



# The LINC00957/miR-17-5p axis regulates the cell cycle and migration in glioblastoma via the cuproptosis-related gene nephronectin

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**Background:** Glioblastoma (GBM) is characterized by poor prognosis and a high malignancy. The competing endogenous RNA (ceRNA) network formed by long non-coding RNA (lncRNA) and microRNA (miRNA) can regulate the incidence of GBM. Therapeutic strategies targeting cuproptosis-related genes (CRGs) have helped reduce drug resistance in patients. However, the regulatory mechanism underlying the ceRNA network related to cuproptosis in GBM remains unclear. Therefore, we aim to explore the ceRNA regulatory axis associated with cuproptosis in GBM and provide a new protocol for therapy.

**Methods:** The ceRNA network related to CRG was constructed by bioinformatics. Dual-luciferase reporter assay and other experiments were used to prove the conclusion.

**Results:** We found that the LINC00957/miR-17-5p axis drove nephronectin (NPNT) expression to promote the malignant progression of GBM. First, by measuring the copper ion concentration and reactive oxygen species (ROS) levels, we found that inhibiting NPNT could promote cuproptosis. Meanwhile, the results of enrichment analysis and phenotypic experiments demonstrated that the LINC00957/miR-17-5p/NPNT axis can regulate the cell cycle and migration in GBM. In terms of mechanistic evidence, findings from reporter gene experiments suggested that LINC00957 acted as a ceRNA to regulate the expression of NPNT via miR-17-5p. In addition, findings from rescue experiments confirmed that the regulation of malignant GBM progression by LINC00957 depended on NPNT to an extent.

**Conclusions:** Our findings indicate that the ceRNA regulatory network is related to cuproptosis in GBM and provide novel potential targets for the diagnosis and treatment of GBM.

**Keywords:** Glioblastoma (GBM); cuproptosis; competing endogenous RNA (ceRNA); nephronectin (NPNT); cell cycle

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## Introduction

Glioblastoma (GBM), an aggressive form of brain cancer, is known for its poor prognosis and high malignancy (1). GBM arises from astrocytes, the supportive cells in the brain, and rapidly grows and invades surrounding brain tissue (2). The types of treatments typically include surgery, radiation, and chemotherapy, mostly with the drug temozolomide (3). However, despite these interventions, the survival rate of patients with GBM remains low, with an average of approximately 15 months (4). The resistance of the disease to conventional therapies is a significant challenge, largely owing to the tumor's complex genetic mutations, its capacity for adaptation and evolution, and the challenges in administering effective treatments through the blood-brain barrier (5). Therefore, it is imperative to look for novel potential therapeutic targets.

Non-coding RNAs, such as microRNAs (miRNA) and long non-coding RNAs (lncRNA), regulate gene expression and play a crucial role in cellular functions and diseases (6). MiRNAs, which are small non-coding RNA segments, regulate gene expression after transcription, either by degrading messenger RNA (mRNA) or inhibiting its translation (7). In tumors, these segments function as oncogenes or tumor suppressors, influencing cell proliferation, apoptosis, and metastasis (8). In GBM, miRNAs regulate gene expression, affecting tumor growth, invasion, and resistance to therapy, and have been investigated as a potential target for novel therapeutic strategies and biomarkers (9). MiR-17-5p is a single miRNA that can specifically act on the G1/S phase cell cycle boundary. MiR-17-5p regulates the cell cycle, apoptosis, and invasion pathways acting as a tumor suppressor in GBM

(10,11). MiR-17-5p is down-regulated in our findings and that this agrees with previous studies (10,11). Thus, miR-17-5p may be a promising therapeutic target and biomarker for GBM and other tumors.

LncRNAs, which are longer RNA molecules, primarily function by modulating gene expression at different stages, including chromatin modification, transcription, and post-transcriptional processing (12). In tumors, lncRNAs can serve as oncogenes or suppressors, influencing cancer initiation, progression, and metastasis (13). In GBM, lncRNAs contribute to tumor growth, drug resistance, and recurrence (14). They are involved in tumor progression and have potential as a target for novel therapeutic strategies (15). The expression of LINC00957 is strongly correlated with the staging and chemotherapy results of certain tumors (16). In recent years, the role of LINC00957 in the prognosis of GBM has been reported in studies, which also illustrates its potential importance (17). Competing endogenous RNAs (ceRNAs) control gene expression by capturing miRNAs, thus influencing their activity (18). Tumors, including GBM, contribute to oncogenesis and progression by disrupting normal miRNA-mediated gene regulation, offering novel insights into cancer biology and potential therapeutic targets.

Regulated cell death (RCD) is a vital biological process involving various mechanisms like apoptosis, necroptosis, and autophagy, which are crucial for maintaining cellular homeostasis and eliminating damaged or harmful cells (19). Cuproptosis is a novel type of RCD, distinct from other types owing to its unique reliance on copper-dependent pathways (20). Unlike apoptosis or necroptosis, which involve protein oligomerization or degradation, cuproptosis is driven by metabolic disruption (21). In tumors, cuproptosis presents a promising therapeutic target. Multiple cancers exhibit altered copper metabolism, and exploring this through cuproptosis could help selectively target cancer cells, especially cells resistant to conventional RCD pathways like apoptosis (22). Owing to its aggressive nature and resistance to standard therapies, GBM can particularly benefit from cuproptosis-based treatments (23). Research is being conducted to explore the sensitivity of GBM to copper-dependent cell death. Targeting cuproptosis in GBM may offer a novel approach to circumvent the typical resistance mechanisms these tumors typically exhibit, which could help develop more effective therapies against this devastating disease (24). The ongoing investigation into cuproptosis in GBM and other cancers opens new frontiers in cancer treatment strategies.

### Highlight box

#### Key findings

- LINC00957/miR-17-5p/nephronectin (NPNT) axis promote the malignant progression of glioblastoma (GBM).

#### What is known and what is new?

- The competing endogenous RNA (ceRNA) network and cuproptosis can regulate the incidence of GBM.
- NPNT could promote cuproptosis.
- LINC00957 acted as a ceRNA to regulate NPNT via miR-17-5p.

#### What is the implication, and what should change now?

- Cuproptosis-related ceRNA axis regulates GBM. It is expected that animal model experiments will further verify.

The nephronectin (NPNT) gene encodes NPNT, a component of the extracellular matrix (25). It plays a key role in cell adhesion and signaling, influencing cellular behaviors crucial for tissue development and repair (25). In tumors, NPNT has been implicated in cell cycle and cell migration regulation (26). Its expression can affect tumor growth and metastasis by modulating cell interactions and the surrounding environment (27). The effect of NPNT on the cell cycle can alter the proliferation rate of cancer cells, whereas its role in cell migration is of significance in cancer invasion and metastasis (28). However, the mechanism of action of NPNT in GBM has not been investigated thoroughly, and its relationship with cuproptosis has never been reported.

In this study, we found that in GBM, LINC00957 uses a ceRNA for miR-17-5p, thereby influencing the expression of the cuproptosis-related gene (CRG) NPNT. Based on the assessment of copper ion concentration and reactive oxygen species (ROS) levels, our initial findings indicated that NPNT inhibition enhances cuproptosis. Furthermore, findings from enrichment analysis and phenotypic experiments revealed that the LINC00957/miR-17-5p/NPNT axis significantly regulates the cell cycle and cell migration in GBM. Mechanistic insights from reporter gene experiments suggested that LINC00957 modulates NPNT expression by serving as a ceRNA for miR-17-5p. We present this article in accordance with the MDAR reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-24-450/rc>).

## Methods

### Data acquisition

We obtained glioma data and clinical information from The Cancer Genome Atlas (TCGA) database (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>), which included the expression levels of mRNAs, miRNAs, and lncRNAs. GSE63319 was used to retrieve miRNA expression data from normal brain and glioma tissues from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

### Differentially expressed gene (DEG) analysis

We used R package limma to analyze the DEGs of normal

and tumor cells in the TCGA and GEO databases. CRGs were also identified based on the different levels of cuproptosis in patients with glioma using cluster analysis. Results with  $\log_2$ fold change (FC)  $\geq 1$  and adjusted  $P < 0.05$  were considered meaningful.

### Univariate regression analysis

Univariate Cox regression analysis was performed to evaluate the prognostic value of the mRNAs, miRNAs, and lncRNAs of 170 patients with glioma in the TCGA database.

### Gene set enrichment analysis (GSEA)

R package clusterprofiler was used to analyze the GSEA results of the DEGs of lncRNAs, miRNAs, and mRNAs in patients with glioma. The  $P$  value  $< 0.05$  was significant.

### Construction of the ceRNA axes

We used the obtained differentially expressed lncRNAs, miRNAs, and cuproptosis-related mRNAs to construct the ceRNA axis. First, the relationship between lncRNA and miRNA was determined using the miRcode database. Second, the potential target mRNAs were predicted based on the miRNA in TargetScan, miRDB, miRTarbase, and ENCORI databases. Lastly, we filtered the results based on the survival analysis results of lncRNAs, miRNAs, and mRNAs and constructed the final ceRNA network. The software Cytoscape (version 3.9.1) was used to visualize the regulatory relationships. A Sankey diagram was visualized using R package ggalluvial and ggplot2.

### Pearson correlation analysis

We performed Pearson correlation analysis using  $\log_2$ [transcripts per million (TPM) +1] values of lncRNAs and mRNAs and  $\log_2$ [reads per million mapped reads (RPM) +1] values of miRNAs.  $R$ -squared  $\geq 0.3$  and  $P$  value  $< 0.05$  were considered relevant.

### Cell culture

We cultured human glioma U87 (catalog number: HTB-14) and LN229 (catalog number: CRL-2611) cells for the study. The cells were obtained from the American Type

Culture Collection (ATCC). U87 cells were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37 °C in 5% CO<sub>2</sub>. LN229 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine serum albumin (BSA), 100 µg/mL penicillin, and 100 µg/mL streptomycin at 37 °C in 5% CO<sub>2</sub>.

### *Transfection*

A miRNA mimic and a miRNA inhibitor were obtained from Biomics (Nantong, China). Short hairpin RNAs (shRNAs) of NPNT were constructed on the PLKO.1 plasmid. The shRNA sequence was shRNA NPNT (shNPNT) 5'-GCTACTGTCTGAATGGCTACA-3'. The antisense oligonucleotides (ASOs) of lncRNA LINC00957 were purchased from RiboBio (Guangzhou, China). The miRNA mimic, inhibitor, shRNA, and ASOs were transfected into U87 and LN229 cells using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions.

### *RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay*

We extracted the total RNA and converted it into complementary DNA (cDNA) using reverse transcriptase. We then measured the levels of RNA and miRNA using a qPCR kit. The primers used are listed in [Table S1](#).

### *Cell Counting Kit-8 (CCK8) assay*

We cultured the cells in 96-well plates and used CCK8 reagent to measure the proliferation potential of the cells at 450 nM using a microplate reader.

### *Cell cycle assay*

The U87 cells with shRNA negative control (shNC) or stable NPNT knockdown ( $1 \times 10^6$ ) were cultured in 6-well plates. The cells were washed with phosphate-buffered saline (PBS) and fixed overnight with 70% ethanol at 4 °C. Finally, the cells were stained with propidium iodide (PI) and detected by flow cytometry.

### *Transwell assay*

ASO NC and ASO LINC00957 U87 cells were cultured in serum-free medium. Following this,  $1 \times 10^4$  cells were cultured in the upper layer of the chamber (BD Biosciences, Shanghai, China), and serum-containing medium was added to the lower layer. After 48 h, the cells were washed with PBS, fixed with 0.1% crystal violet solution, and observed randomly under a microscope in five fields.

### *ROS assay*

The production of ROS was detected using a ROS assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. shNC and shNPNT U87 and LN229 cells were seeded in 12-well plates. After 24 h, the cells were treated with different concentrations of elesclomol (0, 10, and 20 µM) for 24 h at 37 °C. Next, the cells were incubated for 20 min with a dichlorodihydrofluorescein diacetate probe (10 µM) at 37 °C. ROS fluorescence signals were measured using BD C6 (BD Biosciences).

### *Measurement of intracellular copper*

U87 and LN229 cells were cultured in 6 cm plates for 24 h and treated with a cuproptosis inducers (elesclomol) for 24 h. Following this, the cells were collected and disrupted ultrasonically to detect intracellular copper according to the manufacturer's instructions (E-BC-K775-M, Elabscience, Houston, TX, USA).

### *Luciferase reporter assay*

We used PGL4.15 to construct plasmids containing wild-type and mutated LINC00957. A Dual-Luciferase Reporter Assay System Kit (E1910; Promega, Madison, WI, USA) was used according to the manufacturer's instructions.

### *Statistical analysis*

We used univariate regression analysis and Kaplan-Meier (KM) and receiver operating characteristic (ROC) curves to evaluate the patient survival status. We used GraphPad Prism 8 to analyze the statistical significance of the experimental results. Unpaired *t*-test was used to evaluate

the results between the two groups. P values <0.05 were considered statistically significant.

## Results

### *Identification of lncRNAs, miRNAs, and mRNAs associated with GBM progression*

Tumor markers are genes that play a vital role in tumor occurrence and development and show significant changes in their expression levels in tumors. In this study, differential mRNA and lncRNA analysis was performed on five pairs of GBM samples and normal brain tissue samples from the TCGA database. Similarly, we performed differential miRNA analysis on four pairs of GBM samples and normal brain tissue samples from the GEO database (GSE63319). A total of 4,694 differentially expressed mRNAs (2,286 upregulated and 2,408 downregulated), 536 lncRNAs (380 upregulated and 156 downregulated), and 50 miRNAs (43 upregulated and 7 downregulated) (Figure 1A,1B and Figure S1) were identified. Subsequently, univariate Cox analysis was performed using 170 GBM tumor samples from the TCGA database, using which we identified 838 oncogenes and 192 tumor suppressor genes (Figure 1C). To further investigate the significance of these genes in GBM tumors, the mRNAs were subjected to GSEA. Interestingly, these DEGs were found to be expressed at high levels in the cell proliferation and cell migration pathways (Figure 2A and Figure S2A-S2C). Results of the gene ontology (GO) enrichment analysis yielded similar results, indicating that these genes were primarily involved in the proliferation and cell migration functions of GBM tumors (Figure 2B and Figure S2D-S2F). Collectively, the genes regulating the malignant progression of GBM tumors showed significant enrichment in pathways related to the cell cycle and cell migration.

### *Consensus clustering analysis of CRGs*

Cuproptosis is a copper-dependent programmed cell death pathway. However, the function of CRGs in GBM remains unclear. Hence, we performed clustering analysis using the expression matrix of CRGs to categorize 170 patients from the TCGA database into k groups. The optimal classification was achieved when patients were divided into two groups based on ConsensusClusterPlus (Figure 3A and Figure S3A,S3B). Additionally, DEGs were identified in the mRNA data of the two subgroups, with 256 upregulated and 678 downregulated genes (Figure 3B and Figure S3C).

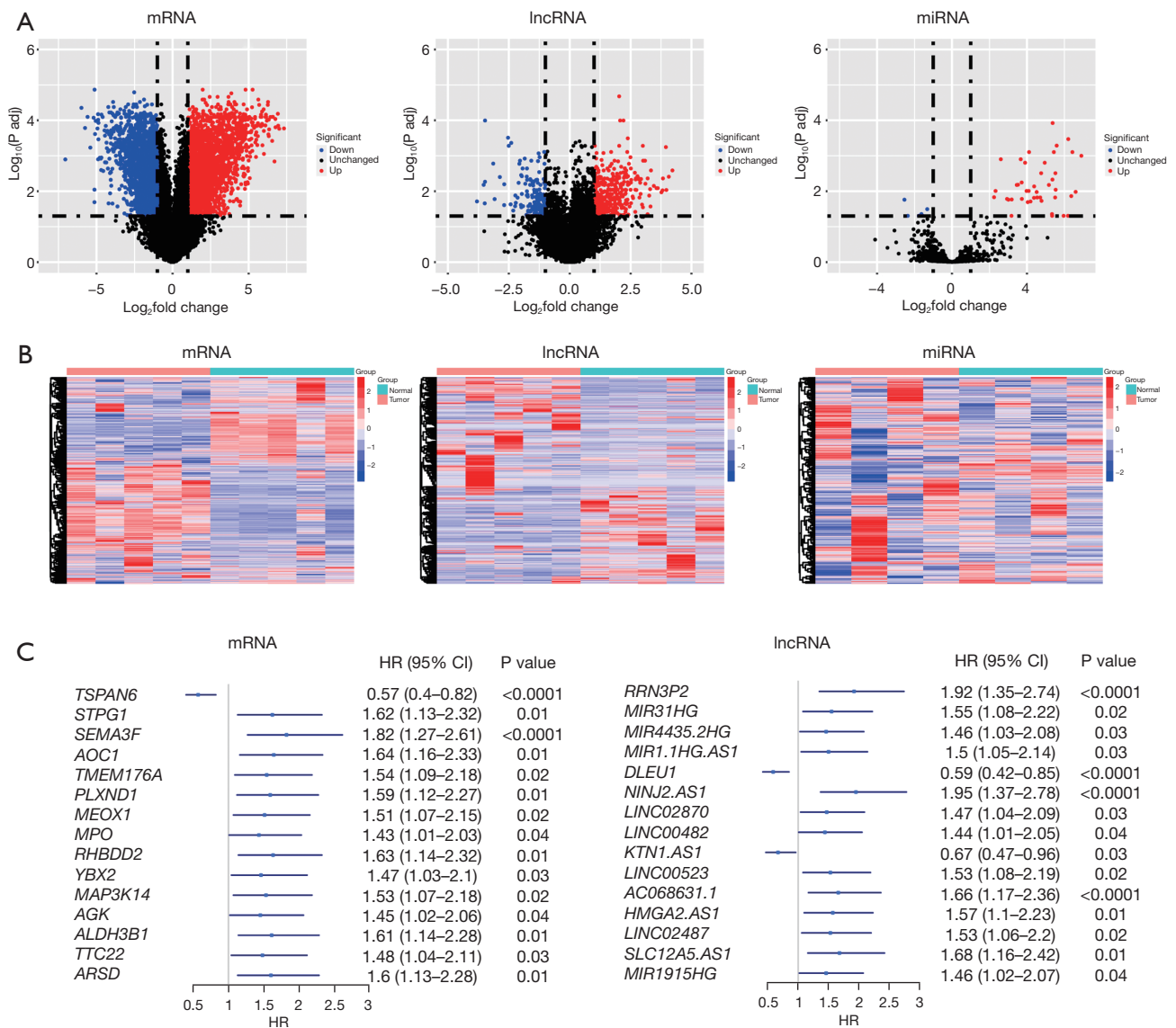
Furthermore, GO analysis of these DEGs revealed their significant enrichment in the cell cycle and cell migration pathways (Figure 3C,3D and Figure S3).

### *Identifying cuproptosis-related ceRNA axis in GBM tumorigenesis*

Based on the above results, we constructed a ceRNA network associated with the cuproptosis-related regulation of malignant progression in GBM, using differentially expressed mRNA, lncRNA, and miRNA, which included 24 lncRNAs, three miRNAs, six mRNAs, and 50 interacting axes (Figure 4A and Figure S4A,S4B). Surprisingly, we identified NPNT, a potential prognostic biomarker for GBM. Literature reports suggest that NPNT can promote cancer cell proliferation, migration, and invasion. However, its mechanism of action in GBM remains unclear. Therefore, we focused on the ceRNA axes associated with NPNT. The results indicated that 15 lncRNAs may be involved in the regulation of NPNT expression (Figure 4B). Among them, AL365361.1, AL591848.3, and LINC00957 exhibited higher expression levels in GBM tumors, suggesting their greater potential functionality. Consequently, we selected these lncRNAs for further screening.

### *NPNT knockdown enhances the sensitivity of GBM cells to cuproptosis inducers*

Based on previous findings, NPNT was observed to be associated with the progression of GBM. However, the potential involvement of NPNT in cuproptosis pathways has not been reported. To better understand the relationship between NPNT and cuproptosis, we treated GBM cells with the cuproptosis inducer elesclomol and measured its half-maximal inhibitory concentration (IC<sub>50</sub>) value using the CCK8 assay (Figure 5A). Subsequently, we treated NPNT knockdown cells and control cells with varying concentrations (0, 10, and 20 nM) of elesclomol and assessed the changes in the cellular ROS levels using flow cytometry. Lower concentrations of elesclomol significantly reduced the ROS levels in NPNT knockdown cells compared to those in the control group (Figure 5B). To further confirm the association between ROS levels and cuproptosis, we measured the Cu<sup>2+</sup> concentrations in both groups of cells, and found that knockdown of NPNT resulted in an increase in intracellular copper ion concentration when GBM cells were induced at low concentrations of elesclomol (Figure 5C). Collectively, the



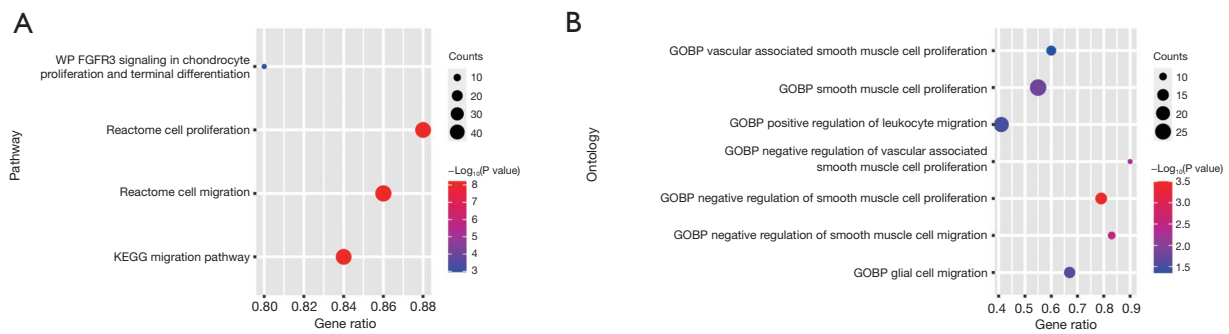
**Figure 1** The differentially expressed lncRNA, miRNA, and mRNA in normal and tumor glioma patients. (A) The volcano plots of the DEGs in normal and tumor glioma patients. (B) The heatmaps of the DEGs in normal and tumor glioma patients. (C) The top 15 results of univariate Cox regression results in mRNAs and lncRNAs. mRNA, messenger RNA; lncRNA, long non-coding RNA; miRNA, microRNA; HR, hazard ratio; CI, confidence interval; DEG, differentially expressed gene.

results indicated that NPNT knockdown enhanced the sensitivity of GBM cells to elesclomol and provided further evidence supporting the link between ROS levels and cuproptosis.

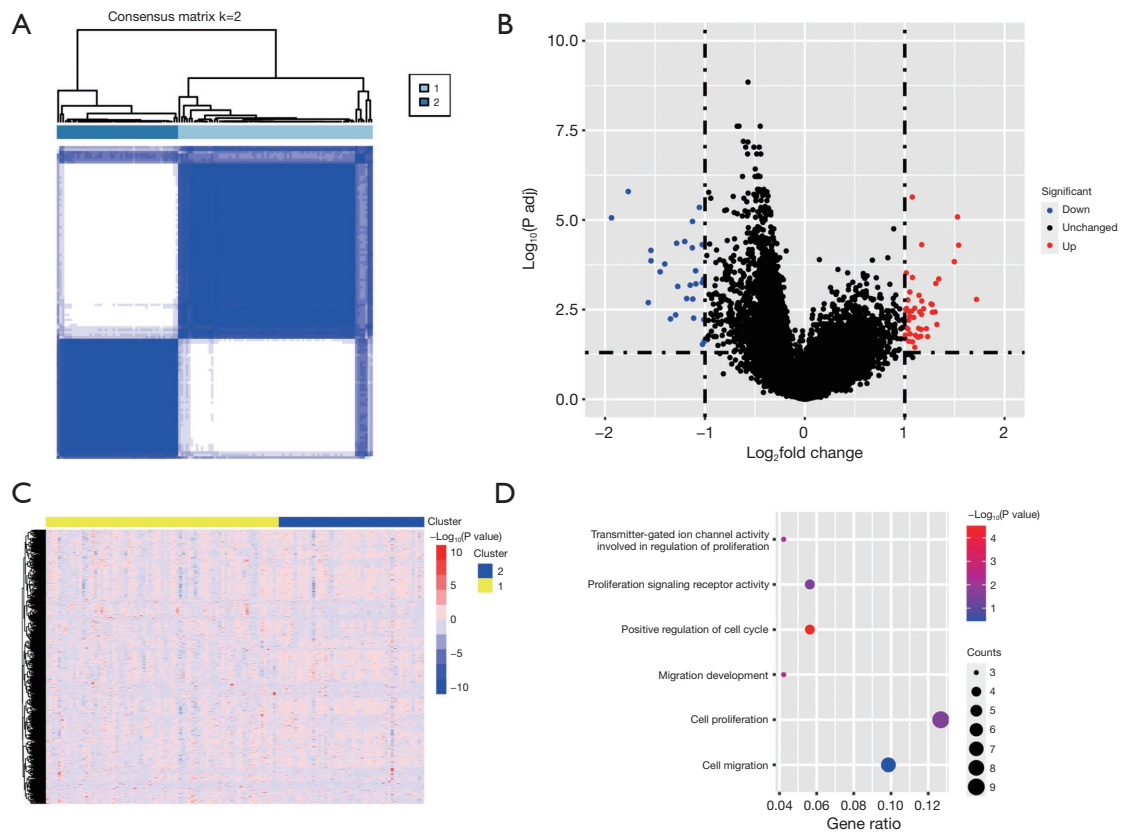
### *LINC00957 potentially regulates the nucleic acid levels of NPNT*

To determine the primary lncRNA regulating NPNT, we

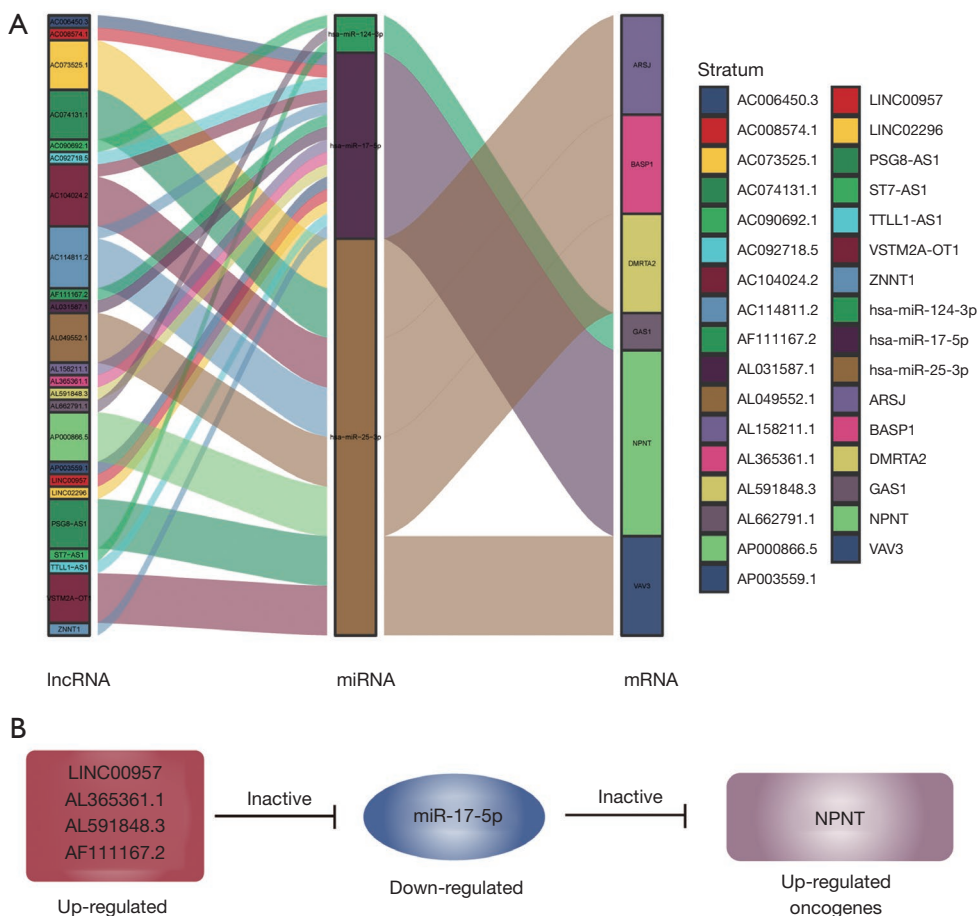
conducted an ROC analysis. The area under the curve (AUC) indicated a significant association between the expression of LINC00957 and the survival rate (Figure 6A). To further refine our findings, we performed knockdown experiments using ASOs targeting AL365361.1, AL591848.3, AF111167.2, and LINC00957, and measured the changes in NPNT mRNA levels. LINC00957 knockdown led to a significant alteration in NPNT expression, whereas no statistically significant differences were observed with the



**Figure 2** Pathway enrichment of genes associated with GBM progression. (A) KEGG pathway analysis of up- and down-regulated differential mRNAs. (B) GO analysis of up- and down-regulated differential mRNAs. The text on the left indicates enriched KEGG terms, the size of the ball indicates the number of enriched genes, and the color indicates the level of enrichment. GBM, glioblastoma; KEGG, Kyoto Encyclopedia of Genes and Genomes; mRNA, messenger RNA; GO, gene ontology; BP, biological process.



**Figure 3** DEG analysis of glioma patients based on cuproptosis-associated clusters. (A) The consensus matrix of all samples when k=2. (B) Volcano plot and (C) heatmap of cuproptosis-associated DEGs between cluster 1 and cluster 2. (D) GSEA of DEGs between the two cluster groups. DEG, differentially expressed gene; GSEA, gene set enrichment analysis.



**Figure 4** Construction of cuproptosis-related ceRNA network by integrated analysis in GBM. (A) LncRNA-miRNA-mRNA regulatory axes extracted from this ceRNA network. (B) The potential lncRNA/miRNA/mRNA axis. LncRNA, long non-coding RNA; miRNA, microRNA; mRNA, messenger RNA; NPNT, nephronectin; GBM, glioblastoma; ceRNA, competing endogenous RNA.

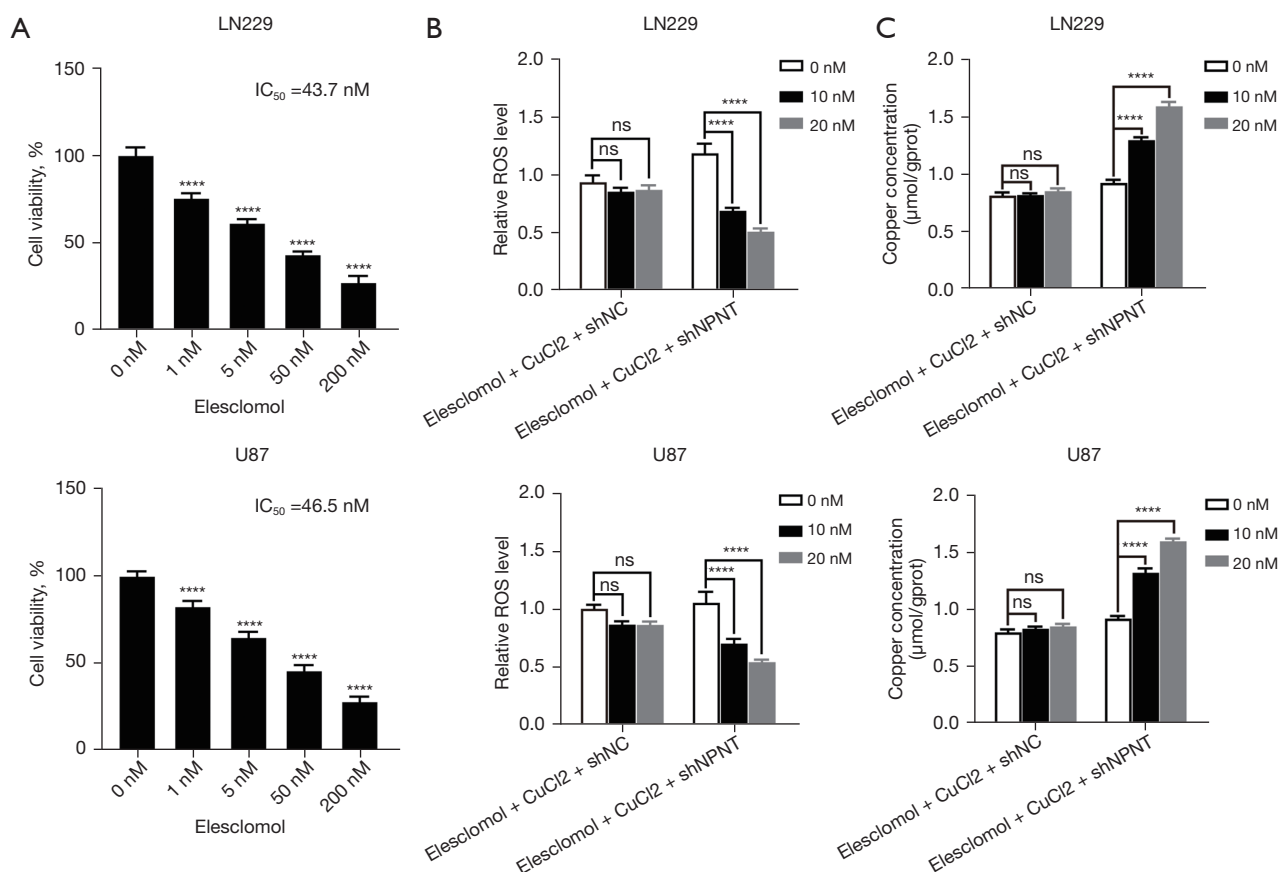
knockdown of the other lncRNAs (Figure 6B). Collectively, these findings emphasize the significant role of LINC00957 in regulating NPNT expression.

**Regulation of GBM malignant progression by LINC00957, miR-17-5p, and NPNT protein**

According to the aforementioned predictions, NPNT/miR-17-5p/LINC00957 may be a ceRNA network that regulates the development of GBM, but the specific roles of the network in promoting the malignant progression of GBM remain unclear. Hence, we divided GBM samples into two groups based on the expression levels of LINC00957. We performed differential expression analysis and enrichment analysis (similar analyses were performed for NPNT).

Our findings revealed that the target genes regulated by LINC00957 were primarily enriched in cell migration, while NPNT was enriched in cell cycle (Figure 7A and Figure S5A-S5C). To further validate the regulatory roles of NPNT and LINC00957 in GBM malignant progression, we conducted knockdown experiments targeting NPNT and LINC00957 individually. Transwell assays and cell cycle analyses were performed, revealing that the knockdown of NPNT and LINC00957 inhibited GBM cell cycle progression and migration, consistent with the findings of the enrichment analysis (Figure 7B-7D). These results confirmed that NPNT and LINC00957 can indeed regulate the cell cycle and cell migration in GBM and supported the involvement of these molecules in the malignant progression of the disease.



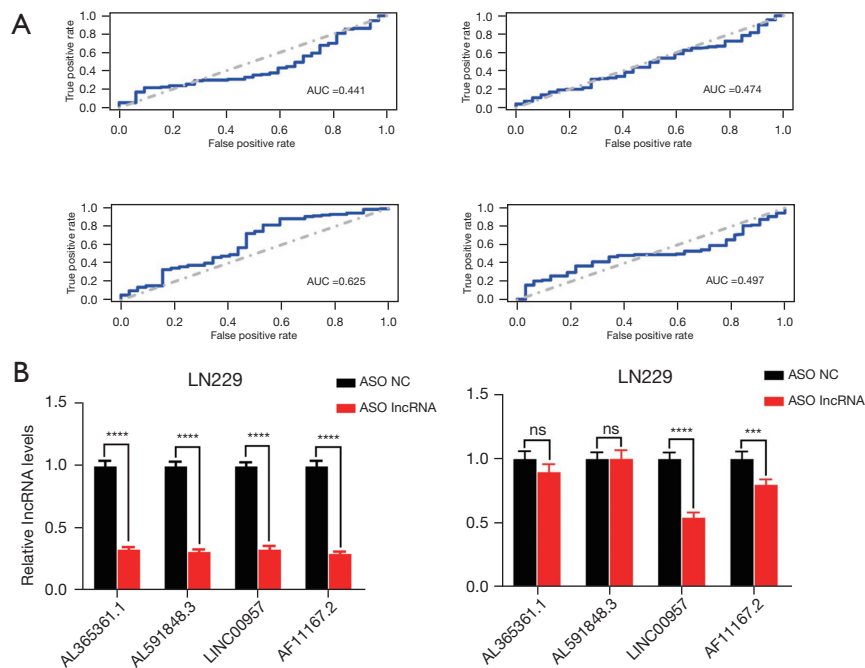


**Figure 5** Knocking down NPNT enhances the sensitivity of glioma cells to cuproptosis inducers. (A) The CCK8 assay of U87 and LN229 cells treating with elesclomol. (B) The ROS levels of shNC or shNPNT U87 and LN229 cells treating with elesclomol and CuCl<sub>2</sub>. (C) The Copper concentration of shNC or shNPNT U87 and LN229 cells treating with different concentration of elesclomol (10 and 20 nM). Data were expressed as the mean ± standard deviation of three independent experiments (n=3). ns, not significant; \*\*\*\*, P<0.0001. IC<sub>50</sub>, half-maximal inhibitory concentration; ROS, reactive oxygen species; NPNT, nephronectin; CCK8, Cell Counting Kit-8; shNC, shRNA negative control; shNPNT, shRNA NPNT; shRNA, short hairpin RNA.

### *MiR-17-5p as a ceRNA for LINC00957 regulates NPNT protein expression to influence malignant GBM progression*

To further investigate the relationship among LINC00957, miR-17-5p, and NPNT, we conducted Pearson correlation analysis. The results showed a high correlation among the expression of these molecules, indicating a significant association between the three genes (Figure 8A). Next, we altered the expression of LINC00957 or miR-17-5p and used RT-PCR to measure the expression levels of the three genes. LINC00957 and miR-17-5p could regulate NPNT expression in GBM, which was consistent with the ceRNA axis hypothesis (Figure 8B). To verify whether LINC00957 acts as the ceRNA of miR-17-5p, we conducted the dual

luciferase reporter assay was conducted after introducing complementary mutations in the predicted binding sites between LINC00957 and miR-17-5p. Our findings revealed that the luciferase activity of wild-type LINC00957 was regulated by miR-17-5p, but the mutant form of LINC00957 did not show any changes (Figure 8C,8D). Furthermore, we assessed the DEGs co-regulated by LINC00957 and NPNT in GBM (Figure S6A,S6B). We conducted proliferation in GBM cells with LINC00957 knockdown in both control and shNPNT groups. The findings indicated that LINC00957 knockdown suppressed tumor cell proliferation specifically in the shNPNT group, but not in the control group (Figure 8E). These results suggest that LINC00957 acts as a ceRNA for miR-17-



**Figure 6** LncRNA LINC00957 and miR-17-5p can regulate NPNT protein in GBM tumors. (A) ROC curve of the AL365361.1, AL591848.3, LINC00957, and AF111167.2. (B) NPNT mRNA levels and AL365361.1, AL591848.3, LINC00957, and AF111167.2 in LN229 cells with ASO knockdown four potential lncRNAs was detected. Data were expressed as the mean  $\pm$  standard deviation of three independent experiments (n=3). ns, not significant; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ . AUC, area under the curve; ASO, antisense oligonucleotide; NC, negative control; lncRNA, long non-coding RNA; NPNT, nephronectin; GBM, glioblastoma; ROC, receiver operating characteristic; mRNA, messenger RNA.

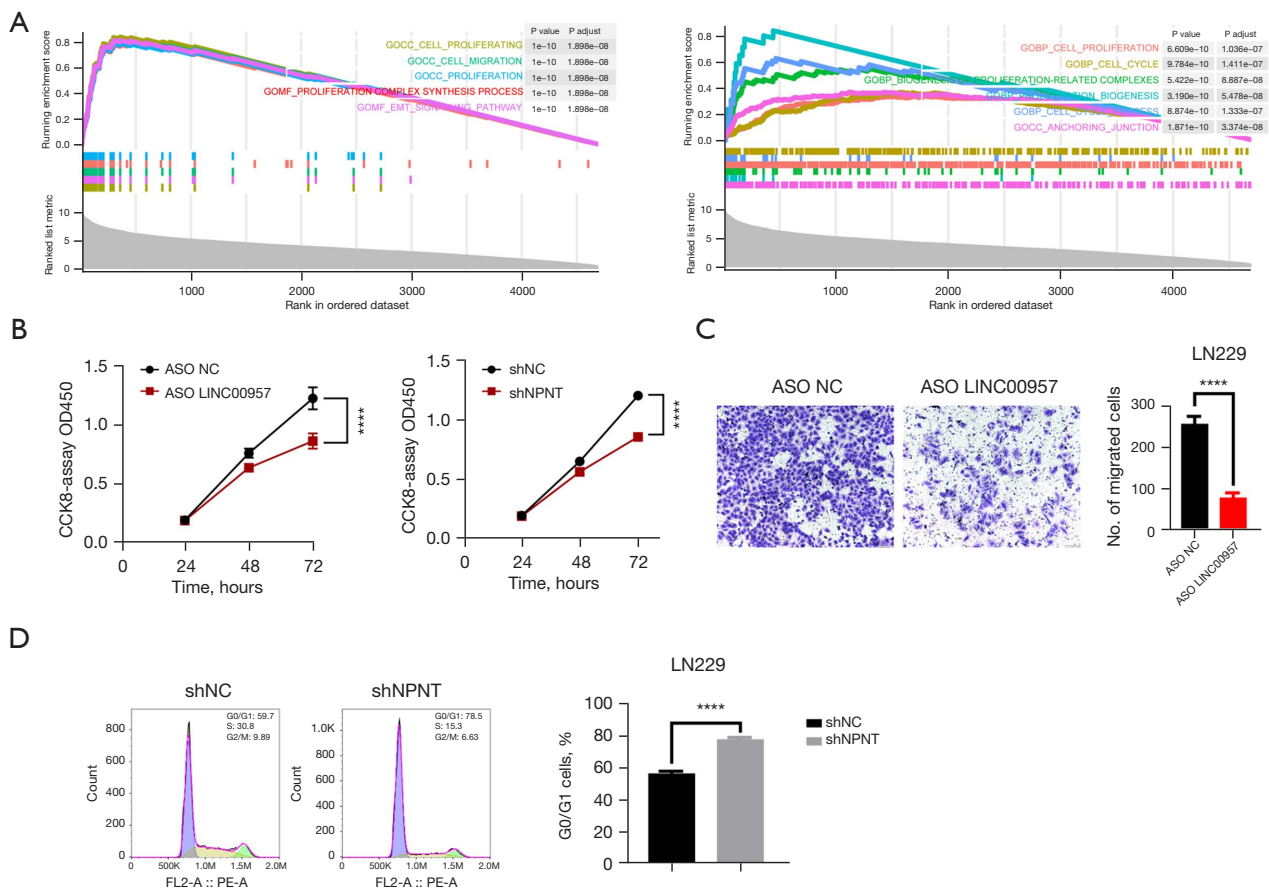
5p, thereby regulating NPNT protein expression and influencing the malignant progression of GBM tumors.

## Discussion

GBM is the most aggressive and lethal form of brain cancer, characterized by poor prognosis, high malignancy, and a dire lack of effective treatments. However, despite significant advancements in surgery, radiation, and chemotherapy, the survival rate in GBM remains dismally low, emphasizing the indispensable requirement for novel diagnostic and therapeutic approaches. The complex biology of GBM, including its rapid growth, invasion of adjacent brain tissues, and resistance to conventional therapies, poses significant challenges in treatment. An expanding area of interest in GBM research is the function of non-coding RNAs, particularly lncRNAs and miRNAs, in its pathogenesis. These molecules are crucial regulators in the occurrence and development of GBM, functioning primarily via the ceRNA mechanism. The ceRNA hypothesis suggests that lncRNAs can act as molecular

sponges, sequestering miRNAs and preventing them from downregulating their target mRNAs (29). This intricate system of lncRNA-miRNA-mRNA interactions contributes to the regulation of gene expression, thereby impacting key processes in cancer biology like cell proliferation, apoptosis, and metastasis (30). CeRNA function in GBM has been the focus of several studies. A study examined the lncRNA, miRNA, and mRNA-associated ceRNA networks to identify potential prognostic indicators for GBM, highlighting the importance of ceRNA network genes in GBM diagnosis and treatment (31). Collectively, the findings from these studies contribute to the understanding of the regulatory mechanisms and functions of ceRNA networks in the pathogenesis of GBM (31). In this study, we developed a ceRNA regulatory network specific to GBM. We confirmed that LINC00957, acting as a ceRNA for miR-17-5p, shows significance in regulating the malignant progression of GBM. This study substantially expands our understanding of the ceRNA regulatory mechanisms in GBM.

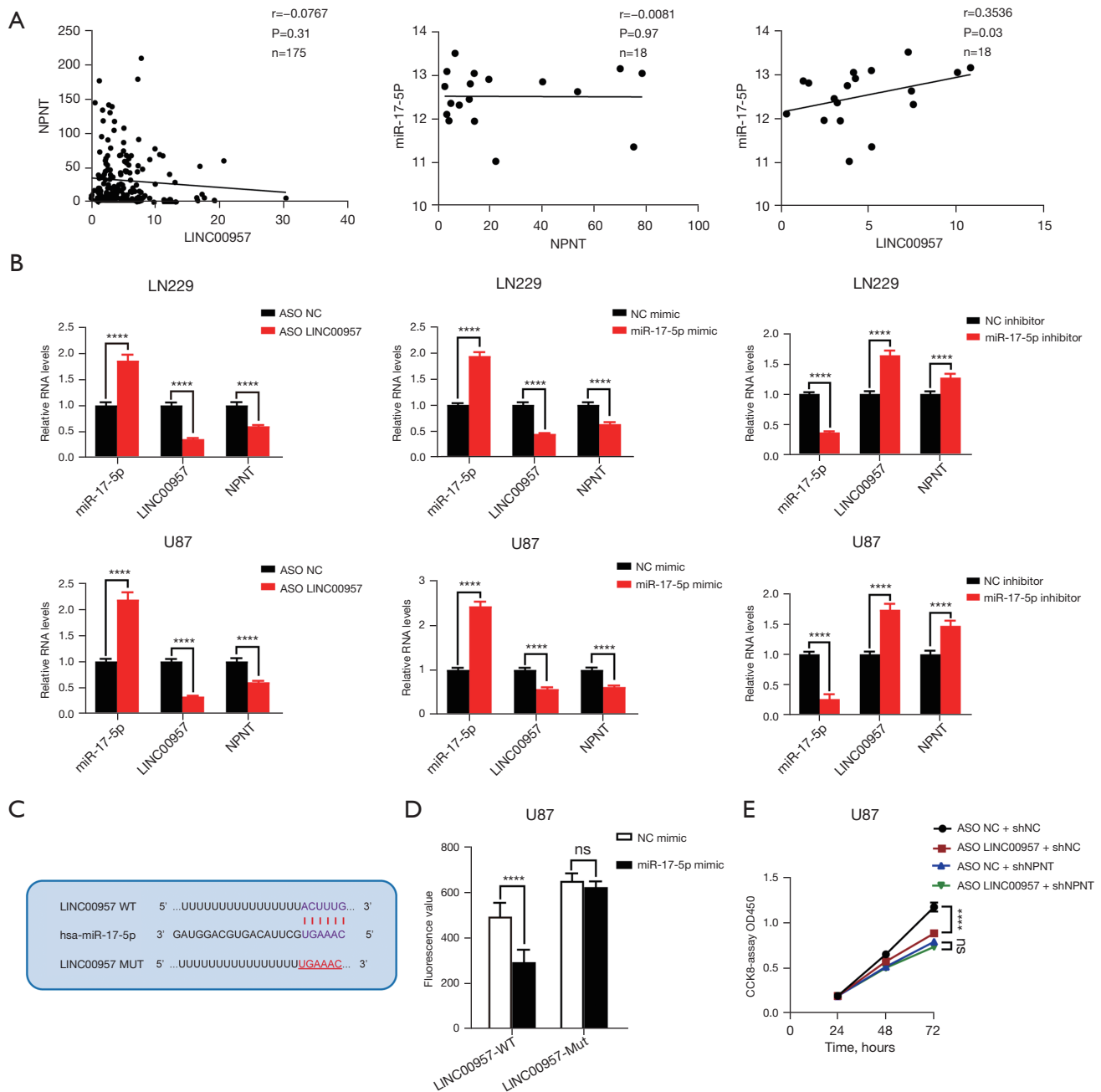
Cuproptosis is a specific cell death pathway mediated via copper accumulation within cells. Cuproptosis is



**Figure 7** The LINC00957, miR-17-5p, and NPNT regulate the malignant progression of GBM. (A) The GO functional analysis of the related biological processes of DEGs in low expression group and high expression group among LINC00957 and NPNT. (B) The CCK8 results in U87 cells with knockdown of LINC00957 and NPNT. (C) The transwell results of crystal violet staining at 40× magnification in LN229 cells with knockdown of LINC00957. (D) The cell cycle results in LN229 cells with knockdown of NPNT. Data were expressed as the mean ± standard deviation of three independent experiments (n=3). ns, not significant; \*\*\*\*, P<0.0001. ASO, antisense oligonucleotide; NC, negative control; CCK8, Cell Counting Kit-8; OD450, optical density at 450 nm; shNC, shRNA negative control; shNPNT, shRNA NPNT; shRNA, short hairpin RNA; NPNT, nephronectin; GBM, glioblastoma; GO, gene ontology; DEG, differentially expressed gene.

distinct from apoptosis or necrosis in that it entails the direct interaction of copper with lipoylated elements of the tricarboxylic acid (TCA) cycle (32). This interaction leads to the accumulation of lipoylated proteins, which consequently disrupts iron-sulfur cluster proteins that are essential for mitochondrial function (33). This disruption leads to the loss of mitochondrial respiration, leading to cell death. In cancer, where cells often exhibit altered copper metabolism and mitochondrial function, the exploitation of cuproptosis presents a unique therapeutic avenue (34). By selectively inducing cuproptosis, it is possible to target tumor cells, offering a potential strategy for cancer treatment, particularly in case of tumors that have developed resistance

to traditional treatments (35). The effect of CRGs on GBM has been a subject of interest in recent research. For example, a systematic analysis and bioinformatics studies highlighted the link between CRG expression and immune infiltration in GBM and its potential implications for prognosis (36). In another study, a cuproptosis network was developed to determine the interactions and prognostic implications of CRGs in GBM, providing insights into the role in the disease (37). Furthermore, the activation of cuproptosis was associated with prognosis in GBM, with the expression of CRGs being linked to tumor immune infiltration and the immune microenvironment (38). These results enhance our understanding of the unique impact



**Figure 8** The lncRNA LINC00957 and miR-17-5p can regulate NPNT protein in GBM tumors. (A) Correlation analysis between NPNT, LINC00957, and miR-17-5p. (B) The RNA levels of NPNT, LINC00957, and miR-17-5p in U87 and LN229 with ASO knockdown LINC00957 was detected; the RNA levels of NPNT, LINC00957 and miR-17-5p in U87 and LN229 treated with miR-17-5p mimic was detected; the RNA levels of NPNT, LINC00957, and miR-17-5p in U87 and LN229 treated with miR-17-5p inhibitor was detected. (C) Predicted binding site between LINC00957 and miR-17-5p. (D) Luciferase reporter gene test was used to test the relationship between LINC00957 and miR-17-5p treated with miR-17-5p mimic in U87 cell. (E) The CCK8 results in ASO NC or ASO LINC00957 U87 cells treated with shNC or shNPNT. Data were expressed as the mean  $\pm$  standard deviation of three independent experiments ( $n=3$ ). ns, not significant; \*\*\*\*,  $P < 0.0001$ . NPNT, nephronectin; ASO, antisense oligonucleotide; NC, negative control; WT, wild-type; MUT, mutated; shNC, shRNA negative control; shNPNT, shRNA NPNT; shRNA, short hairpin RNA; lncRNA, long non-coding RNA; GBM, glioblastoma; CCK8, Cell Counting Kit-8.

of cuproptosis on GBM and its potential influence on disease prognosis and therapeutic strategies. Nevertheless, the precise role of cuproptosis in the evolution and advancement of GBM warrants additional research, given the existing ambiguities in this area. In this context, GBM samples were classified according to the expression levels of 13 CRGs, which facilitated the segregation of patients into two different groups characterized by varying degrees of cuproptosis. Subsequent differential analysis helped identify specific genes associated with cuproptosis in GBM. These insights offer promising targets for developing targeted therapies for cuproptosis in GBM treatment.

The NPNT gene encodes NPNT, which plays a crucial role in tumor cell apoptosis and migration (39). NPNT reduces the migration and invasive potential of cancer cells, such as ovarian and cervical cancer cells, by suppressing specific pathways and decreasing protein expression in cell motility (40). Additionally, various studies have demonstrated the ability of certain compounds to influence cancer cell proliferation, migration, and apoptosis by targeting signaling pathways, including the PI3K/AKT pathway, which is central to the control of cell physiology (41,42). Therefore, NPNT and its associated signaling pathways play a crucial role in regulating tumor cell behavior, making them potential targets for therapeutic intervention. However, its mechanism of action in GBM has not been clarified, and a relationship with cuproptosis has never been reported. In the ceRNA network, NPNT may form an integral part of a complex regulatory mechanism in cancer biology (43). In the case of GBM, understanding how ceRNA interactions regulate NPNT can help gain insights into tumor progression and potential therapeutic targets. For example, if a particular lncRNA is found to regulate NPNT expression through a ceRNA mechanism, targeting the lncRNA could be a strategy to control NPNT levels and, consequently, impact GBM progression. The abovementioned studies established a co-regulatory network of NPNT and non-coding RNA in GBM, providing a theoretical basis for targeted NPNT diagnosis and treatment.

## Conclusions

Herein, we discovered that LINC00957 serves as a ceRNA for miR-17-5p in GBM, thereby influencing the expression of the CRG NPNT. We first assessed copper ion levels and ROS, determining that NPNT inhibition enhances

cuproptosis. Further, enrichment analyses and phenotypic tests revealed that the LINC00957/miR-17-5p/NPNT axis impacts the GBM cell cycle and migration. From a mechanistic perspective, the findings of our reporter gene assays indicate that LINC00957 modulates NPNT expression by acting as a ceRNA for miR-17-5p. Our findings reveal a ceRNA network linked to cuproptosis in GBM, offering novel insights for GBM diagnosis and therapy.

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## Footnote

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