LncRNA KTN1-AS1 drives tumor progression in non-small cell lung cancer through the microRNA-153-3p/KLF5 axis

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Abstract

Introduction: The most typical kind of lung cancer is non-small cell lung cancer (NSCLC). Surgery, targeted therapy, chemotherapy, and immunotherapy are all options for the treatment of NSCLC. LncRNA KTN1-AS1 is significantly elevated in NSCLC, and it modulates the expression of microRNAs and downstream genes, promoting NSCLC progression.
Material and methods: TCGA was employed to predict lncRNA KTN1-AS1 expression in NSCLC. The starBase database was utilized to predict downstream microRNAs of KTN1-AS1. The RNA22 database was employed to predict corresponding binding sites. Subcellular localization of KTN1-AS1 was forecasted using the lncLocator database, and the results were validated by FISH. qRT-PCR was used to test KTN1-AS1, microRNA-153-3p, and KLF5 expression. CCK-8, flow cytometry, and Transwell assay were used to determine cell viability, proliferation, migration, and invasion. Western blot was used to test KLF5 and Ki67 protein levels, and dual-luciferase assay was used to assess binding of KTN1-AS1 with microRNA-153-3p, and KLF5 with microRNA-153-3p.
Results: KTN1-AS1 was significantly upregulated in NSCLC cells. Silencing KTN1-AS1 significantly repressed the proliferation, migration, and invasion of NSCLC cells. KTN1-AS1 bound to microRNA-153-3p, and KLF5 was a direct target of microRNA-153-3p. Inhibition of microRNA-153-3p or overexpression of KLF5 restored the stimulatory impact of KTN1-AS1 knockdown on NSCLC cell proliferation and migration.
Conclusions: KTN1-AS1 drove proliferation, migration, and invasion of NSCLC cells by regulating the microRNA-153-3p/KLF5 axis.

Key words: non-small cell lung cancer, lncRNA KTN1-AS1, microRNA-153-3p, KLF5, tumor progression.

Introduction

The most prevalent and deadly disease in the world is lung cancer (LC) [1]. Non-small cell LC (NSCLC), which accounts for approximately 85% of all cases of LC diagnosed, has a 5-year overall survival rate of only 20% [2]. Therefore, it is necessary to further explore new biomarkers to provide potential therapeutic targets for NSCLC patients.

LncRNAs are a subclass of RNA molecules longer than 200 nucleotides that do not encode proteins [3]. Typically, lncRNAs participate in transcription, post-transcriptional regulation, and epigenetic processes

[4]. Previous research has shown that lncRNAs participate in modulating LC-associated signaling, thereby affecting LC progression. LncRNA KTN1-AS1 acts as an oncogene in various cell types [5]. Moreover, KTN1-AS1 inhibits autophagy in LC cells through the microRNA-130a-5p/PDPK1 signaling pathway [6], highlighting the significant role of KTN1-AS1 in regulating LC progression. In NSCLC, however, the molecular mechanism of KTN1-AS1 is intricate and remains unknown.

Therefore, this study aimed to investigate the characteristics of lncRNA KTN1-AS1 in regulating malignant progression of NSCLC cells and molecular mechanisms of lncRNA KTN1-AS1, so as to provide reliable therapeutic targets for NSCLC.

Material and methods

Bioinformatics prediction

The R package limma was used for differential analysis of data from the NSCLC chip GSE4077 in the GEO database to obtain the top 3500 significantly differentially expressed genes. The Cancer Genome Atlas (TCGA) dataset was subjected to GEPIA (http://gepia.cancer-pku.cn/) to extract the top 3500 significantly expressed genes related to NSCLC. The GENCODE database provided 17,937 human lncRNA gene names, and the intersection of the obtained genes was taken. KTN1-AS1 expression in tumor and adjacent normal samples was obtained through GEPIA and the expression data from chip data were analyzed. miRDB (https://mirdb.org/) and starBase (https://ngdc.cncb.ac.cn/) were utilized to forecast downstream microRNAs of KTN1-AS1 and corresponding binding sites were forecasted through the RNA22 (cm.jefferson.edu/data-t) database.

Cell culture and transfection

Human NSCLC cells H1975, H1299, and A549 were obtained from ATCC (USA), and the bronchial epithelial cell line BEAS-2B was obtained from BNCC (China). H1975 and H1299 cells were cultured in RPMI-1640 complete medium, A549 cells were cultured in F-12K complete medium, and BEAS-2B cells were cultured in DMEM-H complete medium. All the above media contained 10% fetal bovine serum (FBS), 0.1 mg/ml streptomycin, and 100 U/ml penicillin. Cells were maintained under conditions of 37°C and 5% CO_2 in a cell culture incubator.

oe-KLF5, sh-KTN1-AS1, microRNA-153-3p inhibitor, and microRNA-153-3p mimic, and negative controls, were obtained from Ribobio (China). The above vectors were transfected in 2 µg quantities into NSCLC cells with Lipofectamine 2000 (Invitrogen). Following transfection, cells were incubated for 48 h, and transfection efficiency was tested through qRT-PCR.

Cell proliferation assay

Transfected cells were plated in 96-well plates (10^3 cells/well). CCK-8 assay was recommended for proliferation detection. At 1, 2, 3, and 4 days of culture, 10 µl of CCK-8 reagent (Beyotime, China) was added to each well, followed by absorbance measurement at 450 nm using a microplate reader. The cell growth proliferation curve was obtained [7].

Transwell assay

Transwell assay was recommended for examination of migration and invasion of the transfected cells. For the migration assay, NSCLC cells (2×10^4 cells/well) in 200 µl of serum-free medium were added to the upper chamber (BD Bioscience, USA), and the medium containing 10% FBS was added to the lower one. Twenty-four hours later, cells on the upper surface were gently wiped off with a cotton swab, and the bottom cells were fixed with methanol for 30 min and stained with 0.1% crystal violet. Cell migration was recorded by photography.

For invasion assay, transfected NSCLC cells (2×10^4 cells/well) were plated in the upper chamber coated with Matrigel in serum-free culture medium, and the subsequent procedures were the same as the migration assay. Finally, cell invasion was recorded by photography [7].

Cell apoptosis detection

Cell apoptosis was assayed via flow cytometry. Transfected cells were harvested after stable cultivation. Cells were treated with an apoptosis detection kit (BD Bioscience, USA), and the distribution characteristics of the cells after treatment were detected and recorded by flow cytometry (BD Bioscience, USA). The apoptosis status of the cells was documented. Flowjo software was used to analyze the data [7].

Western blot analysis

Total proteins were isolated from NSCLC cells by radioimmunoprecipitation assay (RIPA) lysis buffer with protease inhibitors. Protein concentration was quantified using a BCA kit (Beyotime, China). Samples were prepared using the up-sampling buffer, PAGE gels were prepared and placed in an electrophoresis tank with added electrophoresis solution, and marker and protein samples (25 µg per well) were added. The protein samples were separated by SDS-PAGE and transferred to a PVDF membrane. After being sealed with 5% skim milk for 1 h, the membrane was incubated overnight at 4°C with rabbit anti-KLF5 (Abcam, ab137676, UK), rabbit anti-Ki67 (Abcam, ab16667, UK) and rabbit

anti-GAPDH (Abcam, ab9485, UK) primary antibodies. After washing three times with TBST, the membrane was incubated with HRP-conjugated goat anti-rabbit secondary antibodies for 2 h. Protein bands were detected with an ECL detection kit (Pierce Biotechnology, USA) and imaged with a chemiluminescence imaging system [8].

Dual-luciferase reporter assay

Potential binding sites of microRNA-153-3p with KTN1-AS1 or KLF5 were predicted using the starBase database and miRDB. The binding was validated using a dual-luciferase reporter assay. First, pmirGLO-KTN1-AS1-WT, pmirGLO-KTN1-AS1-MUT, pmirGLO-KLF5-WT, and pmirGLO-KLF5-MUT plasmids were constructed. The constructed dual-luciferase reporter plasmids were co-transfected with microRNA-153-3p to mimic or mimic NC into NSCLC cells. Forty-eight hours later, luciferase activity was assessed with the Dual-Luciferase Assay Kit on a GloMax 20/20 Luminometer (Promega) [7].

Fluorescence in situ hybridization (FISH)

Subcellular localization prediction of KTN1-AS1 was performed using the lncLocator database. Subsequently, FISH was used to determine the subcellular localization of KTN1-AS1. The FISH kit used (RiboBio, China) is recommended for hybridization in situ. NSCLC cells were briefly rinsed in PBS and fixed in 4% formaldehyde for 10 min. Cells were permeabilized for 5 min at 4°C in PBS containing 0.5% Triton X-100 before being rinsed 3 times in PBS for 5 min each time. Prehybridization was conducted at 37°C for 30 min. Hybridization was completed overnight at 37°C in the dark using probes against KTN1-AS1, U6, or 18S. On the next day, cells were counterstained with DAPI, and imaged with a confocal laser scanning microscope (Carl Zeiss). The imaging results were recorded [9].

qRT-PCR

TRIzol reagent (Thermo Fisher, USA) was employed for total RNA isolation. 1 µg of total RNA was reverse transcribed into cDNA with the PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan). LncRNA, microRNA, and mRNA expression levels were analyzed using TB Green Premix Ex Taq and the 7500 Real-Time PCR System (Thermo Fisher, USA). KTN1-AS1, mRNA, and microRNA levels were normalized to 18S RNA, GAPDH mRNA, and U6, respectively. The $2^{-\Delta\Delta Ct}$ technique was utilized to determine the expression of KTN1-AS1, microRNA-130a-5p, and PDPK1. GenePharma (China) manufactured each primer. Table I lists certain primer sequences [7].

Data analysis

Statistical analysis was conducted using GraphPad Prism 8.0. All data were presented as mean ± SD. The *p*-values for intergroup differences were determined by Student's *t*-test for two-group or one-way ANOVA for multi-comparisons. A *p*-value < 0.05 was considered statistically significant.

Results

High expression of KTN1-AS1 in NSCLC promotes tumor progression

Through bioinformatics analysis, seven significant lncRNAs (LINC00472, ADAMTSL4-AS1, MIR22HG, LINC00921, C22orf34, TMEM99, KTN1-AS1) were identified (Figure 1 A). Subsequently, the KTN1-AS1 level in tumor and adjacent non-tumor samples was compared using GEPIA, and it was found to be higher in tumors (Figure 1 B). Similarly, the KTN1-AS1 level from GSE44077 was significantly higher in tumor samples than in adjacent samples (Figure 1 C). Also, the KTN1-AS1 level was significantly higher in NSCLC cells than in BEAS-2B cells (Figure 1 D). A549 and H1975 cell lines were selected, and sh-KTN1-AS1 plasmids were transfected into the cell lines. KTN1-AS1 level after transfection was measured via qRT-PCR. KTN1-AS1 was significantly inhibited in the sh-KTN1-AS1 group compared to the sh-NC group (Figure 1 E). Subsequently, the CCK-8 assay showed that cell viability was significantly suppressed in the sh-KTN1-AS1 group compared to the sh-NC group (Figure 1 F). Western blot results also showed

Table I. Primer information

Primer sequence (5′-3′)		
Gene	**Forward**	**Reverse**
KLF5	ACCTGGAGAAACGCCGCATC	AGTGTGAGTCCTCAGGTGAG
18S RNA	CGTTCTTAGTTGGTGGAGCG	CCGGACATCTAAGGGCATCA
U6	TGCGGGTGCTCGCTTCGGCAGC	GTGCAGGGTCCGAGGT
KTN1-AS1	AGGGAAATTTGGGCAGAAGT	GTTACCCGTGTGAGCCTGAT
microRNA-153-3p	ACACTCCAGCTGGGTGTGCATAGTCAAA	CAGTGGTGTCGTGGAGT
GAPDH	CACCCACTCCTCCACCTTTG	CCACCACCCTGTTGCTGTAG

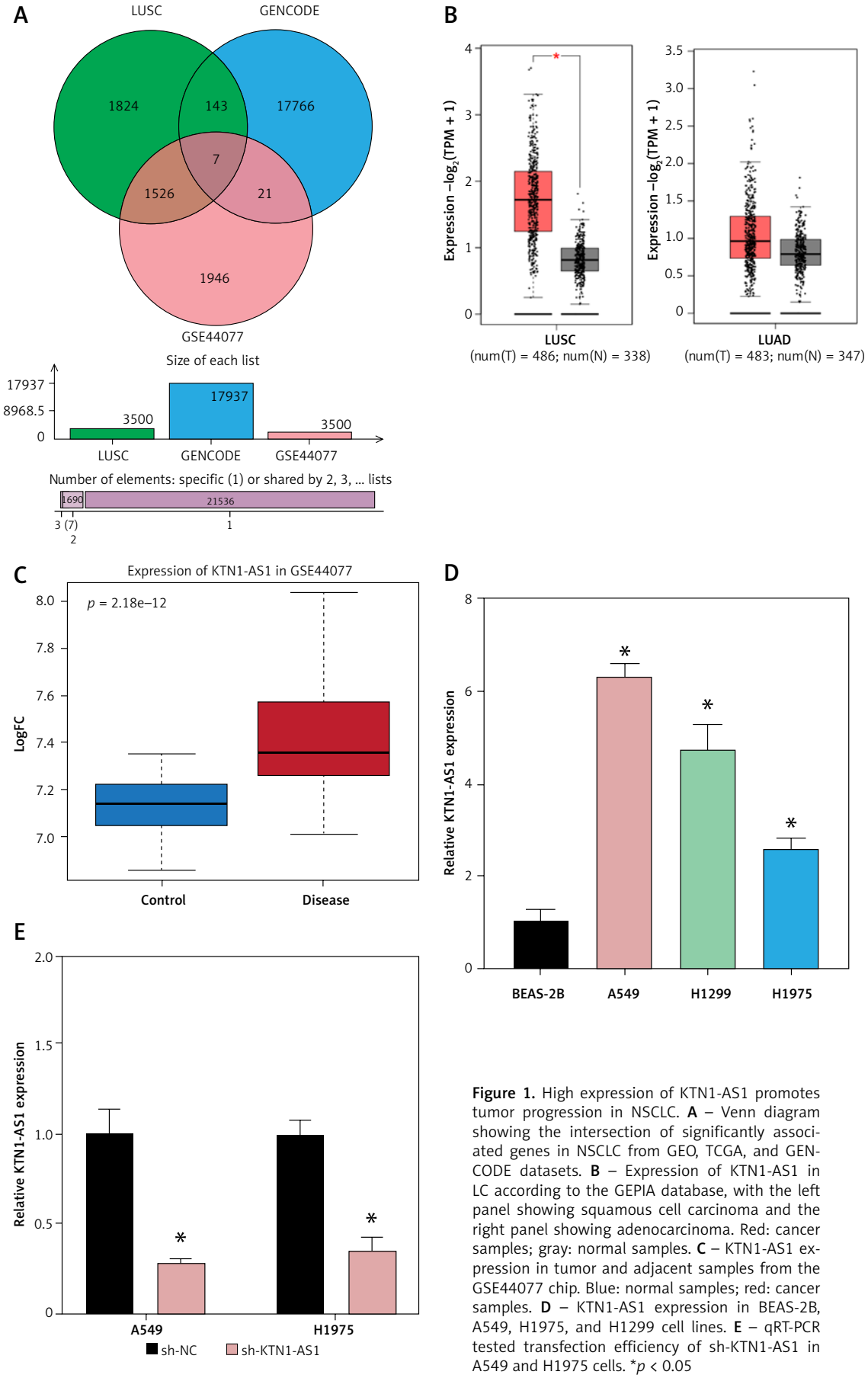


Figure 1. High expression of KTN1-AS1 promotes tumor progression in NSCLC. **A** – Venn diagram showing the intersection of significantly associated genes in NSCLC from GEO, TCGA, and GENCODE datasets. **B** – Expression of KTN1-AS1 in LC according to the GEPIA database, with the left panel showing squamous cell carcinoma and the right panel showing adenocarcinoma. Red: cancer samples; gray: normal samples. **C** – KTN1-AS1 expression in tumor and adjacent samples from the GSE44077 chip. Blue: normal samples; red: cancer samples. **D** – KTN1-AS1 expression in BEAS-2B, A549, H1975, and H1299 cell lines. **E** – qRT-PCR tested transfection efficiency of sh-KTN1-AS1 in A549 and H1975 cells. *$p < 0.05$

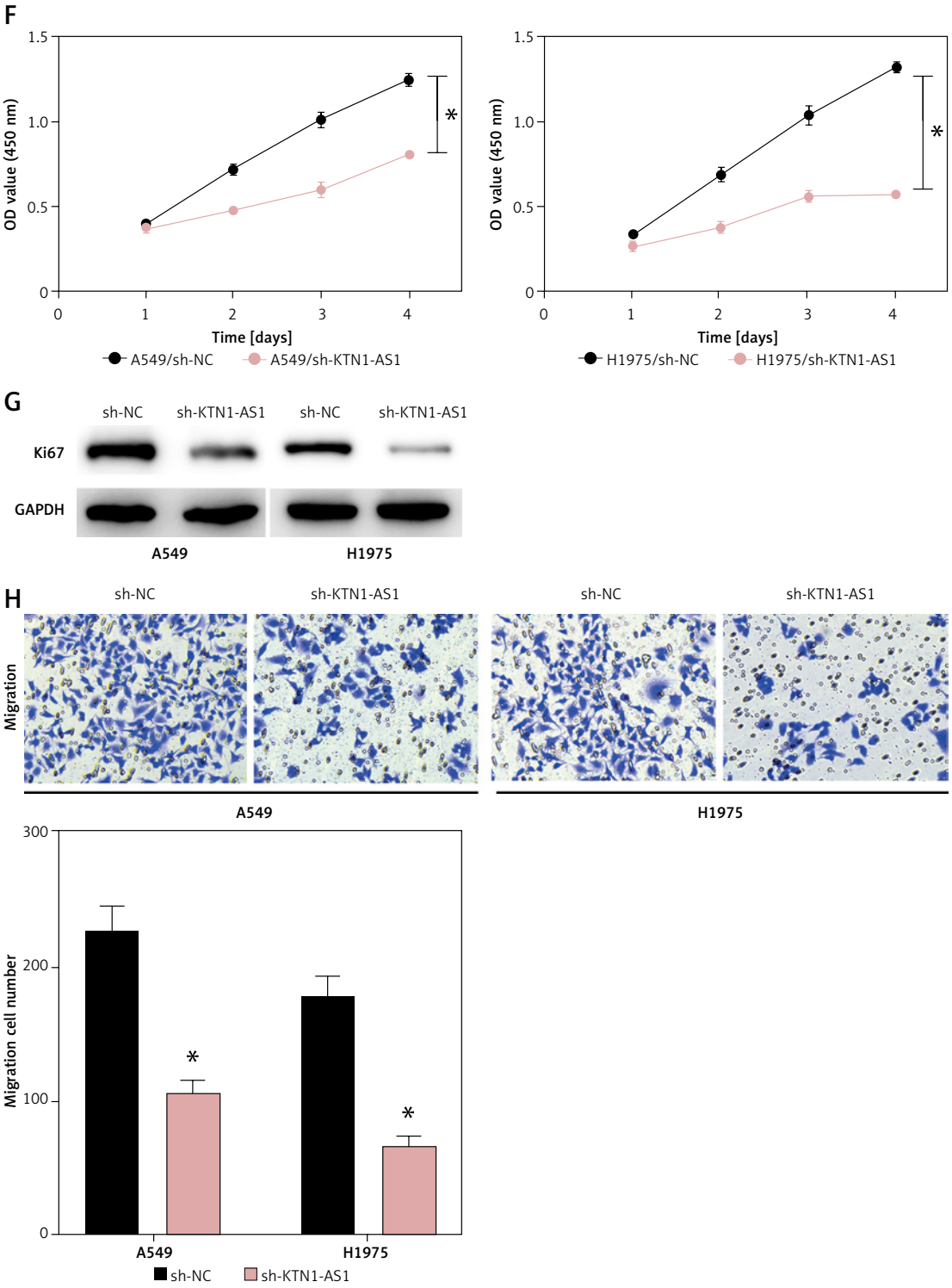


Figure 1. Cont. **F** – CCK-8 assayed the effect of KTN1-AS1 knockdown on the proliferation of A549 and H1975 cells. **G** – Western blot tested protein levels of Ki67 in A549 and H1975 cells after KTN1-AS1 knockdown. **H**, **I** – Transwell assayed migration and invasion of cells after KTN1-AS1 knockdown

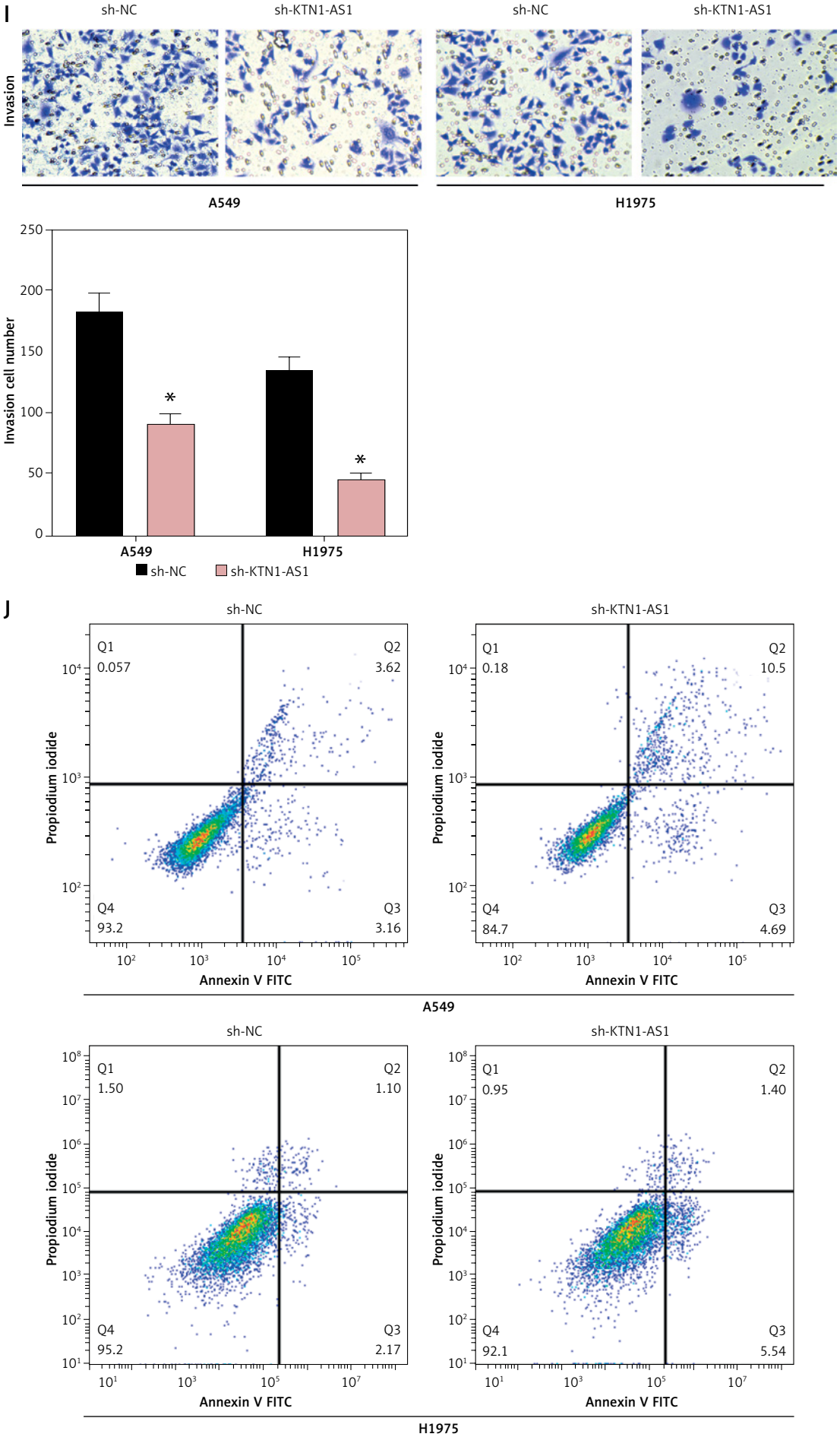


Figure 1. Cont. **I** – Transwell assayed migration and invasion of cells after KTN1-AS1 knockdown. **J** – Flow cytometry detected A549 and H1975 cell apoptosis after sh-KTN1-AS1 transfection. *$p < 0.05$

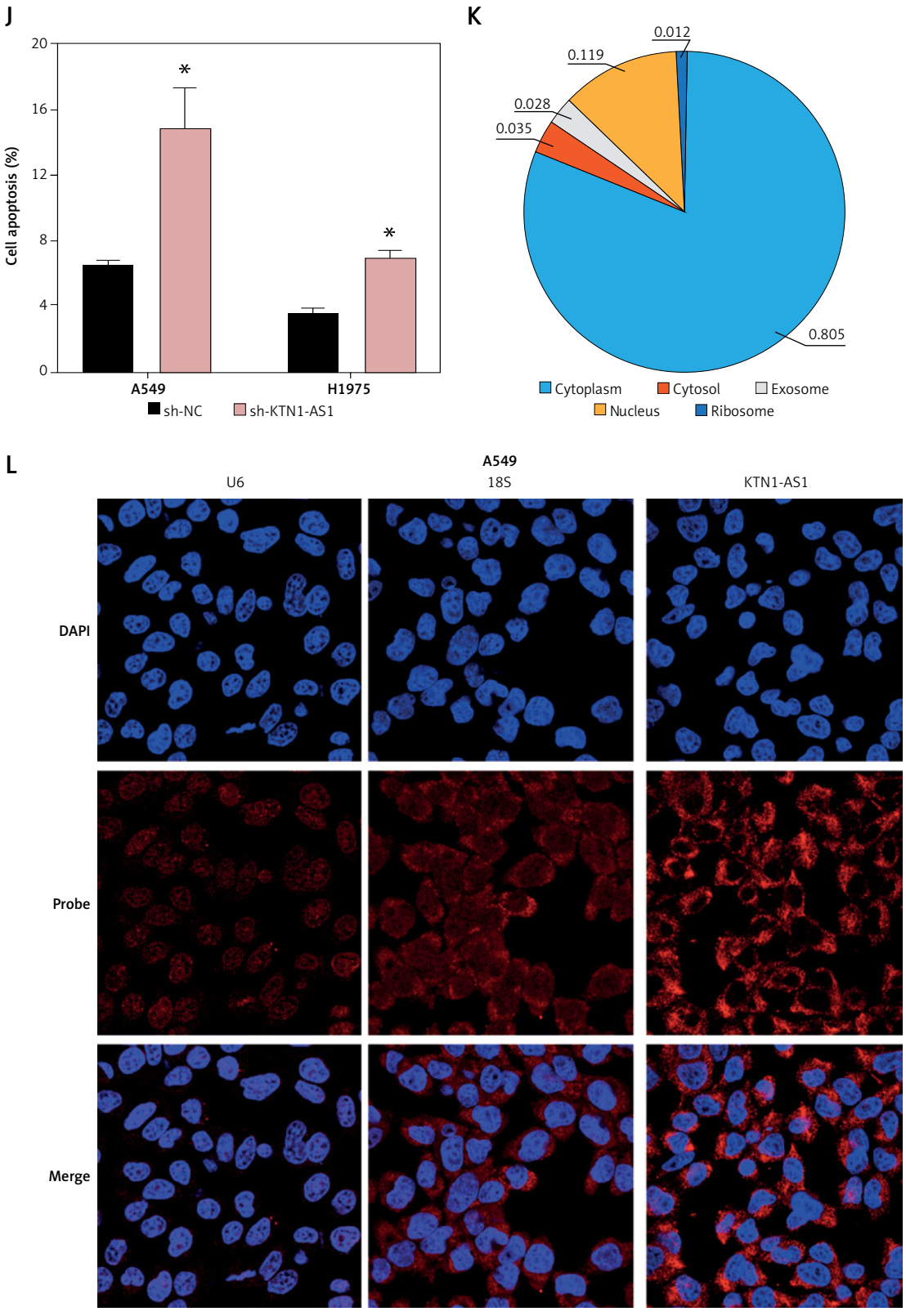


Figure 1. Cont. **J** – Flow cytometry detected A549 and H1975 cell apoptosis after sh-KTN1-AS1 transfection. **K** – Subcellular localization prediction of KTN1-AS1 using the lncLocator database. **L** – FISH assay validated localization of KTN1-AS1 in A549 cells. *$p < 0.05$

that the expression level of Ki67 protein in the sh-KTN1-AS1 group was significantly decreased (Figure 1 G). Results of Transwell assays revealed that, compared with the sh-NC group, the number of cells migrating and invading in the sh-KTN1-AS1 group was reduced by about 40%-50%, and the migration and invasion abilities of cells were significantly inhibited (Figures 1 H, I). Furthermore, flow cytometry revealed that the apoptosis rate of NSCLC cells increased by about 50% after silencing KTN1-AS1 (Figure 1 J).

LncRNA KTN1-AS1 could act as a ceRNA by binding to downstream microRNAs and regulating the expression of target genes. Therefore, investigating the subcellular localization of KTN1-AS1 is essential for understanding its mechanism. As predicted by the lncLocator database, KTN1-AS1 was primarily distributed in the cytoplasm (Figure 1 K). Furthermore, we validated the cytoplasmic localization of KTN1-AS1 using FISH (Figure 1 L). Taken together, KTN1-AS1 drove malignant behaviors of NSCLC cells and was predominantly distributed in the cytoplasm.

Regulation of NSCLC development by KTN1-AS1/microRNA-153-3p

The cytoplasmic localization of KTN1-AS1 was confirmed by FISH, suggesting its role as a ceRNA that could bind to microRNAs to mediate downstream signaling. Subsequently, using starBase, we further predicted ten downstream microRNAs of KTN1-AS1 (microRNA-130a-5p, microRNA-23a-3p, microRNA-23b-3p, microRNA-23c, microRNA-519c-3p, microRNA-519b-3p, microRNA-519a-3p, microRNA-153-3p, microRNA-3144-3p, microRNA-505-3p). Clinical studies have revealed the promoting role of microRNA-153-3p in LC development [10]. Additionally, other literature reports demonstrated the repressive effects of microRNA-153-3p on tumor proliferation and invasion in lung adenocarcinoma [11]. Hence, microRNA-153-3p was selected as downstream microRNA. By performing qRT-PCR, we observed a significantly higher level of microRNA-153-3p in BEAS-2B cells than in NSCLC cells (Figure 2 A). Subsequently, qRT-PCR was used to measure levels of microRNA-153-3p in NSCLC cells transfected with sh-NC and sh-KTN1-AS1, revealing a significant increase in microRNA-153-3p expression upon KTN1-AS1 silencing (Figure 2 B). Binding sites between KTN1-AS1 and microRNA-153-3p were obtained from the RNA22 database (Figure 2 C), and a dual-luciferase assay validated the binding of microRNA-153-3p to KTN1-AS1. A significant reduction was observed in the luciferase activity of wild-type KTN1-AS1 under microRNA-153-3p mimic conditions, while the activity of mutant-type KTN1-AS1 remained unaffected (Figure 2 D). The impact of KTN1-AS1/microRNA-153-3p on H1975 and A549 cell proliferation was verified through the CCK-8 assay and western blot. The results showed that KTN1-AS1 knockdown significantly inhibited the viability of NSCLC cells and the expression of Ki67 protein, but miR-153-3p-inhibitor inhibited the above effects (Figures 2 E–G). KTN1-AS1 knockdown significantly inhibited NSCLC cell migration by 56% and NSCLC cell invasion by 40%, while increasing the apoptosis rate by 6.2%. However, these effects were also rescued by the microRNA-153-3p inhibitor (Figures 2 H–J). Overall, our experiments validated the promoting role of the KTN1-AS1/microRNA-153-3p axis in the malignant progression of NSCLC cells.

microRNA-153-3p negatively regulates KLF5 as its target

To dissect downstream regulatory target genes of microRNA-153-3p, the miRDB database was utilized to forecast target genes, resulting in 777 relevant genes. Among them, six genes (KCNQ4, KLF5, HEY2, SERTAD2, UNC5C, FEM1C) showed the highest binding strength with microRNA-153-3p. Based on relevant literature, KLF5 was selected as a downstream target of microRNA-153-3p [12]. qRT-PCR revealed significantly higher expression of KLF5 in NSCLC cells than in BEAS-2B cells (Figure 3 A). Binding sites between microRNA-153-3p and KLF5 were obtained from the starBase database (Figure 3 B). Dual-luciferase assay was used to confirm the targeted relationship between microRNA-153-3p and KLF5. The data showed that luciferase activity was reduced by about 50% in the presence of microRNA-153-3p mimic and wild-type KLF5, but luciferase activity of mutant-type KLF5 remained unaffected (Figure 3 C). Further experiments were performed by transfecting microRNA-153-3p mimic into NSCLC cells, and qRT-PCR (Figure 3 D) and western blot (Figure 3 E) assessed mRNA and protein expression of KLF5. The results demonstrated a significant reduction in both mRNA and protein levels of KLF5 in the mimic-microRNA-153-3p group compared to the mimic-NC group. In conclusion, these experimental results illustrated that microRNA-153-3p negatively regulated KLF5 expression.

KTN1-AS1 exerts oncogenic effects by inhibiting microRNA-153-3p-mediated KLF5

The connection of the KTN1-AS1/microRNA-153-3p/KLF5 signaling axis was experimentally validated. To elucidate the role of KTN1-AS1 in the development of NSCLC within this signaling axis, different treatments were applied to NSCLC cell lines, including sh-NC, sh-KTN1-AS1, sh-KTN1-

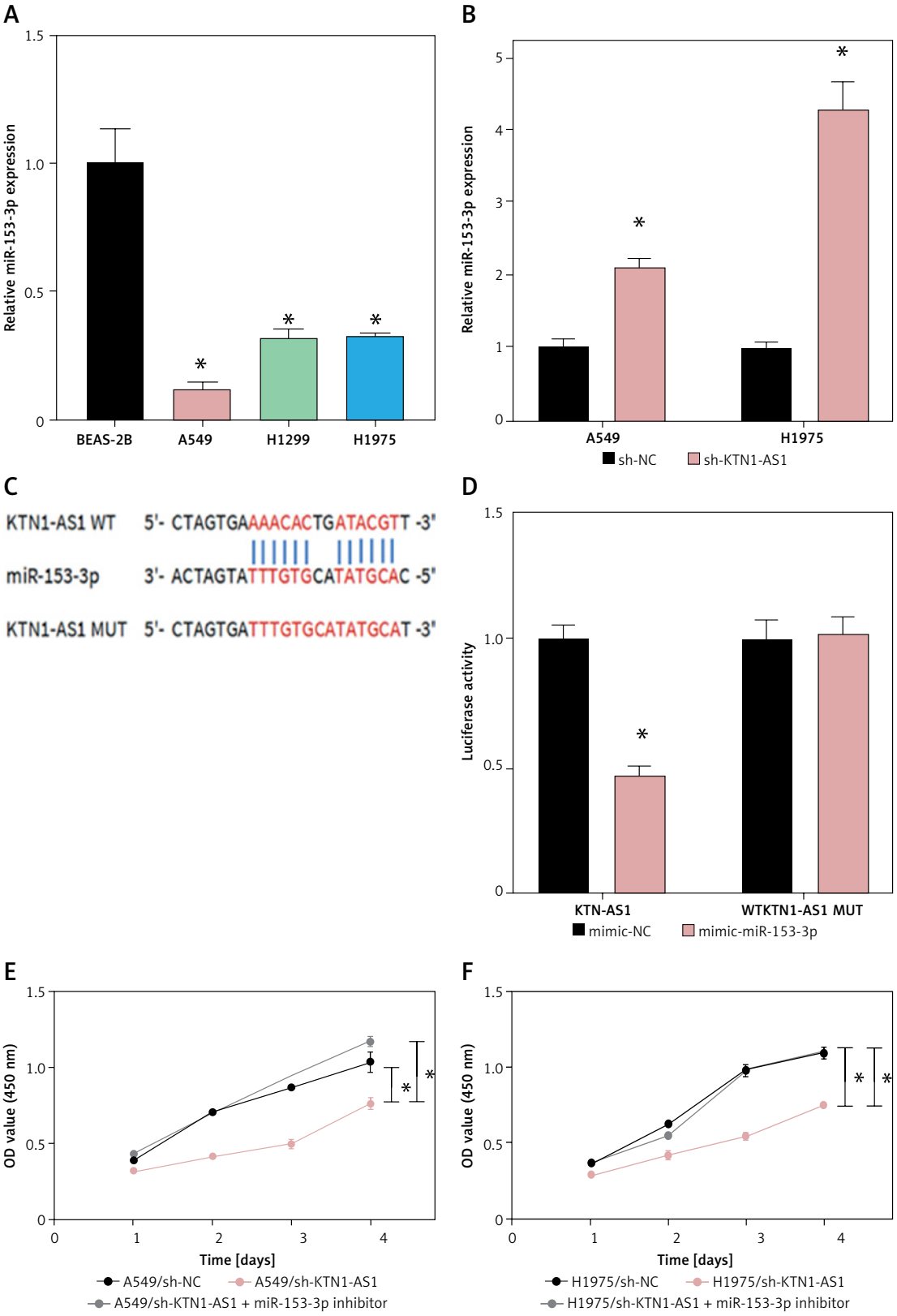


Figure 2. KTN1-AS1/microRNA-153-3p regulates the development of NSCLC. **A** – qRT-PCR assayed expression of microRNA-153-3p in BEAS-2B cells and NSCLC cells. **B** – qRT-PCR assayed microRNA-153-3p expression in A549 and H1975 cells after sh-KTN1-AS1 transfection. **C** – Prediction of binding sites of KTN1-AS1 to microRNA-153-3p using RNA22 database. **D** – Dual-luciferase assay assessed targeting interaction between KTN1-AS1 and microRNA-153-3p. **E**, **F** – CCK-8 assessed the proliferation of H1975 and A549 cells. *$p < 0.05$

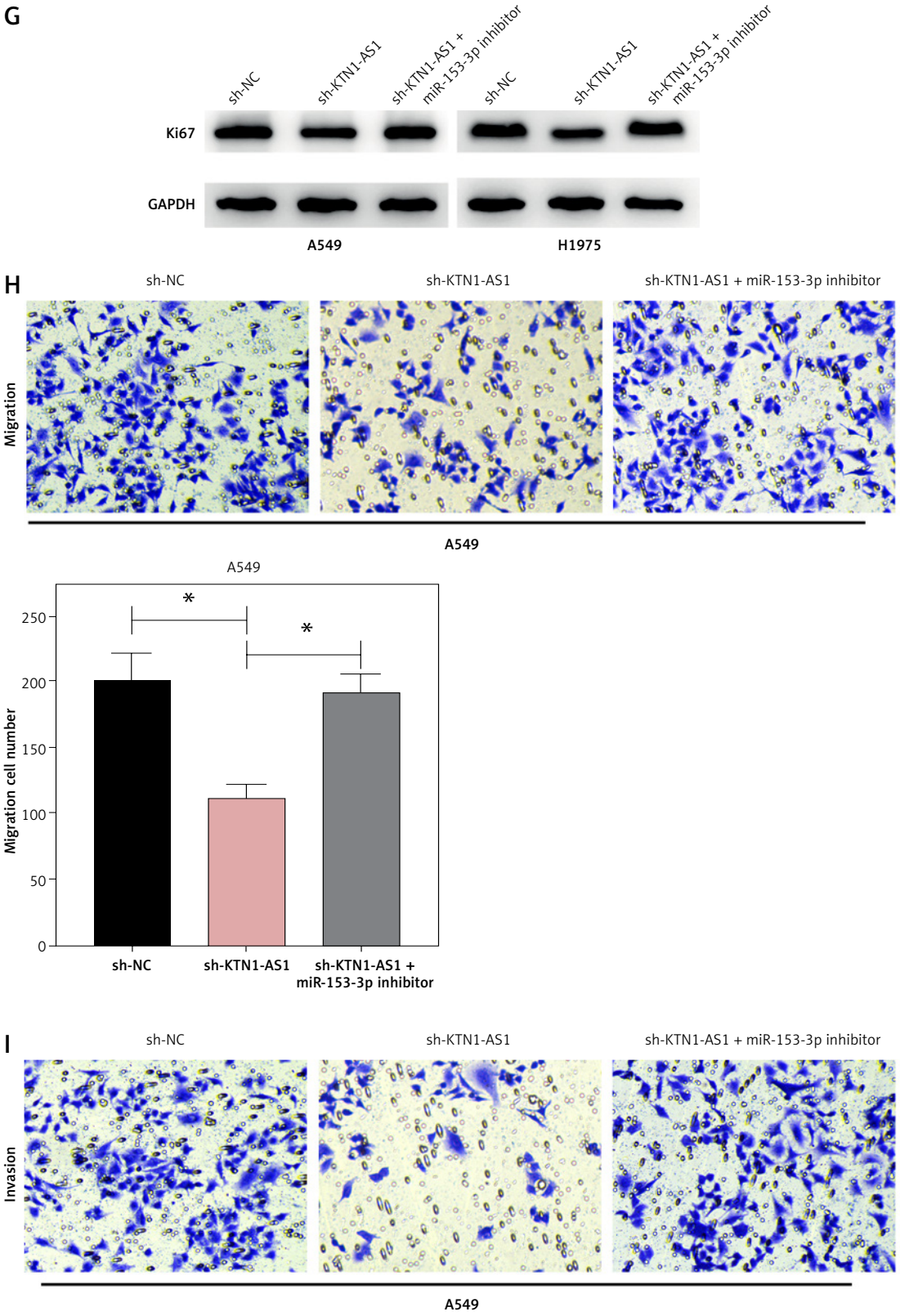


Figure 2. Cont. **G** – Western blot tested protein levels of Ki67 in A549 and H1975 cells. **H**, **I** – Transwell assay examined migration and invasion of A549 cells. *$p < 0.05$

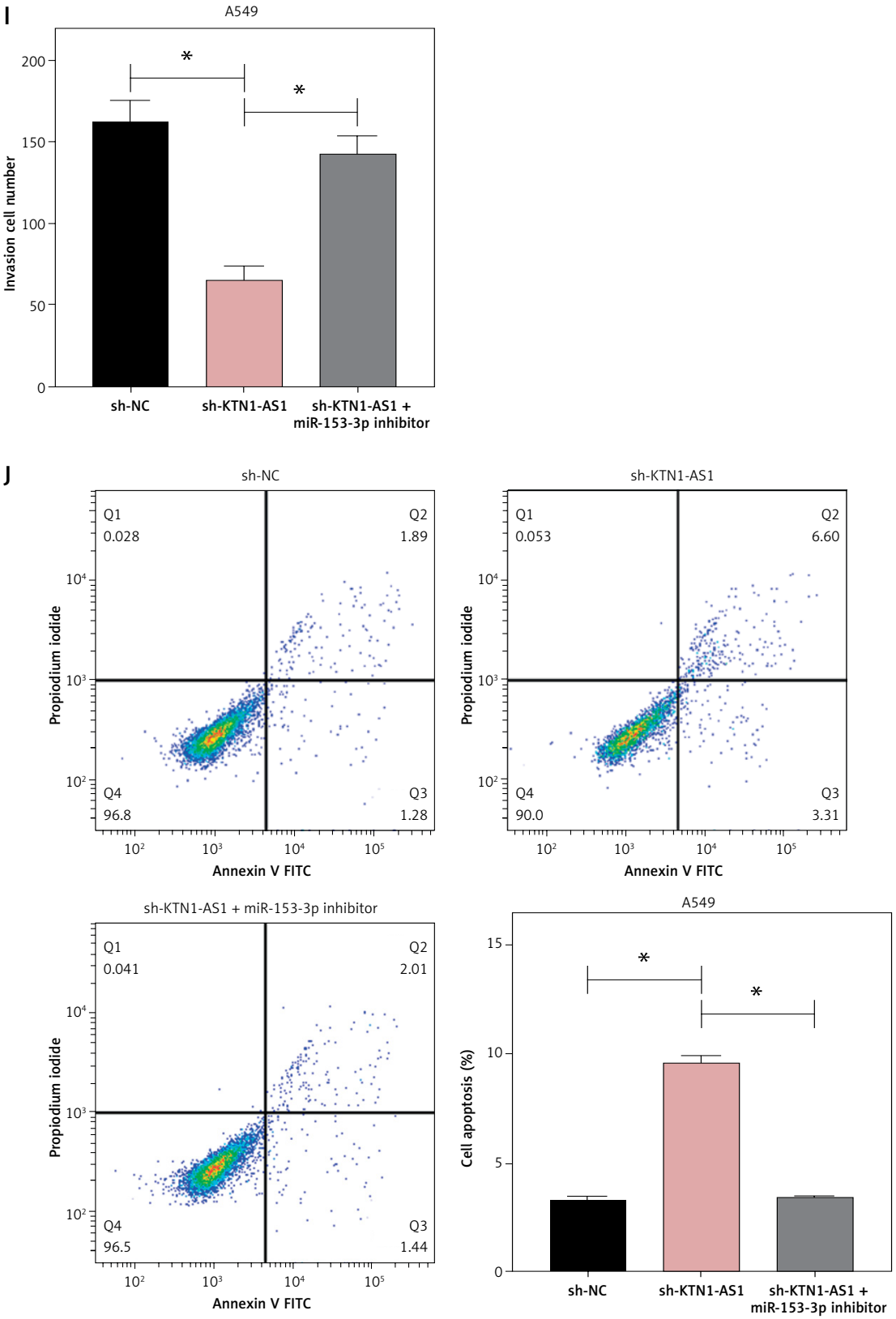


Figure 2. Cont. **I** – Transwell assay examined migration and invasion of A549 cells. **J** – Flow cytometry evaluated apoptosis of A549 cells. *$p < 0.05$

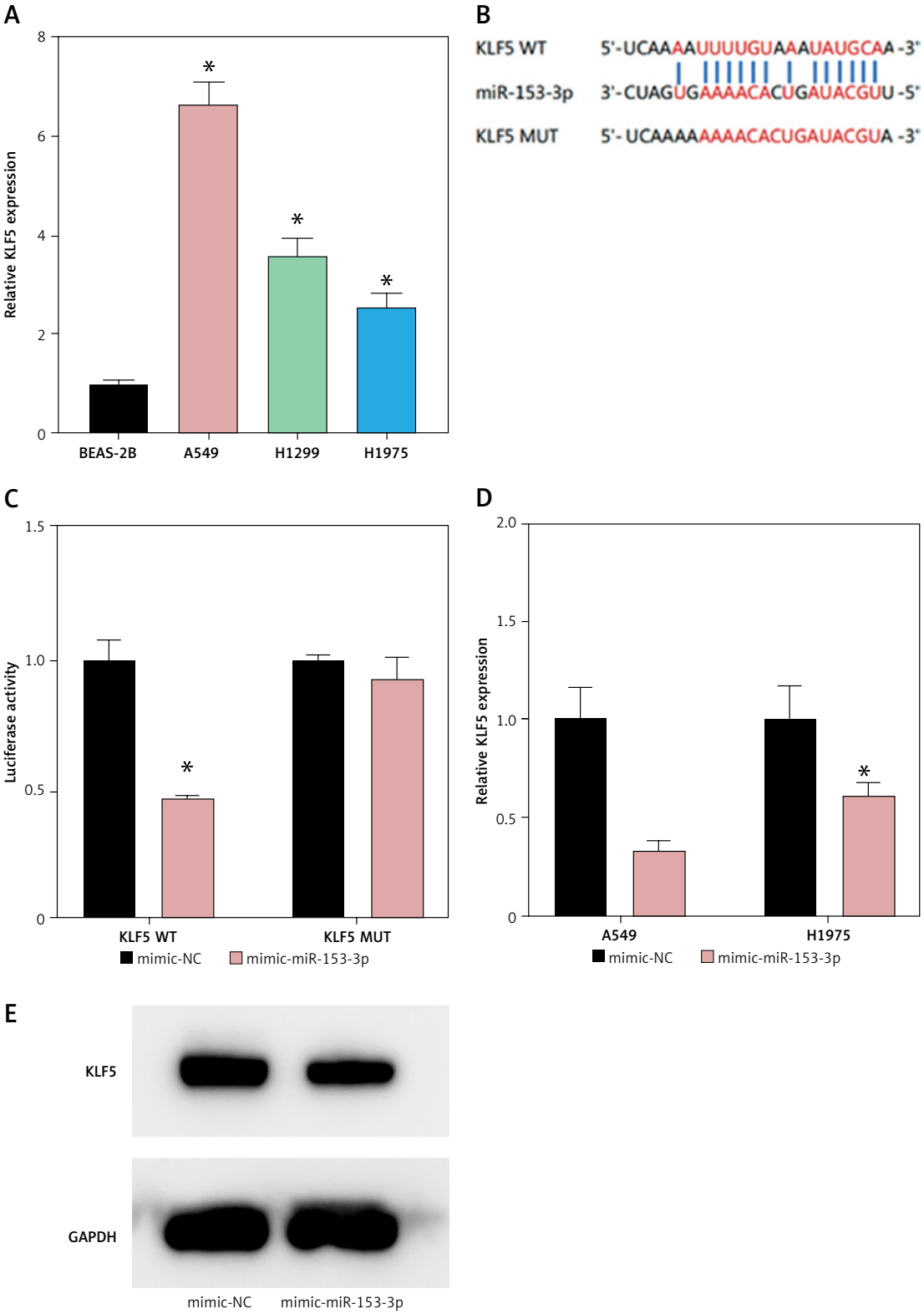


Figure 3. microRNA-153-3p negatively regulates KLF5. **A** – qRT-PCR measured levels of KLF5 in BEAS-2B cells and NSCLC cells. **B** – Prediction of binding sites of microRNA-153-3p to KLF5 using the starBase database. **C** – Dual-luciferase assay assessed the impact of microRNA-153-3p mimic on luciferase activity of wild-type and mutant KLF5. **D**, **E** – qRT-PCR and western blot tested mRNA and protein levels of KLF5 in A549 and H1975 cells after microRNA-153-3p mimic transfection. *$p < 0.05$

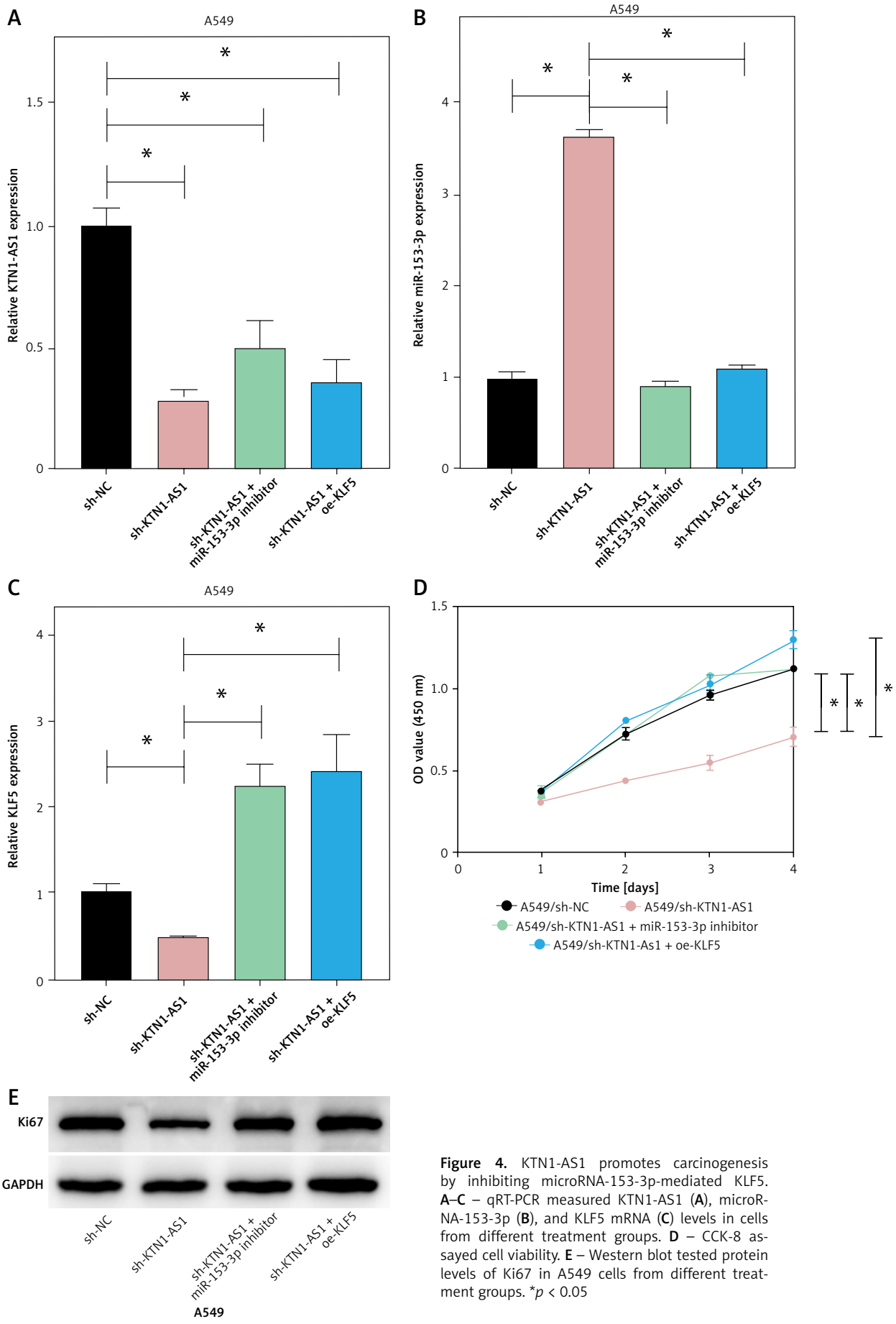
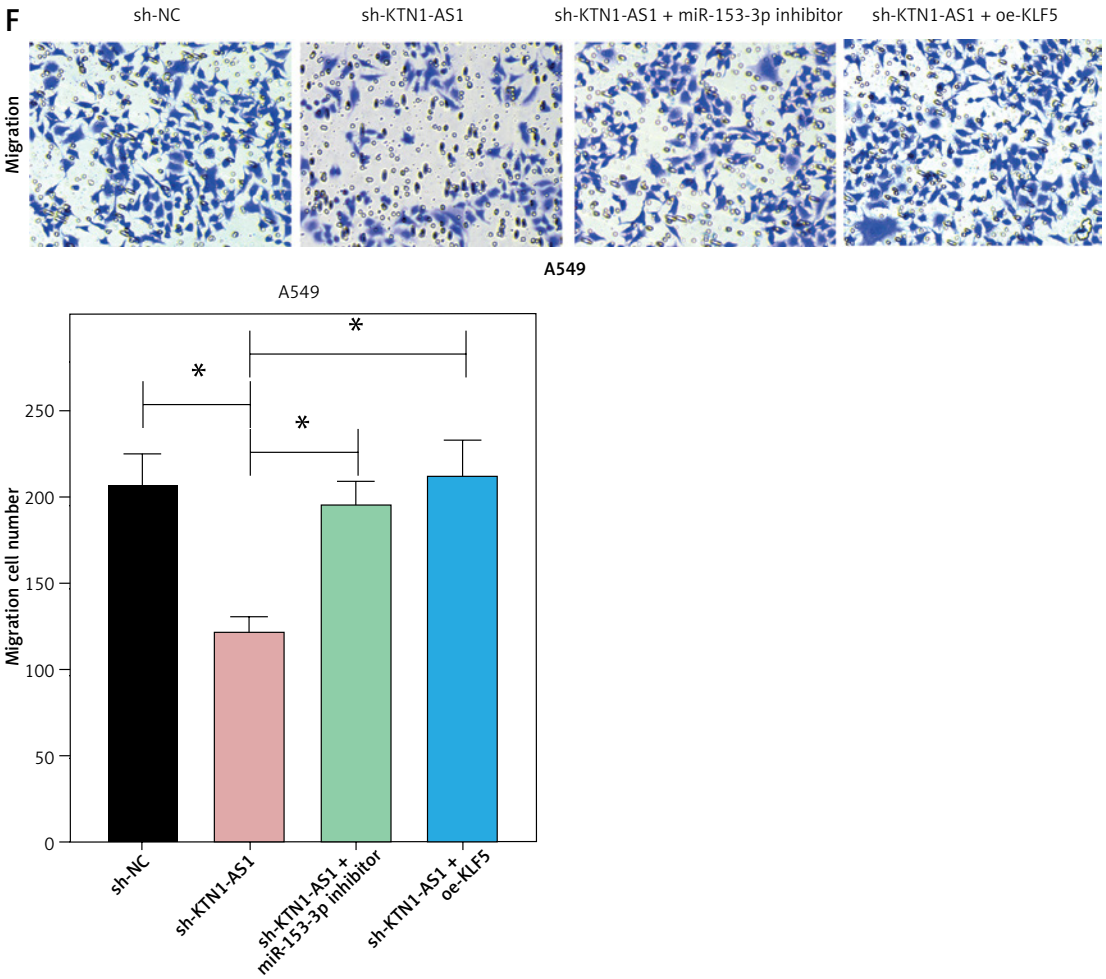


Figure 4. KTN1-AS1 promotes carcinogenesis by inhibiting microRNA-153-3p-mediated KLF5. **A–C** – qRT-PCR measured KTN1-AS1 (**A**), microRNA-153-3p (**B**), and KLF5 mRNA (**C**) levels in cells from different treatment groups. **D** – CCK-8 assayed cell viability. **E** – Western blot tested protein levels of Ki67 in A549 cells from different treatment groups. *$p < 0.05$



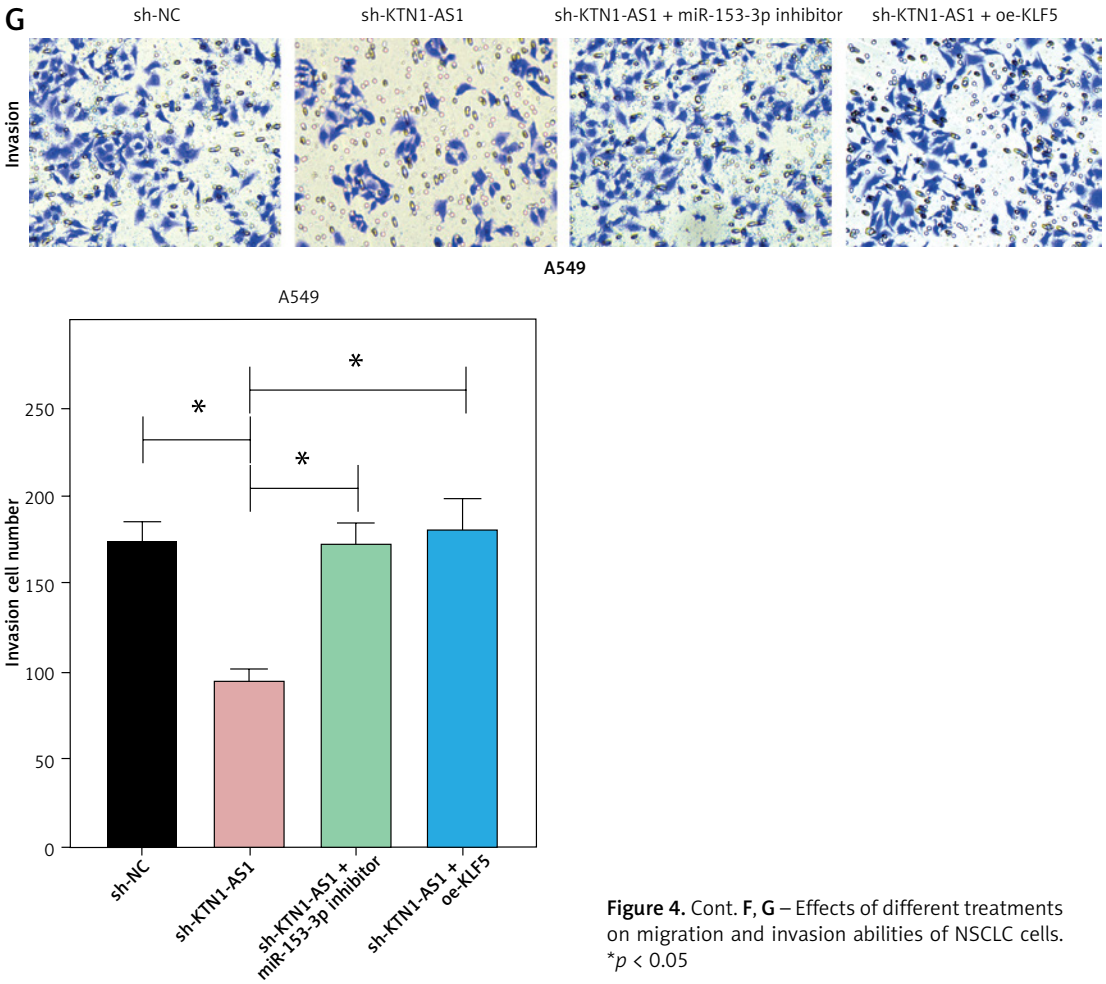


Figure 4. Cont. **F**, **G** – Effects of different treatments on migration and invasion abilities of NSCLC cells. *$p < 0.05$

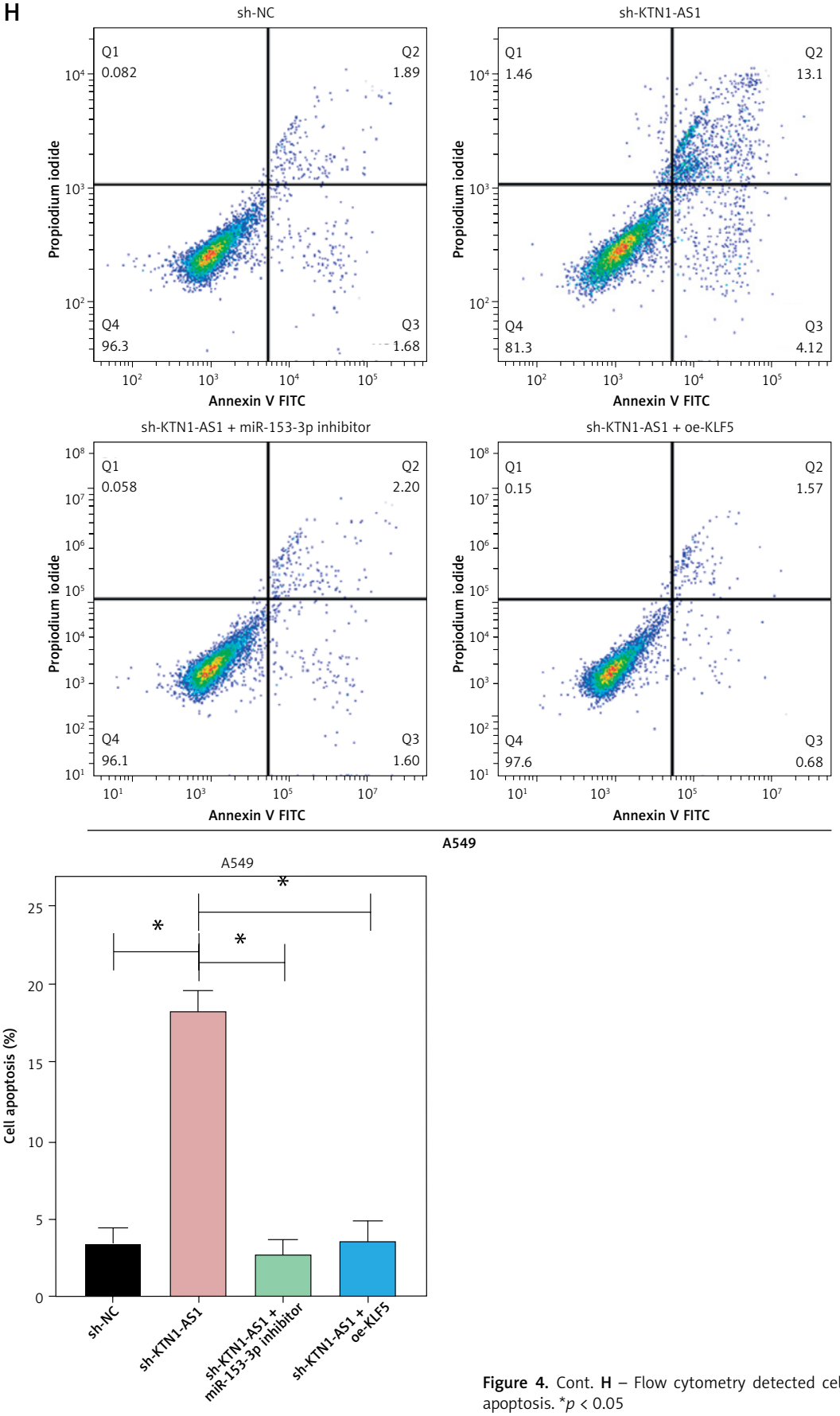


Figure 4. Cont. **H** – Flow cytometry detected cell apoptosis. *$p < 0.05$

AS1 + microRNA-153-3p inhibitor, and sh-KTN1-AS1 + oe-KLF5. qRT-PCR was used to assay KTN1-AS1 (Figure 4 A), microRNA-153-3p (Figure 4 B), and KLF5 mRNA (Figure 4 C) levels in cells from different treatment groups. Cell proliferation in NSCLC cells under various conditions was assessed using CCK-8 assay, revealing that cell proliferation was inhibited in the sh-KTN1-AS1 group. However, cell proliferation was restored in sh-KTN1-AS1 + microRNA-153-3p inhibitor and sh-KTN1-AS1 + oe-KLF5 groups (Figure 4 D). Similarly, the expression of Ki67 protein was significantly reduced in the sh-KTN1-AS1 group, but the expression returned to the control level in the sh-KTN1-AS1 + microRNA-153-3p inhibitor and sh-KTN1-AS1 + oe-KLF5 groups (Figure 4 E). Transwell experiments were conducted to measure the migration and invasion capabilities of NSCLC cells under different treatment conditions. The results showed that the number of migrating cells in the sh-KTN1-AS1 group decreased by 58%, while the number of invading cells decreased by 53%; that is, the cell migration and invasion abilities decreased, while these abilities were partially reversed in the sh-KTN1-AS1 + microRNA-153-3p inhibitor and sh-KTN1-AS1 + oe-KLF5 groups (Figures 4 F, G). Flow cytometry demonstrated that the apoptosis rate of the sh-KTN1-AS1 group was 15% higher than that of the sh-NC group. However, the extent of apoptosis was partially reversed in sh-KTN1-AS1 + microRNA-153-3p inhibitor and sh-KTN1-AS1 + oe-KLF5 groups (Figure 4 H). Taken together, the results of these experiments indicated that KTN1-AS1 exerted oncogenic effects by inhibiting microRNA-153-3p-mediated KLF5 expression.

Discussion

NSCLC is a disease with a high mortality rate and the main cause of cancer-related fatalities globally [13]. There is increasing proof that lncRNAs can act as diagnostic and therapeutic markers for NSCLC [14]. For instance, lncRNAs DLEU2 and XIST have been shown to promote tumor development in NSCLC [15, 16]. LncRNA KTN1-AS1 is a type of lncRNA associated with the progression, metastasis, and invasion of NSCLC6. KTN1-AS1 is elevated in glioma and hepatocellular carcinoma, and drives cell proliferation and invasion via modulation of microRNA, thereby facilitating cancer progression [17, 18]. In NSCLC cells, KTN1-AS1 also regulates tumor progression by modulating microRNAs. Furthermore, KTN1-AS1 acts as a prognosticator and is upregulated in NSCLC patients with poor prognosis, and it regulates the microRNA-23b/DEPDC1 axis in the progression of NSCLC [7]. In this work, the effects of KTN1-AS1 expression on NSCLC cell malignant behaviors were validated through the construction of KTN1-AS1-silenced NSCLC cells and *in vitro* cell culture. Additionally, differential expression of KTN1-AS1 between tumors and adjacent tissues was analyzed using databases. The modulatory impact of the KTN1-AS1/microRNA-153-3p/KLF5 axis on NSCLC cells was further investigated through experimental research.

Research has confirmed that lncRNAs can function as ceRNAs and competitively bind to microRNAs, thus regulating the expression of related target genes [19]. When lncRNAs act in tumors, they can help control biological processes [20]. microRNA-153-3p can target KTN1-AS1 [21]. Moreover, inhibition of microRNA-153-3p has been found to promote cancer development in NSCLC [11], which is consistent with our research. Based on the ceRNA principle, this study identified significantly increased expression of KTN1-AS1 and KLF5 in NSCLC through the starBase database, suggesting a regulatory role of the KTN1-AS1/microRNA-153-3p/KLF5 axis in NSCLC. The binding of KTN1-AS1 to microRNA-153-3p was validated through dual-luciferase assays. Furthermore, under conditions of KTN1-AS1 inhibition and simultaneous microRNA-153-3p suppression, NSCLC cell proliferation, migration, and invasion abilities were reversed, indicating that KTN1-AS1 could regulate tumor cell malignant progression by modulating microRNA-153-3p.

In this study, miRDB was utilized to forecast downstream targets of microRNA-153-3p, and KLF5 was selected as the target gene in the regulatory axis. Krüppel-like factor 5 (KLF5) is a type of transcription factor regulating cell fate and tumor progression [12]. Firstly, KLF5 is elevated in NSCLC and implicated in the unfavorable prognosis in NSCLC patients [22]. KLF5 has been reported to encourage GDF15 expression and C5a-induced cell proliferation in NSCLC [23]. Secondly, KLF5 modulates malignant progression in cancers including breast cancer and esophageal squamous cell carcinoma by binding to microRNAs [24, 25]. The binding of KLF5 to microRNA-153-3p was validated through dual-luciferase assays. Furthermore, NSCLC cell lines with silenced KTN1-AS1 were established, and the modulatory impact of KTN1-AS1 on the microRNA-153-3p/KLF5 axis was studied under conditions of microRNA-153-3p inhibition and KLF5 overexpression. Knockdown of KTN1-AS1 inhibited cell malignant behaviors, while inhibiting microRNA-153-3p and overexpressing KLF5 reversed these effects in NSCLC cells. Therefore, it was postulated that the regulatory role of the KTN1-AS1/microRNA-153-3p/KLF5 axis in NSCLC progression was achieved through the suppression of microRNA-153-3p expression by KTN1-AS1, leading to increased expression of KLF5 and promoting tumor progression. This is consistent with the similar promotion of malignant behaviors of colon and gastric cancer cells by KLF5 [26, 27].

In conclusion, we proposed the regulatory role of the KTN1-AS1/microRNA-153-3p/KLF5 axis in NSCLC. Further experimental validation confirmed the mutual targeting interaction within the regulatory axis and its modulatory effects on cell proliferation, migration, and invasion in NSCLC. However, there is limited research on the detailed regulatory mechanisms of the KTN1-AS1/microRNA-153-3p/KLF5 axis. Finally, our research raises the possibility that the KTN1-AS1/microRNA-153-3p/KLF5 axis may modulate NSCLC malignant progression, offering novel targets for NSCLC-targeted treatment.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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