

RETRACTED ARTICLE: The lncRNA LINC01194/miR-486-5p Axis Facilitates Malignancy in Non-Small Cell Lung Cancer via Regulating CDK4

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Background: This experimental design was based on lncRNA LINC01194 to explore the pathogenesis of NSCLC.

Methods: RT-qPCR was used to detect the expression of lncRNA LINC01194 and miR-486-5p in NSCLC tissues and cell lines. Wound healing, colony formation, and transwell assays were used to examine the effects of lncRNA LINC01194 and miR-486-5p on NSCLC cell proliferation and migration. For target gene prediction and screening, luciferase reporter assays were used to verify downstream target genes for lncRNA LINC01194 and miR-486-5p. The protein expression of CDK4 was detected using Western blotting. The tumor changes in mice were detected by *in vivo* experiments in nude mice.

Results: lncRNA LINC01194 was highly expressed in NSCLC tissues and NSCLC lines (A549, H1299, H460 cells, H1975), and lncRNA LINC01194 significantly promoted cell proliferation and migration of NSCLC cells. MiR-486-5p was identified as a potential target for LINC01194, and miR-486-5p was expressed at a low level in NSCLC tissues and NSCLC lines (A549, H1299, H460 cells, H1975). CDK4 was identified as a potential target for miR-486-5p. lncRNA LINC01194 was able to inhibit miR-486-5p expression and upregulate the expression level of CDK4. Finally, the results of *in vivo* animal models confirmed that lncRNA LINC01194 promoted NSCLC progression by modulating the miR-486-5p/CDK4 axis.

Conclusion: lncRNA LINC01194 promoted the progression of NSCLC by modulating the miR-486-5p/CDK4 axis.

Keywords: lncRNA LINC01194, miR-486-5p, CDK4, non-small cell lung cancer, proliferation, invasion

Introduction

Lung cancer is the leading cause of cancer death in both developed and developing countries.^{1,2} Among them, 85% are NSCLC cases.^{3,4} Although the diagnosis of lung cancer has been researched intensively in recent years, the 5-year survival rate has not been significantly improved.^{5,6} The key cause of high mortality is the metastatic potential of the tumor.⁷ Therefore, a better understanding of the mechanisms of tumor cell proliferation is a necessary condition for more effective NSCLC management. However, no tumor molecular markers have been reported to accurately predict the metastasis of lung cancer. Therefore, identification of the biomarker related to NSCLC metastasis is an important part of the NSCLC research process.

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Previous studies have reported that programmed the coding gene is closely related to the metastasis of malignant tumors.^{8,9} Recent advances indicated that another important type of gene, non-coding RNA, may also become new participants in cancer progression.¹⁰ Long non-coding RNA (lncRNA) is an RNA molecule that does not have the ability to encode proteins.^{11,12} It is known that lncRNA generally affects gene expression by regulating the transcriptional and post-transcriptional levels of gene expression.¹² In normal cells, lncRNA are key regulators of the physiological processes of cells and pathological processes.¹³ Studies have found that lncRNA are abnormally expressed in NSCLC, and they regulate the development of NSCLC by stimulating or inhibiting biological processes.^{14,15} lncRNA LINC01194 was discovered as a new lncRNA. Studies have confirmed that LINC01194 levels in breast cancer tissues are significantly lower. Based on the survival curve of patient follow-up data, the expression level of LINC01194 was negatively correlated with the prognosis of patients.¹⁶ However, there is a lack of literature on the regulation of NSCLC by LINC01194.

MicroRNAs have the biological roles of targeting mRNAs for cleavage or translational inhibition. In recent years, miRNAs have been considered to be capable of regulating gene expression, and to synergistically interact with other transcription factors to form a complex gene expression regulatory network system.¹⁷ Increasingly, studies have shown that miRNAs in human tumor cells and tumor tissues are significantly different from those in normal cells and tissues, suggesting that miRNAs are involved in the development of tumors and play a role in tumor promotion or inhibition factors.^{19,20} miR-486-5p is located on chromosome 18, and it acts as a tumor suppressor in various tumors.²¹ miR-486-5p was first cloned from the embryonic liver, and its downregulation often leads to malignant tumors. For example, miR-486 was the most downregulated miRNA in tumors compared with adjacent uninvolved lung tissues, and miR-486 directly targets components of insulin growth factor (IGF) signaling including insulin-like growth factor 1 (IGF1), IGF1 receptor (IGF1R), and phosphoinositide-3-kinase regulatory subunit 1 (alpha) (PIK3R1 or p85a). This miRNA functions as a potent tumor suppressor of lung cancer both in vitro and in vivo.²³ However, there are few reports on the specific effects of miR-486-5p on NSCLC.²⁴ In recent years, several studies have found that lncRNA may regulate downstream target genes

through miRNA adsorption processes.²⁵ Studies have found that cell cycle-dependent kinase 4 (CDK4) plays a critical role in the development of tumors and is highly expressed in NSCLC.²⁶ Our study aimed to investigate whether LINC01194 was involved in cancer stem cells of NSCLC. DGCR5 was increased while miR-486-5p was decreased in NSCLC cells. It was speculated that LINC01194 can promote NSCLC-like properties by modulating the miR-486-5p/CDK4 axis.

Materials and Methods

Research Objects

NSCLC and adjacent non-lesional tissue were collected from 26 patients who underwent primary surgical resection in Hebei Chest Hospital. Diagnosis was based on pathological evidence. All patients were not receiving pre-operative treatments for cancer, such as radiotherapy, chemotherapy, or immunotherapy. Tissues of patients confirmed to be NSCLC by pathology were immediately chilled in tissue nitrogen. All patients provided written informed consent. The study was approved by the Ethics Committee of Hebei Chest Hospital.

Cell Culture

Human lung cancer A549, H1299, H460, H1975 cells and human normal lung epithelial cells (BES-2B) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). A549 and H1299 cells were subcultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA). H460, H1975, and BES-2B cells were sub-cultured in Dulbecco's modified Eagle medium (DMEM, Gibco). All cells were incubated at 37°C in a cell incubator containing 5% CO₂.

Transfection

The miR-486-5p mimetic, miR-486-5p inhibitor (anti-miR-486-5p), and the corresponding negative control miR (miR-NC, anti-miR-NC) were purchased from RiboBio (Guangzhou, People's Republic of China). The LINC01194 cDNA sequence was amplified and introduced into a pcDNA vector (ABM, Richmond, Canada) to construct plasmid complementary DNA LINC01194. The shRNA sequences targeting LINC01194 and sh-NC were purchased from Genepharma Co., Ltd (Shanghai, People's Republic of China). LINC01194 sequence was GAA-CATGCAGTCTAGGAACCGGCAT. The CDK4 cDNA was cloned into pcDNA3.1 (Invitrogen,

Carlsbad, CA, USA). Cells were transiently transfected with RNAiMax and Lipofectamine 3000 with Plus Reagent (Thermo Fisher Scientific).

RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)

Total RNA in cells was extracted using TRIzol reagent (Invitrogen). After the reverse transcription reaction, qRT-PCR was performed using a ViiATM 7 real-time PCR system (Life Technologies, Grand Island, NY, USA). qRT-PCR analysis conditions were as follows: 55°C for 10 min, 40 cycles of 95°C for 30 s, 55–59°C for 30 s, and 72°C for 42 s. The expression levels of lncRNA LINC01194 and miR-486-5p was calculated using the $2^{-\Delta\Delta CT}$ method. The expression levels of miRNA and lncRNA/target genes were standardized by U6 and GAPDH, respectively. Quantitative real-time PCR (qRT-PCR) specific experimental methods were performed with reference to the literature.²⁷ The sequence of primers is shown in Table 1.

Cell Proliferation Detection

The transfected cells were trypsinized and seeded at 2×10^3 cells/well in 96-well plates. After 24 h, 20 μ L of CK8 (Sigma-Aldrich Co., St Louis, MO, USA) solution (5 mg/mL) in phosphate-buffered saline (PBS) was added. Plates were incubated for another 3 h. The optical density for each well was measured using a microculture plate reader (BioTek Instruments Inc., Winooski, VT, USA) at a wavelength of 490 nm.

Colony Formation Test

The transfected cells were made into a single dispersed cell suspension, plated in an incubator for 7 d. After the medium was discarded, they were fixed with Wright's stain for 5 min. They were stained for 10 min with a mixture of Giemsa dye solution and Sorensen phosphomolybdic acid buffer solution at a ratio of 1:9. After washing, colonies containing 50 cells or more were counted under a microscope.

Transwell Assays

Transwell assays were used to detect cell invasion and metastasis. The upper basement membrane of the Transwell chamber was pre-coated with 20 μ g Matrigel and cultured overnight in a 24-well plate. The cell suspension was added to the upper chamber. After 12 h of culture, it was washed with the PBS 3 times. Then it was fixed with 90% formaldehyde, and stained in the crystal violet solution for 15 min. After that, the photograph was taken under an inverted microscope. In the cell migration experiment, the upper chamber of Transwell chamber was free of matrigel coating, and the rest of the operation was the same as the invasion experiment.

Xenograft Model

Ten female athymic BALB/c nude mice were randomly divided into three groups. To construct a mouse xenograft model, 2×10^7 A549 cells containing sh-lncRNA LINC01194, si-NC was injected intravenously into the flanks of each mouse (n=5 per group). Tumor size was measured twice a week. All nude mice were sacrificed after 2 weeks and tumors were dissected for qPCR or Western blot analysis experiments. Ethical and legal approval was obtained from Hebei Chest Hospital prior to the commencement of the study. All procedures performed in studies involving animals were in accordance with the ethical standards of Hebei Chest Hospital in which the studies were conducted.

Biotinylated RNA Pull-Down Assay

In order to determine the possible interaction between lncRNA LINC01194 and miR-486-5p, the T7-lncRNA LINC01194 nucleic acid probe sequence was constructed as TAATACGACTCAC TATAGGGAGGCACT TGGCACC CAGTT, GCGGCCGCGAAATCTTTAATATTGTGA. The RNA pull-down assay was performed by the above T7-lncRNA LINC01194 nucleic acid probe, and the pull-down product was obtained by Western blot and corresponding specific antibody test. For the specific steps refer to Barnes and Kanhere.²⁸

Table 1 Primer Sequences Used in Quantitative Real-Time PCR

Gene	Sense Primer 5'–3'	Antisense Primer 5'–3'
lncRNA LINC01194	AGGCCTGACACGTTTACTAA	AGGTCGTAGTGTTAGTGCAT
miR-486-5p	CATTGTGCTGTTCTGTCAGTTAA	CCCTCCAGGAATTGGCCTGTCTT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTGATGAG
CDK4	GCAGCGACTATGCACAACGA	CCAGAGTGGTGACGGAGACA

Luciferase Reporter Assay

The wildtype full-length 3'UTR fragment of the LINC01194 or CDK4 gene was obtained using PCR from human genomic DNA. The predicted seed sequences were mutated from the 3'UTR of Dock1 using the overlapping PCR method. PCR products were cloned into the pGL3 vector (Promega, Madison, WI, USA).

Synthetic wild type (WT) or mutant (MUT) CDK4 bound to hsa-miR-486-5p was subcloned into the pGL3 Basic vector (Promega). Cells were co-transfected with these reporter plasmids and miR-486-5p inhibitors or mimetics, respectively. After 48 h of transfection, luciferase activity was measured using a dual luciferase assay system (Promega).

RNA-Binding Protein

Immunoprecipitation Assay

RNA-binding protein immunoprecipitation assay was carried out using Magna RIP Kit (EMD Millipore, Billerica, MA, USA). A549 cells were lysed in RIP lysis buffer, and the cell lysate was incubated with magnetic beads conjugated to human anti-Ago2 antibody (Millipore) or isotype-matched control antibody (normal mouse IgG; Millipore). qRT-PCR was performed to detect LINC01194 and hsa-miR-486-5p expression.

Ki67 Immunohistochemical Staining

The immunohistochemical staining SP method was used to stain Ki67 (Ki67 monoclonal antibody, 1:100, Myr Company). Conventional paraffin sections were deparaffinized; 3% H₂O₂ was added to the sections for 10 min; antigen repairing; normal goat serum sealing solution was added for 20 min; one anti-50 mL was added for 1 h; two anti-50 mL was added for 1 h at room temperature; DAB was colored for 5 min, hematoxylin was re-stained, conventional paraffin was dehydrated.

Western Blot

The transfected cells were collected, total protein was extracted, and the protein concentration was quantified using the BCA Protein Assay Kit. Then it was incubated with anti-CDK4 antibody (1:1000, Proteintech, Chicago, IL, USA) and anti-GAPDH antibody (1:1000, Abcam, Cambridge, UK) overnight at 4°C. Then anti-rabbit secondary antibody (1:1000, Cell Signaling Technology, Boston, MA, USA) was added to incubate for 1 h. GAPDH was used as an internal reference, and relative protein expression was expressed as the ratio of the gray value of the target band/GAPDH band. The specific experimental methods for

Western blot analysis were performed with reference to the literature.²⁹

Statistical Methods

The monitoring data were analyzed by SPSS19.0 statistical software. Data were expressed as the mean $\pm$ SD. Statistical comparisons were made between two groups with the *t* test. A value of $P < 0.05$ was considered significant.

Results

Biological Role of lncRNA LINC01194 in NSCLC Tumorigenesis

As shown in Figure 1A, the expression level of lncRNA LINC01194 was significantly increased in NSCLC tissues compared with that in adjacent normal tissues ($P < 0.05$). After analyzing the relationship between lncRNA LINC01194 expression and other general clinical data of patients, it was found that there were significant differences in the expression levels of lncRNA LINC01194 for gender, tumor size, TNM stage and lymph node metastasis ($P > 0.05$, [supplementary Table 1](#)). As shown in Figure 1B, compared with the BES-2B cells, lncRNA LINC01194 was significantly increased in the NSCLC line (A549, H1299, H1975) ($P < 0.05$). There was no significant difference in the expression level of LINC01194 in the NSCLC, so A549 cells were chosen for further experiments.

In order to further analyze the carcinogenic effect of lncRNA LINC01194, A549 cells were transfected with sh-NC or sh-LINC01194 or pc-NC or pc-LINC01194. As shown in Figure 1C, compared with the control group, the expression level of sh-LINC01194 or pc-LINC01194 in the LINC01194 group was abnormally expressed, indicating successful transfection. As shown in Figure 1D and E, LINC01194 silencing significantly inhibited cell proliferation compared with the control group, while LINC01194 overexpression significantly induced cell proliferation ($P < 0.05$). In addition, compared with the control group, LINC01194 silencing significantly inhibited the migration and invasion of A549 cells, while overexpression of LINC01194 significantly promoted migration and invasion in A549 cells (Figure 1F and G) ($P < 0.05$). These data indicated that LINC01194 was capable of promoting the proliferation and metastasis of NSCLC.

MiR-486-5p Was the Target of LINC01194

The results are shown in Figure 2A. Compared with BES-2B cells, the expression level of miR-486-5p in the NSCLC line

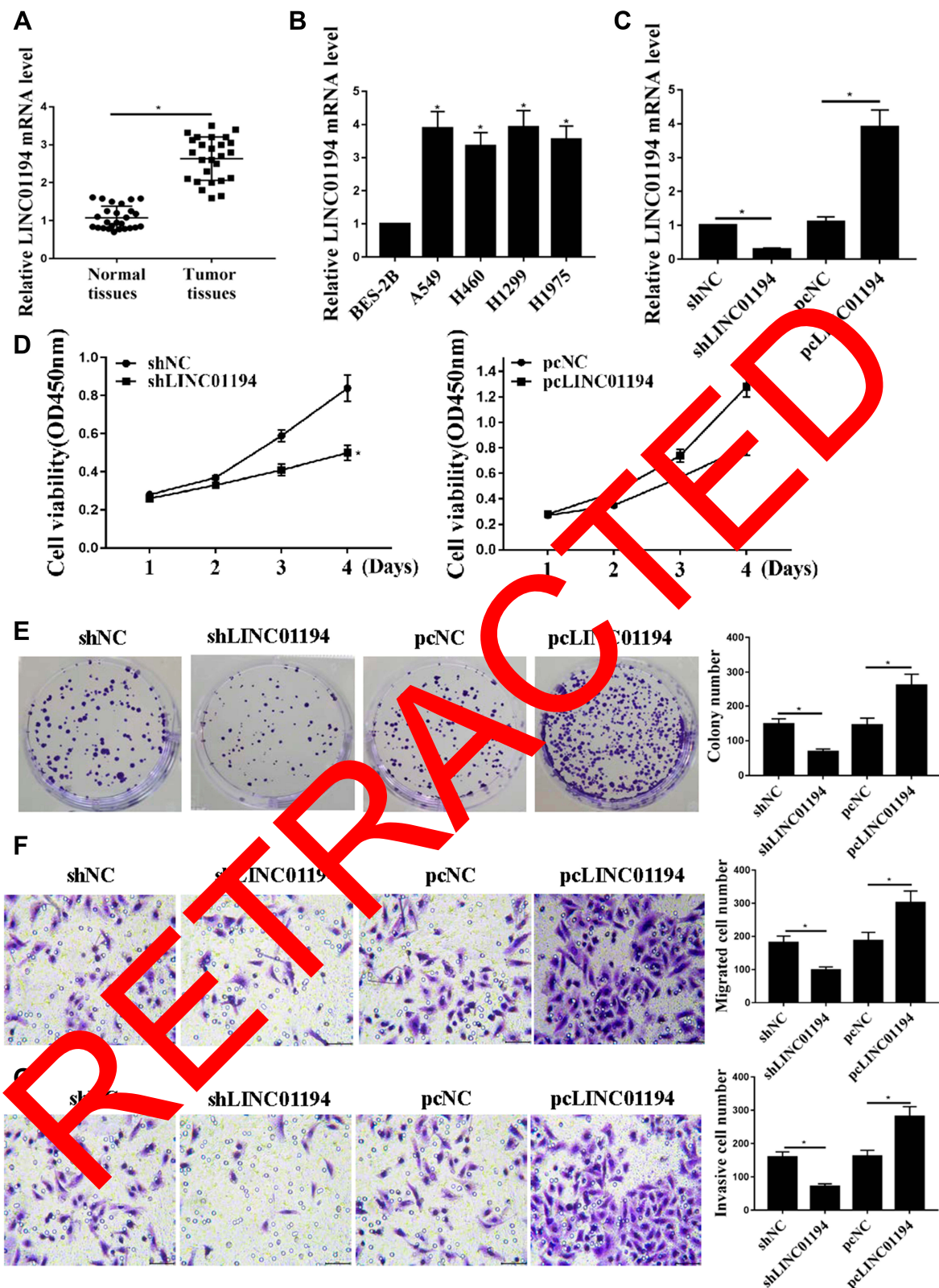


Figure 1 Biological role of lncRNA LINC01194 in NSCLC. **(A)** Relative expression of NSCLC in NSCLC tissues and adjacent normal tissues (n=26). **(B)** lncRNA LINC01194 mRNA expression level in NSCLC cell lines. **(C)** lncRNA LINC01194 mRNA levels under different treatment conditions. **(D)** CCK8 measured cell viability. **(E)** Colony formation measured cell proliferation. **(F, G)** Transwell measured the number of cell invasion and migration. *P<0.05, n=3.

(A549, H1299, H460 cells, H1975) was significantly reduced ($P < 0.05$). It was predicted by searching StarBase v.2.0 and miR-486-5p was identified as a potential target for LINC01194 (Figure 2B). In addition, miR-486-5p expression levels were abnormally expressed in the miR-486-5p over-expression group or the miR-486-5p inhibitor group compared with the control group, indicating successful

transfection (Figure 2C). WT-LINC01194 or mutant (mut)-LINC01194 luciferase reporter plasmid for luciferase reporter gene assay was used to validate the predicted results. The luciferase activity of pGL3-REPOR-LINC01194-WT was reduced by miR-486-5p mimetics, while there was no significant change in the luciferase activity of pGL3-REPOR-LINC01194-mut (Figure 2D). As shown in Figure 2E, the

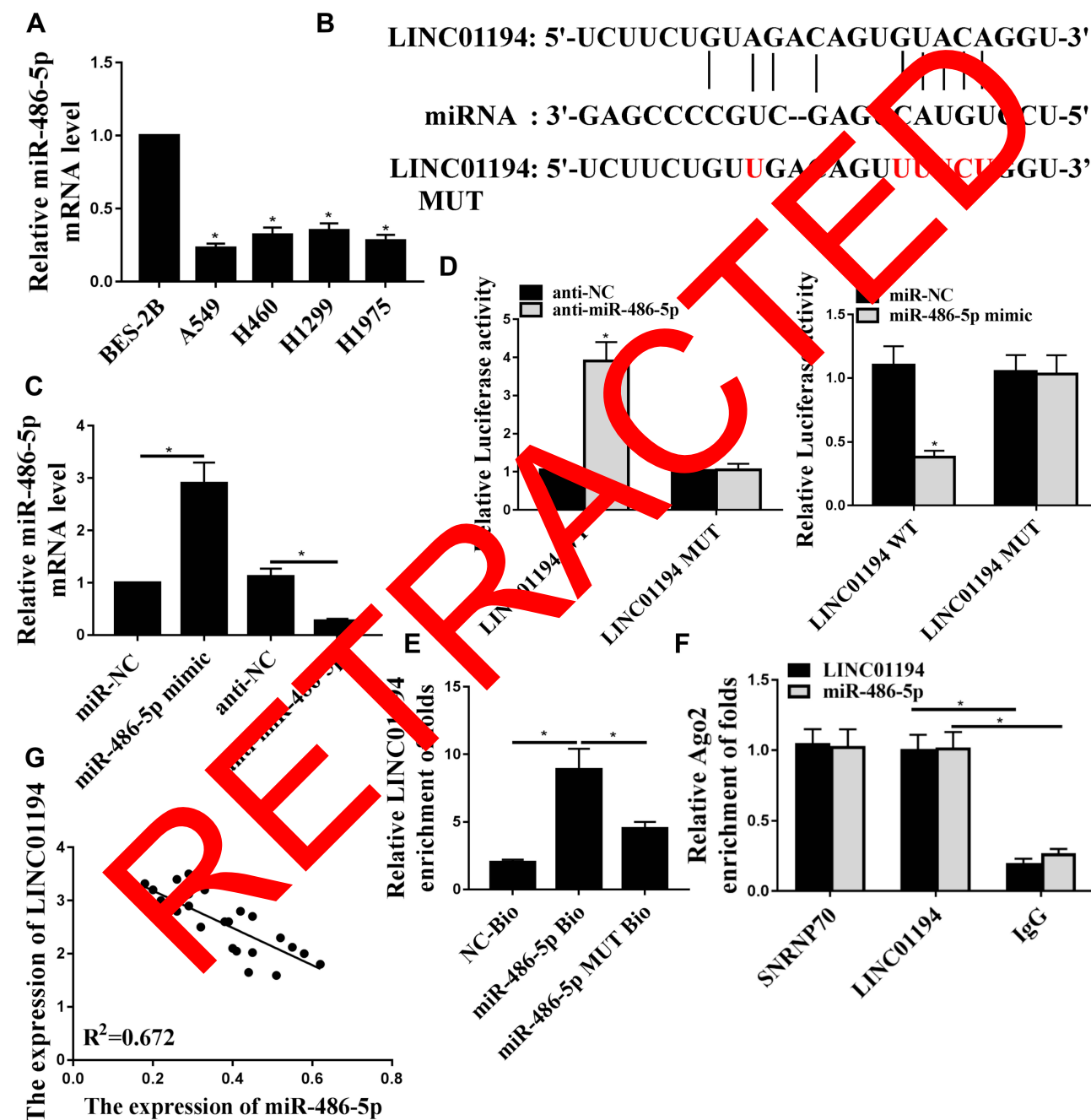


Figure 2 LINC01194 regulated the expression of miR-486-5p in NSCLC cells. (A) Expression of miR-486-5p mRNA levels in NSCLC cell lines. (B) Putative target sequence of miR-486-5p on the 3'-UTR of LINC01194. (C) miR-486-5p mRNA levels in A549 cells under different treatment conditions. (D) Detection of luciferase activity by luciferase reporter assay. (E) LINC01194 expression levels in samples by biotinylated miR-486-5p or negative control. (F) Correlation between LINC01194 and miR-486-5p levels was using detecting RNA pull down. (G) Pearson's correlation analysis of LINC01194 and miR-486-5p in NSCLC tissues ($n=26$) ($r=-0.672$, $P < 0.01$). * $P < 0.05$, $n = 3$.

level of LINC01194 was significantly higher than that of the NC-bio or hsa-miR-486-5p probe. As shown in Figure 2F, the anti-Ago2 IP experiments confirmed binding of LINC01194 to miR-486-5p. In addition, a significant negative correlation between LINC01194 and miR-486-5p was observed (Figure 2G). These results indicated that LINC01194 may exert its biological function through miR-486-5p.

The Effects of LINC01194 Were Mediated by miR-486-5p in NSCLC Cells

In order to further analyze whether LINC01194 acts on NSCLC cells via miR-486-5p, the miR-486-5p inhibitor, and sh-LINC01194 were transfected into the A549 cell line. Silencing of LINC01194 significantly inhibited the proliferation of A549 cells, and the miR-486-5p inhibitor significantly induced the proliferation of A549 cells. Co-transfection with miR-486-5p inhibitor partially eliminated the effect of LINC01194 silencing on cell proliferation ($P<0.01$) (Figure 3A–C). As shown in Figure 3D–G, silencing of LINC01194 significantly inhibited migration and invasiveness of A549 cells, and the miR-486-5p inhibitor significantly increased cell migration and invasiveness in A549 cells. Co-transfection with miR-486-5p inhibitors partially eliminated the effect of LINC01194 silencing on cell migration and invasion ($P<0.01$). These results indicated that LINC01194 promoted proliferation, invasion, and migration of NSCLC cells by producing miR-486-5p.

CDK4 Was a Direct miR-486-5p Target

From bioinformatics predictions, CDK4 was identified as a potential target for miR-486-5p (Figure 4A). Luciferase reporter assays were performed using the WT-miR-486-5p or mutant (mut) miR-486-5p luciferase reporter plasmid to validate the predicted results. As shown in Figure 4B, the ectopic expression of CDK4 significantly inhibited the luciferase activity of WT-miR-486-5p, but it had no significant effect on the luciferase activity of Mut miR-486-5p. In addition, in A549 cells, miR-146a-5p overexpression significantly reduced the expression level of CDK4 protein and miRNA in A549 cells compared with the control group, while miR-146a-5p inhibitor significantly increased the expression level of CDK4 protein and miRNA ($P<0.01$) (Figure 4C and D). In addition, compared with the control group, shLINC01194 significantly reduced CDK4 protein levels, and co-transfection with anti-miR-486-5p can reverse the effect of LINC01194 on CDK4 protein levels

($P<0.01$) (Figure 4E). These results indicated that CDK4 was a direct target of miR-486-5p.

CDK4 Mediated the Effects of LINC01194/miR-486-5p Axis in NSCLC Cells

As shown in Figure 5A and B, miR-486-5p mimic and sh-LINC01194 significantly inhibited cell proliferation compared with that in the control group. Co-transfection with si-pc CDK4 partially eliminated the effect of miR-486-5p mimic and sh-LINC01194 on cell proliferation ($P<0.01$). As shown in Figure 5C and D, compared with the control group, miR-486-5p mimic, and sh-LINC01194 was significantly inhibited cell migration and invasiveness, and co-transfection with pc-CDK4 partially eliminated the effect of miR-486-5p mimic and sh-LINC01194 on cell migration and invasion ($P<0.01$).

LINC01194 Promotes NSCLC Cell Tumor Growth in vivo

The effect of LINC01194 on the progression of NSCLC in vivo was further determined through in vivo experiments. As shown in Figure 6A–C, the tumor volume and weight of mice in the LINC01194 knockdown group were significantly reduced compared to in the control group, and co-transfection with anti-miR-486-5p reversed the effect of LINC01194 knockdown on tumor volume and weight of the mouse ($P<0.01$). In addition, Ki-67 immunohistochemical staining showed that there were fewer Ki67-positive cells in the tumor tissue of the LINC01194 knockdown group, while Ki67-positive cells in tumor tissue were significantly increased after co-transfection with anti-miR-486-5p (Figure 6D). In addition, the protein levels of CDK4 in the LINC01194 knockdown group were significantly reduced compared to those in the control group, and co-transfection with anti-miR-486-5p reversed the effect of LINC01194 knockdown on the protein level of CDK4 ($P<0.01$, Figure 6E).

Discussion

The current study found that lncRNA can play a role in miRNA molecular sponge and regulate gene transcription.^{30,31} It is widely believed that lncRNA is involved in a variety of cellular functions, including apoptosis, differentiation, and various physiological and pathological processes from cell growth to carcinogenesis.³²

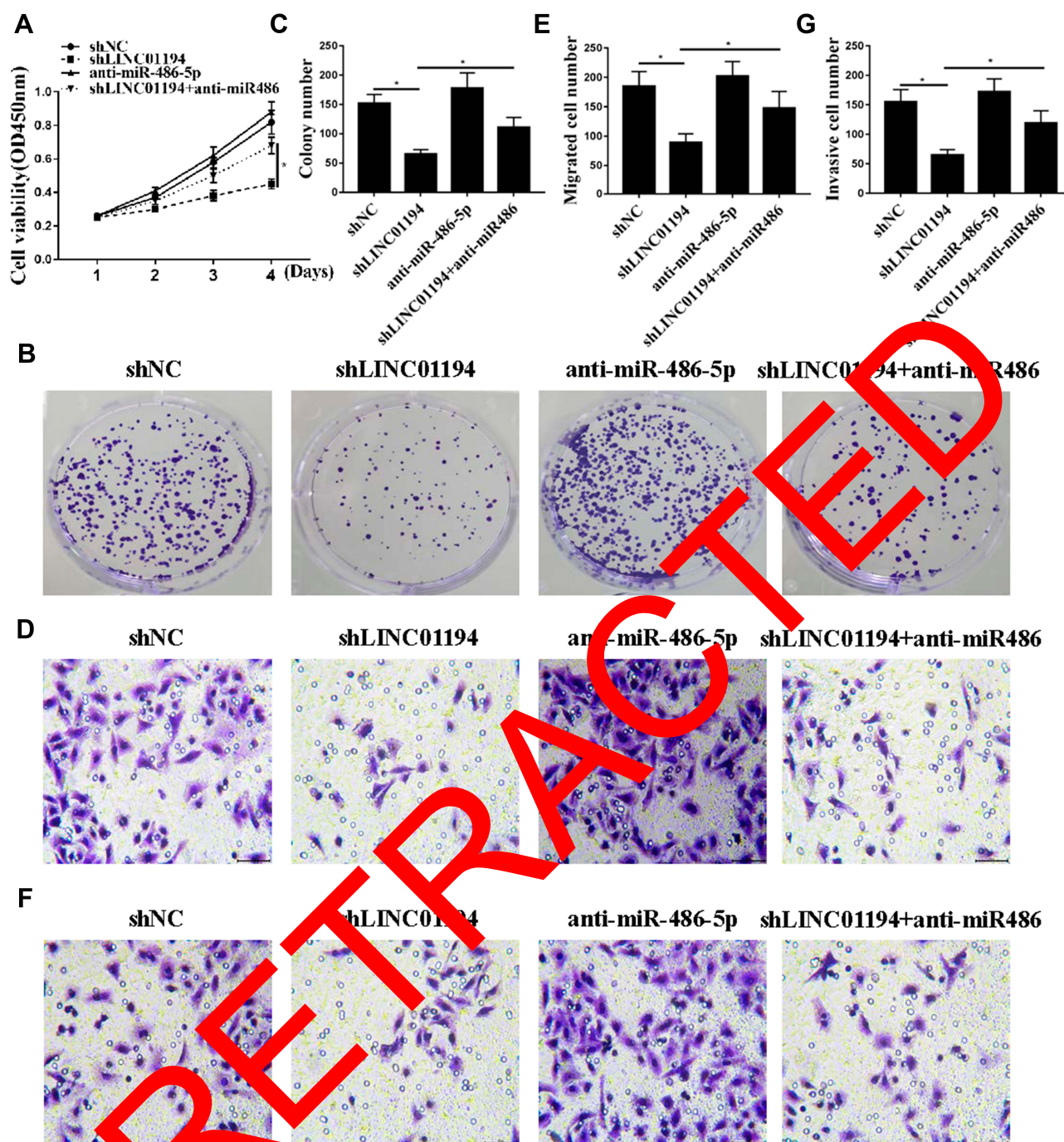


Figure 3 LINC01194 promoted a biological effect on NSCLC cells via miR-486-5p. (A) CCK8 measured the viability of A549 cells. (B, C) colony formation assay cell proliferation. (D, E) Transwell measured the number of cell invasions. (F, G) Transwell measured the amount of cell migration. * $P < 0.05$, $n = 3$.

Importantly, lncRNA expression is dysregulated in a wide range of diseases including cancers, leading to abnormal cell proliferation, migration, and apoptosis, which contribute to the progression and outcomes of human tumor. One of the major reasons for aberrant lncRNA expression is epigenetic regulation. For example, high expression of lncRNA colon cancer-associated transcript-1 (CCAT1) in esophageal squamous cell

carcinoma is caused by H3K27 acetylation-mediated activation, whereas decreased expression of p53-induced lncRNA TP53 target 1 (TP53TG1) is due to high methylation of its gene promoter.³³ Like other tumors, some abnormally expressed lncRNAs were found in cancer tissues of primary lung cancer, and some of them were highly expressed in NSCLC.^{34,35} LINC01194 is an up-and-coming lncRNA that

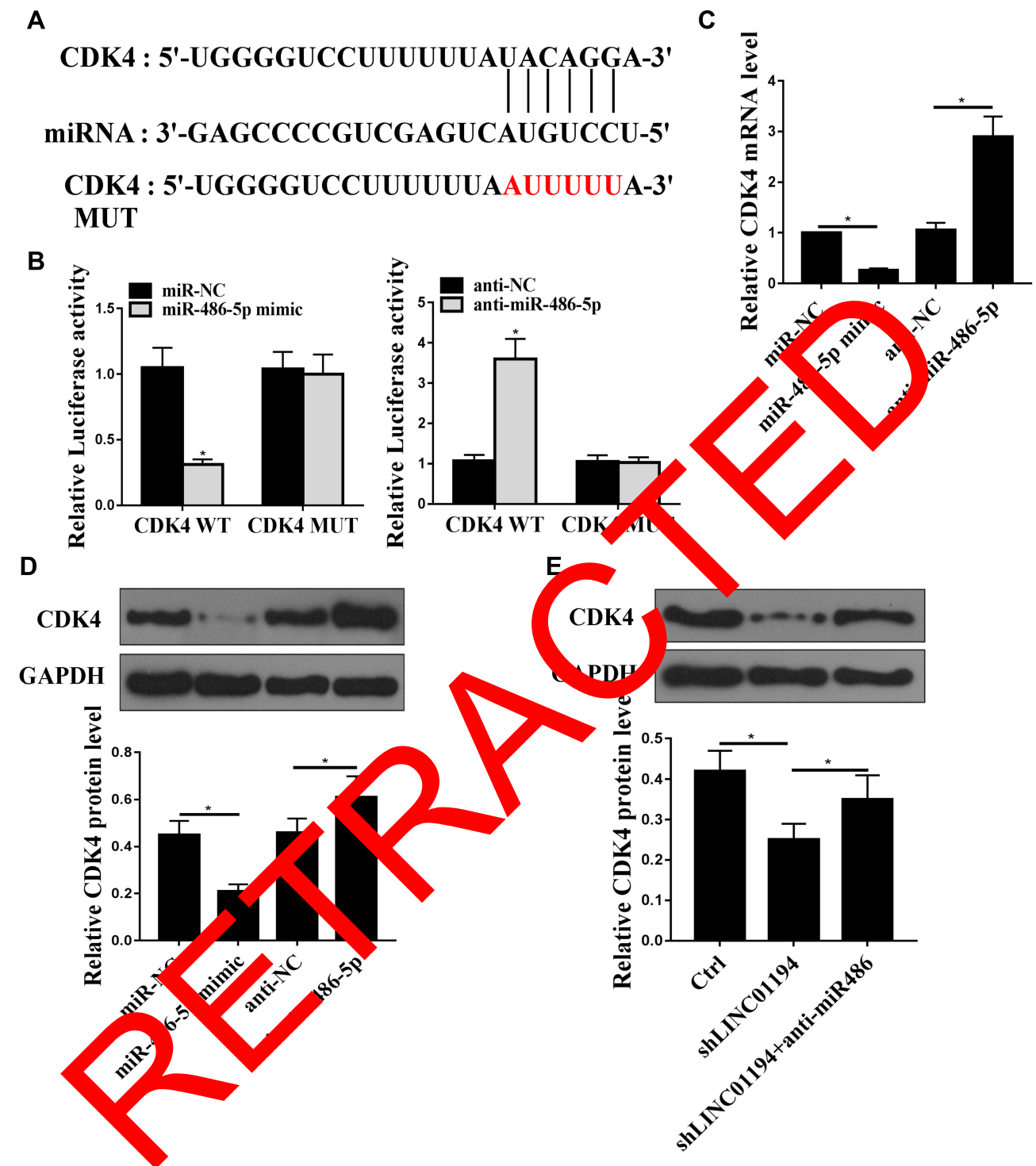


Figure 4 CDK4 was a direct miR-486-5p target. (A) Putative target sequence of miR-486-5p on the 3'-UTR of CDK4. (B) Detection of luciferase activity by luciferase reporter assay. (C) RT-PCR and Western blot analysis were used to determine (C and D) CDK4 mRNA and protein levels. (E) Western blot analysis to determine CDK4 protein levels. *P<0.05, n=3

has received increasing attention.¹⁶ Previous studies have found that LINC01194 is involved in the development of liver cancer and other tumors. It has been found that LINC01194 antagonizes the tumor suppressor miR-195, and it regulates the development of Wilms tumor through IKK α .³⁶

In this study, it was found that the expression of lncRNA LINC01194 in NSCLC was significantly increased, and lncRNA LINC01194 silencing inhibited NSCLC cell proliferation and migration and invasiveness, while the lncRNA LINC01194 overexpression group had the opposite results. In

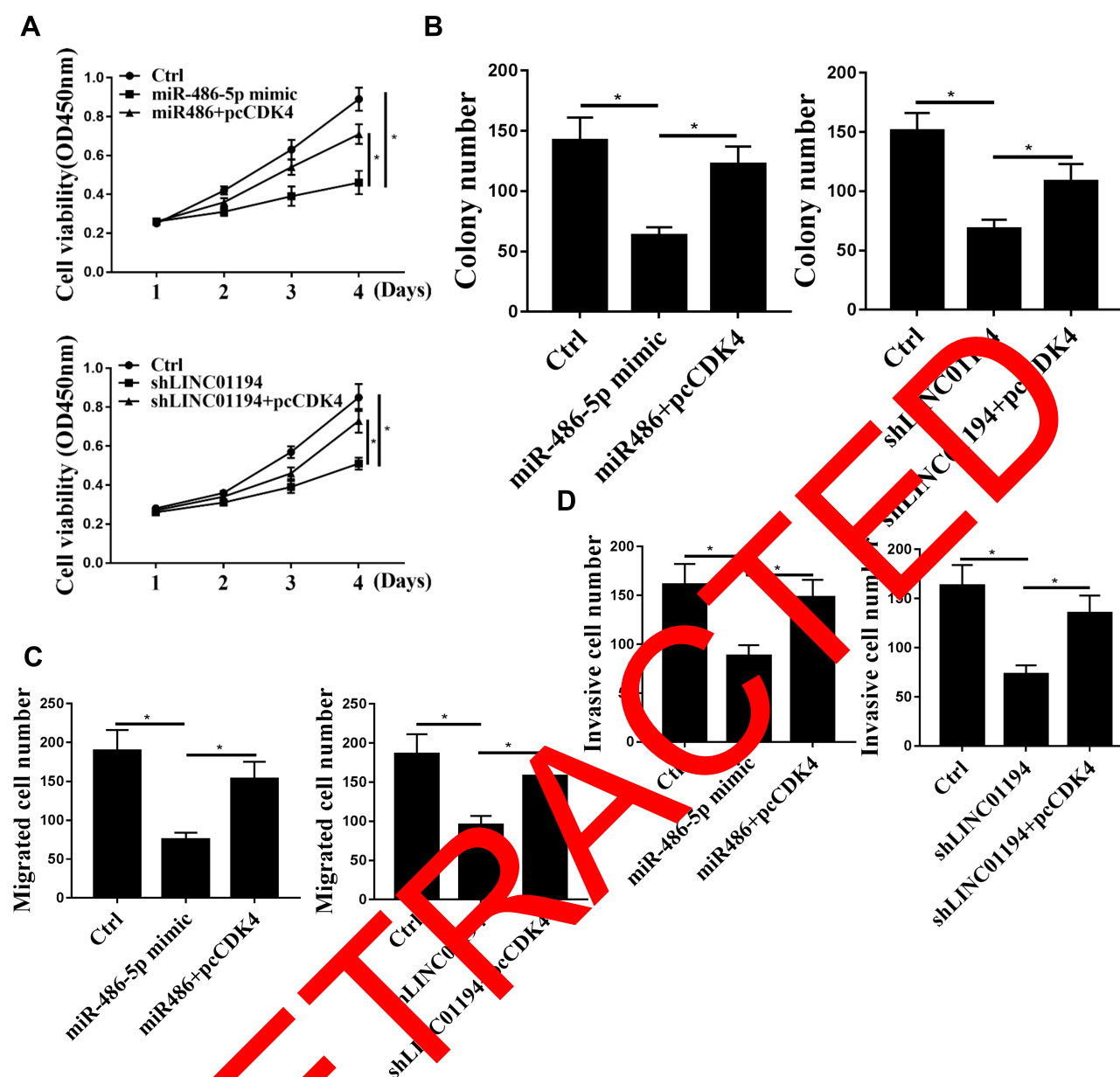


Figure 5 CDK4 mediated the role of the LINC01194-miR-486-5p axis in NSCLC cells. (A) CCK8 measured cell viability. (B) Colony formation assay cell proliferation. (C, D) Transwell measured cell invasion and migration. * $P < 0.05$, $n = 3$.

vivo experiment showed that lncRNA LINC01194 silencing inhibited tumor growth in mice. Our findings are consistent with a range of previous studies, showing that by suppressing the expression of LINC01194 in colon cancer cell lines, cell proliferation and migration were inhibited. Therefore, lncRNA LINC01194 can be used as an oncogene to control the development of NSCLC by inhibiting its expression.

As a target of lncRNA, miRNA is the most widely studied non-coding RNA, and can regulate cell proliferation, differentiation, and apoptosis by degrading target miRNA or inhibiting its translation.³⁷ Studies have found

that microRNAs, as upstream regulators of multi-gene multi-target regulation, are involved in the regulation of post-transcriptional gene expression.³⁸ At present, a variety of microRNAs were found to be dysregulated in NSCLC, and are associated with the development of NSCLC, gene mutation, pathological typing, targeted drug resistance, and prognosis.³⁹ Studies have found that miR-486-5p was one of the most significant and highly expressed miRNAs in human lung squamous cell carcinoma.⁴⁰ Its expression in sputum and peripheral blood has important clinical value for the diagnosis of NSCLC. Further studies

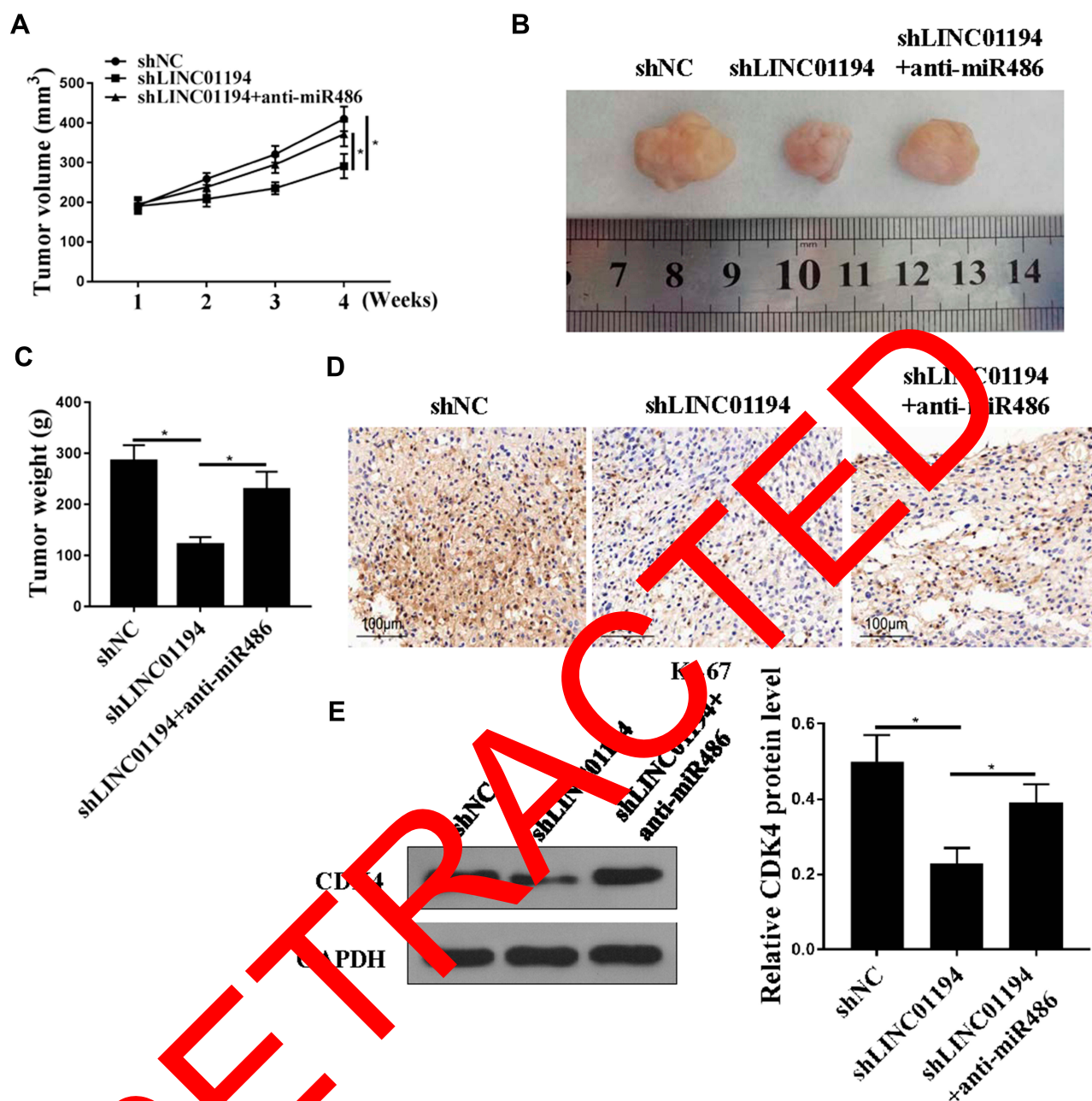


Figure 6 LINC01194 promoted tumor growth in vivo. (A) Tumor volume. (B) Image of tumor tissue. (C) Tumor weight. (D) Ki67 immunohistochemistry. (E) Western blot analysis to determine CDK4 protein levels. * $P < 0.05$.

have shown that miR-486-5p plays a tumor suppressor gene role in NSCLC. Its low expression contributes to cell proliferation, invasion, and metastasis, and participates in the regulation of NSCLC formation and progression.⁴¹ Our study was consistent with the literature; miR-486-5p was the target gene for lncRNA LINC01194. The expression of miR-486-5p was significantly reduced in NSCLC cells, and lncRNA LINC01194 regulated its expression by targeting the 3'UTR of the miR-486-5p gene. LncRNA LINC01194 knockdown significantly increased the expression of

MiR-486-5p. Conversely, lncRNA LINC011948 was negatively correlated with MiR-486-5p. MiR-486-5p inhibitors partially eliminated the effects of sh-LINC01194 on cell proliferation, migration, and invasion. These results suggested that LINC01194 may promote NSCLC cell growth by regulating miR-486-5p.

In recent years, there have been reports of interactions between miRNA and lncRNA, targeting and regulating related pathways, thus affecting the occurrence and development of tumors.⁴² Cyclin-dependent kinase 4

(CDK4) is a silk/threonine protein kinase that localizes to the 12q13 region of the chromosome.⁴³ Recent studies have shown that CDK4 also plays a critical role in the process of tumor development.⁴⁴ Studies have found that the oncogene CDK4, which plays a critical role in the progression of the cell cycle, is directly targeted by miR-486-5p, and miR-486-5p can inhibit non-small cell lung cancer by downregulating the expression of CDK4.⁴⁵ In this study, it was found that CDK4 was a potential target of miR-486-5p and that miR-486-5p regulated its expression by targeting the 3'UTR of the CDK4 gene. In addition, miR-486-5p overexpression reduced CDK4 expression, and co-transfection of shLINC011948, and miR-486-5p mimicked partially the effect of sh-LINC011948 on CDK4 protein levels. MiR-486-5p mimic and sh-LINC011948 inhibited cell proliferation and migration, and co-transfection with pc-CDK4 partially eliminated the effect of miR-486-5p mimic and sh-LINC011948 on cell proliferation and migration. These results indicated that lncRNA LINC011948 can promote the proliferation of NSCLC by regulating the miR-486-5p/CDK4 axis.

Conclusion

LncRNA LINC011948 promoted the proliferation of NSCLC by regulating the miR-486-5p/CDK4 axis, suggesting that lncRNA LINC011948 may be a potential oncogene of NSCLC.

Disclosure

The authors report no conflict of interest in this work.

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