Original Article

miR-325 Supresses Cell Proliferation and Migration in Non-Small Cell Lung Cancer via Targeting DNA Ligase 1 (LIG1)

(DNA ligase 1 / LIG1 / NSCLC / miR-325 / proliferation / migration)

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Abstract. DNA ligase 1 (LIG1) plays a key role in DNA synthesis and DNA damage repair pathways. LIG1 has been shown to be up-regulated in human non-small cell lung cancer (NSCLC); however, its role and molecular regulatory mechanism in NSCLC cell proliferation are still not fully understand. In this study, we aimed to explore the role of LIG1 and post-transcripional regulators in NSCLC. Utilizing bioinformatic tools and qRT-PCR, our investigation substantiated the up-regulation of LIG1 within NSCLC cell lines and tumour tissues. Remarkably, individuals exhibiting elevated levels of LIG1 had diminished survival rates. Functionally, the depletion of LIG1 inhibited cell proliferation and migration, contrasting with the increased proliferation and migration upon LIG1 over-expression. Prediction from the TargetScanHuman database and results of dual luciferase reporter assays indicated that miR-325 could directly bind to and negatively regulate LIG1. Moreover, our findings demonstrated that the mimicry of miR-325 decreased cell viability, whereas its inhibition correspondingly increased viability, indicative of the tumour-suppressive role of miR-325 through the down-regulation of LIG1. Collectively, our findings show that LIG1 could promote tumour progression and knockdown of LIG1 could exert suppressive effects on NSCLC. As the post-transcriptional factor of LIG1, miR-325 could negatively regulate the expression of LIG1 to inhibit tumour progression *in vitro*. These findings suggest that LIG1 and miR-325 might be potential therapeutic targets for NSCLC treatment.

Introduction

Lung cancer stands out as one of the most lethal malignancies worldwide, claiming a significant number of lives annually (Molina et al., 2008). Histologically, it is broadly categorized into small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), with NSCLC constituting approximately 85 % of all lung cancer cases (Morgensztern et al., 2010; Torre et al., 2015). Within NSCLC, adenocarcinoma (ADC) and squamous cell carcinoma (SQCC) are predominant, collectively representing over 70 % of NSCLC diagnoses (Osmani and Li, 2017). Despite advancements in treatment modalities over the past two decades, the five-year overall survival rate for lung cancer patients remains dismally low at 22 % (Siegel et al., 2021). Consequently, there is a pressing need to identify novel therapeutic targets for NSCLC.

Tumour proliferation is a hallmark characteristic of cancer, reliant on DNA synthesis and regulated by numerous pathways (Ren et al., 2023). Within eukaryotic cells, DNA ligase 1 (LIG1) plays a pivotal role in this process, participating in DNA replication, repair and recombination (Johnson and O'Donnell, 2005; Li et al., 2015; Tian et al., 2015; Lu et al., 2016; Howes et al., 2017). Given its central involvement in DNA metabolism, LIG1 emerges as a potential candidate implicated in lung cancer susceptibility. How is the expression of LIG1 regulated molecularly? Recently, it has been reported that the oncogenic RNA-binding protein SRSF1 binds to LIG1 mRNA and regulates its expression by increasing its mRNA stability and enhancing its translation in an mTOR-dependent manner in NSCLC cell lines (Martínez-Terroba et al., 2018), suggesting positive regu-

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Abbreviations: 3'-UTR 3'– untranslated region, CCK8 – Cell Counting Kit-8, LIG1 – DNA ligase 1, miRNAs – microRNAs, NSCLC – non-small cell lung cancer, qRT-PCR – quantitative real-time polymerase chain reaction, SCLC – small-cell lung cancer.

lation of the expression of LIG1 post-transcriptionally. However, it is still not fully understood what the negative regulators of LIG1 post-transcriptionally are.

MicroRNAs (miRNAs), small non-coding RNAs approximately 22–26 nucleotides in length, exert mainly negative regulatory control of gene expression by binding to the 3'-untranslated region (3'-UTR) of target genes post-transcriptionally (Rupaimoole and Slack, 2017). Numerous studies have underscored the critical roles of miRNAs in cellular processes such as proliferation, differentiation and apoptosis, all of which are intimately linked to tumorigenesis (Billeter et al., 2012; Barger and Nana-Sinkam, 2015; Bersimbaev et al., 2020). Specifically, miR-325 has been implicated in various cancers, exhibiting tumour-suppressive effects in gastric cancer (Huang et al., 2023), glioma (Xiong and Su, 2021) and bladder cancer (Sun et al., 2020). In the context of NSCLC, miR-325 has been reported to repress proliferation by targeting KIF2C and HMGB1 (Yao et al., 2015; Gan et al., 2019); it is reasonable and critical to identify the new targets of miR-325 in the context of NSCLC.

In our study, we observed up-regulation of LIG1 in NSCLC tissues compared to normal tissues. Functional investigations revealed that modulation of LIG1 expression, either through knockdown or over-expression, impacted NSCLC cell proliferation and migration. Most importantly, mechanistic insights further unveiled miR-325 as an upstream regulator of the expression of LIG1 post-transcriptionally in the context of NSCLC. These findings collectively shed light on a novel regulatory axis involved in NSCLC pathogenesis and suggest potential therapeutic targets for clinical intervention.

Material and Methods

Human non-small-cell lung cancer samples

Surgically resected NSCLC tissues and corresponding normal lung tissue were obtained from 24 Chinese patients at Shaanxi Provincial Hospital of Chinese Medicine between 2010 and 2019. The samples were histologically confirmed to be tumour or non-tumour tissues, and then were quickly frozen in liquid nitrogen before use. Clinical parameters, including pathological features and clinical stage, were retrospectively collected and listed in Table 1. The study protocol was approved by the ethics committees of Shaanxi Provincial Hospital of Chinese Medicine with No. AF/SQ-04/01.3. The written consents were obtained from each patient. Freshly harvested samples were immersed in Trizol (Life Technologies, Carlsbad, CA) before snap freezing within 30 minutes after surgery.

Cell culture

The NSCLC cell lines (A549, H1299, HCC827, BEAS-2B and H1975) and human bronchial epithelial cell line BEAS-2B were obtained from the Cell Bank of Shanghai Institutes for Biological Sciences Cell Resource Center with their catalogue information: A549 (cat. SCSP-503), H1299 (cat. SCSP-589), HCC827 (cat. SCSP-538), BEAS-2B (GNHu27), H1975 (cat. SCSP-597). SPC-A1 cells were obtained from Hengyuan Biotechnology Co., Shandong, China (cat. HYCC10059). Cells were cultured in Dulbecco's Modified Eagle's Medium containing 10 % foetal bovine serum (Hyclone, Logan, UT), 100 IU/ml penicillin and 100 IU/ml streptomycin (ST488S, Beyotime, Jiangsu, China). All cells were maintained at 37 °C under an atmosphere of 5 % CO_2.

RNA extraction

Total RNA was extracted from tissues and cells using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA concentration and purity were determined using a model ND-2000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). Only samples with absorbance ratios 260 nm/280 nm of ~2.0, and 260 nm/230 nm of 1.9–2.2 were considered for inclusion in the study.

Table 1. Clinicopathological characteristics of LIG1 expression in NSCLC patients

Features	Number	LIG1 level (relative to the mean value of control) (mean ± SD)	P value
Gender			
Male	14	14.56 ± 4.33	P > 0.05
Female	10	12.19 ± 3.17	
Age			
≥ 60	13	13.17 ± 2.99	P > 0.05
< 60	11	10.87 ± 2.47	
TNM stage			
I–II	14	17.36 ± 1.89	P < 0.05
III	10	10.87 ± 4.74	
Tumour size			
< 3 cm	16	15.74 ± 2.78	P > 0.05
> 3cm	8	13.17 ± 3.85	
Lymph node metastasis			
Yes	12	17.09 ± 1.15	P < 0.05
No	12	11.48 ± 2.45	

Quantitative RT-PCR

Quantitative RT-PCR analysis was used to determine the relative level of LIG1 and miR-325. Five hundred ng total RNA was used for reverse transcription using the RevertAid first-stand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA). Quantitative PCR using SYBR Green PCR master mix (Thermo Fisher Scientific) was employed to determine the LIG1 level with PCR cycle conditions of 95 ℃ 10 min, 95 °C 15 s, and 60 °C 1 min, for 40 cycles. The relative expression was calculated using the $2^{-\Delta\Delta CT}$ method. *GAPDH* and *U6* were used as loading controls. All experiments were performed in triplicate and each sample was plated in duplicate.

Primer sequences were:
LIG1-QF: 5'-CTTCCTGCTGGCCTCCTAC-3'
LIG1-QR: 5'-CACTGAAGCCAGTTCCAAGC-3'
GAPDH-QF:5'-CAAGAGCACAAGAGGAAGAGAG-3'
GAPDH-QR: 5'-CTACATGGCAACTGTGAGGAG-3'
miR-325-QF: 5'-GTAGGTGTCCAGTAAGTG-3'
miR-325-QR: 5'-GAACATGTCTGCGTATCTC-3'
U6-QF: 5'-GCTTCGGCAGCACATATACTAAAAT-3'
U6-QR: 5'-CGCTTCACGAATTTGCGTGTCAT-3'

Western blot analysis

Total proteins were extracted from cells using ice-cold RIPA buffer (cat: 89901, Thermo Fisher Scientific) containing protease inhibitors (cat: 87786, Thermo Fisher Scientific) and quantified using a bicinchoninic acid (BCA) protein quantification kit (cat: P0010, Beyotime). Protein was separated using 10 % sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). Membranes were incubated with primary antibodies and GAPDH. Immunoreactive bands were visualized using the Pierce ECL Western Blotting Substrate (cat: sc-2048, Santa Cruz Biotechnology, Dallas, TX).

Cell migration assay

CA549 cells were transfected with siRNA for 48 h. Cells in an amount of 1×10^5 were seeded in 3.5-cm wells and attached on the plates overnight. The scratch test was performed using a sterile 100 µl filter tip. Medium was replaced twice to remove floating cells. Three pictures for each condition were taken at 10× magnification every 18 hours. The size of the wound was measured using ImageJ bundled with 64-bit Java 8 (Schneider et al., 2012). Measurements were performed in triplicate for each condition in at least three independent experiments and normalized to 0 h values (100 %).

Cell proliferation assay

Cell proliferation was detected using the CCK-8 assay kit (cat: CK04-11, Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. Briefly, A549 cells were seeded into 96-well plates at low density (2×10^3/well) in DMEM medium and allowed to attach overnight. The cells were then transfected with one of LIG1-specific siRNAs, with scrambled sequence RNA as control. At the last 12 hours of culture of different time points, 10 µl CCK-8 solution was added into each well. Then, the absolute fluorescence value at OD 450 nm of different time points was determined. All determinations were performed in duplicates.

Luciferase reporter assay

To construct dual luciferase reporter plasmids, the theoretical binding sequence of miR-325 in LIG1 (LIG1 WT) and its mutated sequence (LIG1 Mut) were cloned into pmirGLO Dual-luciferase vectors, respectively (GenePharma, Shanghai, China). A549 cells were co-transfected with wild-type pmirGLO-LIG1 (LIG1 WT) or the mutated LIG1 (LIG1 Mut) reporter plasmid and miR-325 mimics or negative control using Lipofectamine 2000 (cat:11668019, Invitrogen). After 48 h, luciferase activity was detected using the dual-luciferase reporter kit following the manufacturer's instruction (Promega, Madison, WI). The relative firefly luciferase activity was calculated by normalizing to the renilla luciferase activity.

Plasmid constructs and cell transfection

Specific siRNA oligonucleotides against LIG1 (si-LIG1) and negative control (si-NC) were purchased from GenePharma. The complementary DNA encoding LIG1 was PCR-amplified and inserted into pcDNA3.1 (NovoPro Bioscience Inc, Cat. V012531#, Shanghai, China). miR-325 mimics, miR-325 inhibitors and negative control were purchased from Life Technologies (Ambion, Grand Island, NY). The transfection of cells was carried out using Lipofectamine 2000.

Si-LIG1-1 sense: 5-GACAGUGAGGAGCUGCAGGTT-3
Anti-sense: 5-CCUGCAGCUCCUCACUGUCTT-3

Bioinformatic analysis

The LIG1 level in normal and NSCLC (LUSC and LUAD) tissues was extracted from the GEPIA database (http://gepia.cancer-pku.cn/) (Tang et al., 2017). LUSC is short for lung squamous cell carcinoma) and LUAD is short for lung adenocarcinoma. $P < 0.05$ was considered as significant. ImageJ was downloaded from https://imagej.net/ij/ij/download.html, version ImageJ bundled with 64-bit Java 8. miRNA targeting prediction was from TargetScanHuman (https://www.targetscan.org/vert_80/) (Agarwal et al., 2015).

Statistical analysis

Data were analysed using SPSS Statistical Package version 19.0 (SPSS Inc., Chicago, IL). The results were analysed by paired *t*-test. A two-tailed P value < 0.05 was considered significant.

Results

The expression of LIG1 was up-regulated in NSCLC

To determine the potential association of LIG1 with the progression of NSCLC, analysis of LIG1 expression was conducted utilizing the GEPIA database (http://gepia.cancer-pku.cn/). Given that SQCC and ADC collectively represent over 70 % of NSCLC cases, datasets for both subtypes available in the database were utilized. The analysis revealed a significant increase ($P < 0.05$) in LIG1 expression in tumour tissues compared to normal tissues in both lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) (Fig. 1A). To corroborate these findings, we further investigated LIG1 expression in NSCLC tissues obtained from our hospital by qRT-PCR. Consistently, significant up-regulation ($P <$

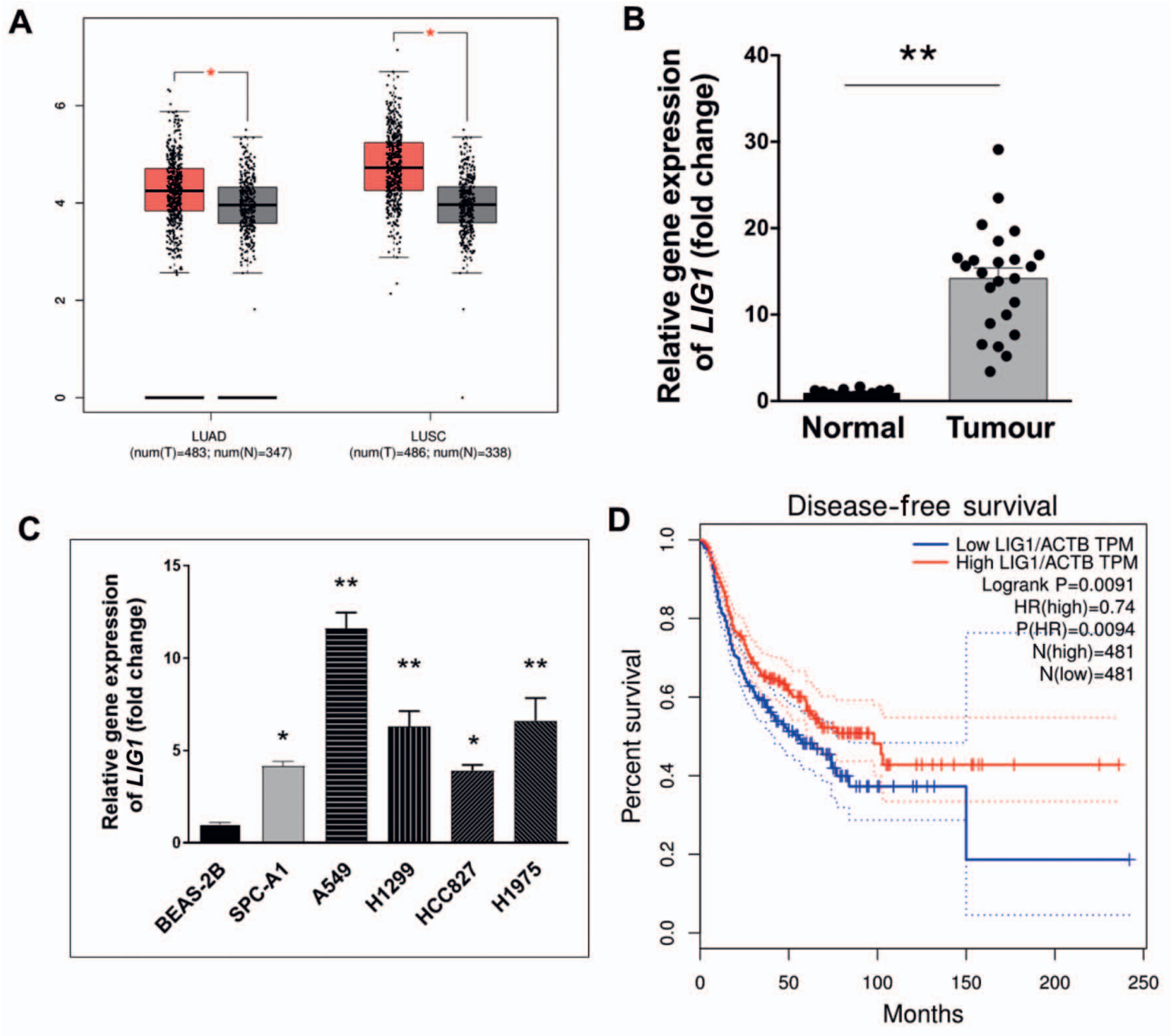

Fig. 1. The expression of LIG1 was up-regulated in NSCLC.
(**A**) The expression level of LIG1 in normal and NSCLC (LUSC and LUAD) tissues was extracted from the GEPIA database. num(T) is the tumour group and num(N) is the normal group.
(**B**) The expression of LIG1 was determined by qRT-PCR in 12 pairs of NSCLC tissues (Tumour) compared with adjacent non-tumour tissues (Normal).
(**C**) The expression of LIG1 was examined by qRT-PCR in five NSCLC cell lines (A549, SPC-A1, H1299, HCC827, and H1975) and human bronchial epithelial cell line BEAS-2B, which was used as a normal cell control.
(**D**) The Kaplan-Meier survival analysis from the GEPIA database showed that NSCLS patients with high LIG1 expression had a poor overall survival compared to patients with low LIG1 expression. LUSC: lung squamous cell carcinoma, LUAD: lung adenocarcinoma, $^{*}P < 0.05$, $^{**}P < 0.01$.

0.01) of LIG1 expression in NSCLC tissues compared to adjacent non-tumour tissues was observed (Fig. 1B). Moreover, LIG1 expression was elevated ($P < 0.05$) in five NSCLC cell lines (A549, SPC-A1, H1299, HCC827, and H1975) relative to the human bronchial epithelial cell line BEAS-2B (Fig. 1C). Additionally, analysis of NSCLC patient data from the GEPIA database revealed that individuals with high LIG1 expression exhibited poorer overall survival compared to those with low LIG1 expression (Fig. 1D). Taken together, these findings suggest a potential key role for LIG1 in the development and progression of NSCLC.

Knockdown of LIG1 suppressed NSCLC cell proliferation and migration in vitro

To elucidate the oncogenic potential and functional role of LIG1 in NSCLC, A549 cells were transfected with siRNA targeting LIG1 (si-LIG1). Subsequently, the knockdown efficiency was assessed via qRT-PCR 48 hours post-transfection. LIG1 expression was significantly reduced ($P < 0.01$) at both mRNA and protein levels in cells transfected with si-LIG1 compared to those transfected with control siRNA (si-NC) (Fig. 2A–2B). To evaluate the impact of *LIG1* knockdown on cell viability, CCK-8 assay showed a substantial decrease ($P < 0.01$) in cell vitality at 72 and 96 hours post-si-LIG1 transfection compared to the si-NC group (Fig. 2C). Next, the migration assay demonstrated that the si-LIG1 group exhibited a significantly reduced migration distance compared to the si-NC group ($P < 0.01$, Fig. 2D–2E), suggesting an impaired migratory capacity upon LIG1 depletion. Altogether, these findings underscore the crucial role of LIG1 in NSCLC cell proliferation and migration.

Over-expression of LIG1 increased NSCLC cell proliferation and migration in vitro

To further investigate the function of LIG1 in NSCLC cells, A549 cells were transfected with pcDNA-LIG1 plasmid to over-express *LIG1* or pcDNA-GFP control plasmid. Forty-eight hours later, qPCR revealed a significant increase ($P < 0.01$) in LIG1 expression at both mRNA and protein levels in cells transfected with pcDNA-LIG1 compared to those transfected with pcDNA-GFP (Fig. 3A–3B). Remarkably, over-expression of *LIG1* led to a significant promotion ($P < 0.05$) in cell vitality at 72 and 96 hours post-vector transfection compared to the pcDNA-GFP control group (Fig. 3C). Furthermore, *LIG1* over-expression exhibited an increased migration distance compared to the control group ($P < 0.01$, Fig.

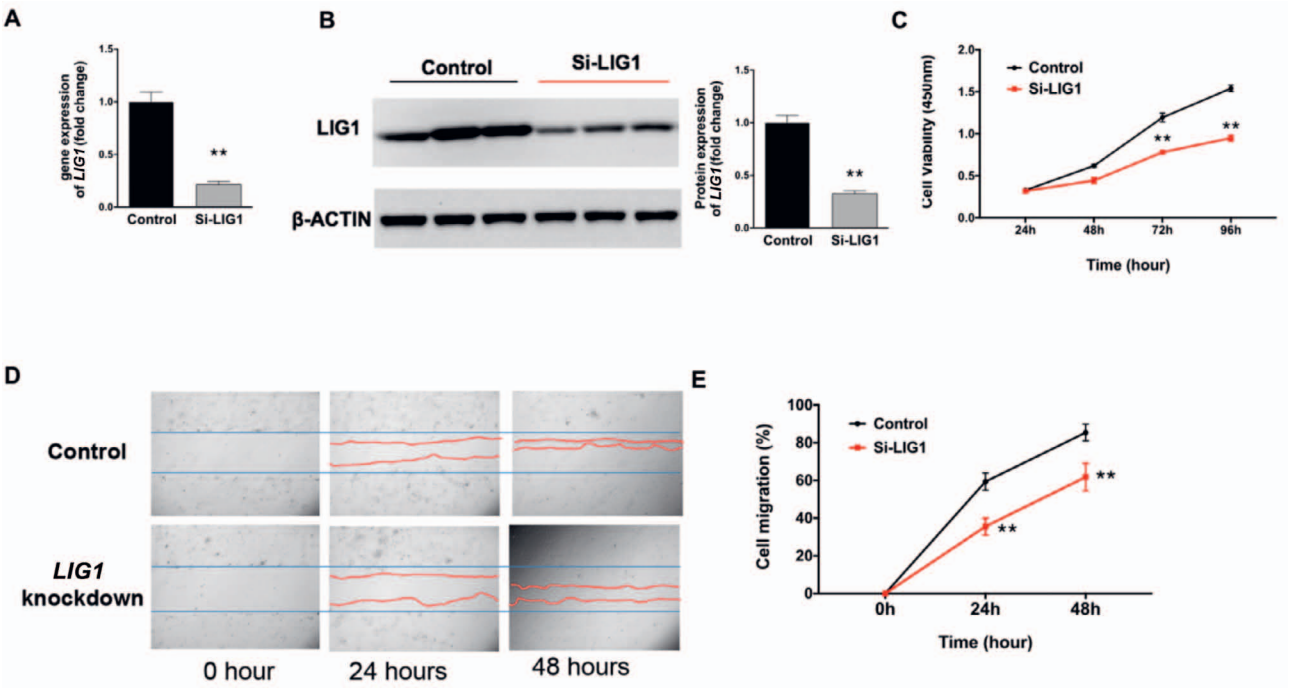

Fig. 2. Knockdown of *LIG1* suppressed NSCLC cell proliferation and migration *in vitro*.
(**A**) Relative gene expression of *LIG1* after A549 cells were transfected with si-LIG1 or si-NC. Si-LIG1 is siRNA knockdown of LIG1, si-NC is a negative control.
(**B**) Protein expression of LIG1 after A549 cells were transfected with si-LIG1 or si-NC. (**C**) The effect of si-LIG1 on the proliferation of A549 cells was detected by CCK-8 assay.
(**D**, **E**) The effect of si-LIG1 on the migration of A549 cells was detected by the wound closure assay. In panel D, red lines are cell migration borders. $^{*}P < 0.05$, $^{**}P < 0.01$.

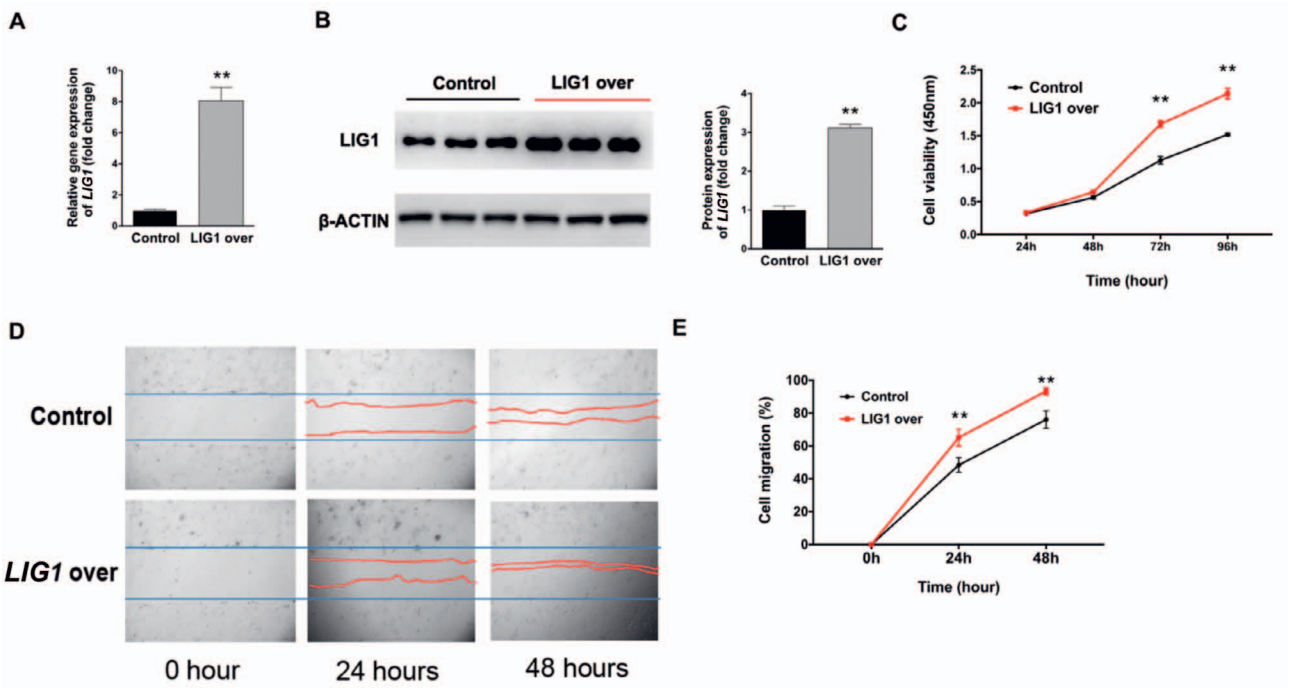

Fig. 3. Over-expression of *LIG1* increased NSCLC cell proliferation and migration *in vitro*.
(**A**) Relative gene expression of *LIG1* after A549 cells were transfected with LIG1 or vector.
(**B**) Protein expression of LIG1 after A549 cells were transfected with LIG1 or vector.
(**C**) The effect of *LIG1* over-expression on the proliferation of A549 cells was detected by CCK-8 assay.
(**D**, **E**) The effect of *LIG1* over-expression (*LIG1* over) on the migration of A549 cells was detected by the wound closure assay. In panel D, red lines are cell migration borders. $^{*}P < 0.05$, $^{**}P < 0.01$.

3D–3E), indicating enhanced migratory capacity upon *LIG1* over-expression. Collectively, these functional findings strongly suggest that LIG1 plays a pivotal role in promoting A549 cell proliferation and migration.

miR-325 was a negative regulator of LIG1 post-transcriptionally

Utilizing the bioinformatic tool 'TargetScan Human', we identified miR-325 as the sole conserved miRNA predicted to regulate LIG1, indicating its potential significance (Fig. 4A). To validate this prediction, dual luciferase reporter assays were conducted, revealing that transfection with miR-325 mimics significantly attenuated ($P < 0.01$) the luciferase activity of LIG1 WT, while no effect was observed on LIG1 Mut, confirming the regulatory interaction of miR-325 and LIG1 mRNA. These data also strongly indicated that there might be a direct binding of miR-325 onto LIG1 mRNA (Fig. 4B). Moreover, our investigation of clinical specimens demonstrated significant down-regulation ($P < 0.01$) of miR-325 expression in NSCLC tissues compared to normal counterparts (Fig. 4C). Similarly, miR-325 expression was notably decreased ($P < 0.05$) in five NSCLC cell lines (A549, SPC-A1, H1299, HCC827, and H1975) relative to the human bronchial epithelial cell line BEAS-2B (Fig. 4D). Correlation analyses further revealed a significant negative correlation between miR-325 and LIG1 expression in NSCLC tissues ($P < 0.0001$, $r = -0.7832$, Fig. 4E). Collectively, these findings suggest that miR-325 acts as a negative regulator of LIG1 in NSCLC cells.

miR-325 exerted its functions on lung cancer cells via targeting LIG1

To examine the function of miR-325 in NSCLC cell proliferation, A549 cells were transfected with either miR-325 mimic or inhibitor, and the expression of miR-325 was determined by qPCR (Fig. 5A). The results demonstrated significant down-regulation ($P < 0.01$) of LIG1 expression following transfection with the miR-325 mimic, whereas LIG1 expression was markedly up-regulated ($P < 0.01$) in the miR-325 inhibitor group (Fig. 5B). Notably, this regulatory pattern was consistent at the protein level, with LIG1 expression exhibiting concordant changes ($P < 0.05$) following transfection with the miR-325 mimic or inhibitor (Fig. 5C–5D). Next, the function of miR-325 modulation on cell proliferation was investigated. The CCK-8 assay revealed a significant decrease ($P < 0.01$) in the proliferation capacity of A549 cells upon treatment with the miR-325 mimic (Fig. 5E), whereas treatment with the miR-325 inhibitor led to a significant increase ($P < 0.01$)

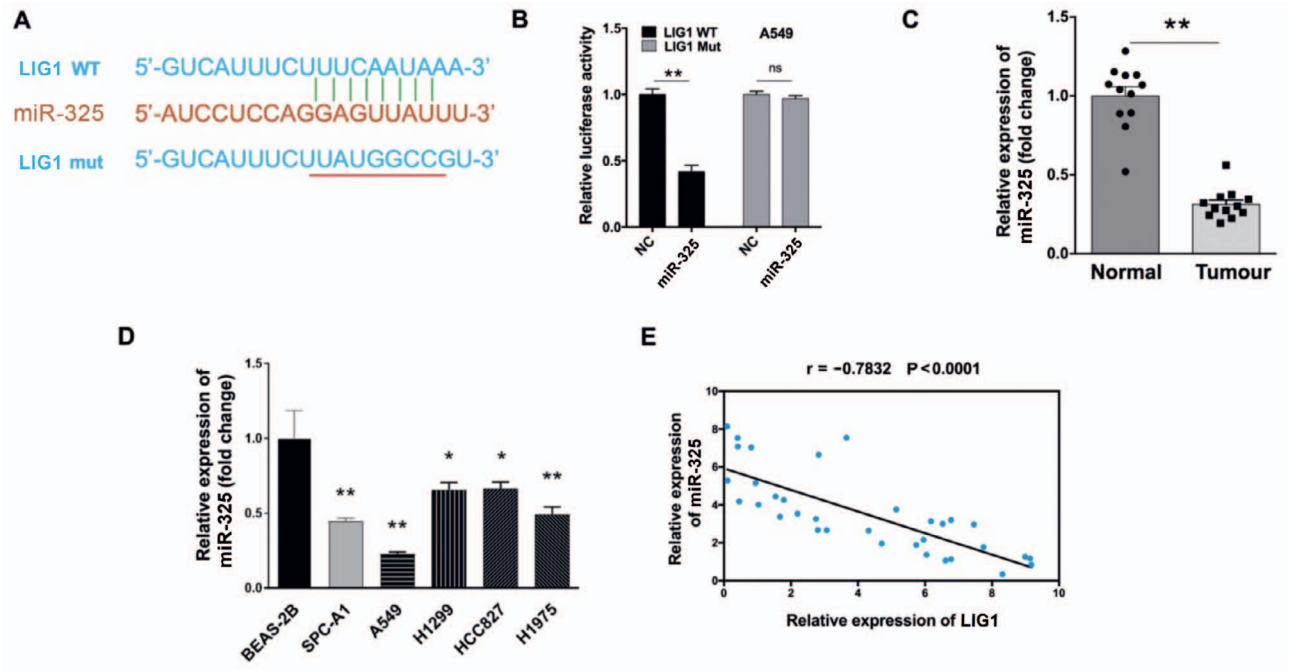

Fig. 4. Direct interaction between LIG1 and miR-325.
(**A**) LIG1 harboured the miR-325 binding site, which was predicted by bioinformatic tool "TargetScanHuman".
(**B**) Dual luciferase reporter assays revealed that miR-325 could negatively regulate the luciferase activities of LIG1 WT, rather than LIG1 Mut in A549 cells.
(**C**, **D**) qRT-PCR assays were utilized to detect the miR-325 expression in lung cancer tissues (**C**) and cell lines (**D**).
(**E**) Correlation analysis between the expression of miR-325 and LIG1. Pearson's correlation test (r, 95 % CI). $^{*}P < 0.05$, $^{**}P < 0.01$.

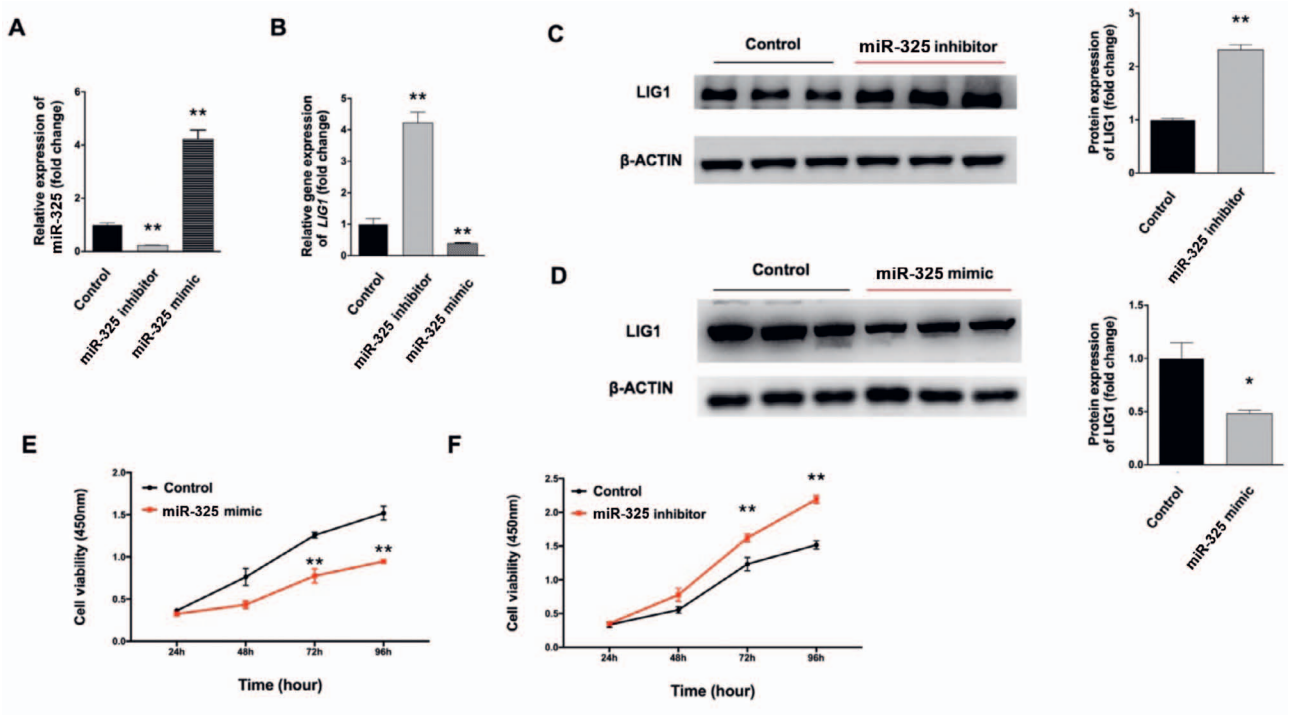

Fig. 5. miR-325 exerted its functions on lung cancer cells via targeting LIG1.
(**A**) Relative expression levels of miR-325 in A549 cells after transfecting with miR-325 mimic or inhibitor.
(**B–D**) Gene expression and protein levels of LIG1 in A549 cells after transfection with miR-325 mimic or inhibitor.
(**E**, **F**) The effect of miR-325 mimic and inhibitor on the proliferation of A549 cells was detected by CCK-8 assay. $^{*}P < 0.05$, $^{**}P < 0.01$.

in cell proliferation (Fig. 5F). Together these data indicate that the observed effects of miR-325 on cell proliferation were partially mediated via targeting LIG1.

Discussion

Despite advancements in diagnosis and treatment, lung cancer remains the leading cause of mortality worldwide (Siegel et al., 2021). NSCLC accounts for the majority of lung cancer cases, with ADC and SQCC representing nearly 70 % of NSCLC cases (Herbst et al., 2008; Perez-Moreno et al., 2012). Hence, our study utilized datasets specific to ADC and SQCC to comprehensively investigate NSCLC.

Cell proliferation and migration are critical aspects of tumour growth, both requiring DNA synthesis (Ren et al., 2023). In this context, targeting pathways involved in cancer cell proliferation and migration presents a promising approach to NSCLC treatment.

Consistent with previous reports demonstrating elevated LIG1 expression in NSCLC cell lines compared to normal cells (Timson et al., 2000; Sun et al., 2001), as well as the protein level of LIG1 increased in tumour-bearing nude mice (Chen et al., 2008), our analysis of the GEPIA database confirmed significant up-regulation of LIG1 in the lung ADC and SQCC. Furthermore, our examination of multiple NSCLC cell lines revealed consistently elevated LIG1 expression compared to normal bronchial epithelial cells, corroborating previous findings. Additionally, analysis of NSCLC patient data from GEPIA revealed poorer overall survival in patients with high LIG1 expression, suggesting a potential prognostic value of LIG1 expression in NSCLC.

Functional assays demonstrated that knockdown of LIG1 in A549 cells resulted in suppressed proliferation and migration, while LIG1 over-expression enhanced these processes. These findings align with previous studies showing that down-regulation of LIG1 inhibits cell proliferation in other cancer cell lines (Sun et al., 2001), indicating a conserved role of LIG1 in promoting cancer cell proliferation and migration.

A previous report has demonstrated that the oncogenic RNA-binding protein SRSF1 binds to LIG1 mRNA and regulates its expression by increasing its mRNA stability and enhancing its translation in an mTOR-dependent manner in NSCLC cell lines, which offers insights into the complex interplay between the RNA metabolism, DNA repair mechanisms, and cancer progression (Martínez-Terroba et al., 2018). However, the negative regulatory mechanism controlling LIG1 expression is unknown. To further elucidate the upstream regulatory mechanisms of LIG1, we employed bioinformatic methods to predict miRNAs targeting LIG1. Our analysis identified miR-325 as a conserved miRNA with predicted binding sites on LIG1. Luciferase reporter assays validated the regulatory interaction between miR-325 and LIG1 mRNA. Furthermore, down-regulation of miR-325 was observed in NSCLC tissues and cell lines, correlating negatively with LIG1 expression. Functional assays demonstrated that modulation of miR-325 levels affected NSCLC cell proliferation and migration, partly via regulating LIG1.

While our study provides valuable insights into the role of LIG1 and miR-325 in NSCLC progression, some limitations exist. Our investigation was limited to *in vitro* assays, and further studies in NSCLC animal models are warranted to validate our findings *in vivo.*

In conclusion, our findings highlight the significance of LIG1 in NSCLC proliferation and migration and identify miR-325 as a regulator of LIG1 expression. Targeting the LIG1/miR-325 axis may have therapeutic potential for NSCLC treatment.

Competing interest

All the authors declared that there is no competing interest.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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