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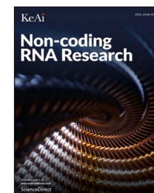

Original Research Article

Targeting LINC00665/miR-199b-5p/SERPINE1 axis to inhibit trastuzumab resistance and tumorigenesis of gastric cancer via PI3K/AKt pathway

Bingyu Wang, Wenbo Liu, Buyun Song, Yong Li, Yingying Wang, Bibo Tan *

The Third Department of Surgery, The Fourth Hospital of Hebei Medical University, 050017, Shijiazhuang, China

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A B S T R A C T

Long noncoding RNAs (lncRNAs) serve as critical mediators of tumor progression and drug resistance in cancer. Herein, we identified a lncRNA, LINC00665, associated with trastuzumab resistance and development in gastric cancer (GC). LINC00665 was highly expressed in GC tissues and high expression of LINC00665 was correlated with poor prognosis. LINC00665 knockdown was verified to suppress migration, invasion, and resistance to trastuzumab in GC. Furthermore, we found that LINC00665 participates in the infiltration of naive B cells, mast cells, and T follicular helper (Tfh) cells. Mechanistically, LINC00665 was confirmed to regulate tumorigenesis and trastuzumab resistance by activating PI3K/AKt pathway. LINC00665 sponged miR-199b-5p to interact with SERPINE1 expression, resulting in the increase of phosphorylation of AKt, thus participating in the PI3K/AKt pathway. To summarize, LINC00665 facilitated the tumorigenesis and trastuzumab resistance of GC by sponging miR-199b-5p and promoting SERPINE1 expression, which further activated PI3K/AKt signaling; this finding reveals a new mechanism by which LINC00665 modulates tumor development and drug resistance in GC.

1. Introduction

Gastric cancer (GC), the third leading cause of cancer death and the fifth most common malignancy in the world [1], is a major threat to human health. Although great progress has been made in diagnosis and therapeutics, the prognosis of GC patients remains unsatisfactory [2]. Chemotherapy combined with targeted therapy has been reported to effectively prolong overall survival and improve the outcomes of GC patients, especially patients with advanced stage disease [3]. Trastuzumab, a monoclonal antibody against HER2, is the first-line agent for targeted therapies. More than 20 % of gastric tumors harbor high expression of HER2 receptors [4]. However, the emergence of drug resistance eventually causes treatment failure and poor prognosis [5,6]. Therefore, exploring the mechanism of drug resistance and guiding the development of promising targets are imperative for GC treatment.

Long noncoding RNAs (lncRNAs), which are more than 200 nucleotides in length, lack the capacity to encode proteins [7–9]. Accumulating evidence has indicated that lncRNAs are involved in precise cancer tratement and targeted therapy [10,11]. For example, the lncRNA HOTAIR exerts its effect on cell growth, metastasis, and apoptosis in breast cancer through the miR-20a-5p/HMGA2 pathway [12]. Wu et al. found that lncRNA MEG3 is downregulated in prostate cancer and influences the development of prostate cancer [13]. Moreover, lncRNAs play a critical role in mediating drug resistance in diverse tumors [14–17]. LINC00665 has been identified as a key regulator that is overexpressed in breast cancer and induces the tumorigenesis of breast cancer [18]. Additionally, the increased expression level of LINC00665 in ovarian cancer regulates the proliferative, migratory and invasive functions of ovarian cancer cells and poor prognosis [19]. However, the role of LINC00665 in mediating progression and drug resistance in GC has not been well investigated, making it a research focus worthy of further exploration.

SERPINE1 (also known as PAI-1), a member of the serpin protease inhibitor superfamily, serves as an essential inhibitor of plasminogen activators and is associated with poor prognosis in many cancers [20–22]. Moreover, SERPINE1 plays a critical role in mediating drug resistance [23,24], which holds promise as a treatment strategy for GC. According to previous studies, SERPINE1 is closely related to the

Abbreviations: lncRNAs, Long noncoding RNAs; GC, gastric cancer; Tfh, T follicular helper; PI3K/AKt, phosphatidylinositol-3-kinase/protein kinase B; miRNAs, microRNAs; ceRNAs, competing endogenous RNAs; MUT, mutant; WT, wild type; DEGs, differentially expressed genes; OS, overall survival; EGFR, epidermal growth factor receptor; ASOs, antisense oligonucleotides.

* Corresponding author.
E-mail address: tanbibo@hebmu.edu.cn (B. Tan).

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phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKt) pathway, which is involved in diverse physiological and pathological processes [25]. The impact of SERPINE1 in GC, however, remains to be ascertained.

LncRNAs perform their regulatory function by sponging microRNAs (miRNAs) as competing endogenous RNAs (ceRNAs), thereby participating in several processes [26]. As a key factor in tumor onset and progression, inflammation has been confirmed to be associated with various types of cancers [27,28] and interestingly, LINC00665, miR-199b-5p, and SERPINE1 were all found to participate in inflammatory conditions [29–31]. Here, we found that LINC00665 is upregulated in GC and sponges miR-199b-5p to affect SERPINE1 levels, resulting in the induction of tumorigenesis and trastuzumab resistance in GC. Together with previous findings, we identified that this axis exerts its effect through the PI3K/AKt pathway. In conclusion, we revealed a novel molecular mechanism for trastuzumab resistance and tumor progression in GC, which may provide evidence to develop new therapeutic targets for GC patients with trastuzumab resistance.

2. Materials and methods

2.1. Clinical samples

A total of 30 paired GC tissues and adjacent normal tissues were collected from patients who were diagnosed with GC and underwent surgery at the Fourth Hospital of Hebei Medical University from April 2021 to July 2021, and informed consent was obtained from all patients. All samples were preserved in lipid nitrogen and sorted at −80 °C for further use. Two experts confirmed histological and pathological diagnosis. The study was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (approval number 2019ME0039) and was reported in accordance with the Declaration of Helsinki.

2.2. Prediction of downstream miRNAs and target genes

Downstream miRNAs of LINC00665 were predicted through the GEO database (http://www.ncbi.nlm.nih.gov/geo/) and miRcode database (https://cancergenome.nih.gov/) [32], and the target genes of hsa-miR-199b-5p were obtained from the TargetScan database (www.targetscan.org), DisGeNET database (https://www.disgenet.org/), and GEPIA database (http://gepia.cancer-pku.cn/index.html) [33]. The lncRNA–miRNA-mRNA network was constructed using the miRDB database (http://mirdb.org/) [34] and RNAhybrid database (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) [35].

2.3. Construction of the trastuzumab-resistant NCI-N87/TR and MKN-45/TR cell lines

The human gastric cancer cell line NCI-N87 and MKN-45 were purchased from the National Infrastructure of Cell Line Resource (NICR) and cultured in RPMI-1640 (Sigma, USA) supplemented with 10 % fetal bovine serum at 37 °C in 5 % CO_2. The induction of NCI-N87/TR cells was described previously [36]. NCI-N87 and MKN-45 cells in the exponential growth phase were seeded into 25 cm^2 culture bottles, and trastuzumab (12 μg/mL) was added during the mitotic phase. When the cells exhibited stable growth, the concentration of trastuzumab was gradually elevated until it reached 3500 μg/mL, which lasted for approximately 150 days. Trastuzumab-resistant NCI-N87/TR cells and MKN-45/TR cells were obtained and used in our study.

2.4. Transfection

The siRNA for LINC00665 was purchased from GenePharma Company (Shanghai, China), and transfection was performed using Lipofectamine 2000 (Invitrogen). 60 nmol/L LINC00665-siRNA was chosen to perform further transfection. The miR-199b-5p mimic/inhibitor and NC mimics/inhibitor were purchased from GenePharma Company (Shanghai, China). LINC00665-shRNAs and NC-shRNAs were purchased from GenePharma Company (Shanghai, China) for knockdown of LINC00665 using Lipofectamine 2000 (Invitrogen, USA). Then, a plasmid including psPAX2, pMD2G, and pcDNA3.1/LINC00665 was cotransfected into 293T cells to generate lentivirus. Lentivirus was harvested after 48 h of transfection.

2.5. qRT–PCR

Total RNA was extracted using TRIzol method (Invitrogen, USA) according to manufacturer's instructions from either GC tissues or cells. RNA was reversed-transcribed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). qRT-PCR was carried out through Quantstudio™ DX system (Applied Biosystems, Singapore). The relative amounts of transcript were calculated with $2^{-\Delta\Delta CT}$ method relative to U6. The primer sequences were listed in Table 1.

2.6. Cell proliferation and trastuzumab sensitivity assays

CCK8 assays were performed to assess cell proliferation rates. Cells that were cultured in 96-well plates at 37 °C for 24, 48, and 72 h in an incubator containing 5 % CO_2 were treated with different concentrations of trastuzumab. Then, 10 μL CCK8 reagent (APExBIO, USA) was added at different time points. Absorbance at 450 nm was recorded and calculated with GraphPad Prism 8.0 Software.

2.7. Wound healing and Transwell assays

Wound healing and Transwell assays were conducted as previously described [37].

2.8. Luciferase reporter assay

The 3′ UTRs of LINC00665 and SERPINE1 were inserted into a pGL3 vector (Promega, USA). The luciferase reporter vectors pGL3-LINC00665-3′ UTR mutant (LINC00665-MUT) and pGL3-SERPINE1-3′UTR mutant (SERPINE1-MUT) were then synthesized at the same position. GC cells were cotransfected with pGL3-LINC00665-3′ UTR wild type (LINC00665-WT) or LINC00665-MUT and miR-199b-5p mimic or control mimic by Lipofectamine 2000. Meanwhile, pGL3-SERPINE1-3' UTR wild type (SERPINE1-WT) or SERPINE1-MUT and miR-199b-5p mimic or control mimic were transfected into GC cells. The transfected cells were harvested 36 h after transfection, and the luciferase activity was detected using the Dual-Luciferase Reporter assay system (Promega, USA).

2.9. Western blot

Proteins extracted from tissues and cells were separated by SDS-polyacrylamide gels and transferred onto PVDF membranes (Millipore, USA). Then the membranes were treated with TBST at room temperature for 1 h and incubated with primary antibodies overnight at 4 °C. After washing with TBST, the membranes were incubated with secondary

Table 1
List for primers sequences used for qRT-PCR.

qRT-PCR primiers	sequences
LINC00665 forward	TGCTGGGATTACAGGTCCA
LINC00665 reverse	TCTGGTCTTCAGGTCTCCTCAC
SERPINE1 forward	ACTTCTTCAGGCTGTTCCG
SERPINE1 reverse	TTGTGTGTGTCTTCACCCAG
miR-199b-5p forward	CCCAGTGTTCAGACTACCTGTT
miR-199b-5p versus	GTCGTATCCAGTGCAGGGT
U6 forward	GAAACACCGTGCTCGCTT
U6 reverse	TGCTAATCTTCTCTGTATCGTTCC

antibodies for 2 h at room temperature and the signals were visualized via Odyssey infrared scanner (Lincoln, USA).

2.10. Animal study

4–6 weeks old BALB/c nude mice were purchased from SPF Biotechnology (Beijing, China) and randomly dived into two groups (n = 3). We implanted male BALB/c nude mice with NCI-N87/TR cells infected with LINC00665-shRNA or NC-shRNA. After two weeks, tumor volumes were measured and calculated using Equation $V = 0.5 \times length \times width^2$ every 4 days. After 24 days, mice were euthanized through injection of amobarbital, and xenograft tumors were excised and weighted. All animal experiments were conducted at the Experimental Animal Center of the Fourth Hospital of Hebei Medical University according to the ARRIVE guidelines and approved by the Committee on the Ethics of Animal Experiments of Hebei Medical University (approval number IACUC-4th Hos Hebmu-2022256).

2.11. Statistical analysis

The experimental results were analyzed by Student's *t*-test (unpaired, two tailed) and one-way ANOVA. The data conformed to normal distribution and are presented as the mean ± standard deviation (SD). $P < 0.05$ was regarded to indicate statistical significance. All statistical analyses were performed using GraphPad Prism 8.0 Software. Biological triplicate samples were required for each experiment.

3. Results

3.1. Construction of the LINC00665/miR-199b-5p/SERPINE1 axis

To identify the regulatory axis LINC00665/miR-199b-5p/SERPINE1, we conducted a series of studies, as shown in Fig. 1A. We obtained GC-related genes from GSE95667 and GSE109476 in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) and carried out principal component analysis (PCA) to analyze the overall differences between the two different gene datasets (Fig. 1B and C). Then, we utilized the package "limma" and set the cutoff as $P < 0.05$ and $|logFC| \geq 1$ to extract 1250 differentially expressed genes (DEGs) in GSE95667 and 1636 DEGs in GSE109476 (Fig. 1C, D and Supplementary Tables 1 and 2). We took the intersection of the 1250 DEGs in GSE95667 and 1636 DEGs in GSE109476 and obtained 203 genes (Fig. 1E and Supplementary Table 3), including 25 lncRNAs (Supplementary Table 4). As mentioned earlier, we obtained 114 gastric cancer-related miRNAs from GSE93415 (Supplementary Table 5) and 113 miRNAs from GSE78091 (Supplementary Table 6) in the GEO database and took the intersection to obtain 27 miRNAs (Fig. 1F and Supplementary Table 7).

To further explore the ceRNA network, the miRcode database (https://cancergenome.nih.gov/) was utilized to predict the interaction between the 114 GC-related lncRNAs and 1113 GC-related miRNAs, which indicated the underlying interaction between 13 lncRNAs and 14 miRNAs (Fig. 1G and Supplementary Table 8). Next, we used the TargetScan database (www.targetscan.org) to predict the downstream genes of the 14 miRNAs and obtained 8751 genes (Supplementary Table 9). Furthermore, a total of 3720 genes associated with GC targets (Supplementary Table 10) from the DisGeNET database (https://www.disgenet.org/) and the 50 most differentially expressed survival genes (Supplementary Table 11) from the GEPIA database (http://gepia.cancer-pku.cn/index.html) intersected with the former 8751 genes, thereby obtaining 7 genes (Fig. 1H): SERPINE1, ERBB4, NRP1, SLC52A3, DYNC1I1, AKR1B1 and RAI14. Among them, we found that SERPINE1 was closely related to the prognosis of GC (Fig. 1I); thus, we speculated that SERPINE1 was a core factor in GC progression.

Furthermore, we used the miRDB database (http://mirdb.org/) to identify the upstream miRNAs of SERPINE1 and obtained 118 miRNAs (Supplementary Table 12) that intersected with the 14 miRNAs mentioned before, which ultimately resulted in 3 miRNAs (Fig. 1J): miR-199a-5p, miR-199b-5p and miR-19a-3p. The subsequent analysis of the RNAhybrid database (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid) proved that miR-199b-5p was most likely to bind to SERPINE1 (Fig. 1K). As shown in Figs. 1G and 7 lncRNAs were linked to miR-199b-5p: FTX, LINC01133, LINC00665, LINC00486, SNHG20, LINC00378 and LINC00184. Among them, LINC00665 has been verified to be an oncogene in diverse cancers and to participate in resistance to cisplatin [37] and apatinib [38]. In total, these findings demonstrated the existence of the LINC00665/miR-199b-5p/SERPINE1 axis.

3.2. LINC00665 is overexpressed in GC and participates in drug resistance

We first analyzed the expression of LINC00665 in GC from the GEPIA database (http://gepia.cancer-pku.cn/index.html), and our results demonstrated that LINC00665 was upregulated in GC (Fig. 2A). To further explore the role of LINC00665 in GC, we detected LINC00665 levels in tumor and adjacent normal tissues through qRT−PCR assays, which indicated that LINC00665 was highly expressed in GC (Fig. 2B). According to LINC00665 expression, we grouped TCGA data (http://cancergenome.nih.gov/) into two groups and identified 300 DEGs (Supplementary Table 13). GO and KEGG analyses showed that these DEGs were associated with various processes (Fig. 2C and D). Subsequently, further BP enrichment showed that those DEGs were enriched in the drug response (Fig. 2E). Additionally, through the Cibersort database (https://cibersortx.stanford.edu/), we found that LINC00665 was positively related to the infiltration of naive B cells and Tfh cells but negatively correlated with mast cell infiltration (Fig. 2F, G, H), which may be a potential mechanism underlying GC development [39]. Moreover, we grouped TCGA data on the basis of SERPINE1 level into two groups and obtained 225 DEGs. Further KEGG analysis showed that these DEGs were involved in the PI3K/AKt pathway, JAK/STAT pathway, and IL17 signaling pathway (Fig. 2I). It is worth noting that the PI3K/AKt pathway plays a crucial role in mediating resistance to trastuzumab [40]. Thus, we speculated that LINC00665 participated in trastuzumab resistance by targeting SERINE1 through the PI3K/AKt pathway.

3.3. LINC00665 sponges miR-199b-5p and upregulates SERPINE1 expression

In the above results, we confirmed the existence of the LINC00665/miR-199b-5p/SERPINE1 axis. However, we did not find sufficient evidence to verify the regulatory relationship among them. As presented in Fig. 2A, LINC00665 was overexpressed in GC, and we detected the levels of miR-199b-5p and SERPINE1 in GC tissues and adjacent normal tissues. As revealed by qRT−PCR assay (Fig. 3A and B), miR-199b-5p was downregulated in GC tissues, while the expression of SERPINE1 in GC tissues was higher than that in adjacent normal tissues. Using the RNAhybrid database (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid), we identified the potential binding sites of LINC00665 and miR-199b-5p (Fig. 3C), and miR-199b-5p targeted SERPINE1, as demonstrated in Fig. 1K. Correlation analysis demonstrated that LINC00665 was positively associated with SERPINE1 but negatively associated with miR-199b-5p (Fig. 3D and E). In the luciferase assay, we found that miR-199b-5p was the downstream target of LINC00665 in GC. LINC00665-WT but not LINC00665-MUT suppressed the 3' UTR reporter gene activity of miR-199b-5p (Fig. 3F). In addition, miR-199b-5p inhibited SERPINE1-WT but not SERPINE1-MUT, indicating that miR-199b-5p was the upstream gene of SERPINE1 (Fig. 3G).

3.4. LINC00665 induces GC proliferation, migration, and invasion via SERPINE1 through the PI3K/AKt pathway

Subsequently, we investigated the function of LINC00665 in GC. The migration and invasion abilities of GC cells were obviously undermined

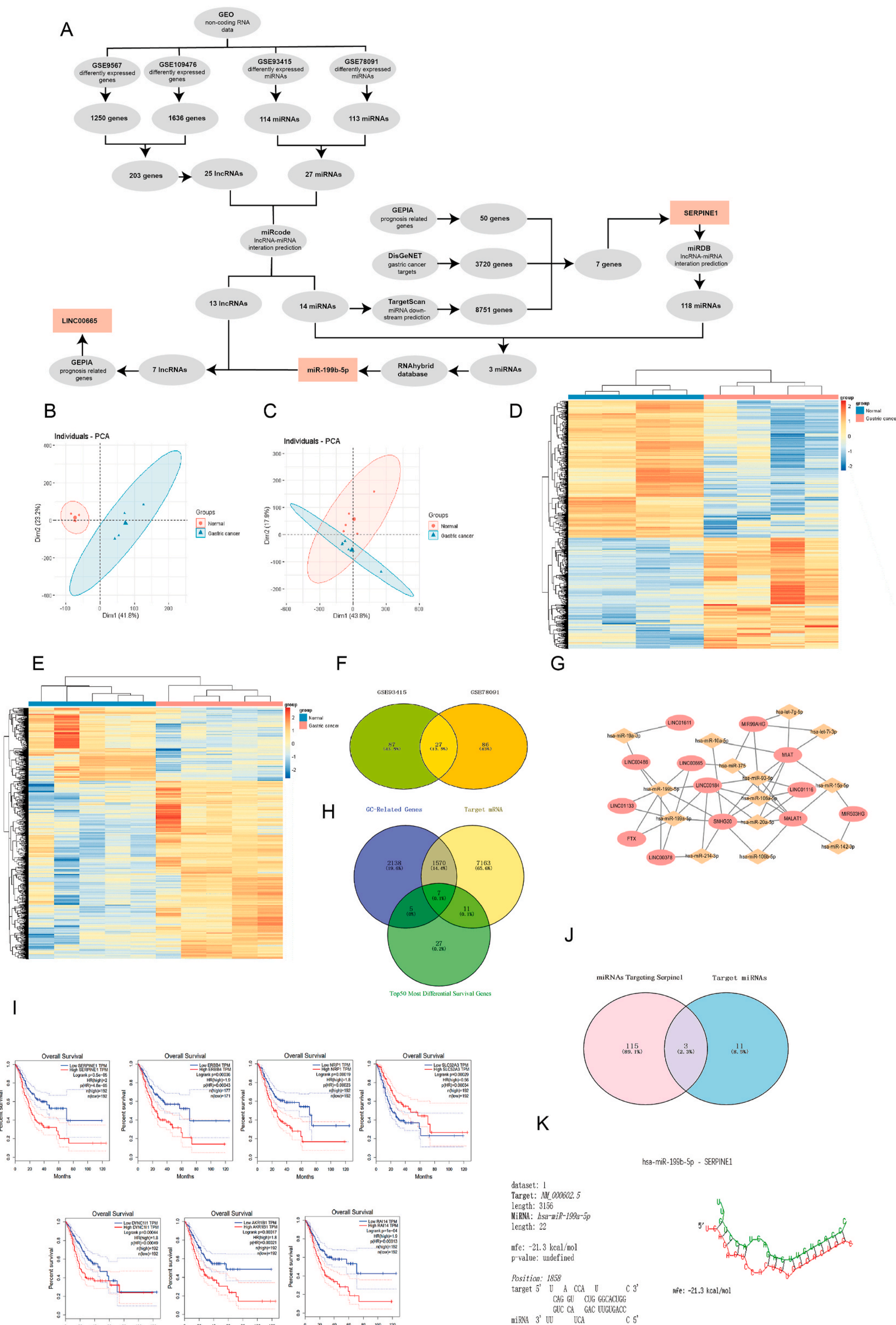

Fig. 1. Identification of LINC00665/miR-199b-5p/SERPINE1 pathway. **A** Schematic diagram of the process design. **B** PCA shows overall differences between normal tissue and GC in GSE95667. **C** PCA shows overall differences between normal tissue and GC in GSE109476. **D** Heatmap of DEGs from GSE95667. **E** Heatmap of DEGs from GSE109476. **F** Venn diagram of common DEGs from GSE109476 and GSE95667. **G** The lncRNA-miRNA network predicted by the miRcode database. **H** Venn diagram of target genes from GC-related genes, target mRNA, and TOP 50 most differential survival genes. **I** The correlation between the expression of target genes and overall survival rate of GC patients: SERPINE1 is negatively related to the overall survival rate of GC patients. **J** Venn diagram of upstream gene of SERPINE1 from the TargetScan and miRDB databases. **K** The potential binding site of SERPINE1 predicted by the RNAhybrid database.

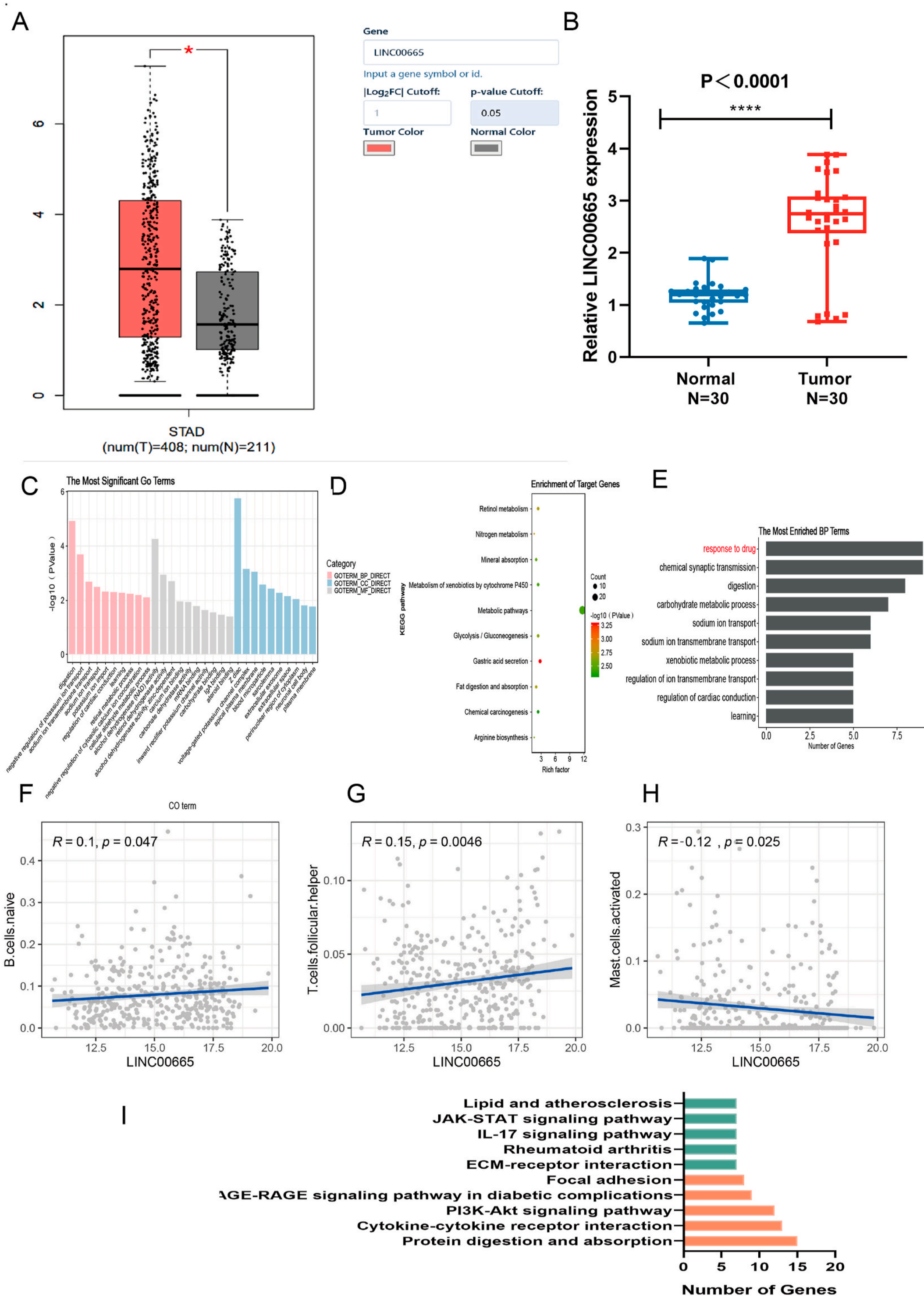

Fig. 2. LINC00665 is upregulated in GC tissues. A LINC00665 is overexpressed in GC tissues analyzed by the GEPIA-STAD database. **B** The mRNA level of LINC00665 is higher in paired GC tumor (n = 30) than that in adjacent normal tissues (n = 30). **C** GO analysis shows that LINC00665 participated in multiply processes (Biological Process, BP; Cellular Component, CC; Molecular Function, MF). **D** KEGG analysis indicates LINC00665 exerted its function in GC tumorgenesis via various pathways. **E** Top 10 BP enrichment suggested that LINC00665 is involved in the drug resistance. **F-H** Correlation between the expression level of LINC00665 And the infiltration levels of naive B cells, Tfh cells, and mast cells. I KEGG analysis shows that SERPINE1 was involved in diverse pathways. *P < 0.05; ****P < 0.0001.

by silencing LINC00665 (Fig. 4A). PCNA and p16 are cell proliferation-related proteins, while MMP-2, E-cadherin, and N-cadherin are proteins related to cell migration and invasion. Furthermore, WB assays demonstrated that LINC00665 knockdown increased the protein levels of p16 and E-cadherin but decreased the protein levels of PCNA, MMP-2, N-cadherin, Vimentin, SERPINE1, and *p*-AKt (Fig. 4B). Additionally, in the next experiment, we explored whether LINC00665 could mediate GC progression through miR-199b-5p. We divided the cells into four groups: the control, NC-siRNA, LINC00665-siRNA, and LINC00665-siRNA combined with miR-199b-5p inhibitor groups. Downregulation of

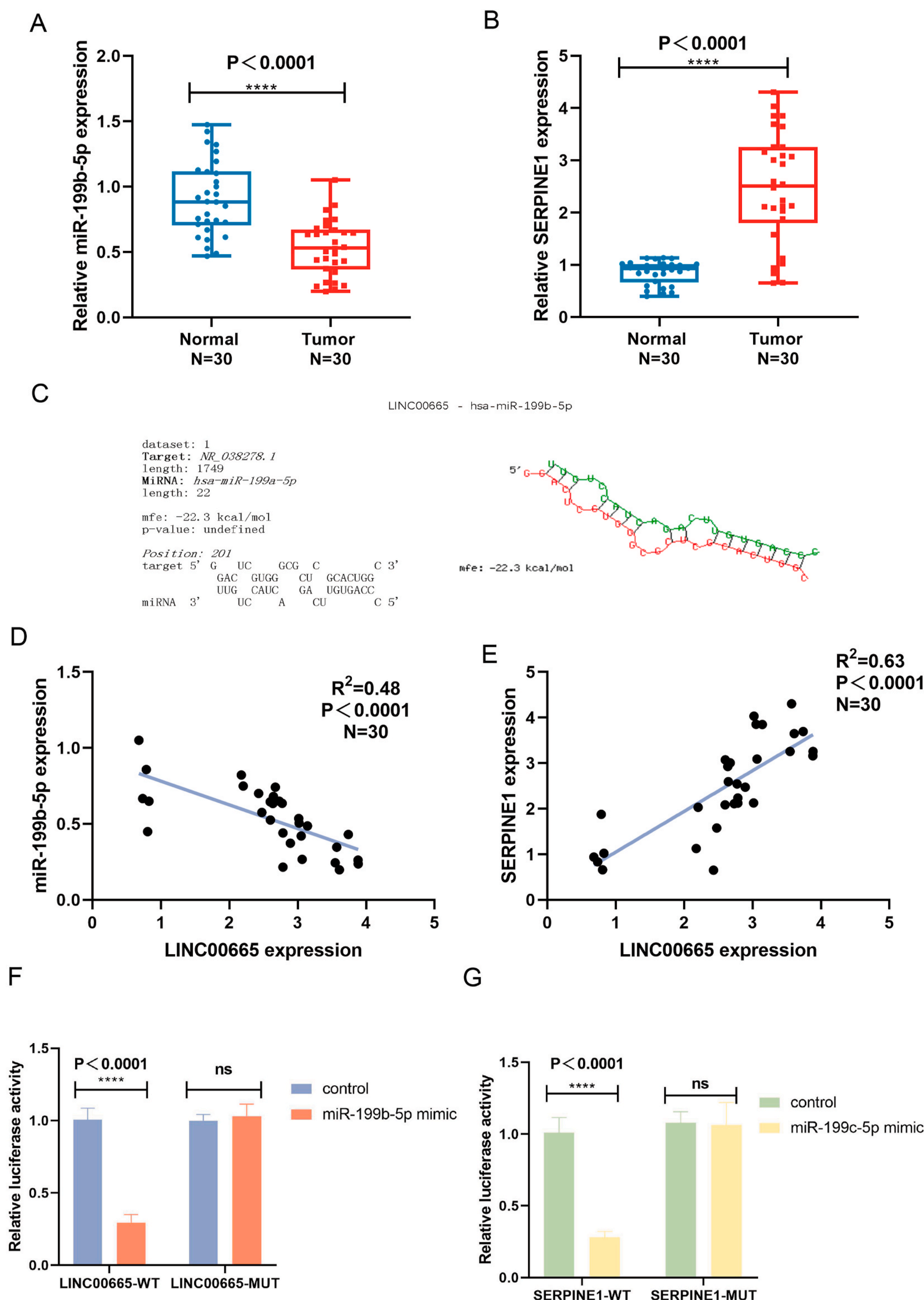

Fig. 3. LINC00665 targets miR-199b-5p to induce SERPINE1 level. A The level of miR-199b-5p is lower in paired GC tumor (n = 30) than that in adjacent normal tissues (n = 30). **B** SERPINE1 is highly-expressed in GC tissues. **C** The biding site of LINC00665 and miR-199b-5p predicted by the RNAhybrid database. **D** Correlation of the expression of LINC00665 and miR-199b-5p in clinical GC tissues. **E** Correlation of the expression of LINC00665 and SERPINE1 in clinical GC tissues. **F** A dual-luciferase reporter assay was performed to verify that LINC00665 obviously inhibits the luciferase activity of the WT 3′-UTR but not the MUT 3′-UTR of miR-199b-5p. **G** A dual-luciferase reporter assay was conducted to prove that miR-199b-5p significantly suppresses the luciferase activity of the WT 3′-UTR but not the MUT 3′-UTR of SERPINE1. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

LINC00665 facilitated the inhibition of cell proliferation, migration and invasion. Importantly, the suppressive effect of LINC00665 inhibition was dramatically mitigated by the miR-199b-5p inhibitor (Fig. 4C, D, E). Subsequent WB assays also verified that the regulatory effect of LINC00665 on the related proteins mentioned earlier was strikingly eliminated by the miR-199b-5p inhibitor (Fig. 4F). Overall, our results suggested that LINC00665 sponged miR-199b-5p to elevate SERPINE1 expression and affect PI3K/AKt signaling, resulting in the promotion of the proliferation, migration, and invasion of GC cells.

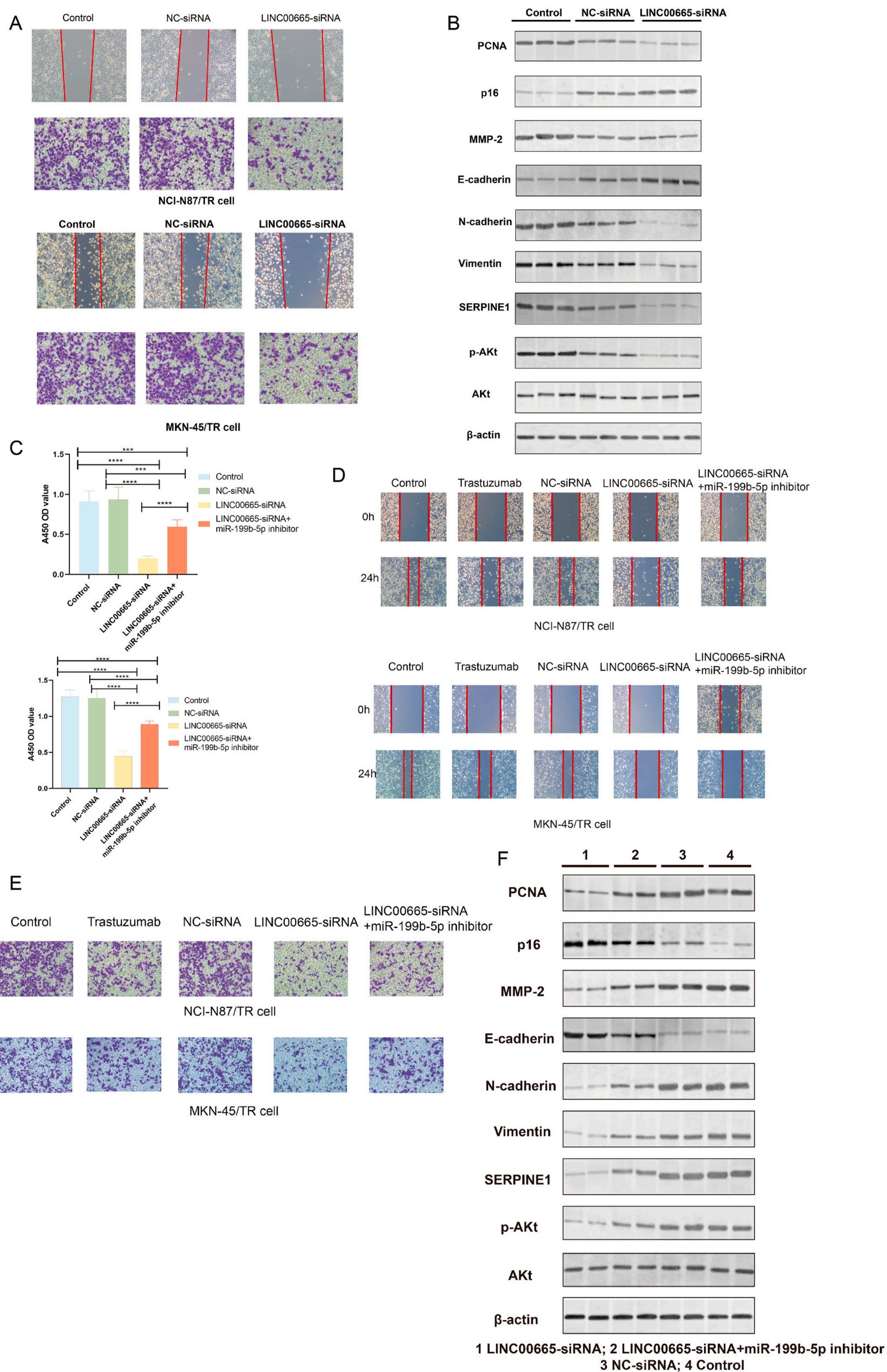

Fig. 4. LINC00665 contributed to the tumorgenesis of GC via SERPINE1. A Scratch assay (scale bar, 200 μm) and Transwell assay (scale bar, 150 μm) evaluate the migration and invasion ability of GC cells with LINC00665 knockdown. **B** WB assay show expression of tumour progression-related proteins (PCNA, p16, MMP-2, E-cadherin, N-cadherin, and Vimentin) and PI3K/AKt signaling-associated proteins (*p*-AKt and AKt) in GC cells with LINC00665 knockdown or NC-siRNA or control. **C** CCK-8 assay demonstrated miR-199b-5p inhibitor restore cell proliferation in LINC00665 knockdown cells. **D** Scratch assay indicated miR-199b-5p inhibitor restored cell migration ability in LINC00665 knockdown cells. Scale bar, 200 μm. **E** Transwell assay showed that miR-199b-5p inhibitor restore cell invasion capacity in LINC00665 knockdown cells. Scale bar, 150 μm. **F** WB assay revealed the expression of proteins mentioned above in cells transfected as indicated. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

3.5. LINC00665 enhances the trastuzumab resistance of GC cells via SERPINE1 through the PI3K/AKt pathway

To assess how LINC00665 exerts its effect on trastuzumab resistance in GC, trastuzumab resistance assays were carried out in the NC-shRNA, trastuzumab, NC-shRNA + trastuzumab, LINC00665-shRNA, and LINC00665-shRNA + trastuzumab groups. The results showed that the sensitivity to trastuzumab was significantly reduced in cells treated with LINC00665-shRNA compared to that of cells in other groups (Fig. 5A). Similar results were obtained from the tests in BALB/c nude mice. As presented in Fig. 5B, the growth rate of subcutaneously transplanted tumors in the LINC00665-shRNA group was distinctly lower than that in the NC-shRNA group. The volume and weight of subcutaneously transplanted tumors in the LINC00665-shRNA group were also lower than those in the NC-shRNA group (Fig. 5C and D). Moreover, compared to the NC-shRNA group, silencing LINC00665 reduced the protein levels of PCNA, MMP-2, SERPINE1, and *p*-AKt while increasing the level of p16 (Fig. 5E). Overall, these findings proved that LINC00665 contributed to trastuzumab resistance in GC by affecting SERPINE1 levels to upregulate *p*-AKt expression in the PI3K/AKt pathway.

4. Discussion

In GC patients, aggressive characteristics and drug resistance severely exacerbate poor outcomes. It has been reported that the average overall survival (OS) after chemotherapy is 7.5–12.0 months [41]; therefore, molecular targeted therapy has gained widespread attention in recent years. HER2, which belongs to the epidermal growth factor receptor (EGFR) family, is the most widely used in the clinic and performs its function through heterodimer and tyrosine kinase

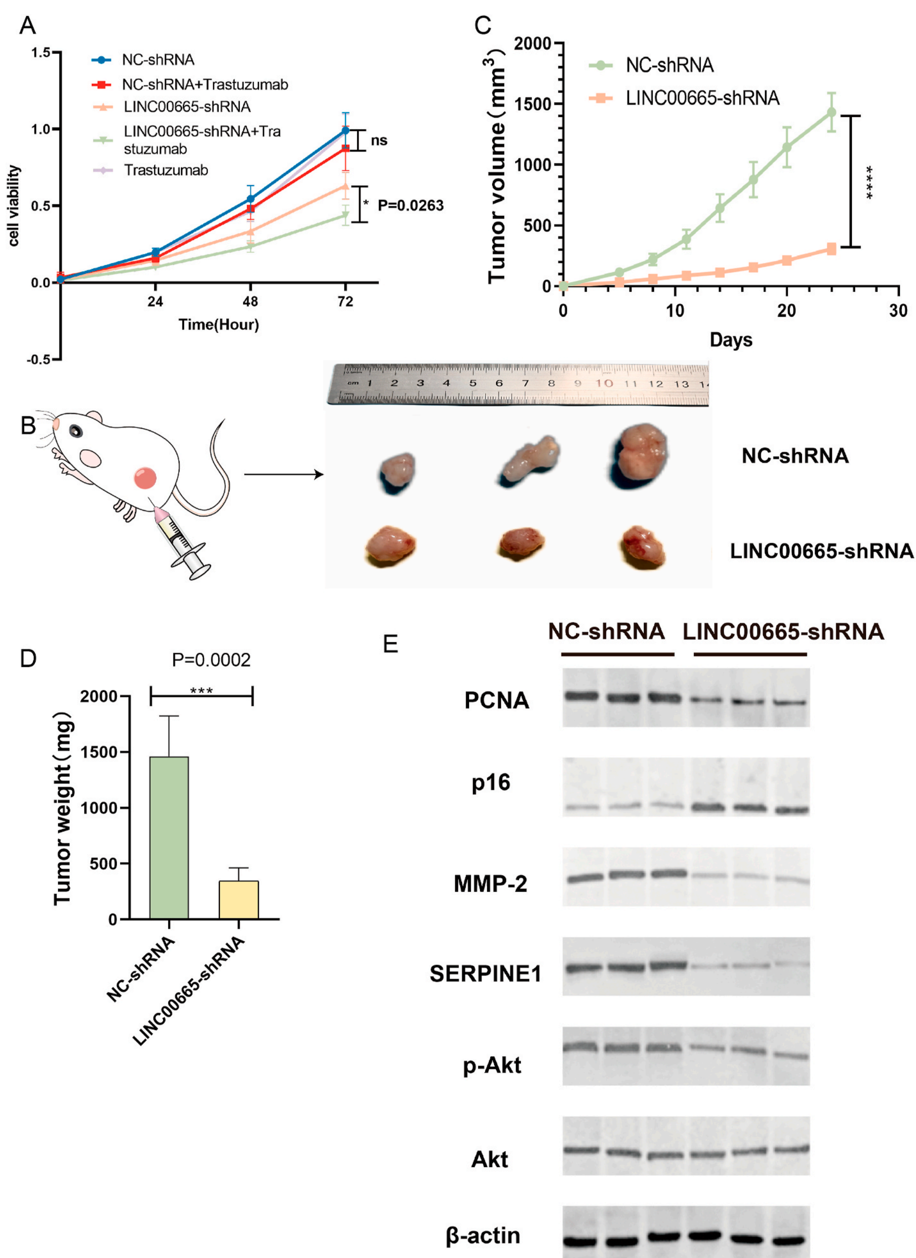

Fig. 5. LINC00665 promotes trastuzumab resistance through PI3K/AKt pathway. A The cell viability of cells treated with trastuzumab with or without LINC00665-shRNA. **B** Schematic diagram of subcutaneous xenograft model and representative images of tumors from nude mice inoculated with NCI-N87/TR cells. **C** The volume of subcutaneously transplanted tumors in 2 groups: LINC00665-shRNA and NC-shRNA. **D** The weight of subcutaneously transplanted tumors in the 2 groups. **E** The protein level of the subcutaneously transplanted tumors in the 2 groups. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

autophosphorylation, thus ultimately influencing signal transduction [42,43]. Hence, it is of great importance to reverse resistance to targeted therapy and develop a corresponding therapeutic strategy. Here, we identified a potential mechanism targeting trastuzumab resistance: the LINC00665/miR-199b-5p/SERPINE1 axis regulated drug resistance and tumorigenesis of GC through PI3K/AKt signaling.

We found that LINC00665 was overexpressed and positively correlated with naive B-cell and Tfh cell infiltration but exhibited a negative correlation with mast cell infiltration. Mounting evidence suggests that LINC00665 has miRNA binding capacity and functions through the ceRNA network [44,45]. Silencing LINC00665 restricted trastuzumab resistance, cell proliferation, migration, and invasion in GC; however, silencing LINC00665 in combination with miR-199b-5p significantly undermined this suppressive effect. At the molecular level, we identified that LINC00665 could bind to miR-199b-5p as a sponge to repress its expression and therefore promote the expression of SERPINE1. Subsequently, LINC00665 knockdown reduced the protein level of p-AKt, the key factor in PI3K/AKt signaling, which was previously reported to affect p-AKt expression.

Although we explored the function of LINC00665 in GC, a major limitation of this study is the lack of the combination treatment with LINC00665 knockdown and trastuzumab to further prove the efficacy of inhibiting LINC00665 on trastuzumab. Moreover, the molecular mechanism how LINC00665 influenced immune infiltration was still unclear. Whether LINC00665 could be involved in GC immunity by regulating immune infiltration remains to be further investigated.

5. Conclusion

In conclusion, we demonstrated that LINC00665 induced tumorigenesis and trastuzumab resistance through LINC00665/miR-199b-5p/SERPINE1 signaling. Thus far, no agent targeting lncRNAs has been successfully applied for the clinical treatment of GC; however, the interventions as antisense oligonucleotides (ASOs) and siRNA that retard or degrade lncRNAs molecules make exploiting lncRNAs possible. Our research sheds light on the theoretical basis for discovering novel agents as the small-molecule inhibitor of LINC00665. It is our novel study that LINC00665 facilitated tumor progression and targeted therapy resistance via functioning as a ceRNA against miR-199b-5p and promoting SERPINE1 expression, which led to trastuzumab resistance driven by PI3K/AKt pathway. The discovery indicated LINC00665 as a candidate treatment target to overcome trastuzumab resistance in the future.

Ethics approval and consent to participate

All patients provided consent and the study was approved by our ethics committee of the Fourth Hospital of Hebei Medical University (approval number 2019ME0039). All animal studies described herein were approved by our institutional animal ethics committee of Hebei Medical University (approval number IACUC-4th Hos Hebmu-2022256). The study was reported in accordance with ARRIVE guidelines.

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Data availability statement

The datasets generated and analyzed during the current study are available in the GEO repository (http://www.ncbi.nlm.nih.gov/geo/), miRcode repository (https://cancergenome.nih.gov/), TargetScan repository (www.targetscan.org), DisGeNET repository (https://www.disgenet.org/), GEPIA repository (http://gepia.cancer-pku.cn/index.html), miRDB repository (http://mirdb.org/), RNAhybrid repository (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid), TCGA repository (http://cancergenome.nih.gov/), Cibersort repository (https://cibersortx.stanford.edu/).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Bingyu Wang: Writing – original draft, Methodology. **Wenbo Liu:** Resources, Data curation. **Buyun Song:** Software. **Yong Li:** Supervision, Funding acquisition. **Yingying Wang:** Data curation. **Bibo Tan:** Supervision, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2024.07.004.

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