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ORIGINAL RESEARCH

LncRNA *DLX6-AS1* promotes the proliferation, invasion, and migration of non-small cell lung cancer cells by targeting the *miR-27b-3p/GSPT1* axis

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Background: Non-small cell lung cancer (NSCLC) has a significant impact on human health. The aim of this study was to explore the role of long non-coding RNA *DLX6-AS1* in the proliferation, migration, and invasion of NSCLC cells.

Methods: The expression of *DLX6-AS1* in NSCLC tumor tissues and cell lines was examined by qRT-PCR. The effects of *DLX6-AS1* knockdown on cell proliferation, migration, and invasion were assessed by Cell Counting Kit-8, wound healing, and transwell assays, respectively. Bioinformatics analyses, luciferase reporter assays, and RNA pull-down assays were employed to examine the mechanism by which *DLX6-AS1* exerted its oncogenesis effects in NSCLC. The anti-tumor effect of silencing *DLX6-AS1* in vivo was also evaluated.

Results: *DLX6-AS1* was over-expressed in NSCLC tumor tissues and cell lines and its level of expression was found to be associated with tumor size and advanced clinical stage in patients with NSCLC. Downregulation of *DLX6-AS1* inhibited cell proliferation, cell clone formation, migration, and invasion of NSCLC cells. *DLX6-AS1* was found to interact with *miR-27b-3p/GSPT1*. *DLX6-AS1* expression was negatively correlated with *miR-27b-3p* expression, but positively correlated with GSPT1 expression in NSCLC samples. *DLX6-AS1* knockdown also effectively suppressed tumor growth in an in vivo xenograft model.

Conclusion: *DLX6-AS1* regulated NSCLC progression by targeting the *miR-27b-3p/GSPT1* axis, which may provide novel insights for NSCLC prognosis and therapy.

Keywords: *DLX6-AS1*, NSCLC, *miR-27b-3p*, GSPT1, invasion

Introduction

Lung cancer is one of the most common malignant tumors worldwide and has the highest morbidity and mortality rates of all malignant tumors.[1] Non-small cell lung cancer (NSCLC) includes squamous cell carcinoma (SCC), adenocarcinoma, and large cell carcinoma.[2,3] NSCLC accounts for approximately 80% of all lung cancer cases. Approximately 75% of NSCLC patients are diagnosed at an advanced stage and therefore, the 5-year survival rate is very low.[2,3] A better understanding of the mechanism of NSCLC invasion and metastasis will enable more effective prevention or treatment of lung cancer. Recent research has focused on the function of non-coding RNAs in the occurrence and progression of NSCLC.[5,6]

Long non-coding RNA (lncRNA) is a class of RNA with a transcript length of more than 200 nucleotides and no protein-coding function. However, these lncRNAs

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play an important role in epigenetic, cell cycle, and cell differentiation regulation, in addition to many other physiological activities.[7] There have been recent reports that abnormal levels of lncRNAs in the tissues and blood of NSCLC patients, play a crucial role in regulating the proliferation, migration, invasion, and apoptosis of tumor cells and are closely related to the occurrence and development of NSCLC.[8] Exploring the biological functions and mechanisms of lncRNAs in NSCLC is helpful for the development of tools for the early diagnosis, targeted therapy, and prognosis of NSCLC. LncRNA *DLX6-AS1* has been reported to be over-expressed in many types of cancers.[9] Li et al reported that *DLX6-AS1* expression was upregulated in lung adenocarcinoma and *DLX6-AS1* expression levels were significantly correlated with histological differentiation and TNM stage.[14] However, the effect of *DLX6-AS1* on the proliferation, migration, and invasion of NSCNC cells and the associated mechanisms were unclear.

In the present study, we measured the expression of *DLX6-AS1* in NSCLC tumor tissues and cell lines. The effect of *DLX6-AS1* on the proliferation, migration, and invasion of NSCLC cell lines was also assessed in vivo and in vitro. Furthermore, the underlying mechanism by which *DLX6-AS1* regulated the phenotype of NSCLC cells was investigated.

Patients and methods

Patients and tissue samples

This research included a total of 51 NSCLC patient samples. All tissue specimens were obtained from surgical tumor resections at the Affiliated Hospital of Jining Medical University. Adjacent normal lung tissue specimens were also collected from these patients as negative controls. Ethical approval for the study was provided by the Ethics Committee of the Affiliated Hospital of Jining Medical University. Written informed consent was obtained from all study subjects, and this work was conducted in accordance with the Declaration of Helsinki. Preoperative clinical and pathological follow-up data were available for all patients.

Cell culture and transfection

NSCLC cell lines (CALU3, CALU6, A549, and H1299) and human bronchial epithelial cells (HBE) were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) and 100× penicillin-streptomycin solution (Invitrogen), in an incubator (Thermo Fisher Scientific Inc., Waltham, MA, USA) set to 37 °C, 100% humidity, and 5% CO_2. A small interfering RNA (siRNA) targeting *DLX6-AS1* (siDLX6-AS1), *miR-27b-3p* mimics, and negative control RNAs were constructed in pLKO.1. Plasmid constructs were transfected into cells at 70–90% confluency using Lipofectamine 2000 (Invitrogen) and were transfected again 24 h later. After an additional 24 h, the transfected cells were collected and processed for further studies.

RNA isolation and quantitative reverse transcription PCR (qrt-PCR)

Total cellular RNA was extracted using TRIzol reagent (Invitrogen), according to the manufacturer's protocol. SYBR Green I (Molecular Probes, Invitrogen) was used to quantify PCR amplification and real-time PCR was performed using a 7500 Fast Real-Time Sequence Detection System (Applied Biosystems, Foster City, CA, USA). miRNA was quantified using Bulge-Loop™ miRNA qRT-PCR Primer Sets (one RT primer and a pair of qPCR primers for each set) specific for *DLX6-AS1, miR-27b-3p* and *GSPT1*, designed by RiboBio (Guangzhou, China). Relative miRNA expression levels were calculated as $2^{-[(\text{Ct of miRNA}) - (\text{Ct of U6})]}$ after normalization to the expression of small nuclear RNA *U6*. The primers used for stem-loop reverse-transcription PCR for *miR-27b-3p* and *U6* were purchased from RiboBio. Gene expression levels were normalized to *GAPDH* expression and were calculated as $2^{-[(\text{Ct of GENES}) - (\text{Ct of GAPDH})]}$. The following primers were used: *DLX6-AS1* forward, 5′-GAA GCTCCTACGCCTTTG-3′ and reverse, 5′-TCCTCCCTTC AACATTCTG-3′; *miR-27b-3p* forward, 5′-AGGGTTCAC AGTGGCTAAG-3′ and reverse, 5′-GAGAGGAGAGGAA GAGGGAA-3′; *GSPT1* forward, 5′-GAGGAAAGTGC CCATGAA-3′ and reverse, 5′-CATCTACGTGCCCAATGA -3′; *U6* forward, 5′-CGGGTGCTCGCTTCGCAGC-3′ and reverse, 5′-CCA GTGCAGGGTCCGAGGT-3′; *GAPDH* forward, 5′-TCCTCTGACTTCAACAGCGACAC-3′ and reverse, 5′-CACCCTGTTGCTGTAGCCAAATTC-3′.

Cell counting kit-8 (CCK-8) assay

NSCLC cells ($1{\times}10^5$ cells per well) were seeded in 96-well plates and cultured for 24 h prior to analysis of cell proliferation using the CCK-8 assay (Dojindo Molecular Technologies, Gaithersburg, USA). Cells in different groups were then cultured for a further 24, 48, or 72 h. Subsequently, all cells were incubated with 10 μL of CCK-8 solution at 37 °C for 4 h. To obtain cell growth curves, plates were read at 450 nm using an iMark microplate absorbance reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). All experiments were performed in triplicate.

Colony formation assay

Cells were seeded into 6-cm tissue culture dishes (0.5×10^3 cells per well) and cultured for 14 d. They were then fixed with 10% formaldehyde for 15 min and subsequently stained with 1.0% crystal violet for 5 min. The number of colonies formed was counted in 10 different fields.

Cell migration and invasion assay

Cell migration was evaluated using a wound-healing assay. In brief, 48 h after transfection, cells were cultured in 6-well plates (5×10^4 cells per well). After reaching 90–95% confluence, the monolayer of cells was scratched with a sterile plastic micropipette tip and cells were then cultured under standard conditions for 24 h. Following several washes, recovery of the wound was observed and imaged using an X71 inverted microscope (Olympus Corporation, Tokyo, Japan).

A transwell invasion assay was performed to assess cell invasion. Transfected cells (1×10^5) were seeded into the upper chamber of Matrigel-coated inserts in serum-free medium. Medium supplemented with 10% FBS was added to the lower chamber as a chemoattractant. Cells were allowed to invade for 48 h at 37°C with 5% CO_2. Those that invaded to the lower surface of the filter were fixed in 70% ethanol for 30 min and stained with 0.1% crystal violet for 10 min at 25°C. The number of cells that migrated to the lower side was counted in five randomly selected fields under an X71 inverted microscope.

Luciferase reporter assay

To identify the *DLX6-AS1*- and GSPT1-binding sites in the *miR-27b-3p* promoter, *miR-27b-3p* promoter reporter constructs with wild-type or mutated *DLX6-AS1*- or GSPT1-binding sites were transfected with pRL-SV40 Renilla luciferase vectors into HEK293T cells using the LT1 Transfection Reagent (Mirus, Madison, WI, USA). Forty-eight hours after transfection, luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA). Measurements from triplicate transfections were analyzed after normalization to firefly luciferase activity.

Protein preparation and western blotting

Total protein lysates were resolved by 10% SDS-PAGE and transferred to polyvinyl difluoride membranes (EMD Millipore, Billerica, MA, USA). Following blocking with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T) for 30 min at 37°C, membranes were washed four times in TBS-T and incubated with primary antibodies overnight at 4°C. All primary antibodies were obtained from Abcam (Cambridge, UK) and used at the following dilutions: anti-GSPT1 (cat. no. ab126090; 1:1,000). Following extensive washing, membranes were incubated with a horseradish peroxidase-conjugated goat polyclonal anti-rabbit IgG secondary antibody (cat. no. 7074; Cell Signaling Technology, Danvers, MA, USA), at a dilution of 1:2,000, for 1 h at room temperature. Immunoreactivity was detected by enhanced chemiluminescence (Pierce; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and visualized using a ChemiDoc XRS imaging system and analysis software (Bio-Rad Laboratories, Inc., Hercules, CA, USA). GAPDH served as a loading control.

Bioinformatics analysis

The target miRNAs of *DLX6-AS1* were predicted with computational algorithms, including starbase (http://starbase.sysu.edu.cn) and miRanda (http://www.microrna.org). *miR-27b-3p* was the highest-ranked predicted target of *DLX6-AS1*. To identify genes targeted by *miR-27b-3p*, we used the online software programs, TargetScan (http://www.targetscan.org/) and miRanda (http://www.microrna.org). From the list of target genes obtained, we extracted all genes that were likely to contribute to NSCLC progression. The 3′-UTR of the *GLPT1* gene was predicted to have *miR-27b-3p*-binding sites.

In vivo xenograft experiments

Male BALB/c nude mice (6 weeks old, n=6) were purchased from Beijing HFK Bioscience Co. Ltd. (Beijing, China) and were maintained under pathogen-free conditions with the approval of the Affiliated Hospital of Jining Medical University. For tumor propagation analysis, 1×10^7 A549 tumor cells, transfected with a short hairpin RNA (shRNA) that targeted *DLX6-AS1* (shDLX6-AS1) or a negative control shRNA (shNC), were subcutaneously injected into BALB/c nude mice. Tumor volume was calculated at the indicated time points using the formula, volume = $\pi ab^2/6$ (a, tumor length; b, tumor width). Tumors were weighed 3 weeks after injection. Animal experiments were approved by the Animal

Care and Use Committee of the Affiliated Hospital of Jining Medical University and were performed in accordance with the relevant guidelines and regulations of the committee. The levels of Ki67 in tumor were detected by immunohistochemical staining.

Statistical analysis

Data are presented as the mean ± standard deviation of at least three biological replicates, unless otherwise noted. A Student's *t*-test or one-way ANOVA was used to analyze data from two or multiple groups, respectively. Pearson's $\chi 2$ test was used to analyze the correlation between clinicopathological features and *DLX6-AS1* expression in NSCLC patients. Spearman's correlation analysis was used to determine the correlations between the levels of *miR-27b-3p* and *DLX6-AS1*/GSPT1 in NSCLC tissues. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LncRNA DLX6-AS1 was up-regulated in NSCLC

To explore the role of *DLX6-AS1* in NSCLC, we first examined its expression in 51 paired clinical NSCLC samples and normal tissues. We found that *DLX6-AS1* was significantly overexpressed in NSCLC tumor tissues compared to normal tissues ($P<0.001$, Figure 1A). We then measured *DLX6-AS1* expression in NSCLC cell lines (CALU3, CALU6, A549 and H1229) and human bronchial epithelial cells (HBE) using qRT-PCR. These results indicated that *DLX6-AS1* expression was significantly higher in NSCLC cell lines compared to HBE cells ($P<0.001$, Figure 1B). In addition, we investigated the clinicopathological significance of *DLX6-AS1* expression level in NSCLC patients. Based on the mean value of *DLX6-AS1* expression, the 51 patients were divided into 2 subgroups: low *DLX6-AS1* group (30 cases) and high *DLX6-AS1* group (21 cases). As shown in Table 1, *DLX6-AS1* expression levels in NSCLC tissues were positively associated with tumor size and advanced clinical stage (Table 1). These results indicated that high levels of DLX6-AS1 expression may have an oncogenic role in the progression of NSCLC.

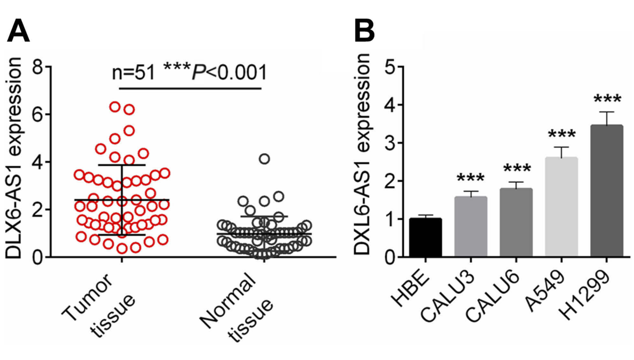

Figure 1 The expression of DLX6-AS1 was up-regulated in NSCLC samples and cell lines. (**A**) The DLX6-AS1 expression in 51 paired NSCLC clinical samples and normal tissues was detected by qRT-PCR. (**B**) The expression of DLX6-AS1 in NSCLC cell lines (CALU3, CALU6, A549, and H1299) and HBE cells were detected by qRT-PCR. Data are shown as the mean ± standard deviation. ***$P<0.001$ vs HBE cell line.
Abbreviations: HBE, human bronchial epithelial; NSCLC, Non-small cell lung cancer.

Downregulation of DLX6-AS1 inhibited proliferation, migration, and invasion of NSCLC cells

Because A549 and H1229 cells exhibited higher *DLX6-AS1* expression than the other cell lines tested, these two cell lines were transfected with pLKO.1-DLX6-AS1 or pLKO.1 plasmids (Figure 2A). Cell proliferation, clonal ability, migration, and invasion were assessed by CCK-8, cell colony formation, wound healing, and transwell assays, respectively. As shown in Figure 2B, proliferation was significantly inhibited in A549

Table 1 Relationship of DLX6-AS1 expression with clinicopathologic characteristics in NSCLC patients

Features	Number	DLX6-AS1 expression		*P*-value
		Low (n=30)	High (n=21)	
Gender				
Male	29	17	12	0.554
Female	21	13	9	
Age				
<65	27	16	11	0.586
≥65	24	14	10	
Size of tumor				
<3 cm	25	19	6	0.015*
≥3 cm	26	11	15	
Grade				
Low	26	18	8	0.105
High	25	12	13	
Lymph node metastasis				
N0	22	16	6	0.070
N1-3	29	14	15	
TNM stages				
I-II	26	19	7	0.034*
III-IV	25	11	14	

Notes: *$P<0.05$.
Abbreviation: NSCLC, non-small cell lung cancer.

and H1229 cells transfected with pLKO.1-DLX6-AS1 compared to cells transfected with pLKO.1 (*P*<0.001). NSCLC cell colony formation was effectively suppressed by pLKO.1-DLX6-AS1 transfection compared to transfection with pLKO.1 (*P*<0.001, Figure 2C). Cell migration and invasion capabilities were also dramatically inhibited in A549 and H1229 cells transfected with pLKO.1-DLX6-AS1, compared to the cells transfected with pLKO.1 (*P*<0.001, Figure 2D–E).

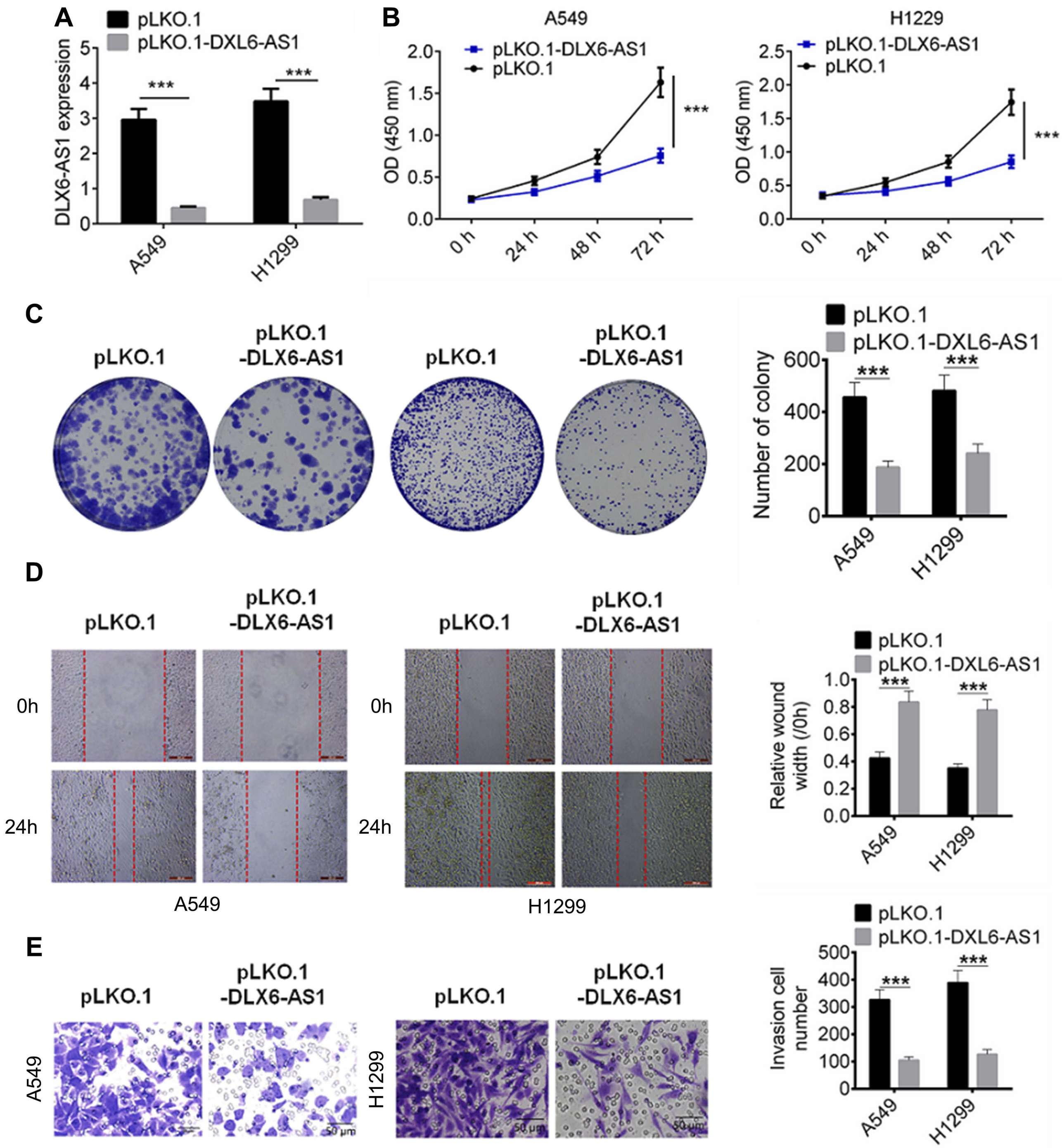

Figure 2 DLX6-AS1 regulated cell proliferation, cloning formation, migration and invasion of NSCLC cells. (**A**) A549 and H1229 cells were transfected with pLKO.1-DLX6-AS1 or pLKO.1 plasmids, the transfection efficiency was evaluated by qRT-PCR analysis. (**B**) Cell proliferation of A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was identified by CCK8 assay. (**C**) Cell cloning capability of A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was examined by colony formation assay. (**D**) Cell migration of A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was examined by wound healing. (E) Cell migration of A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was examined by transwell assay. Data are shown as the mean ± standard deviation, ****P*<0.001.

DLX6-AS1 and GSPT1 were targets of mir-27b-3p

To further explore the mechanism by which *DLX6-AS1* exerted its oncogenic effects in NSCLC, bioinformatics analyses (TargetScan and miRanda) were performed to predict the target miRNAs of *DLX6-AS1*. *miR-27b-3p* ranked the highest among the potential targets identified and the predicted *miR-27b-3p*-interaction site in *DLX6-AS1* is shown in Figure 3A. Furthermore, we constructed luciferase reporters containing the predicted *miR-27b-3p*-binding site (wt-DLX6-AS1) and a corresponding mutant site (mut-DLX6-AS1). As presented in Figure 3B, co-transfection of *miR-27b-3p* and wt-DLX6-AS1 markedly decreased luciferase activity, while co-transfection with *miR-27b-3p* and mut-DLX6-AS1 did not affect luciferase activity. The expression of *miR-27b-3p* in NSCLC cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was then examined by qRT-PCR. *miR-27b-3p* expression increased significantly in NSCLC cells with *DLX6-AS1* knocked down compared to its expression in control cells ($P<0.001$, Figure 3C). The expression levels of *miR-27b-3p* in NSCLC tumor tissues were also determined by qRT-PCR and they showed a negative correlation with *DLX6-AS1* expression levels (Figure 3D). Furthermore, *miR-27b-3p* expression was also significantly downregulated in NSCLC cell lines compared with HBE cells ($P<0.001$, Figure 3E).

Using TargetScan and miRanda, the target mRNA of *miR-27b-3p* was predicted to be *GSPT1* (Figure 3F). To verify this prediction, luciferase reporters containing the predicted *miR-27b-3p*-binding site (wt-GSPT1) and a corresponding mutant site (mut-GSPT1) were constructed. As shown in Figure 3G, co-transfection of *miR-27b-3p* and wt-GSPT1 significantly reduced luciferase activity, while co-transfection with *miR-27b-3p* and mut-GSPT1 did not affect luciferase activity. The mRNA expression of *GSPT1* in clinical NSCLC samples was assessed and was found to be negatively correlated with *miR-195b-3p* expression in NSCLC tumor tissues (Figure 3H). The protein expression of GSPT1 in NSCLC cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was then determined by western blotting. NSCLC cells showed a significant decrease in GSPT1 protein levels after silencing *DLX6-AS1* ($P<0.001$, Figure 3I). These results showed that *DLX6-AS1* interacted with the *miR-27b-3p*/*GSPT1* axis.

Targeting of miR-27b-3p by DLX6-AS1 regulated proliferation, migration, and invasion of NSCLC cells

To determine whether *DLX6-AS1* promoted the proliferation, migration, and invasion of NSCLC cells by targeting *miR-27b-3p*, A549 cells were transfected with pLKO.1 plus NC mimics, pLKO.1-DLX6-AS1 plus NC mimics, or pLKO.1-DLX6-AS1 plus *miR-27b-3p* mimics. Cell proliferation, clonal capability, migration, and invasion were then evaluated by CCK-8, cell colony formation, wound healing, and transwell assays, respectively. As shown in Figure 4A–D, pcDNA 3.1 (+)-DLX6-AS1 significantly inhibited the proliferation, colony formation, migration, and invasion of A549 cells compared to cells transfected with pLKO.1 plus NC mimic. Meanwhile, transfection with the *miR-27b-3p* mimic effectively reversed the effect of *DLX6-AS1* knockdown on A549 cells. These results indicated that the downregulation of *DLX6-AS1* inhibited the proliferation, migration, and invasion of NSCLC cells by regulating *miR-27b-3p* expression.

Knockdown of DLX6-AS1 inhibited tumor growth in vivo

The effect of *DLX6-AS1* on NSCLC development was evaluated in a mouse xenograft model. A549 cells transfected with shNC or shDLX6-AS1 were implanted subcutaneously into nude mice. Tumor growth was then evaluated every 5 d. *DLX6-AS1* knockdown significantly delayed tumor growth in vivo (Figure 5A). As shown in Figure 5B, tumors in the shDLX6-AS1 group weighed significantly less than tumors in the shNC group ($P<0.001$). Moreover, the protein expression of Ki67 and GSPT1 was examined in tumor tissues by immunohistochemical analysis. The protein levels of GSPT1 and Ki67 in tumor tissues were significantly reduced by shDLX6-AS1 transfection, as compared to transfection with shNC (Figure 5C). These results showed that *DLX6-AS1* promoted tumor growth in vivo.

Discussion

In recent years, lncRNAs have been shown to play a crucial role in the occurrence and progression of various cancers. LncRNA *DLX6-AS1* is upregulated in pancreatic cancer,[10] osteosarcoma,[11] glioma,[12] hepatocellular carcinoma,[13] and renal cell carcinoma.[13] Li et al reported that *DLX6-AS1* expression is significantly increased in lung adenocarcinoma and that

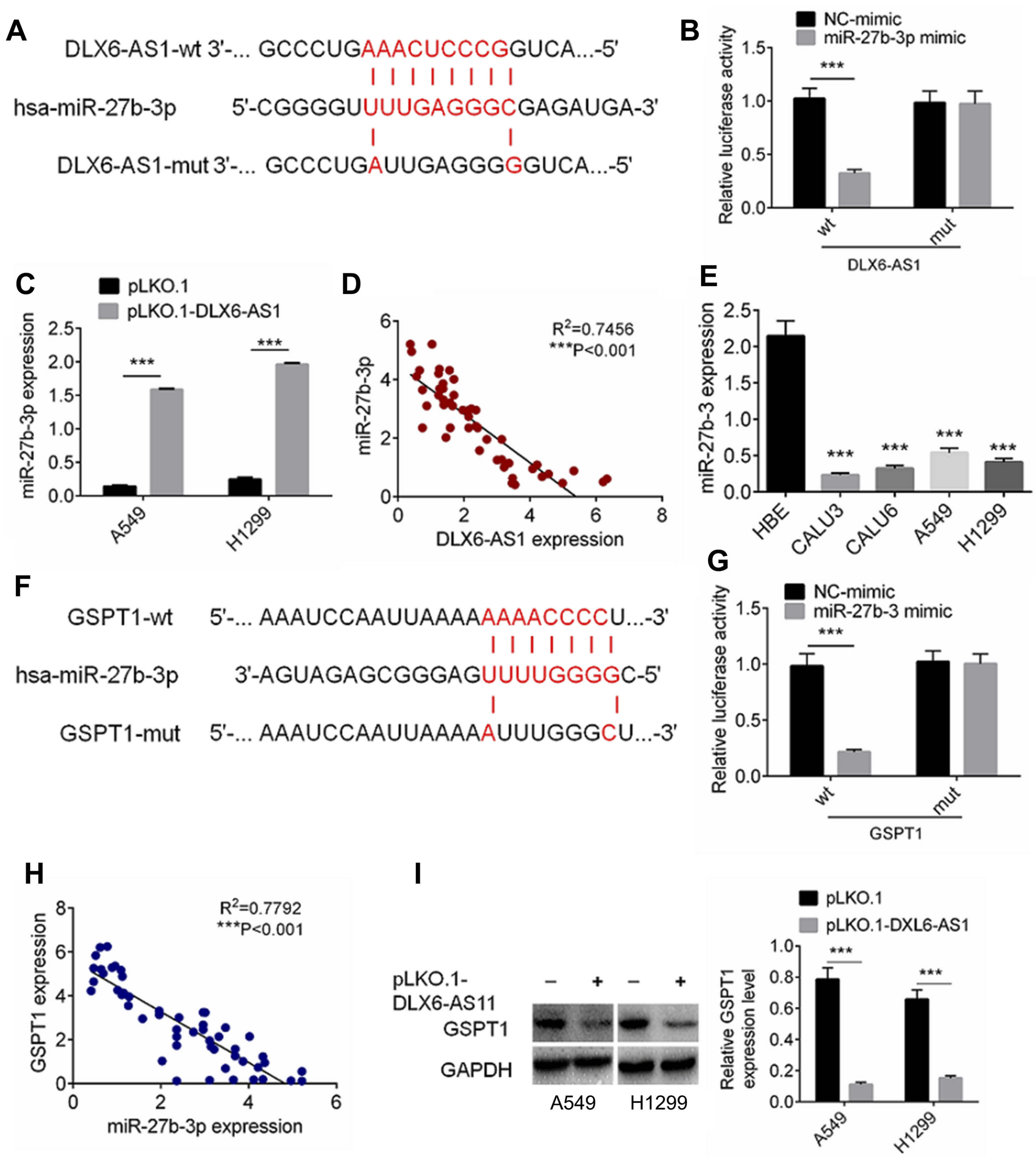

Figure 3 DLX6-AS1 could interact with miR-27b-3p/GSPT1. (**A**) miR-27b-3p binding sites in DLX6-AS1 were predicted by bioinformatics analysis. (**B**) Luciferase reporter assays were performed using HEK293T cells co-transfected with the miR-27b-3p mimic and DLX6-AS1-wt or DLX6-AS1-mut reporter plasmid. (**C**) The expression of miR-27b-3p in A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was examined by qRT-PCR. (**D**) The expression of miR-27b-3p in NSCLC tissues was identified, which showed negative correlation with DLX6-AS1 expression. (**E**) The expressions of miR-27b-3p in NSCLC cell lines and HBE cells were determined by qRT-PCR. (**F**) miR-27b-3p binding sites in GSPT1 were predicted by bioinformatics analysis. (**G**) Luciferase reporter assays were performed using HEK293T cells co-transfected with the miR-27b-3p mimic and GSPT1-wt or GSPT1-AS1-mut reporter plasmid. (**H**) The expression of GSPT1 in NSCLC tissues was identified, which showed negative correlation with miR-27b-3p expression. (**I**) The expression of GSPT1 in A549 and H1229 cells transfected with pLKO.1-DLX6-AS1 or pLKO.1 was examined by western blot analysis. ****P*<0.01.

DLX6-AS1 expression levels were significantly correlated with histological differentiation and TNM stage. However, the effect of *DLX6-AS1* on the proliferation, migration, and invasion of NSCNC cells and the associated mechanisms are unclear. In the present study, we firstly found that *DXL6-AS1* expression was up-regulated in NSCLC tissues and cell lines and that its expression positively correlated with tumor size and advanced clinical stage. These data indicated that *DXL6-AS1* may play a crucial role in the occurrence and development in NSCLC. Previous studies have reported that *DXL6-AS1* regulates the proliferation, migration, and invasion of cancer cells. In our study, NSCLC cells were transfected with

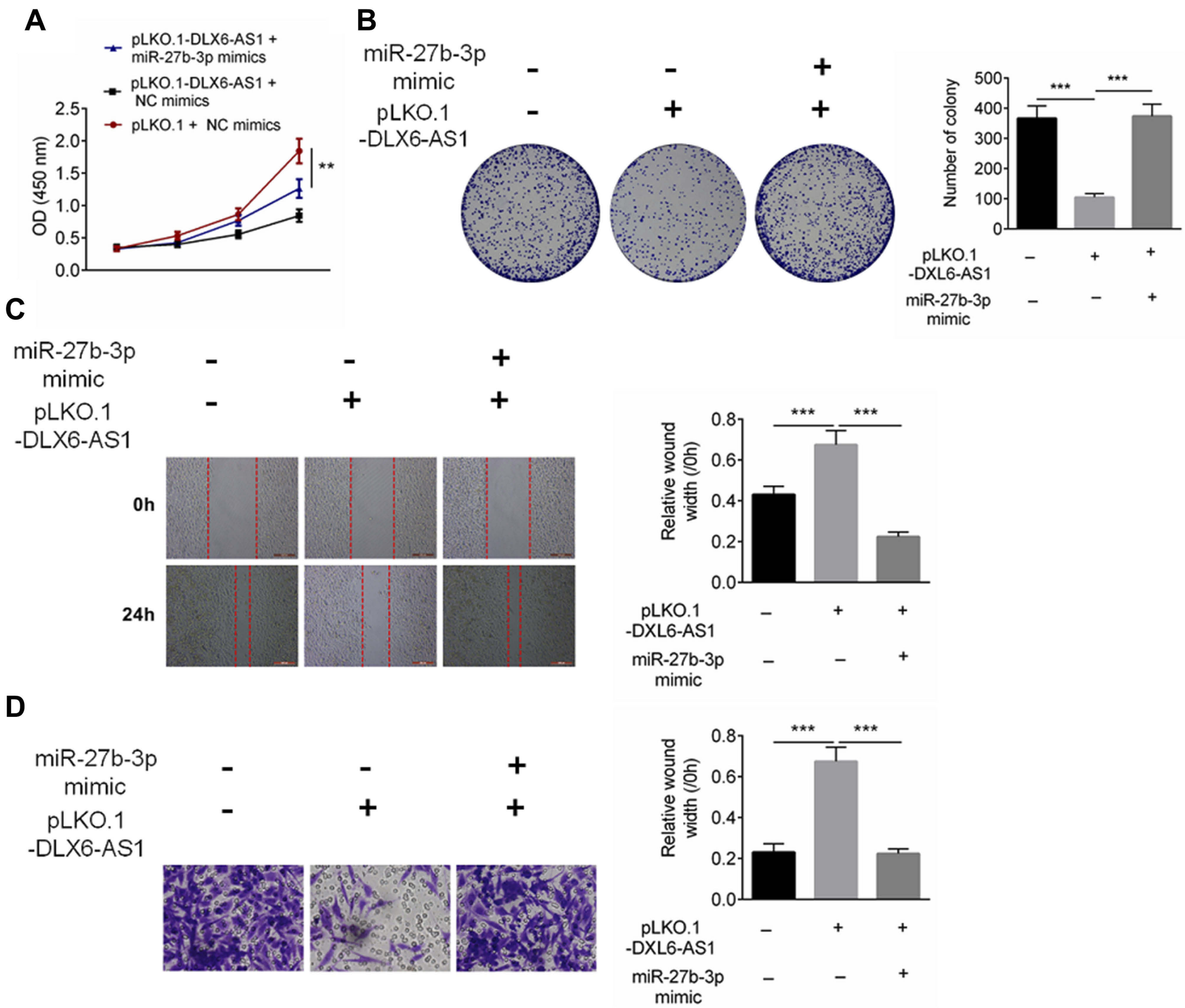

Figure 4 DLX6-AS1 knockdown inhibited cell proliferation, migration and invasion of NSCLC cells through targeting miR-27b-3p. A549 cells were transfected pLKO.1+NC mimics, pLKO.1-DLX6-AS1+NC mimics, pLKO.1-DLX6-AS1+ miR-27b-3p mimics. Cell proliferation (**A**), cloning formation (**B**), migration (**C**) and invasion (**D**) were identified by CCK8, cell cloning formation, wound healing and transwell assays, respectively. Data are shown as the mean ± standard deviation, ****P*<0.001.

siDXL6-AS1 and cell phenotype was then examined. The results showed that *DXL6-AS1* downregulation inhibited the proliferation, colony formation, migration, and invasion of NSCLC cells. Furthermore, the anti-tumor effect of *DXL6-AS1* was confirmed in vivo in a xenograft model. Ki67 is closely correlated with cancer cell proliferation[15] and its expression in tumor tissues was also inhibited by *DXL6-AS1* silencing. These results indicated that *DXL6-AS1* promoted NSCLC tumor growth both in vitro and in vivo.

Previous reports have demonstrated that lncRNAs can exert biological effects through a number of mechanisms, including transcriptional and post-transcriptional regulation. Accumulating evidence indicates that lncRNAs are involved in carcinogenesis and cancer progression by acting as competing endogenous RNAs (ceRNAs) and sponging miRNAs. For example, lncRNA prostate cancer-associated transcript 7 (*PCAT7*) accelerates tumorigenesis by inhibiting *miR-134-5p* in NSCLC and is associated with poor prognosis.[16] LncRNA *LINC00339* promotes the carcinogenesis of NSCLC by targeting FOXM1 and thus, sponging *miR-145*.[17] Zeng et al reported that *DLX6-AS1* promotes cell proliferation, migration, and invasion of renal cell carcinoma by targeting the *miR-26a*/PTEN axis.[9] Li et al reported that *DLX6-AS1* accelerates glioma tumorigenesis by sponging endogenous *miR-197-5p* to decrease its targeting of E2F1.[12] In our study, we confirmed that *miR-27b-3p* was a target of *DLX6-AS1*. Numerous studies have demonstrated that *miR-27b-3p* acts as a tumor suppressor in many types of

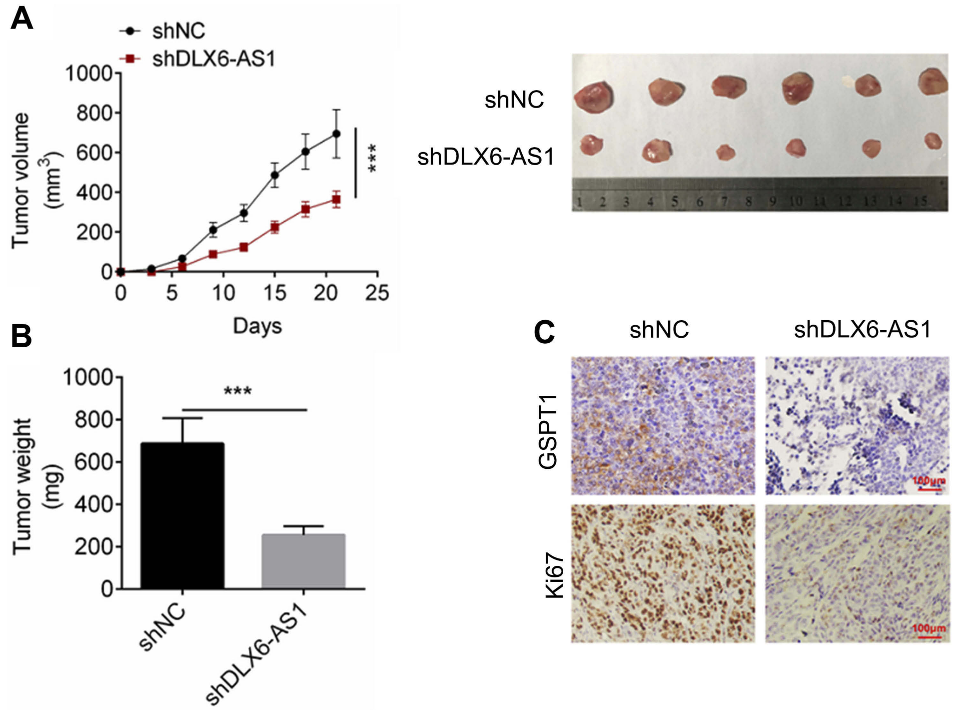

Figure 5 DLX6-AS1 promoted tumor growth in vivo. (**A**) Tumor growth curves were established by measuring tumor volume every 5 for 25 days after injection. (**B**) Tumor weights isolated from nude mice in each treatment group were determined on day 25 after injection. (**C**) GSPT1 and Ki67 expressions in tumor tissue were evaluated by IHC analysis. Data are shown as the mean ± standard deviation, ***P<0.001.

cancers.[18–20] Sun et al reported that *miR-27b-3p* is downregulated in lung cancer tissues and this promotes apoptosis and suppresses cancer cell viability and survival via the downregulation of Fzd7.[21] In our study, *miR-27b-3p* was also shown to be downregulated in NSCLC tissues and its expression was negatively correlated with *DLX6-AS1* expression. Furthermore, *miR-27b-3p* mimics reversed the antitumor effects of *DLX6-AS1* on NSCLC. GSPT1 has been reported as an oncogene in gastric cancer,[22]colorectal cancer,[23] and breast cancer.[24] GSPT1 is a GTPase that associates with eukaryotic Release Factor 1 (eRF1) in a complex mediates that translation termination.[24] GSPT1 has been shown to play several roles in critical cellular processes such as cell cycle regulation, cytoskeleton organization and apoptosis. Sajitha et al reported that nicotine mediated invasion and migration of NSCLC cells by modulating STMN3 and GSPT1.[25] In the present study, *GSPT1* was confirmed as the target mRNA of *DLX6-AS1* and its expression was negatively correlated with *miR-27b-3p* expression in NSCLC samples. GSPT1 expression was significantly decreased in NSCLC cells after siDLX6-AS1 transfection. These results demonstrated that *DLX6-AS1* exerted its tumorigenesis role by targeting *miR-27b-3p*/*GSPT1*.

In conclusion, our study demonstrated that lncRNA *DLX6-AS1* was overexpressed in NSCLC tissues and cell lines and it promoted the proliferation, migration, and invasion of NSCLC cells in vivo and in vitro. *DLX6-AS1* exerted its carcinogenic effects in NSCLC by regulating the *miR-27b-3p*/*GSPT1* axis, which may provide new strategies for the prevention and treatment of NSCLC.

Disclosure

The authors report no conflicts of interest in this work.

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