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LABORATORY INVESTIGATION

Blocking *LINC00152* suppresses glioblastoma malignancy by impairing mesenchymal phenotype through the *miR-612*/AKT2/NF-κB pathway

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Abstract

Introduction Glioblastoma, the most common and mortal primary brain tumor, accompanied with a dismal clinical outcome in adults. The oncogenic functions of long non-coding RNAs (lncRNAs) in glioblastoma have not been completely illuminated. In the present study, we aimed to investigate the potential role of lncRNA *LINC00152* in glioblastoma.

Methods We used bioinformatic method in public databases to select lncRNA *LINC00152* and investigate its clinical value and potential mechanism in glioblastoma. CCK-8, transwell assay, colony formation and wound healing assays were used to explore the role of *LINC00152* in glioblastoma malignant behaviors. PCR, western blot, immunofluorescence, reporter assays and nude mouse tumor intracranial model were employed to further verify the regulatory mechanism of *LINC00152* in glioblastoma.

Results *LINC00152* was closely associated with glioma WHO classification and poor prognosis, and indicated a poor prognosis in glioblastoma patients. Tumor growth and invasion were suppressed both in vitro and vivo after *LINC00152* was blocked. Moreover, *LINC00152* modulated GBM malignant progression and proneural–mesenchymal transition through the *miR-612* dependent AKT2/NF-κB pathway.

Conclusions *LINC00152* acted as a tumor oncogene with prognostic value for patients with glioblastoma through *LINC00152*/*miR-612*/AKT2/NF-κB axis.

Keywords Glioblastoma · *LINC00152* · NF-κB · Proneural–mesenchymal transition · *MiR-612*

Introduction

Glioblastoma (GBM), the most common and mortal primary brain tumor, accompanied with an unfavorable clinical outcome in adults [1]. Despite treatment involving surgery, following chemotherapy and radiation, patients with GBMs only have an average survival of no more than 15 months at the time of the initial diagnosis [2]. The proliferative, invasive and drug resistant hallmarks of GBM account for its poor prognosis [3]. Therefore, an urgent comprehending of the mechanisms of molecular biology involved in GBM malignant behaviors is needed.

AKT/NF-kB signaling plays a critical role in glioblastoma progression. NF-κB could be activated via an AKT-dependent pathway [4]. MK-2206 is a potent allosteric inhibitor of AKT, with anti-proliferative and anti-invasion activity in GBM [5]. The constitutive activation of NF-κB has been detected widespread in human cancers. Migration of GBM cells into the surrounding brain parenchyma is one of the primary reasons for tumor recurrence, and NF-kB

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signaling significantly contributes to this property of GBM cells [6]. Knockdown of p65 induces cytotoxicity in GBM cells, and expression of a p65 shRNA leads to a decrease in GBM xenograft growth [7].

Coding genes and their coding proteins have been thought to be the primary functional effectors of cells [8]. Until recent years, when the roles of noncoding RNAs (ncRNAs), began to be appreciated for their roles in most biological processes [9]. Long non-coding RNAs (lncRNAs) are a class of ncRNAs, which are greater than 200 bp in length and do not code any proteins [10]. LncRNA-mediated biology has been implicated in a wide variety of cellular processes, including histone modification, post-transcriptional processing inactivation and competing endogenous-RNA (ceRNA) [11]. Up to now, lncRNAs like HOTAIR [12], H19 [13], and HOXA11-AS [14], are dysregulated in diseases, especially in GBM. Uncovering GBM-associated lncRNAs would disclose a new level of the mechanism of brain tumor progression.

Although various classification programs have been done, the mesenchymal (MES) and proneural (PN) subtypes are unshakable [15]. Patients in the MES subtype exhibit relatively worse outcome than PN subtype [16]. More importantly, PN subtype has been proved to tend to MES recurrences, indicative of a proneural–mesenchymal transition during glioma progression [17, 18]. Previous studies have verified the occurrence of PN–MES shift in GBM and several major transcription factors, including STAT3 and NF-κB [19].

In this study, we aimed to explore how *LINC00152* regulated the proneural–mesenchymal transition. We revealed that *LINC00152* facilitated transition through *miR-612*/AKT2/NF-κB pathway.

Materials and methods

Clinical samples

The mRNA profiles and relevant clinical parameters were acquired from the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org) and the Cancer Genome Atlas (TCGA, https://genome-cancer.ucsc.edu/). Human glioma samples were obtained from the Department of Neurosurgery, the Second Affiliated Hospital of Harbin Medical University. The grade of human glioma samples used in the present study was confirmed by the pathologist according to WHO standard [15].

Cell culture

LN229 and U87-MG cells were grown in Dulbecco's Modified Eagle's medium (DMEM, Gibco) separately containing 5 and 10% of fetal bovine serum (FBS, BI). N9 which was a patient-derived cell was grown in DMEM/F12 (1:1) (Gibco) and supplemented with 10% FBS and 1% penicillin–streptomycin described in our previous research [20]. All cells were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

Transfection

Lentivirus containing *LINC00152* RNAi segments, *LINC00152* full length and luciferase lentivirus were obtained from Genechem (Shanghai, China). Chemosynthetic siRNA1, 2 and 3 (the siRNA sequences are 5′-GCGGAGCATGGAACTCGACAGTTAA-3′, 5′-CGGAGCATGGAACTCGACAGTTAAA-3′, 5′-GGGCAATAGGCGATACGATGCTTTA-3′) were obtained from Genechem (Shanghai, China). The *miR-612* mimic, inhibitor and negative control (Genechem, Shanghai, China) were used in this study.

Cell proliferation, colony formation, invasion and wound healing assays

Cell proliferation was performed using the Cell-Counting Kit 8 (CCK-8; Dojindo Laboratories) according to the manufacturer's instructions. For colony formation, cells were planted in six-well plates at 200/well for 14 days. Cell colony was fixed with 4% paraformaldehyde and observed by staining with 0.1% crystal violet for 30 s. Invasion assays were performed using Boyden chambers (Corning, NY) and 8-μm-sized pore membranes coated with matrigel. Chambers and plates were incubated at 37 °C with 5% CO_2 for 36 h. Transfected GBM cells were seeded in six-well plates. After the confluence reached ~ 90%, the cell layers were scratched with a tip of a 1 mL pipette. After culture for 24 h, the wound width was measured to evaluate the wound healing ability of the tested cells. Each experiment was performed at least three times.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from frozen normal brain and glioma specimens and cells was extracted using the Trizol reagent (Sigma). qRT-PCR was performed with CFX Connect™ (Bio-Rad) and used to analyze the expression of mRNA in human glioma samples and cell lines. The GAPDH and U6 were used as internal control for mRNAs and miRNAs. *LINC00152* primers are 5′-ATGTGTAGGAGAGTCGGCCT-3′, 5′-ATGCCGTTTTAGGGGGACAG-3′. PDGFRA primers are 5′-CCTCTCGGGTCTCAGTTGAA-3′, 5′-CCACTGTCCGGCTTCTGAC-3′, Olig2 primers are 5′-TCGCATCCAGATTTTCGGGT-3′, 5′-AAAAGGTCATCGGGCTCTGG-3′. Vimentin primers are 5′-GGACCAGCTAACCAA

CGACA-3′, 5′-AAGGTCAAGACGTGCCAGAG-3′. CD44 primers are 5′-CACACCCTCCCCTCATTCAC-3′, 5′-CAC ACCCTCCCCTCATTCAC-3′.

Protein extraction and western blot

RIPA buffer Solarbio (Beijing, China) was used to extract total protein according to the manufacturer's protocol. Nuclear and cytoplasmic protein extraction kit brought from Beyotime Biotechnology (Shanghai, China) was used to extract the different position of the protein in cells. Primary antibodies were P65 (rabbit, 1:1000, CST), phoshorylated-P65 (rabbit, 1:1000, CST), Olig2 (rabbit, 1:1000, Abcam), PDGFRA (rabbit, 1:1000, Abcam), Vimentin (rabbit, 1:1000, CST), CD44 (mouse, 1:1000, CST), AKT2 (rabbit, 1:1000, CST), IKKα (rabbit, 1:1000, CST), p-IKKα (rabbit, 1:1000, CST), p-AKT2 (rabbit, 1:1000, CST), H3 (mouse, 1:1000, CST) and GAPDH (mouse, 1:1000, Proteintech).

Bioinformatics prediction and luciferase reporter assay

The putative *miR-612* target binding sequences in *LINC00152*, AKT2 and its mutant of the binding sites were synthesized and cloned downstream of the luciferase gene in the pmirGLO luciferase vector (Promega). The target gene of *miR-612* was predicted with the support of computeraided algorithms: TargetScan. PGL4.32 (luc2P/NFκB-RE/Hypro Vector), which was purchased from Promega containing NF-κB response element. The plasmids were transfected into cells, and vector Renilla luciferase (Promega) served as an internal control. After 48 h, cells were lysed and tested by the Dual-Luciferase Reporter Assay System (Promega).

Immunofluorescence and microscopy

Immunofluorescence staining was performed using primary antibodies against P65 (1:100 dilutions, Cell Signaling Technology), followed by 1 h of incubation with 594 goat-anti-rabbit IgG (H + L) (Life Technology) at room temperature. All fluorescent results were visualized using the FV-1200 laser scanning confocal microscope.

Intracranial model build, hematoxylin-eosin staining (HE) and immunohistochemistry (IHC)

U87-MG cells were co-transfected with lenti-*LINC00152* RNAi and luciferase lentivirus in vitro for 48 h. A total of 3.0×10^5 U87-MG cells infected with the virus were implanted stereotactically to establish intracranial GBM using cranial crews as the previous description [21]. After 10 and 20 days, tumors were monitored by fluorescent images of whole mice using the Living Imaging system. Paraffin sections (4 μm) were used for HE and IHC staining. Paraffin sections were dewaxed and incubated with the primary antibodies Ki67 (rabbit, 1:50 Santa Cruz), P65 (rabbit, 1:100, CST) and MMP9 (rabbit, 1:100, CST) at 4 °C for 12 h, followed by incubation with biotinylated secondary antibody (Zsbio) for 40 min at room temperature. After washing with PBS, the sections were stained with 3,3′-diaminobenzidine (DAB) for approximately 10–20 s, rewashed in PBS and counterstained with hematoxylin.

Statistical analysis

GraphPad Prism, version 7.0 and SPSS, version 22.0, Gene Cluster 3.0 and Gene Tree View software were used in this study. Univariate and multivariate Cox regression analyses were performed to assess the relative risk for potential factor. Kaplan–Meier curves were used to estimate the survival distributions. Then, the log-rank test was applied to value the statistical significance between different survival groups. Differential lncRNAs between low and high grades were filtrated using significance analysis of microarray (SAM) algorithm. DAVID (http://david.abcc.ncifcrf.gov/) was used for Gene Ontology analysis [22]. Gene set enrichment analysis (GSEA) was conducted using the GSEA software [23]. $P < 0.05$ was considered statistically significant.

Results

Aberrant expression and prognostic value of *LINC00152* for patients with GBMs

We combined differentially expressed lncRNAs in GBMs and low grade gliomas (LGGs) according to Reon et al. [24] and lncRNAs most up- or down-regulated in GBM according to Pastori et al. [25] to obtain 1296 annotated lncRNAs. SAM analysis was performed to compare the differential expressed lncRNAs between the low (WHO II/III) and high (WHO IV) grade glioma samples in CGGA and TCGA databases. Only those lncRNAs (SAM: Fold change > 1.5, Q value < 0.01) were considered as significant lncRNAs. Among those lncRNAs, a lncRNA *LINC00152* towered above for its unknown role in glioma (Fig. 1a). We analyzed *LINC00152* expression levels from whole mRNA profiling of the CGGA and TCGA cohorts. *LINC00152* expression was significantly higher in WHO IV grade samples than WHO III and WHO II samples in CGGA database ($P = 0.0428$ and $P < 0.0001$, Fig. 1b). The same result revealed that *LINC00152* expression was significantly higher in WHO IV glioma than that in other grades in TCGA database ($P < 0.0001$ and $P < 0.0001$, Fig. 1b). To further affirm the expression tendency of *LINC00152* in glioma, we detected the level of *LINC00152*

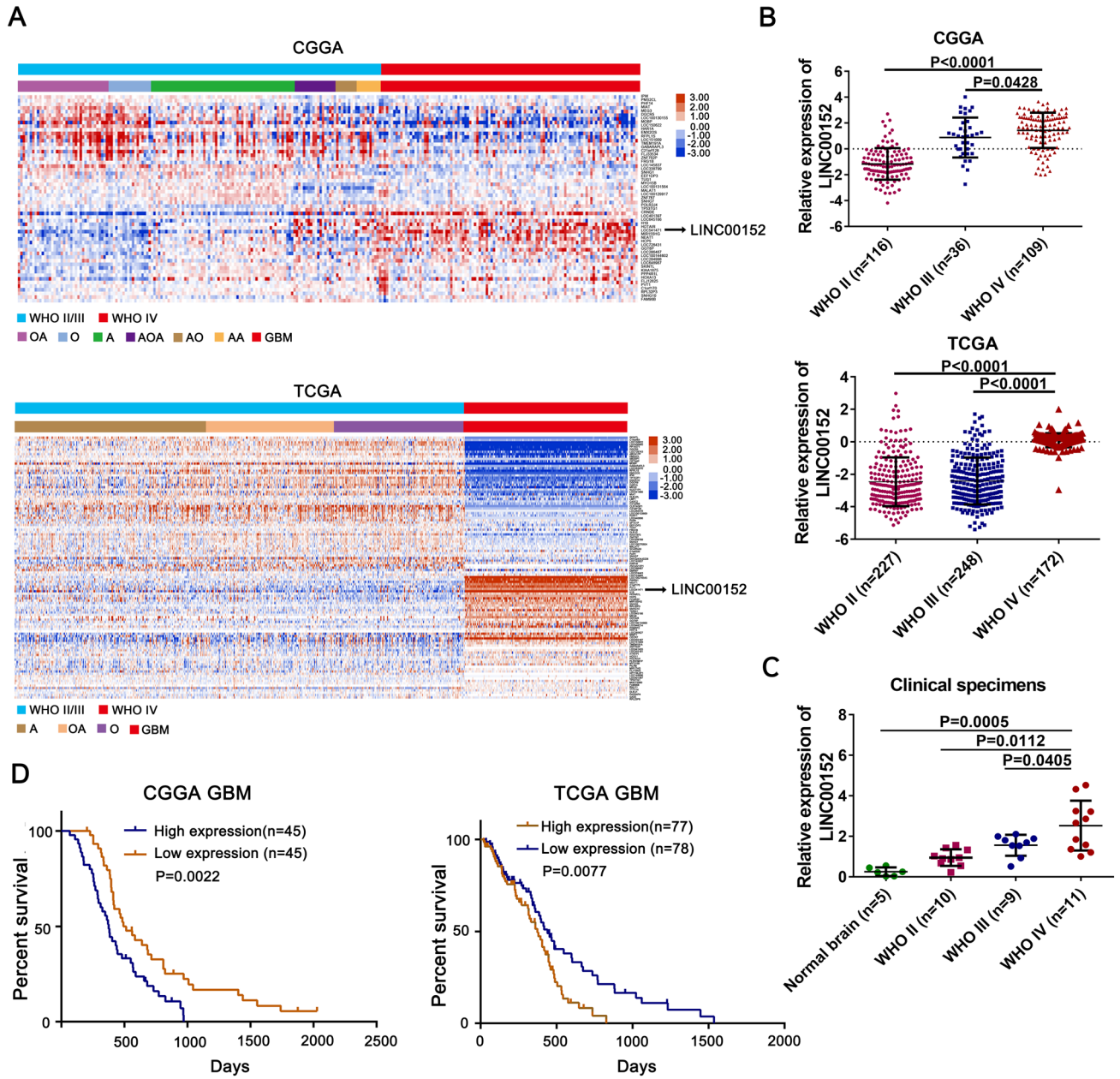

Fig. 1 LncRNA profiling analysis in the CGGA and TCGA databases identifies a lncRNA *LINC00152* in glioma. **a** Heatmaps were constructed using the differential lncRNAs between different grades of glioma identified by significance analysis of microarray algorithm in CGGA and TCGA databases. **b** The levels of *LINC00152* were analyzed in CGGA and TCGA databases. **c** The level of *LINC00152* was analyzed in 35 brain samples. **d** Kaplan–Meier curve was performed to evaluate overall survival time between *LINC00152* high- and low-expression groups in CGGA and TCGA databases

in 35 samples of adult brain tissues by using qRT-PCR. As expected, *LINC00152* had a higher level in WHO IV glioma samples (Fig. 1c). We employed the dichotomization to separate cases for depicting the survival curves according to the median value. Patients with higher *LINC00152* expression had significantly shorter overall survival than those with lower *LINC00152* expression, which highlighted the potential oncogenic character of *LINC00152* for GBM patients (CGGA, P = 0.0022; TCGA, P = 0.0077, Fig. 1d). Next, we analyzed the clinicopathological features of patients through CGGA and TCGA databases, and found that the relative expression of *LINC00152* was associated with TCGA subtype (P = 0.0003) and IDH status (P = 0.0019) in CGGA database and closely related to TCGA subtype (P = 0.0241) and chemotherapy (P = 0.0029) in TCGA database (Tables S1 and S2).

Then, we conducted a univariate Cox regression analysis enrolling clinical and genetic variables of GBM patients from the CGGA and TCGA databases. We found that the expression of *LINC00152*, age, chemotherapy and radiotherapy were statistically associated with overall survival in CGGA database and the expression of *LINC00152*, age, IDH status and chemotherapy were statistically associated with overall survival in TCGA database. We evaluated the factors that contributed to overall survival using a multivariate Cox proportional hazards model. The result revealed that *LINC00152* expression was independently correlated with overall survival (HR = 1.268, P = 0.005; HR = 1.536, P = 0.0005, respectively) after adjusting other factors in CGGA and TCGA databases (Table S3).

Knockdown of *LINC00152* suppresses GBM cell malignant behaviors

LINC00152 decreased in siRNA groups, particularly in the siRNA3 group (Fig. S1A). CCK-8 assay revealed that *LINC00152* knockdown could inhibit the proliferation of LN229 and U87-MG cells (Fig. S1B). Transwell assay also showed that the invasive capacity was suppressed when *LINC00152* decreased in LN229 and U87-MG cells (Fig. S1C). Colony formation assay illustrated that knockdown of *LINC00152* could inhibit the colony formation of LN229 and U87-MG cells in vitro (Fig. S1D). Wound healing results demonstrated that inhibition of *LINC00152* attenuated the LN229 and U87-MG cells migration (Fig. S1E).

Interference of *LINC00152* inhibits NF-κB expression and nuclear transport

To clarify the cellular functions of *LINC00152* in GBM, we clustered positively or negatively genes in the TCGA database according to *LINC00152* expression level (Fig. 2a). GO analysis showed that *LINC00152* positively-related genes were involved in the NF-κB signaling pathway (P < 0.01, Fig. 2b). In order to explore the effect of *LINC00152* on the canonical NF-κB pathway, we detected the P65 and phosphorylated P65 (p-P65) protein levels and found that these two proteins decreased in *LINC00152* down-regulated groups of LN229, U87-MG and N9 cells (Figs. 2c, S2A). In the meantime, the NF-κB transcriptional activity was suppressed when the *LINC00152* decreased (Fig. 2d). P65 and p-P65 distribution in cytoplasm and nucleus decreased after suppressing *LINC00152* in LN229, U87-MG and N9 cells (Figs. 2e, S2B). Furthermore, confocal microscopy analysis suggested that P65 decreased in both cytoplasmic and nuclear components of U87-MG and N9 cells (Figs. 2f, S2C).

The functional role of *LINC00152* involves in the proneural–mesenchymal transition

Previous research found that NF-κB pathway plays an important role in proneural–mesenchymal transition (PN–MES shift) [19], so it was necessary for us to seek the functional influence of *LINC00152* during this transition. We found that the mesenchymal GBM had a higher *LINC00152* expression, whereas the proneural GBM contained a lower *LINC00152* expression (Fig. 3a) in CGGA database. In TCGA cohort, we found that *LINC00152* expression was higher in the mesenchymal subtype than that in the proneural subtype (Fig. 3b). Next, we divided GBM patients from CGGA and TCGA cohorts into two groups on the basis of the expression of *LINC00152*. Similarly, GSEA between different groups reveals that patients with a high-level of *LINC00152* were enriched in mesenchymal-associated gene set (Fig. 3c). It has been reported that in GBM miR-181d/MALT1 modulator axis attenuated mesenchymal phenotype characteristics [26] and miR-34a inhibition in proneural subtype increases the expression of its target PDGFRA and accelerates tumor growth [27], which indicates that ncRNAs are associated with GBM subtype profiles. Downregulation of *LINC00152* induced both mRNA and protein expression of proneural markers (Olig2 and PDGFRA) while restrained the mesenchymal markers (CD44 and Vimentin) in LN229, U87-MG and N9 cells (Figs. 3d, e, S2 D, E).

Knockdown of *LINC00152* could inhibit GBM progression in vivo

In consideration of the oncogenic effects of *LINC00152* in vitro, we decided to extend our research to examine if down-regulated *LINC00152* could suppress GBM progression in vivo. In accordance within vitro experiments, the intracranial tumor decreased (Fig. 4a, b) after inhibiting *LINC00152*. HE staining of xenograft tumors showed that tumor invasion decreased in the down-regulated *LINC00152* group compared with control group (Fig. 4c). Down-regulated *LINC00152* in U87-MG tumors was associated with prolonged survival (Fig. 4d). Immunohistochemistry assay showed that downregulation of *LINC00152* could reduce the Ki-67, P65 and MMP9 protein expression (Fig. 4e).

LINC00152 functions as ceRNA and sponges *miR-612* in GBM cells

In order to investigate how *LINC00152* regulated NF-κB pathway and resulted in the proneural–mesenchymal transition. The RIP assay showed that *LINC00152* and AKT2 could bind Ago2 in LN229 and U87-MG cells (Fig. 5a, b).

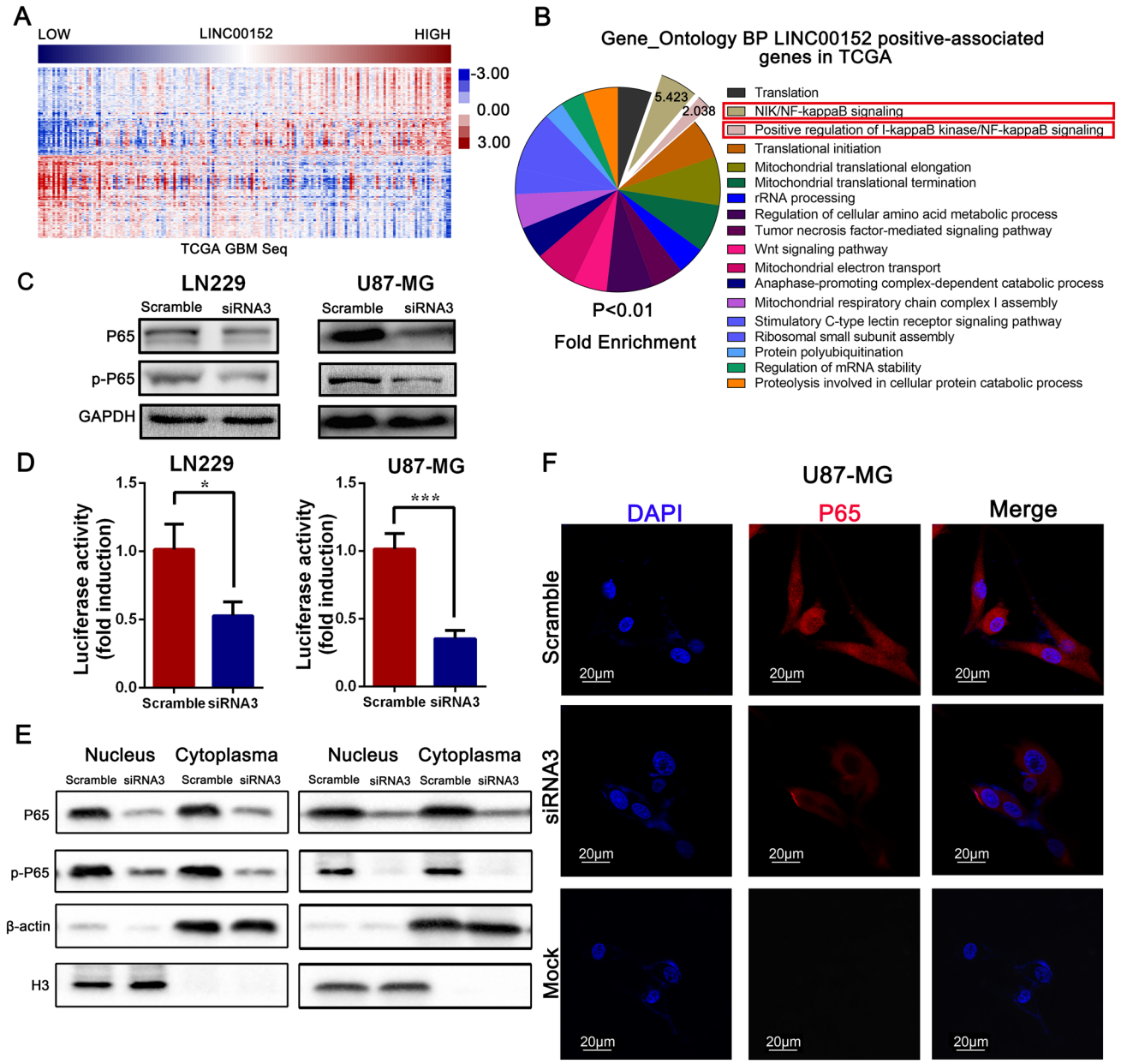

Fig. 2 Knockdown of *LINC00152* inhibits NF-κB expression and nuclear transport. **a**. A heatmap of the *LINC00152* positive-associated genes in GBM tissues sorted by the level of *LINC00152* expression in TCGA database. **b** Gene ontology analysis of *LINC00152* positively associated genes revealed several pathways. **c** The protein levels of P65 and phosphotylated-P65 were detected in siRNA and scramble group. **d** NF-κB luciferase reporter and Renilla plasmids were transfected to measure the influence of *LINC00152* on NF-κB transcriptional activity. **e** The distributions of P65 and p-P65 in cytoplasm and nucleus were tested after suppressing *LINC00152* in GBM cells. **f** P65 expression and the subcellular location were confirmed by confocal microscopy. Scale bars = 20 μm. *P < 0.05, **P < 0.01, ***P < 0.001

We predicted that *LINC00152* and AKT2 as potential targets of *miR-612* (Fig. 5c). AKT2 was a potential target of *miR-612* in previous research [28]. Then, we detected the level of *miR-612* in LN229 and U87-MG cells treated by the scramble and siRNA3. *MiR-612* increased in LN229 and U87-MG cells after *LINC00152* was inhibited (Fig. 5d). Furthermore, the dual luciferase reporter assays revealed that *miR-612* directly bound to the *LINC00152* and *AKT2* 3′UTR regions (Fig. 5e, f). After overexpression of *LINC00152*, luciferase activity decreased in *miR-612* WT groups, revealing that *LINC00152* bound *miR-612* (Fig. 5g).

Moreover, the overexpression of *miR-612* inhibited AKT2 and its downstream expression in LN229 and U87-MG cells. In contrast, *miR-612* inhibitor could restore AKT2 and its

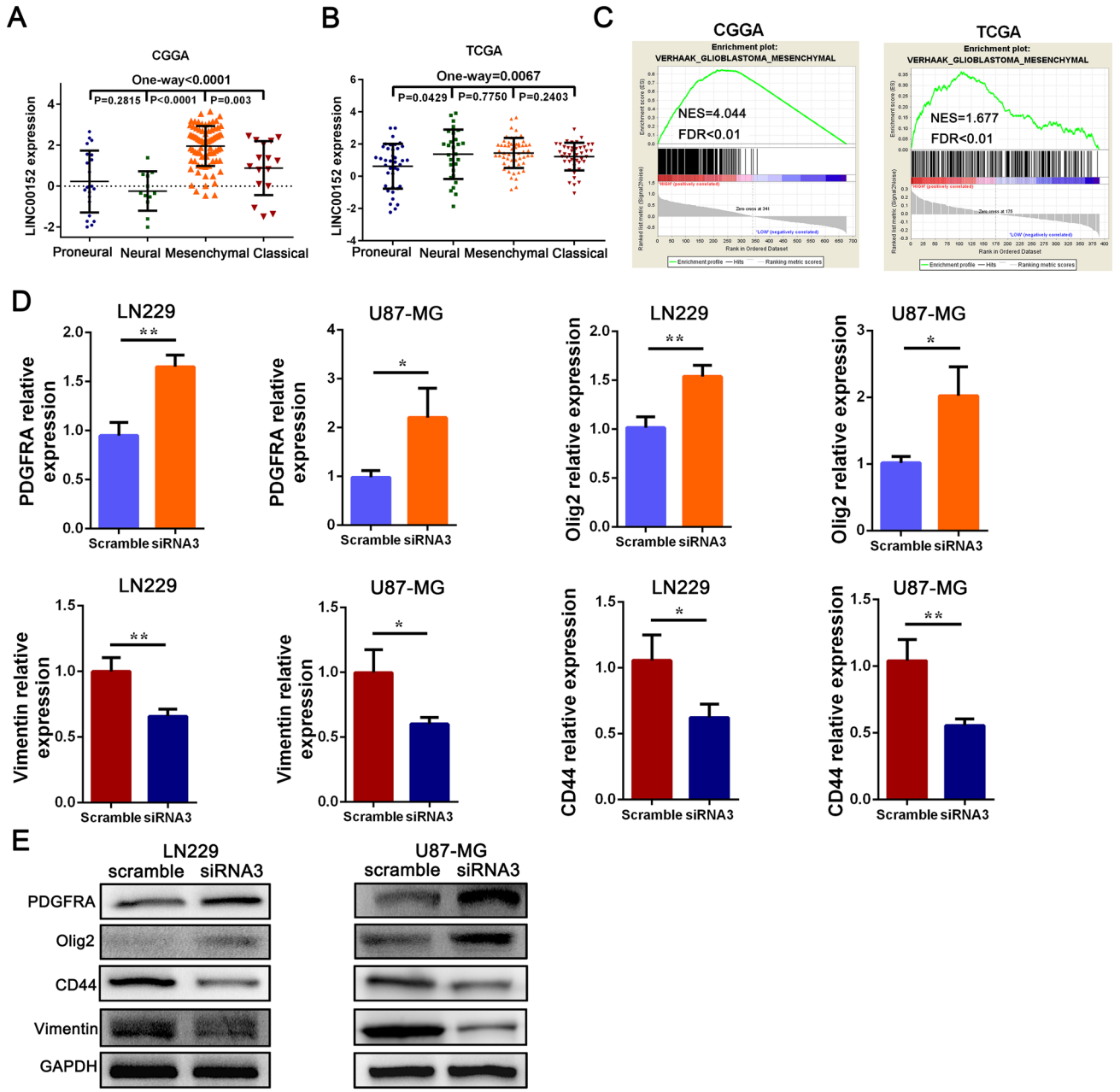

Fig. 3 Knockdown of *LINC00152* attenuates proneural–mesenchymal transition. **a, b** The expression of *LINC00152* was analyzed in subtypes of GBM in the CGGA and TCGA databases. **c** GSEA was conducted to analyze patients with different *LINC00152* expression among GBM subtypes. **d, e** The mRNA and protein levels of PDGFRA, Olig2, Vimentin and CD44 were detected in siRNA and scramble groups. *P < 0.05, **P < 0.01, ***P < 0.001

downstream activity in LN229 and U87-MG knocking down *LINC00152* (Fig. 5h, i). In the meantime, the knockdown of AKT2 could inhibit its downstream activity in LN229 and U87-MG overexpressing *LINC00152* (Fig. 5j).

Discussion

Thousands of lncRNAs have been discovered, benefiting from large sequencing consortia such as ENCODE and FANTOM [29]. However, only a few of these lncRNAs have been successfully established. To manifest aberrant expression patterns of lncRNAs in glioma, we performed an analysis of lncRNAs in CGGA and TCGA

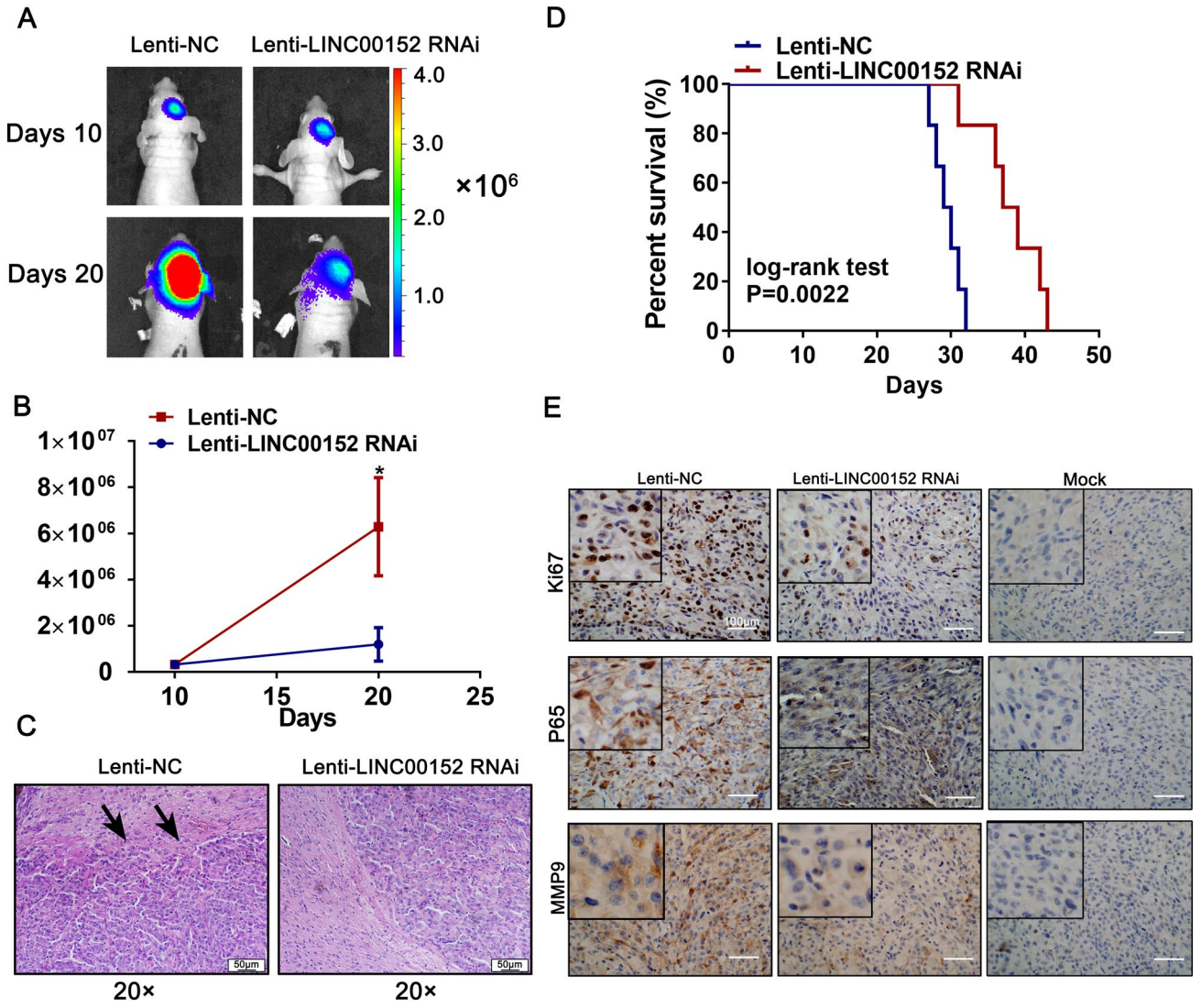

Fig. 4 Knockdown of *LINC00152* inhibits GBM progression in vivo. **a** Luminescence imaging for lenti-NC versus lenti-*LINC00152* RNAi group. **b** Tumor volume in the lenti-*LINC00152* RNAi group was smaller than that in the lenti-NC group. **c** The tissue sections from representative tumors in each group were stained with HE; the black arrows indicated tumor invasion. Scale bars = 50 μm. **d** Kaplan–Meier curve was performed for evaluating overall survival time between lenti-NC and lenti-*LINC00152* RNAi. **e** The levels of Ki67, P65 and MMP9 were analyzed through IHC. Scale bars = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001

mRNA databases. Subsequently, we identified a lncRNA, *LINC00152* in glioma. The abnormal expression level of *LINC00152* observed from the databases was further verified in glioma specimens, suggesting the oncogenic role of *LINC00152*. The high expression pattern of *LINC00152* also predicted a poor outcome of patients. In other tumors, like non-small-cell lung cancer [30], tongue squamous cell carcinoma [31] and gastric cancer [32], *LINC00152* could serve as a clinical prognostic predictor. Cluster analysis revealed gene profile differences between *LINC00152* high versus low-expressing patients, which indicated that *LINC00152* regulated genes involved in the NF-κB signaling pathway as determined via GO analysis. Our experimental results showed that *LINC00152* affected a series of cellular biological functions via NF-κB transcriptional activity. Integrated genomic analysis has explicitly defined the classification of adult GBM into four subtypes [33]. Commonly, GBMs with mesenchymal subtype exhibited elevated levels of NF-kB pathway genes. Activation of NF-kB signaling has also been discovered to promote mesenchymal differentiation of GBM by regulating downstream transcriptional signaling [34]. Considering our experimental results, we suspected that *LINC00152* had an association with GBM subtype and mesenchymal differentiation. Next, *LINC00152* expression was analyzed in GBM subtype and revealed that it was low-expressed

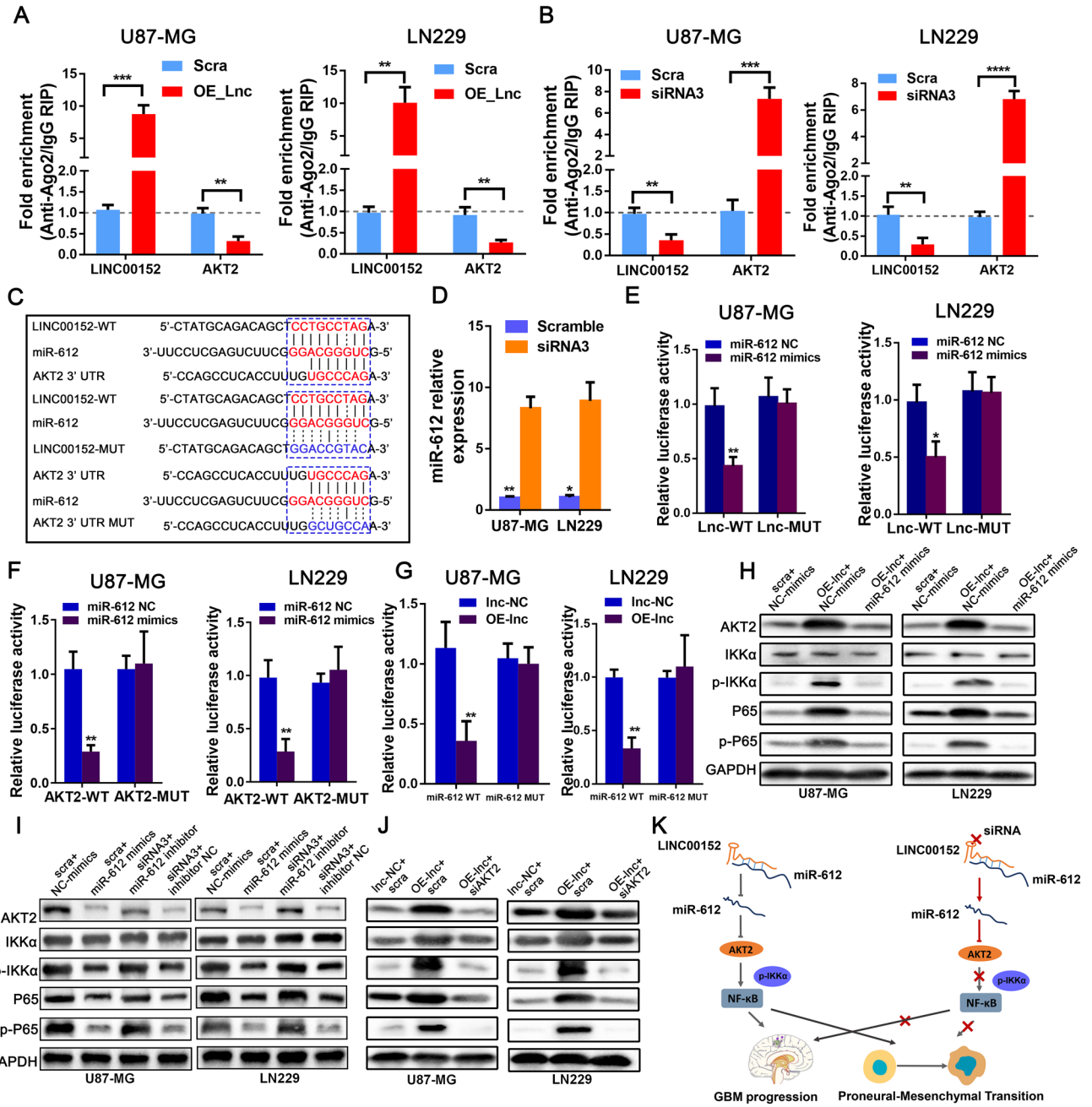

Fig. 5 *LINC00152* functions as ceRNA and sponges *miR-612* in GBM cells. **a** RIP–PCR assay of the enrichment of Ago2 on *LINC00152* and *AKT2* transcripts normalized to IgG in U87-MG and LN229 cells transfected with scramble or *LINC00152*. **b** RIP–PCR assay of the enrichment of Ago2 on *LINC00152* and *AKT2* transcripts normalized to IgG in U87-MG and LN229 cells transfected with scramble or siRNA3. **c** Predicted results showed the sequence of *LINC00152* with highly conserved putative *miR-612* binding sites and *miR-612* with highly conserved putative AKT2 3′-UTR. **d** qRT-PCR analysis of *miR-612* in GBM cells transfected with scramble or siRNA3. **e, f** Luciferase activity of *LINC00152* and AKT2 3′-UTR in GBM cells upon transfection of *miR-612* mimics. **g** Luciferase activity of *miR-612* in GBM cells after overexpression of *LINC00152*. **h** Western blot analysis of indicated proteins in GBM cells after overexpression of *LINC00152* upon transfection with *miR-612* mimics. **i** Western blot analysis of indicated proteins in GBM cells after inhibiting of *LINC00152* upon transfection with *miR-612* mimics and inhibitor. **j** Western blot analysis of indicated proteins in GBM cells after overexpression of *LINC00152* upon transfection with siAKT2. **k** Mechanism diagram of *LINC00152* modulating GBM progression and proneural–mesenchymal transition through *miR-612* dependent on AKT2/NF-κB pathway. *P < 0.05, **P < 0.01, ***P < 0.001

in PN compared with that in MES. MES differentiation induced resistance to chemotherapy is of great importance for GBM progression and recurrence [35]. A long non-coding RNA HIF1A-AS2 maintained mesenchymal features of GBM stem-like cells in the hypoxic microenvironment [36]. However, there is no available research until now that refers to the role of *LINC00152* in the process of MES to PN transition. Our result showed that down-regulated lncRNA *LINC00152* attenuates mesenchymal profile and induced proneural markers.

One of the main functions of lncRNAs is acting as endogenous miRNA sponges to bind to miRNAs and regulate their function of post-transcriptional control. Knockdown of X-inactive specific transcript (*XIST*) exhibits the tumor-suppressive effect in GBM stem cells via regulating *miR-152* [37]. *MALAT1* induces chemoresistance to temozolomide through restraining *miR-203* and promoting synthesis of thymidylate synthase [38]. Taurine upregulated 1 enhances angiogenesis through blocking *miR-299* in human GBM [39]. *RP11-838N2.4* through blocking the functions of *miR-10a* induces the cytotoxic effects of temozolomide in GBM [40]. *LINC00152* accelerates renal cell carcinoma progression through negatively regulating *miR-205* [41] and facilitates gallbladder cancer metastasis by regulating HIF-1α via *miR-138* [42]. *LINC00152* also down-regulates miR-193a-3p to enhance MCL1 expression and promote gastric cancer progression [43]. In triple-negative breast cancer, *LINC00152* accelerates tumor progression by regulating DNMTs [44].

The previous study reported that *miR-612* negatively regulates colorectal cancer growth and metastasis by targeting AKT2 [28]. Besides, AKT/NF-κB axis widely exists in biological process and tumor progression. AKT/NF-κB signaling regulates expression of inflammatory genes [45, 46]. The metastatic ability of human breast cancer cells could be inhibited through the UPR inducer DPP23 by targeting the Akt/NF-κB/MMP-9 axis [47]. It has been proved that blocking AKT2 enhances sensitivity to chemotherapy through NF-κB (P65) in pancreatic carcinoma [48]. MK-2206 an AKT inhibitor suppresses the NF-κB activity in GBM [49]. In our work, *LINC00152* acted as a miRNA sponge for *miR-612* in GBM cells, negatively regulated *miR-612* releases, which resulted in the elevated AKT2, activated NF-κB pathway to promote proneural–mesenchymal transition.

In summary, our research thoroughly showed that *LINC00152* acted as a tumor oncogene with important prognostic value for patients with GBMs, and that *LINC00152* regulated NF-κB/AKT2 pathway by capturing *miR-612* to promote the proneural–mesenchymal transition (Fig. 5e). Our results demonstrate the regulatory mechanisms of *LINC00152* and provide a novel strategy of lncRNA-based therapy in GBM.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval The study involving human participants and animals was approved by the ethics committees of participating hospitals, and all patients provided written informed consent.

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