression and negatively correlated with miR-455-5p expression in glioma tissues.
B

USP3 3' UTR-WT : 5' AGUUCACUUUGAGGCACAUU 3'
miR-455-5p : 3' GCUACAUCCGGUUCGGUGAU 5'
USP3 3' UTR-MUT : 5' AGUUCACUUUGAAGGUGUUU 3'

C

LN229

U251

D

Relative Expression of USP3

E

Relative Expression of USP3

F

USP3
GAPDH

USP3
GAPDH
FIGURE 6 | MiR-455-5p rescued effect on the proliferation and invasion of IncRNA HOXA-AS3 overexpression glioblastoma multiforme (GBM) cells. A, MTT experiments suggested that MiR-455-5p rescued the effect of IncRNA HOXA-AS3 on migration of LN229 cell and U251 cell (**P < .01, ***P < .001). B, Transwell assay was conducted to evaluate the effect of miR-455-5p on the proliferation of IncRNA HOXA-AS3 overexpressed LN229 cells and U251 cells (**P < .01, ***P < .001). C, Colony formation assay showed that miR-455-5p inhibited the cell proliferative capacity rescued by IncRNA HOXA-AS3 (**P < .01). D, Representative images of tumour viewed by IVIS after control group, shRNA IncRNA HOXA-AS3 group and miR-455-5p inhibitor group inoculation. E, Western blot analysis showed protein expression of E-cadherin, N-cadherin, vimentin and USP3 in orthotopic xenograft tumour IncRNA HOXA-AS3 knock-down group and NC control group (*P < .05, **P < .01 and ***P < .001).

(Figure 5D,E). At the same time, the effect of IncRNA HOXA-AS3 and miR-455-5p interaction on USP3 expression was quantified by Western blot analysis (Figure 5F). These evidences indicate that IncRNA HOXA-AS3 can regulate the expression of USP3 in glioma through miR-455-5p.

3.7 | Overexpression miR-455-5p suppresses the effect of IncRNA HOXA-AS3 on proliferation and invasion of GBM

To validate the role of the IncRNA HOXA-AS3/miR-455-5p/USP3 axis, a rescue test was used. The results showed that miR-455-5p reversed the effects of the IncRNA HOXA-AS3 overexpression on GBM cell proliferation and invasion (Figure 6A-C). To further confirm the interaction between miR-455-5p and IncRNA HOXA-AS3, LN229 cells were labelled with luciferase expression and transfected with IncRNA HOXA-AS3 shRNA. Then, the transfected cells were inoculated into the brain of nude mice. After 3 weeks, IVIS results showed that miR-455-5p inhibitor rescued the effect of the down-regulation of IncRNA HOXA-AS3 on the cell proliferation LN229 cells (Figure 6D). Previous studies have shown that USP3 raised GBM progression by regulating EMT.18 We explored the expression of EMT-related genes in GBM orthotopic xenografts from different treatment groups. Western blot analysis showed that down-regulation of IncRNA HOXA-AS3 inhibits USP3 expression and EMT in vivo (Figure 6E).

4 | DISCUSSION

Glioblastoma multiforme treatment is challenging and understanding the molecular mechanisms of malignant glioma is desirable in order to find a new therapy. Recent studies on IncRNA expression profiling have shown that IncRNA expression is significantly altered in glioma tissue, and many IncRNAs have shown as important players in cancer development and metastasis.19-21 However, the functional roles and molecular mechanisms of IncRNA HOXA-AS3 in glioma progression remain largely unknown. In this study, we found that IncRNA HOXA-AS3 is significantly up-regulated in glioma tissue compared to normal adjacent tissue. In addition, IncRNA HOXA-AS3 expression was positively associated with adverse prognosis of malignant glioma patients. Regarding its cellular function, we found that knock-down of IncRNA HOXA-AS3 inhibited the proliferation, invasion and migration of GBM cells in vitro. In contrast, these capacities of GBM cells were significantly enhanced when IncRNA HOXA-AS3 was overexpressed. In addition, we have demonstrated that overexpression of IncRNA HOXA-AS3 can enhance tumorigenesis in vivo. Taken together, these results indicated that IncRNA HOXA-AS3 may play a critical role in the progression of glioma.

Recently, evidence suggests that IncRNA acts as a miRNA sponge or competing endogenous RNA (ceRNA) in the regulatory network.17,22 miRNAs are a class of small non-coding RNAs that regulate gene expression by being forced to locate at target sites in the 3′ non-transferable region (UTR) of the mRNA, thereby controlling their translation and degradation in the cells. Multiple miRNAs take on critical functions in the development and progression of tumours by fine-tuning multiple intracellular signalling pathways that mediate various biological functions such as cell proliferation and migration.23,24 MiR-455-5p has been considered as either an oncogene or a tumour suppressor in certain types of tumour. For example, miR-455-5p acts as an oncogene in lung cancer.25 In contrast, miR-455-5p exerts tumour-suppressive effects in prostate cancer.26 However, the role of miR-455-5p in GBM has not been revealed. Using bioinformatics analysis, we found that miR-455-5p may be a promising target miRNA for IncRNA HOXA-AS3. To further validate the predictions, using qRT-PCR, we revealed a negative correlation between IncRNA HOXA-AS3 and miR-455-5p in LN229, U251 cells and GBM tissues. In addition, we demonstrated the direct binding of IncRNA HOXA-AS3 and miR-455-5p using a dual-luciferase reporting assay, suggesting that IncRNA HOXA-AS3 promotes GBM progression by sponging miR-455-5p. Therefore, we predict that IncRNA HOXA-AS3 can regulate GBM processes through sponge miR-455-5p. To validate this prediction, we performed a rescue experiment, and the results showed that miR-455-5p could significantly rescue the effect of IncRNA HOXA-AS3 on GBM proliferation and metastasis. Using bioinformatics analysis, we found that USP3 may be a promising target gene for miR-455-5p. The dual-luciferase report assay has shown that miR-455-5p can bind to the 3′-UTR region of USP3. These results indicate that IncRNA HOXA-AS3 can promote glioma progression by regulating the miR-455-5p/USP3 axis.

Epithelial-mesenchymal transition is a process in which epithelial cells are transformed into mesenchymal cells and acquire motile and invasive characteristics.27,28 Recent studies have proven that USP3 promotes GBM progress by regulating the EMT process.18,29 Therefore, in order to study the molecular mechanism of IncRNA HOXA-AS3 promoting GBM transfer, we detected the level of EMT-related biomarkers in GBM cells.
overexpressed by IncRNA HOXA-AS3 by Western blot analysis. We found that depression of IncRNA HOXA-AS3 significantly decreased the expression of EMT-related protein, indicating that IncRNA HOXA-AS3 can participate in GBM transfer by regulating HOXA-AS3/miR-455-5p/USP3 axis.

In conclusion, we found an associated IncRNA network that regulates the tumour cell development network, consisting of IncRNA HOXA-AS3, miR-455-5p and USP3. Our research not only contributes to the intensive efforts to elucidate the mechanism of IncRNA HOXA-AS3, but also specifically encourages the development of clinic-based IncRNA anti-glioma diagnostics and therapeutics.

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CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest.

AUTHOR CONTRIBUTION

Wanghao Chen: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (lead); Project administration (equal); Resources (equal); Software (lead); Supervision (equal); Validation (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead). Qiaoyu Li: Funding acquisition (supporting); Hong Wang: Methodology (lead); Project administration (equal); Resources (equal); Software (lead); Supervision (equal); Validation (lead); Visualization (lead); Writing (equal); Funding acquisition (equal); Investigation (supporting); Formal analysis (supporting); Methodology (equal); Project administration (equal); Resources (equal); Software (lead); Supervision (equal); Validation (lead); Visualization (lead); Writing (equal); Funding acquisition (supporting); Project administration (supporting); Resources (equal); Software (lead); Supervision (equal); Validation (lead); Visualization (lead); Writing (equal); Formal analysis (supporting); Methodology (supporting); Project administration (equal); Resources (equal); Software (lead); Supervision (equal); Validation (lead); Visualization (lead); Writing (equal); Funding acquisition (equal); Investigation (supporting); Conceptualization (supporting); Data curation (supporting); Formal analysis (supporting); Methodology (supporting); Project administration (supporting); Resources (equal); Software (lead); Supervision (supporting); Validation (supporting); Visualization (supporting); Zhihan Zhu: Conceptualization (supporting); Data curation (supporting); Funding acquisition (supporting); Project administration (supporting); Writing (supporting); Methodology (supporting); Project administration (supporting); Resources (equal); Software (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing (supporting); Hong Wang: Data curation (supporting); Formal analysis (supporting); Funding acquisition (supporting); Project administration (supporting); Resources (equal); Software (supporting); Supervision (supporting); Validation (supporting); Visualization (supporting); Writing (supporting);

DATA AVAILABILITY STATEMENT

The data sets used or analysed in this study may be obtained from appropriate authors upon reasonable request.

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