

Original Article

LncRNA DNAJC3-AS1 promotes gastric cancer malignancy through miR-576-5p-mediated upregulation of LYPLA1

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Abstract: Long non-coding RNAs (lncRNAs) are involved in tumor progression, but the role of lnc-DNAJC3-AS1 in gastric cancer (GC) remains unclear. This study aimed to investigate the biological function and regulatory mechanism of lnc-DNAJC3-AS1 in GC. Reverse transcription quantitative PCR (RT-qPCR) was used to detect the expression levels of lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1, and Western blot was used to analyze protein expression. The Cancer Genome Atlas (TCGA) database and clinical samples were used to evaluate their clinical relevance. Cell viability, cell cycle distribution, apoptosis, migration, and invasion were assessed using Cell Counting Kit-8 (CCK-8), flow cytometry, wound-healing, Transwell, and immunofluorescence assays. Dual-luciferase reporter assay, RNA immunoprecipitation (RIP), fluorescence in situ hybridization (FISH), and rescue assays were performed to explore the potential regulatory relationship among lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1. A xenograft tumor model was also established to evaluate the role of lnc-DNAJC3-AS1 in vivo. The results showed that lnc-DNAJC3-AS1 and LYPLA1 were upregulated, whereas miR-576-5p was downregulated in GC tissues and cells. Knockdown of lnc-DNAJC3-AS1 inhibited GC cell viability, migration, and invasion, induced G0/G1 phase arrest and apoptosis, and suppressed tumor growth in vivo. Mechanistically, lnc-DNAJC3-AS1 was mainly localized in the cytoplasm and was associated with miR-576-5p-related RNA-induced silencing complex (RISC) complexes. MiR-576-5p targeted LYPLA1, and restoration of miR-576-5p or knockdown of LYPLA1 partially attenuated the effects of lnc-DNAJC3-AS1 overexpression on GC cell phenotypes and LYPLA1 enzymatic activity. These findings suggest that lnc-DNAJC3-AS1 promotes GC progression, at least in part, through the miR-576-5p/LYPLA1 pathway, providing a potential target for GC treatment.

Keywords: Gastric cancer, lnc-DNAJC3-AS1/miR-576-5p/LYPLA1 axis, migration, proliferation, apoptosis

Introduction

Gastric cancer (GC) is a frequently diagnosed malignancy of the gastrointestinal tract and is associated with a heavy global disease burden [1, 2]. Over the past few years, the management of GC has been improved by the development of surgery, chemotherapy, targeted agents, and immunotherapy [3, 4]. However, early-stage GC often lacks obvious symptoms, many patients are not diagnosed until the disease has already progressed substantially [5]. Adding to the challenge, recurrence and metas-

tasis are common during GC progression, which further worsens patient prognosis [6]. Therefore, identifying new treatment targets may help improve GC management.

Long non-coding RNAs (lncRNAs) regulate gene expression by interacting with DNA, RNA, or proteins [7]. In cancer, lncRNAs contribute to tumor cell growth and malignant progression [8-10]. They can promote tumor development through various mechanisms. One well-studied mechanism is the competing endogenous RNA (ceRNA) pathway, in which lncRNAs act as

molecular sponges that bind to specific microRNAs (miRNAs). This binding relieves miRNA-mediated silencing of downstream targets, thus modulating the malignant behavior of tumor cells [11-13]. In various malignancies, Lnc-DNAJC3-AS1 has recently emerged as a tumor-promoting lncRNA, where its expression is abnormally elevated and drives tumor progression [14-16]. For example, one study showed that lnc-DNAJC3-AS1 promotes cell proliferation in hepatocellular carcinoma by binding to miR-27b [17]. Another study reported that it exerts oncogenic effects in clear cell renal cell carcinoma through the miR-27a-3p/PRDM14 axis [18]. Additionally, high expression of lnc-DNAJC3-AS1 has been found in breast cancer tissues and is linked to poor patient prognosis [19]. MiR-576-5p plays a role as an anti-tumor factor in certain cancers, as reported in previous studies [20, 21]. By targeting various oncogenes, it suppresses tumor cell proliferation and metastasis [22].

Lysophospholipase 1 (LYPLA1) belongs to the fatty acyl-thioesterase (APT) family. It is involved in lipid metabolism, where it catalyzes the conversion of lysophosphatidylcholine into fatty acids and glycerophosphatidylcholine [23]. Beyond its role in lipid metabolism, LYPLA1 also possesses depalmitoylation activity, allowing it to remove palmitoyl groups from proteins, which in turn affects protein localization, stability, and intracellular signaling [24, 25]. It participates in cell signaling, cell membrane integrity, and cell proliferation [26]. Not surprisingly, abnormal expression of LYPLA1 is thought to contribute to the progression of a range of malignancies [27, 28]. Specifically, LYPLA1 is abnormally expressed in cervical cancer and non-small cell lung cancer, where it promotes tumor development [29]. Whether miR-576-5p targets LYPLA1 to drive GC progression remains unresolved. Similarly, the role of the lnc-DNAJC3-AS1/miR-576-5p/LYPLA1 regulatory axis in GC awaits further elucidation.

Here, we investigated lnc-DNAJC3-AS1 expression and its clinical relevance in GC, suggested that it upregulates LYPLA1 expression in association miR-576-5p, and evaluated the effects of this regulatory axis on GC cell growth and motility.

Materials and methods

Clinical samples and ethical statement

Paired tumor and non-cancerous tissues were collected from 50 surgically treated GC patients at Qingdao Municipal Hospital. Some samples were used directly for experiments. The rest were frozen in liquid nitrogen and stored at -80°C. Ethical approval was obtained from the Institutional Research Human or Animal Ethics Committee of Qingdao Municipal Hospital (Approval No. 2023-092). All procedures followed the Declaration of Helsinki and relevant regulations. Informed consent was obtained from every patient.

Bioinformatics analysis

Expression data of lnc-DNAJC3-AS1 and LYPLA1 were retrieved from the stomach adenocarcinoma cohort of TCGA (TCGA-STAD). This dataset includes 174 tumor samples and 36 normal gastric tissue samples, allowing comparison of expression differences between the two groups. Wilcoxon rank-sum test compared lnc-DNAJC3-AS1 and LYPLA1 expression between GC and adjacent normal tissues. A nomogram was constructed using LYPLA1 and clinical factors. Nomogram analysis estimated 1-, 3-, and 5-year OS probability. R software (v 4.4.1) with survival (v 3.8.3) and survminer (v 0.5.0) performed all analyses.

Cell lines

GC cell lines (HGC-27 and NCI-N87) and the normal gastric epithelial cell line (GES-1) were obtained from Wuhan Puricella Biotechnology Co., Ltd. STR authentication and mycoplasma-free status were confirmed for all lines. Cells were cultured in RPMI 1640 medium (C2721) with 10% fetal bovine serum (C0226) and 1% penicillin-streptomycin (C0222) at 37°C in 5% CO₂, with regular medium changes and passaging. All culture reagents were purchased from Bio-Techne (Shanghai, China).

Cell transfection

The shRNA construct targeting lnc-DNAJC3-AS1 (sh-DNAJC3-AS1) and its negative control (sh-NC) were obtained from Invitrogen (Thermo Fisher Scientific). Sangon Biotech Co., Ltd. supplied the miR-576-5p mimic, mimic-NC

Table 1. RT-qPCR primers

Gene Name	Primer sequence (5'-3')
<i>Inc-DNAJC3-AS1</i>	Forward: 5'-AGCGATTGTGGAAGACCCT-3' Reverse: 5'-TTTCCCCTGGTAAGCGCAA-3'
<i>miR-576-5p</i>	Forward: 5'-GCGCGATTCTAATTCTCCAC-3' Reverse: 5'-AGTGCAGGGTCCGAGGTATT-3'
<i>LYPLA1</i>	Forward: 5'-ACGTCTGTGGAGGGAGTACA-3' Reverse: 5'-TCCACCTTAAAGTAATTGCGCT-3'
<i>GAPDH</i>	Forward: 5'-AAAGGGTCATCATCTCTG-3' Reverse: 5'-GCTGTTGTCATACTTCTC-3'
<i>U6</i>	Forward: 5'-CTCGCTTCGGCAGCACATA-3' Reverse: 5'-AACGATTCACGAATTGCGT-3'

(NC), si-LYPLA1. We constructed pcDNA-DNAJC3-AS1 by inserting the entire DNAJC3-AS1 sequence into the pcDNA3.1 vector. The indicated plasmids or oligonucleotides were transfected into HGC-27 and NCI-N87 cells using Lipofectamine 3000 (Invitrogen, USA). In the reverse rescue experiments, DNAJC3-AS1-OE was co-transfected with either the miR-576-5p mimic or si-LYPLA1. Forty-eight hours after cell transfection, intracellular RNA and protein expression levels were detected using RT-qPCR and Western blot, respectively, to verify transfection efficiency.

Real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted with TRIzol reagent (Invitrogen, USA). We used the PrimeScript RT Reagent Kit and the Mir-X miRNA First-Strand Synthesis Kit (both from TaKaRa, Japan) for reverse transcription of mRNA/lncRNA and miRNA, respectively. RT-qPCR was run with SYBR Premix Ex Taq II (TaKaRa, Japan) on an ABI 7500 system. The 20 µL PCR reaction system contained 10 µL SYBR Premix Ex Taq II, 0.8 µL forward primer, 0.8 µL reverse primer, 2 µL cDNA template, and 6.4 µL nuclease-free water. The PCR conditions were as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. GAPDH and U6 were used as internal reference genes for mRNA/lncRNA and miRNA, respectively. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression values. Primer sequences appear in **Table 1**. All experiments were independently repeated at least three times.

Western blot

Cells and tumor tissues were lysed in RIPA buffer with protease inhibitors (Bio-Ote, China). We determined protein concentrations with a BCA kit (Thermo Fisher Scientific, USA). Equal protein amounts (30 µg) were resolved by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, USA). The membranes were blocked with 5% nonfat milk at room temperature for 1 h and subsequently incubated overnight at 4°C with primary antibodies: anti-LYPLA1 (ab91606, 1:1000, Abcam), AKT (4691S, 1:1000, CST), p-AKT (4060S, 1:1000, CST), MMP2 (40994S, 1:1000, CST), MMP9 (13667S, 1:1000, CST), and GAPDH (60004-1-Ig, 1:2000, Proteintech). Following TBST washes, membranes were probed with HRP-conjugated secondary antibodies: anti-rabbit IgG (7074S, 1:5000, CST) and anti-mouse IgG (SA00001-1, 1:5000, Proteintech). All experiments were independently repeated at least three times. LYPLA1 enzymatic activity was measured using a commercial enzymatic activity assay kit according to the manufacturer's instructions.

Dual-luciferase assay

We inserted Inc-DNAJC3-AS1 WT, MUT, and the LYPLA1 3'UTR into pmirGLO (Promega, USA). HGC-27 cells were seeded into 24-well plates. Upon reaching 60%-70% confluence, we co-transfected each reporter plasmid together with either miR-576-5p mimic or negative control (NC) per the manufacturer's protocol. At 48 hours, luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega, USA).

RNA immunoprecipitation (RIP)

HGC-27 and NCI-N87 cells were subjected to RIP with the Magna RIP kit (Millipore, USA). For the mutation-based Ago2 RIP assay, the mutant DNAJC3-AS1 construct carried the same miR-576-5p binding-site mutation as that used in the dual-luciferase reporter assay, in which the core sequence was changed from 5'-AUUAGAA-3' to 5'-UAAUCUU-3'. RNA complexes associated with Ago2-containing RNA-

induced silencing complexes (RISC) were immunoprecipitated, and the co-precipitated RNA was extracted. The bound RNA was isolated and detected by RT-qPCR to assess the enrichment of lnc-DNAJC3-AS1 and miR-576-5p. This assay was used to evaluate the association of the target RNAs with Ago2-containing RISC complexes rather than to demonstrate direct RNA-RNA interaction.

Fluorescence in situ hybridization (FISH)

We used a FISH kit (Cat. No. FKT01001, RiboBio, China) to determine the subcellular localization of lnc-DNAJC3-AS1. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. We then performed hybridization overnight at 37°C with Cy3-labeled probes per the kit instructions. Fluorescent images were captured using a confocal microscope (Zeiss, Oberkochen, Germany).

CCK-8

We used the CCK-8 kit (Dojindo, Japan) to evaluate cell proliferation. HGC-27 and NCI-N87 cells were plated in 96-well plates at 3×10^3 cells/well in 100 μ L of complete medium and maintained at 37°C with 5% CO₂. At 48 h, we added 10 μ L of CCK-8 working solution to each well. After 2 h of incubation, absorbance was measured at 450 nm using a microplate reader. Each experiment included at least three replicate wells and was independently repeated three times.

Flow cytometry

HGC-27 and NCI-N87 cells were harvested, rinsed with cold PBS, and fixed in 70% ethanol at 4°C overnight. After staining with PI (50 μ g/mL)/RNase A (100 μ g/mL) solution for 30 min in the dark. Cell cycle distribution was analyzed using a flow cytometer (BD FACSCanto II), and data were analyzed using FlowJo software. We handled HGC-27 and NCI-N87 cells following the Annexin V-FITC/PI kit instructions. A total of 100 μ L of the cell suspension was pipetted, followed by the addition of 5 μ L of Annexin V-FITC and 5 μ L of PI, and the cells were incubated at room temperature in the dark for 15 minutes. Then, 400 μ L of Binding Buffer was added to stop the reaction. Apoptotic cells were identified by flow cytome-

try, and total apoptosis was determined as the sum of early and late apoptotic cells. Both detection reagents were from BD Biosciences, USA.

Cleaved caspase-3 immunofluorescence (IF) staining

HGC-27 and NCI-N87 cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 5% BSA, then incubated overnight at 4°C with anti-cleaved caspase-3 antibody (ab13847, 1:200, Abcam). The next day, we added Alexa Fluor 488-conjugated secondary antibody (1:500) at room temperature. Nuclei were counterstained with DAPI. Fluorescence signals were observed under an Olympus fluorescence microscope.

Wound-healing assay

After HGC-27 and NCI-N87 cells reached about 90% confluence, a scratch was made with a 200- μ L tip. PBS rinsing was used before adding serum-free medium. At the 0 h and 24 h, photographs of the scratch areas were taken at the same fixed positions using an inverted microscope.

Transwell

We evaluated cell invasion using Matrigel-coated Transwell chambers (8- μ m pore size, Corning) in a 24-well format. The upper insert contained 5×10^4 HGC-27 and NCI-N87 cells/well in medium, and the bottom wells contained medium with 10% fetal bovine serum. After 24 h, we removed non-invaded cells from the upper membrane surface with a cotton swab. Methanol-fixed and crystal violet-stained cells on the opposite side, counted in five fields (200 $\times$) per well under an inverted microscope.

Animal

Male BALB/c nude mice aged 4 weeks were supplied by Gemie Pharmaceuticals (Suzhou, China) and maintained in an SPF animal facility. Food and water were available throughout the experiment. Ethical approval was obtained from the Institutional Research Human or Animal Ethics Committee of Qingdao Municipal Hospital (Approval No. PZ20220606), and all animal procedures strictly adhered to the relevant guidelines for animal experimentation. For

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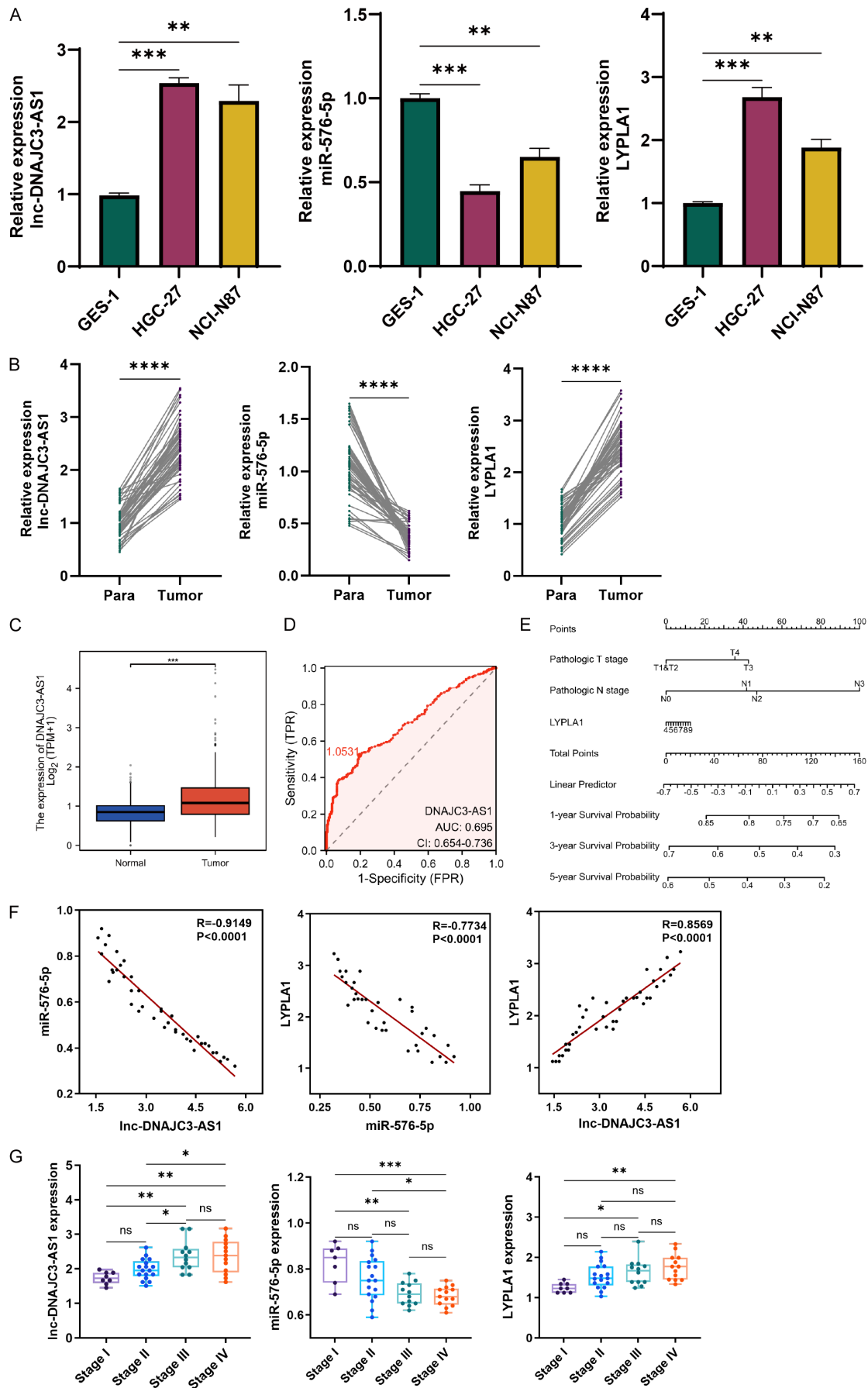


Figure 1. Expression features of lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 in GC. A. RT-qPCR results for lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 in HGC-27, NCI-N87, and normal GES-1 cells. B. RT-qPCR analysis of paired GC and adjacent non-tumor tissues. C. TCGA-STAD dataset showing lnc-DNAJC3-AS1 expression in tumors (n = 174) versus normal tissues (n = 36). D. The curve and 95% confidence interval were indicated on the graph. E. Nomogram estimating 1-, 3-, and 5-year OS using LYPLA1 and clinical factors from TCGA. F. Spearman's correlation analysis among lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 in GC tissues. R values and P values are shown. G. Expression of lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 across TNM stages I-IV in GC. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, ns, not significant.

xenograft assays, HGC-27 cells carrying sh-DNAJC3-AS1 or sh-NC were prepared at 5×10^6 cells/100 μ L PBS and injected subcutaneously into the right flank, with five mice in each group. At the end of the animal experiment, mice were euthanized by carbon dioxide inhalation, followed by cervical dislocation to ensure death. The absence of respiration, heartbeat, and reflex response was confirmed before tumor tissues were collected. Tumor length (L) and width (W) were measured and tumor volume (V) was calculated using the formula $V = (L \times W^2)/2$.

Immunohistochemistry (IHC)

We prepared 4 μ m paraffin sections from tumor tissues fixed in 4% paraformaldehyde. Sections were deparaffinized and rehydrated, then subjected to antigen retrieval by microwave heating in citrate buffer (pH 6.0). After blocking, anti-Ki-67 antibody (ab15580; 1:600, Abcam, USA), was applied, followed by HRP-conjugated secondary antibody. DAB and hematoxylin were used for staining. Ki-67 staining was examined under a light microscope and quantified with ImageJ.

Statistical analysis

We carried out statistical analyses with GraphPad Prism 8.0, and data are expressed as mean $\pm$ SD from ≥ 3 independent experiments. Normality and variance homogeneity were checked by Shapiro-Wilk and Levene's tests. For two-group comparisons, we used Student's t-test (paired t-test for paired data); for multi-group comparisons, we applied one-way ANOVA with Tukey's post-hoc test. Nomogram analysis was performed to estimate OS probability and Spearman's test was used for correlation analysis. All tests were two-sided, with *P* < 0.05 considered significant. Significance levels are indicated as: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Results

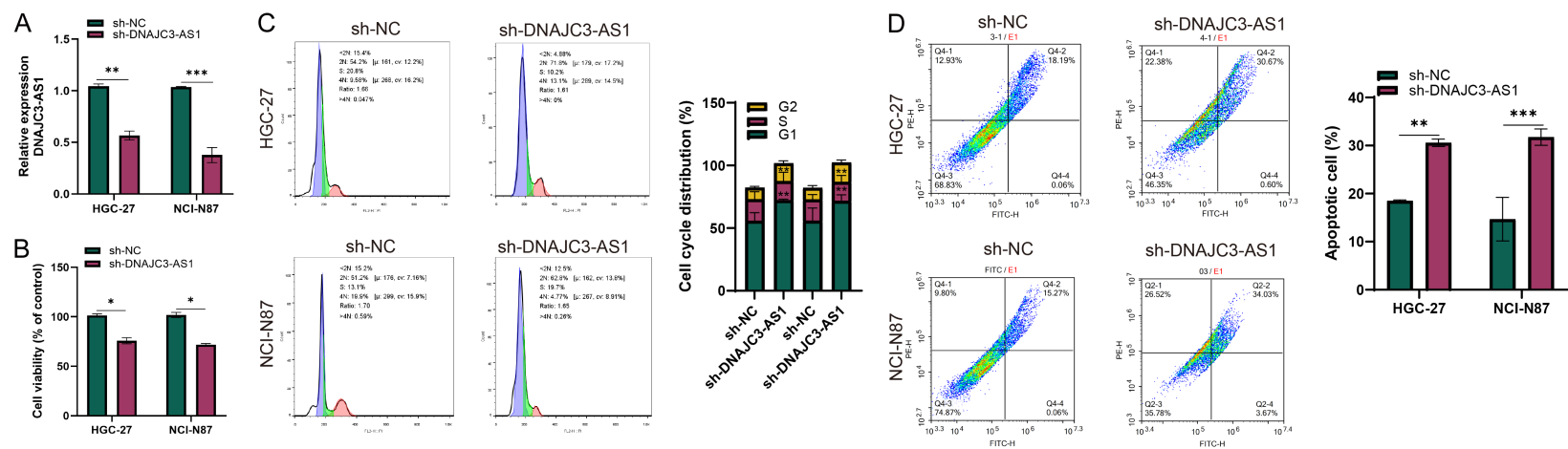
Upregulation of lnc-DNAJC3-AS1 and LYPLA1 and downregulation of miR-576-5p in GC

To investigate the expression pattern of the lnc-DNAJC3-AS1/miR-576-5p/LYPLA1, we measured its levels in GC cells and patient samples. In GC cell lines, both lnc-DNAJC3-AS1 and LYPLA1 showed significant upregulation, while miR-576-5p was downregulated relative to GES-1 cells (**Figure 1A**). A similar pattern was observed in clinical samples (**Figure 1B**). TCGA-STAD analysis also showed increased lnc-DNAJC3-AS1 in tumor samples (**Figure 1C**). The AUC for lnc-DNAJC3-AS1 in separating tumor from normal tissue stood at 0.695 (95% CI: 0.654-0.736), indicating that it showed moderate discriminatory performance (**Figure 1D**). The nomogram model integrates LYPLA1 expression levels and other clinical and pathological factors to provide a visual estimation of the 1, 3, and 5-year OS in GC (**Figure 1E**). Spearman's correlation analysis showed negative correlations for lnc-DNAJC3-AS1/miR-576-5p (*R* = -0.9149) and miR-576-5p/LYPLA1 (*R* = -0.7734), and a positive correlation for lnc-DNAJC3-AS1/LYPLA1 (*R* = 0.8569) in GC tissues (all *P* < 0.0001; **Figure 1F**). lnc-DNAJC3-AS1 and LYPLA1 expression tended to be higher in advanced TNM stages, whereas miR-576-5p expression tended to be lower (**Figure 1G**).

Silencing lnc-DNAJC3-AS1 suppresses the malignant behavior of GC cells

The functional role of lnc-DNAJC3-AS1 in GC cells was examined by knocking down lnc-DNAJC3-AS1 in HGC-27 and NCI-N87 cells. RT-qPCR confirmed efficient knockdown of lnc-DNAJC3-AS1 in both cell lines (**Figure 2A**). Compared with the sh-NC group, lnc-DNAJC3-AS1 knockdown significantly reduced the via-

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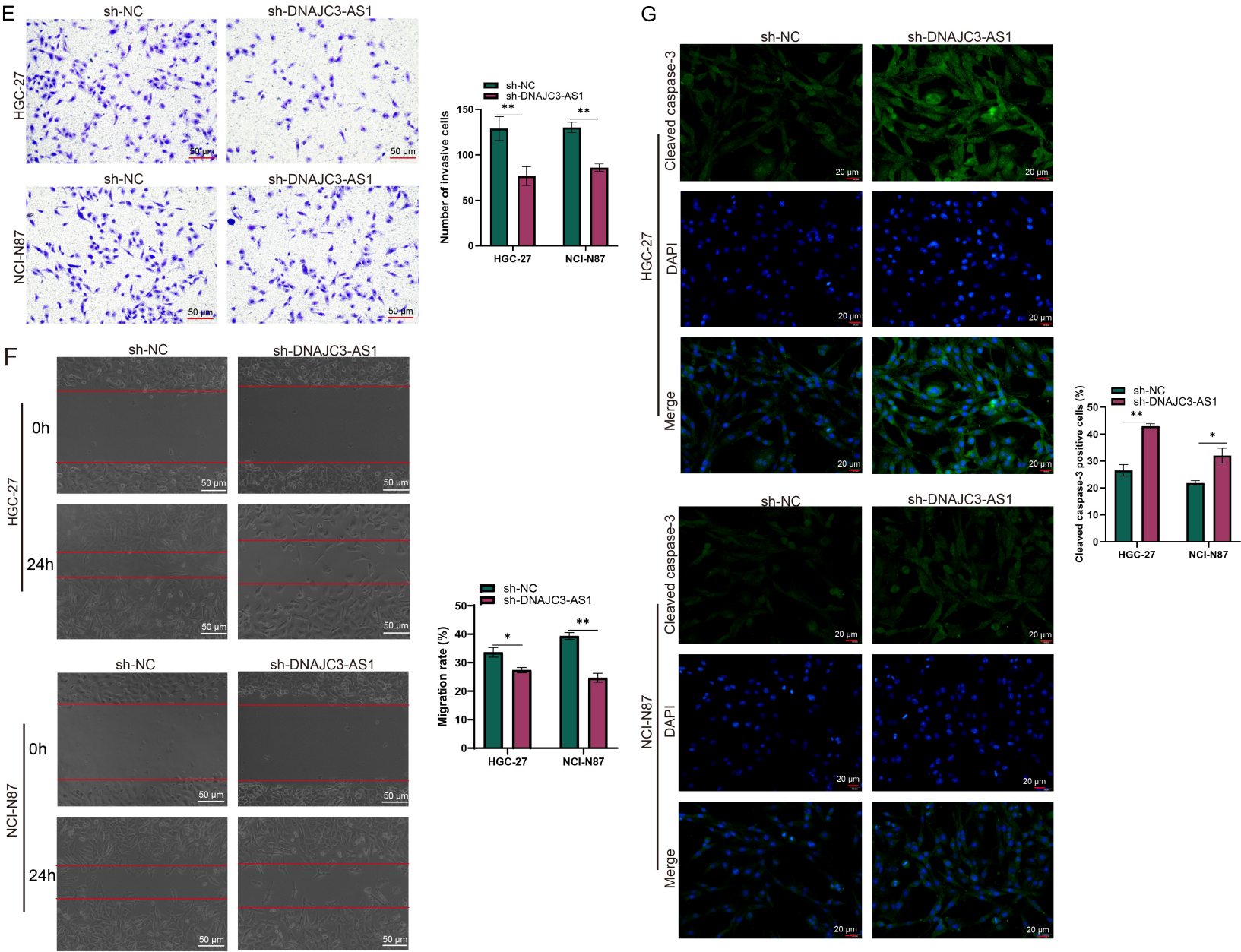


Figure 2. Effects of lnc-DNAJC3-AS1 knockdown on GC cell behavior. (A) RT-qPCR analysis of lnc-DNAJC3-AS1 knockdown efficiency in HGC-27 and NCI-N87 cells. (B) CCK-8 assay of cell viability. (C) Flow cytometry analysis of cell-cycle distribution. (D) Flow cytometry analysis of apoptosis. (E) Transwell assay of cell invasion. (F) Wound-healing assay of cell migration. (G) Immunofluorescence staining of cleaved caspase-3; nuclei were stained with DAPI. Magnification, $\times 40$. Scale bars: 50 μm in (E, F), and 20 μm in (G). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

bility of HGC-27 and NCI-N87 cells (**Figure 2B**). Flow cytometry showed that lnc-DNAJC3-AS1 silencing induced G0/G1 phase arrest (**Figure 2C**) and increased apoptosis in both cell lines (**Figure 2D**). In addition, lnc-DNAJC3-AS1 knockdown decreased the number of invasive cells and reduced the wound closure rate, indicating impaired invasion and migration abilities of GC cells (**Figure 2E, 2F**). IF staining further showed that cleaved caspase-3 fluorescence intensity was increased after lnc-DNAJC3-AS1 knockdown (**Figure 2G**).

Lnc-DNAJC3-AS1 regulates LYPLA1 through miR-576-5p

Bioinformatics analysis and experimental verification supported the regulatory relationship among the lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1. The DIANA tool predicted complementary binding sites between lnc-DNAJC3-AS1 and miR-576-5p. Analysis using miRDB revealed a 7-base pair match between miR-576-5p and the 3'UTR of LYPLA1 (**Figure 3A, 3B**). WT lnc-DNAJC3-AS1 reporter activity was reduced after miR-576-5p mimic transfection, but MUT reporter activity was not affected (**Figure 3A**). Likewise, it suppressed the WT-LYPLA1 3'UTR reporter without impacting the MUT (**Figure 3B**). RIP analysis detected more lnc-DNAJC3-AS1 and miR-576-5p in Ago2 pull-downs than in IgG controls (**Figure 3C**). Meanwhile, after mutation of the predicted miR-576-5p binding site, the enrichment level of AGO2-associated DNAJC3-AS1 was decreased in HGC-27 and NCI-N87 cells (**Figure 3D**). FISH analysis showed that lnc-DNAJC3-AS1 was mainly located in the cytoplasm (**Figure 3E**). Together, cytoplasmic lnc-DNAJC3-AS1 is associated with miR-576-5p-related RISC complexes and participates in LYPLA1 regulation through a potential ceRNA-related mechanism. However, the Ago2 RIP results indicate RISC association rather than direct RNA-RNA binding.

Lnc-DNAJC3-AS1 regulates the miR-576-5p/LYPLA1 axis in GC

Next, we investigated whether it regulates the expression of LYPLA1 through miR-576-5p.

DNAJC3-AS1-OE was transfected alone or together with miR-576-5p mimic or si-LYPLA1 into HGC-27 cells. DNAJC3-AS1-OE effectively increased lnc-DNAJC3-AS1 expression (**Figure 4A**), while decreasing the abundance of miR-576-5p (**Figure 4B**), and promoting LYPLA1 expression (**Figure 4C, 4D**). In addition, DNAJC3-AS1-OE increased LYPLA1 enzymatic activity, whereas miR-576-5p mimic or si-LYPLA1 partially attenuated this effect (**Figure 4E**).

Lnc-DNAJC3-AS1 overexpression promotes GC cell growth through the miR-576-5p/LYPLA1 pathway

Whether the oncogenic effects of lnc-DNAJC3-AS1 depend on the miR-576-5p/LYPLA1 pathway was further examined using reverse rescue experiments in HGC-27 and NCI-N87 cells. lnc-DNAJC3-AS1 overexpression significantly increased cell viability, whereas cotransfection with miR-576-5p mimic or si-LYPLA1 partially attenuated this increase (**Figure 5A**). Flow cytometry analysis showed that lnc-DNAJC3-AS1 overexpression reduced apoptosis in HGC-27 and NCI-N87 cells, and restoration of miR-576-5p activity or LYPLA1 knockdown partially reversed this anti-apoptotic effect (**Figure 5B**). In addition, lnc-DNAJC3-AS1 overexpression reduced the proportion of cells in the G0/G1 phase, indicating enhanced cell-cycle progression; this effect was partially attenuated by miR-576-5p mimic or si-LYPLA1 (**Figure 5C**). The growth advantage and cell-cycle progression induced by lnc-DNAJC3-AS1 overexpression were weakened after miR-576-5p restoration or LYPLA1 knockdown, supporting the involvement of the miR-576-5p/LYPLA1 pathway in this process.

Lnc-DNAJC3-AS1 overexpression enhances GC cell migration and invasion and is associated with AKT/MMP-related protein changes

Next, we examined the effect of lnc-DNAJC3-AS1 overexpression on the motility of GC cells. Compared with the pcDNA3.1+NC group, lnc-DNAJC3-AS1 overexpression increased the number of invasive HGC-27 and NCI-N87 cells.

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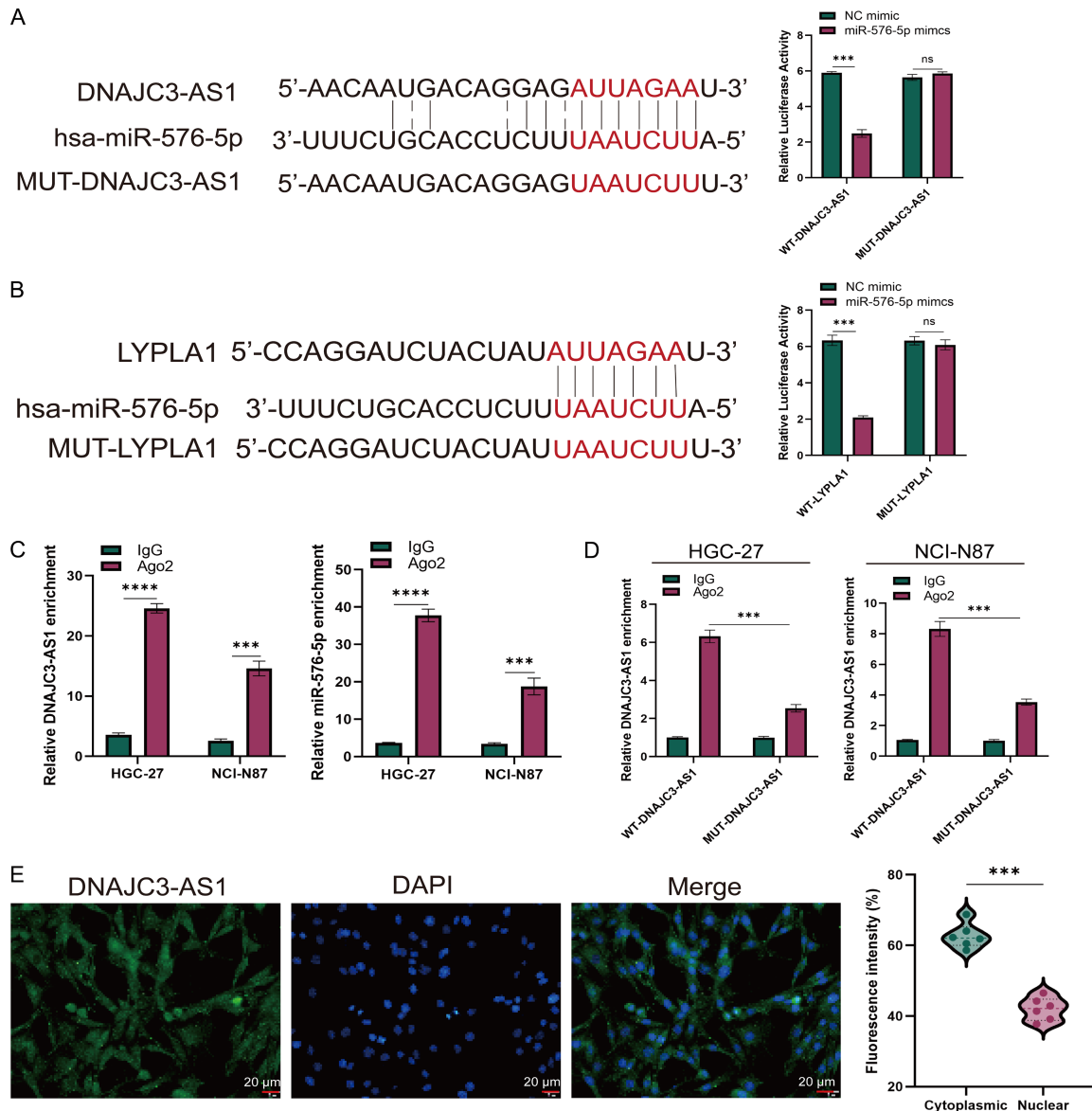


Figure 3. Lnc-DNAJC3-AS1 is associated with the miR-576-5p/LYPLA1 regulatory pathway. **A.** Dual-luciferase reporter assay of wild-type (WT) or mutant (MUT) lnc-DNAJC3-AS1 reporters after miR-576-5p mimic transfection in HGC-27. **B.** Dual-luciferase reporter assay of WT or MUT LYPLA1 3'UTR reporters after miR-576-5p mimic transfection in HGC-27. **C.** RIP analysis of lnc-DNAJC3-AS1 and miR-576-5p enrichment in Ago2 pull-downs compared with IgG controls. **D.** Ago2-associated DNAJC3-AS1 enrichment after mutation of the predicted miR-576-5p binding site in HGC-27 and NCI-N87 cells. **E.** FISH analysis of lnc-DNAJC3-AS1 subcellular localization in HGC-27 cells. Magnification, $\times 40$. Scale bar = 20 μm . $**P < 0.01$, $***P < 0.001$.

This increase was partially attenuated by co-transfection with miR-576-5p mimic or si-LYPLA1 (Figure 6A). Consistently, lnc-DNAJC3-AS1 overexpression increased the wound closure rate, and restoration of miR-576-5p activity or LYPLA1 knockdown weakened this effect (Figure 6B). At the protein level, lnc-DNAJC3-AS1 overexpression was accompanied by increased p-AKT/AKT, MMP2, and MMP9 lev-

els. These protein changes were partially reduced after miR-576-5p mimic transfection or LYPLA1 knockdown (Figure 6C). In addition, lnc-DNAJC3-AS1 overexpression decreased the proportion of cleaved caspase-3 positive cells, and miR-576-5p mimic or si-LYPLA1 partially restored cleaved caspase-3 expression (Figure 6D). lnc-DNAJC3-AS1 therefore appears to contribute to GC cell migration and

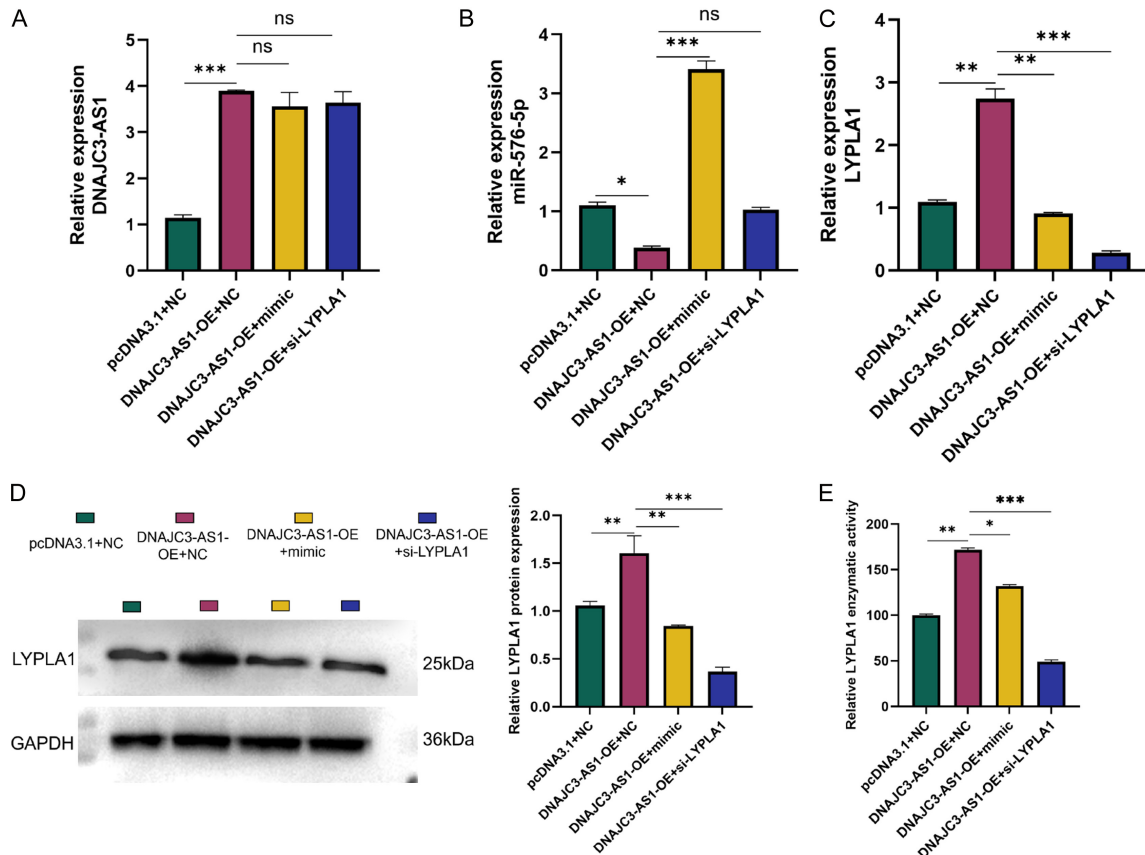


Figure 4. Lnc-DNAJC3-AS1 overexpression regulates the miR-576-5p/LYPLA1 axis in GC. A-C. RT-qPCR results for lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 expression. D. LYPLA1 protein level and Western blot quantification. E. Relative LYPLA1 enzymatic activity in different transfection groups. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

invasion, while miR-576-5p/LYPLA1-associated changes in AKT/MMP signaling proteins may participate in this process.

Knockdown of lnc-DNAJC3-AS1 suppresses tumor progression

We established a xenograft model to examine the in vivo role of lnc-DNAJC3-AS1. Mice received HGC-27 cells carrying sh-DNAJC3-AS1 or sh-NC. Tumors from the sh-DNAJC3-AS1 group showed lower lnc-DNAJC3-AS1 and LYPLA1 levels, together with higher miR-576-5p expression (Figure 7A-D). Tumor volume was reduced after lnc-DNAJC3-AS1 silencing (Figure 7E). Ki-67 and LYPLA1 staining were also weaker in sh-DNAJC3-AS1 tumors (Figure 7F, 7G). In addition, lnc-DNAJC3-AS1 knockdown decreased P-AKT, MMP2, and MMP9 protein levels in xenograft tumor tissues (Figure 7H). Collectively, lnc-DNAJC3-AS1 knockdown inhibited GC tumor growth in vivo, ac-

companied by increased miR-576-5p expression and reduced LYPLA1 expression.

Discussion

This study has confirmed the carcinogenic role of lnc-DNAJC3-AS1 in GC and its mechanism. The expression of lnc-DNAJC3-AS1 and LYPLA1 were both elevated in GC; conversely, the level of miR-576-5p was decreased. Mechanistically, lnc-DNAJC3-AS1 is associated with miR-576-5p and increases LYPLA1 expression, thereby promoting GC cell growth and motility while reducing apoptosis. In vivo, lnc-DNAJC3-AS1 knockdown inhibited tumor growth.

Abnormal expression of tumor-associated lnc-RNAs represents a molecular hallmark of cancer development, often correlating with the clinical and pathological characteristics of tumors as well as patient prognosis [30-32]. Previous work identified that lnc-DNAJC3-AS1

A

HGC-27

Cell viability (% of control)

pcDNA3.1+NC DNAJC3-AS1-OE+NC DNAJC3-AS1-OE+mimic DNAJC3-AS1-OE+si-LYPLA1

NCI-N87

Cell viability (% of control)

pcDNA3.1+NC DNAJC3-AS1-OE+NC DNAJC3-AS1-OE+mimic DNAJC3-AS1-OE+si-LYPLA1

B

pcDNA3.1+NC DNAJC3-AS1-OE+NC DNAJC3-AS1-OE+mimic DNAJC3-AS1-OE+si-LYPLA1

HGC-27

4-1/E1

Q2-1 0.19% Q2-2 5.84% Q2-3 79.80% Q2-4 13.16%

NCI-N87

2-1/E1

Q2-1 0.14% Q2-2 9.03% Q2-3 80.74% Q2-4 10.10%

C

pcDNA3.1+NC DNAJC3-AS1-OE+NC DNAJC3-AS1-OE+mimic DNAJC3-AS1-OE+si-LYPLA1

HGC-27

2-2/E1/P2

Watson RMS: 9.69 Freq G1: 59.09 Freq S: 26.05 Freq G2: 14.87 Mean G1: 1,217,804 Mean G2: 2,199,499 G2/G1: 1.81 CV G1: 4.80% CV G2: 3.93% Freq Sub-G1: -0.01 Freq Super-G2: 0.00

NCI-N87

2-1/E1/P2

Watson RMS: 5.62 Freq G1: 63.45 Freq S: 23.91 Freq G2: 12.65 Mean G1: 1,163,498 Mean G2: 2,170,257 G2/G1: 1.87 CV G1: 5.03% CV G2: 3.98% Freq Sub-G1: -0.01 Freq Super-G2: 0.00

Figure 5. Effects of lnc-DNAJC3-AS1 overexpression on GC cell growth. A. CCK-8 assay of cell viability. B. Flow cytometry analysis of apoptosis. C. Flow cytometry analysis of cell-cycle distribution. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

is highly expressed in various malignancies and drives tumor progression through different molecular mechanisms [33]. The present study found that lnc-DNAJC3-AS1 and LYPLA1 alongside reduced miR-576-5p in GC. This expression pattern aligns with observations in other cancers, implicating lnc-DNAJC3-AS1 as a potential oncogenic driver across malignancies.

The ceRNA regulatory paradigm has garnered substantial attention in cancer research. In ovarian cancer, miR-576-5p overexpression enhances tumor cell growth and aggressiveness [34]. Conversely, in breast cancer, LINC-PINT upregulates MEIS2 expression by interacting with miR-576-5p, thereby inhibiting the NF- κ B pathway and exerting an antitumor effect [35]. The association between lnc-DNAJC3-AS1 and miR-576-5p-related RISC complexes was supported through bioinformatics prediction and multi-pronged experimental validation. In summary, lnc-DNAJC3-AS1 may modulate miR-576-5p-mediated LYPLA1 regulation, supporting a potential ceRNA-related mechanism.

Palmitoylation is an important post-translational protein modification associated with tumor growth, invasion, metastasis, and antitumor immunity [36]. The depalmitoylating enzyme LYPLA1 participates in the regulation of this modification process. In breast cancer, LYPLA1 gene copy number amplification correlates with high mRNA expression, is linked to poor patient prognosis, and enhanced cancer cell proliferation [37, 38]. However, the genetic regulatory mechanisms of LYPLA1 in cancer have not yet been fully elucidated. Here, LYPLA1 was identified as a direct miR-576-5p target, with lnc-DNAJC3-AS1 upregulating its expression through ceRNA competition.

A growing body of evidence suggests that LYPLA1 possesses both depalmitoylase and lysophospholipase activities. For example, LYPLA1/APT1 regulates mitochondrial function and promotes cell proliferation and metastasis by depalmitoylase GPCPD1 [39]. Tang et al. found that LYPLA1 overexpression inhibits CD95-mediated apoptosis by de-palmitoylating the CD95 receptor [40]. Furthermore,

LYPLAL1, a homolog of LYPLA1, can suppress innate immune responses by depalmitoylase cGAS, and targeting LYPLAL1 can enhance the efficacy of anti-tumor immunotherapy [41]. Notably, our study confirmed that knocking down lnc-DNAJC3-AS1 or inhibiting LYPLA1 expression resulted in decreased levels of p-AKT and MMP9/2. This suggests that LYPLA1 may promote GC progression in association with changes in AKT/MMP-related signaling proteins. Given LYPLA1's known substrates (HRAS, NRAS, and EGFR), LYPLA1 may be associated with alterations in signaling pathways related to tumor progression through depalmylation, thereby activating the downstream AKT and ERK pathways. However, whether LYPLA1 directly regulates the palmitoylation status of Ras or other oncoproteins to influence GC progression requires further validation.

lnc-DNAJC3-AS1 overexpression promoted GC cell growth and migration and reduced apoptosis. These effects were partly attenuated by miR-576-5p overexpression or LYPLA1 knockdown. These findings indicated that lnc-DNAJC3-AS1 promoted the malignant phenotype of GC through the miR-576-5p/LYPLA1 axis. In vivo, the silencing of lnc-DNAJC3-AS1 reduced the growth of tumors in nude mice, suggesting its functional importance in GC progression.

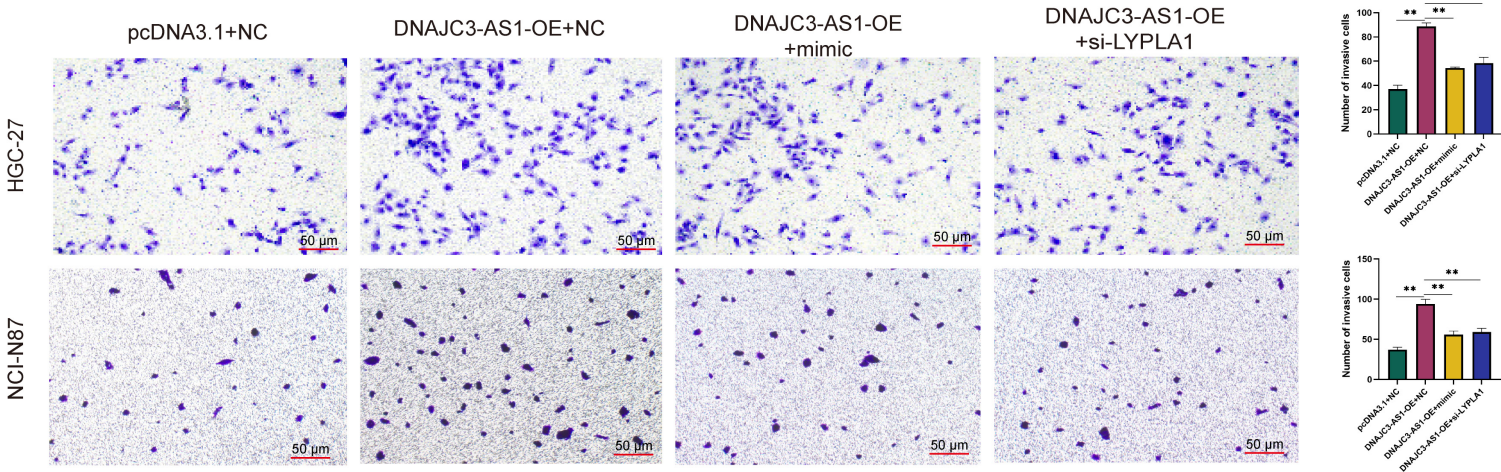
This study has the following limitations: the in vivo experiments only utilized the subcutaneous tumor xenograft model, which did not fully simulate the clinical characteristics of peritoneal metastasis in GC; future research can adopt in situ transplantation or peritoneal metastasis models to further verify. Additionally, although this study confirmed the importance of LYPLA1 in the carcinogenic effect of lnc-DNAJC3-AS1, the specific mechanism by which it regulates lipid metabolism and its causal relationship with the malignant phenotype of GC still require further investigation, especially focusing on the impact of LYPLA1 on the lysophosphatidic acid pathway.

Conclusion

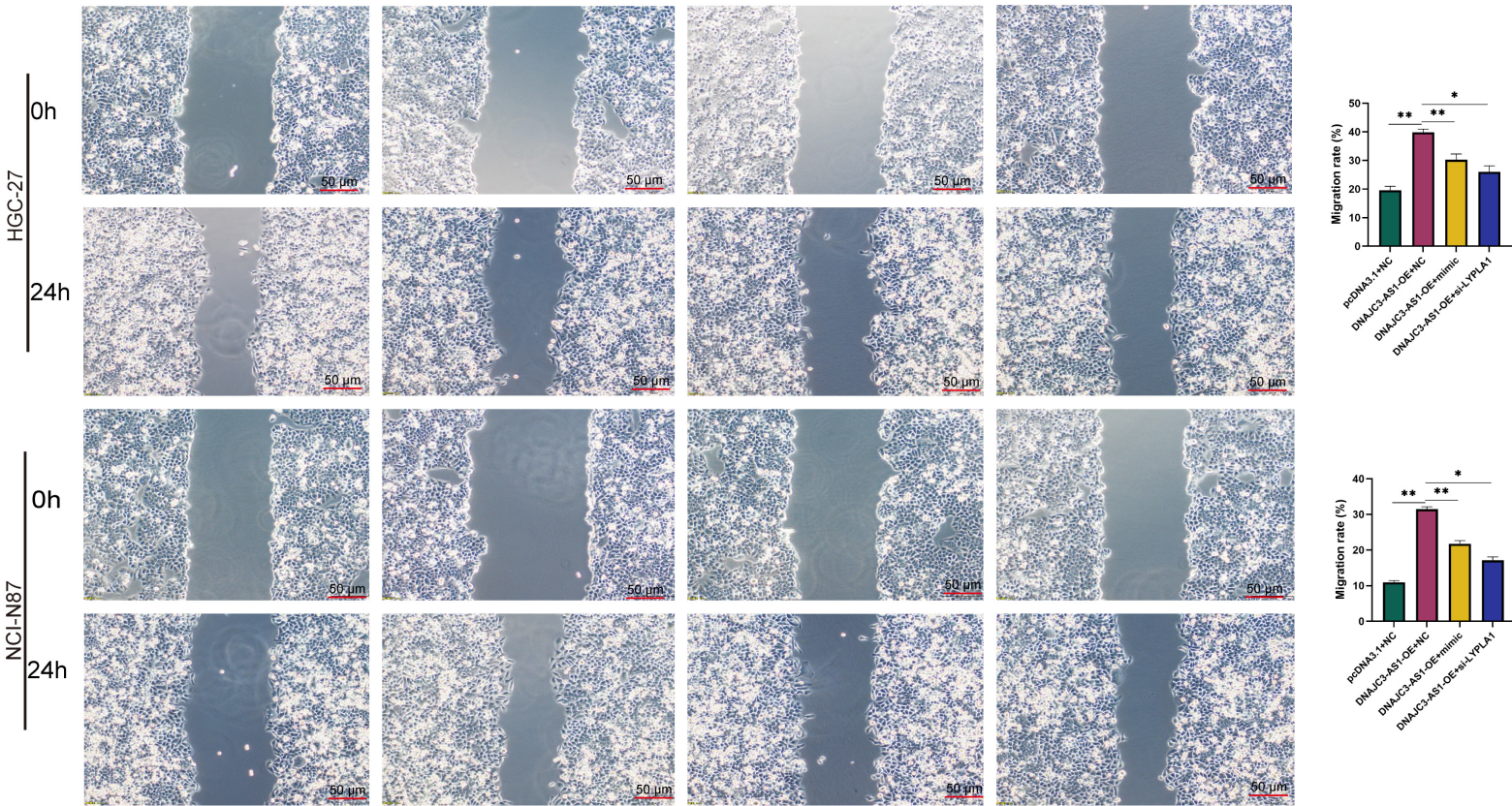
In summary, lnc-DNAJC3-AS1 plays a role in promoting tumor growth in GC. lnc-DNAJC3-

Long non-coding RNA DNAJC3-AS1 promotes gastric cancer progression

A



B



Long non-coding RNA DNAJC3-AS1 promotes gastric cancer progression

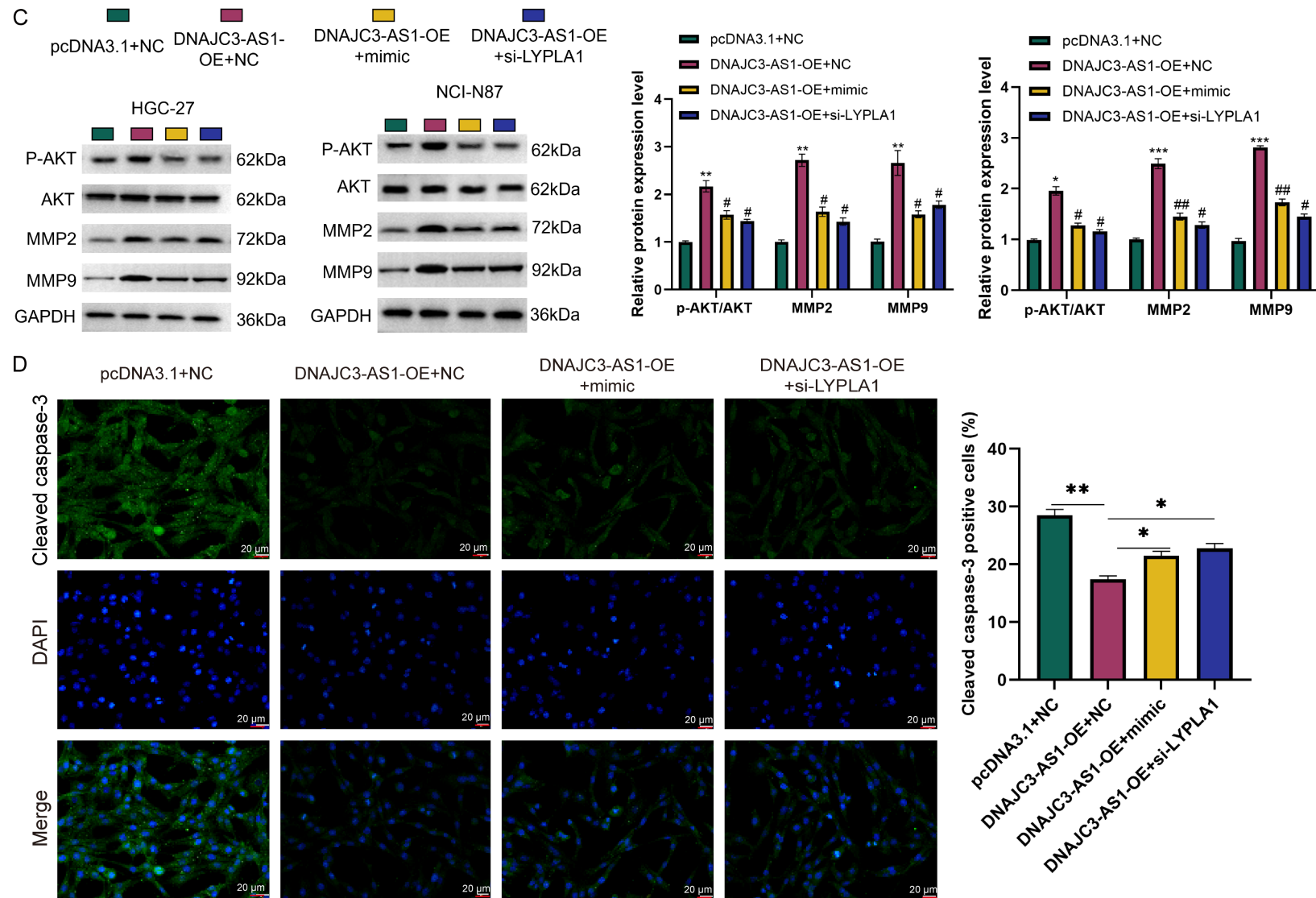


Figure 6. Effects of lnc-DNAJC3-AS1 overexpression on GC cell motility and apoptosis. (A) Transwell assay of cell invasion in HGC-27 and NCI-N87 cells. (B) Wound-healing assay of cell migration at 0 h and 24 h. (C) Western blot analysis of p-AKT/AKT, MMP2, and MMP9 protein levels. (D) Immunofluorescence staining of cleaved caspase-3 in HGC-27 cells; nuclei were stained with DAPI. Magnification, $\times 40$. Scale bars: 50 μ m in (A, B), and 20 μ m in (D). * P < 0.05, ** P < 0.01, *** P < 0.001; # P < 0.05, ## P < 0.01 versus the DNAJC3-AS1-OE+NC group.

Long non-coding RNA DNAJC3-AS1 promotes gastric cancer progression

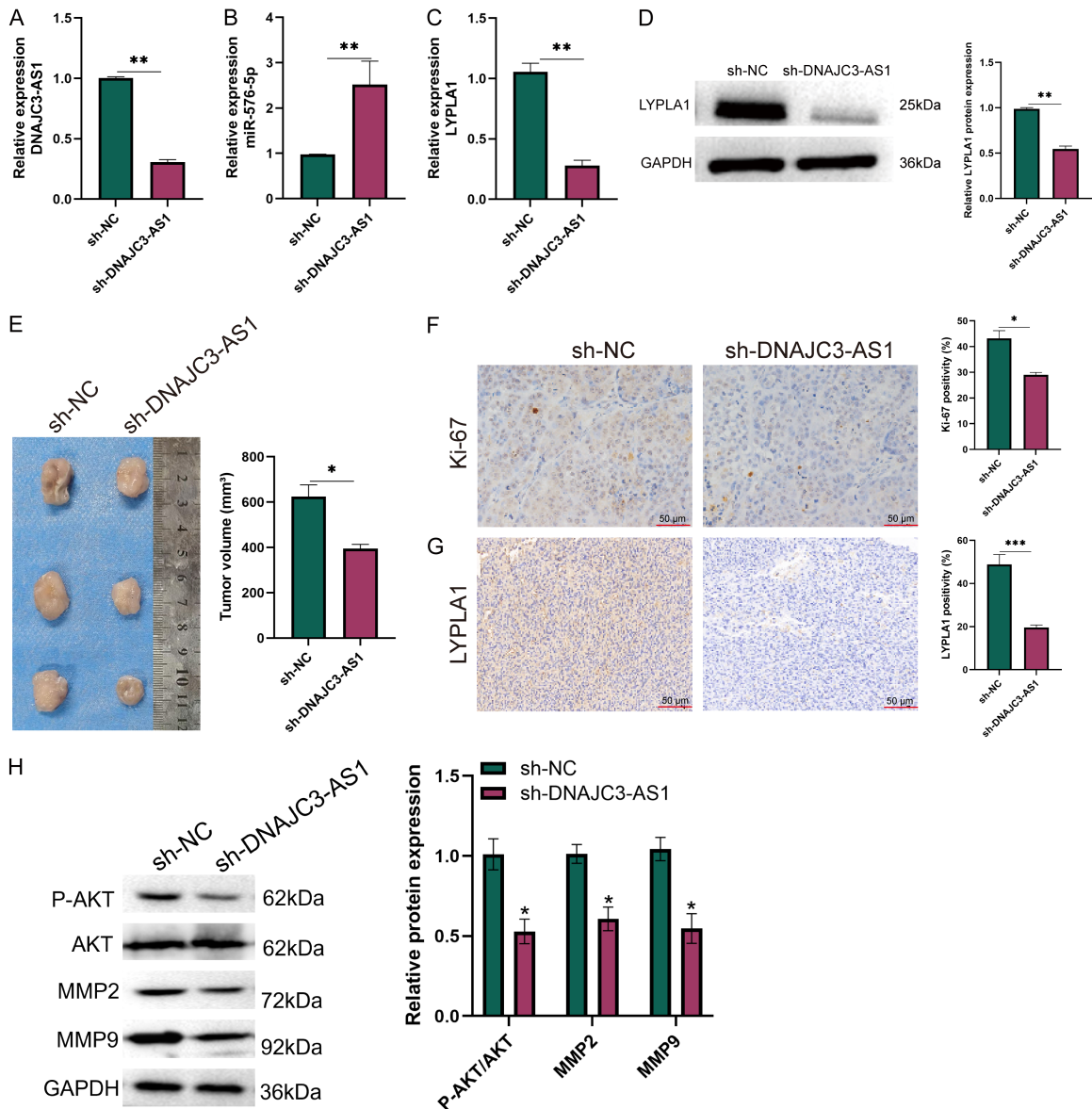


Figure 7. Knockdown of lnc-DNAJC3-AS1 suppresses tumor progression. A-C. RT-qPCR analysis of lnc-DNAJC3-AS1, miR-576-5p, and LYPLA1 mRNA in xenograft tumors. D. Western blot analysis and quantification of LYPLA1 protein expression. E. Representative tumor images and tumor volume measurement. F, G. IHC staining and quantification of Ki-67 and LYPLA1. H. Western blot analysis and quantification of p-AKT, AKT, MMP2, and MMP9 protein expression in xenograft tumor tissues. The P-AKT level was normalized to total AKT, while MMP2 and MMP9 were normalized to GAPDH. Magnification, $\times 40$. Scale bars = 50 μ m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

AS1 upregulates the expression of LYPLA1 by capturing miR-576-5p, thereby promoting GC cell growth and motility and reducing apoptosis. Therefore, therapeutic strategies targeting the lnc-DNAJC3-AS1/miR-576-5p/LYPLA1 axis may offer a new approach for GC treatment.

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Disclosure of conflict of interest

None.

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