Long Non-Coding RNA NEAT1 Accelerates Lung Cancer Development Via MicroRNA-107/FOXK1 Axis

Peng Cao
Huazhong University of Science and Technology
Shan Hu
Huazhong University of Science and Technology
Kangle Kong
Huazhong University of Science and Technology
Peng Han
Huazhong University of Science and Technology
Yu Deng
Huazhong University of Science and Technology
fan Li (lifan0281@sina.com)
Huazhong University of Science and Technology
Bo Zhao
Huazhong University of Science and Technology

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Abstract

Objective: Studies have abstracted the partial mechanism of long non-coding RNA nuclear enriched abundant transcript 1 (NEAT1) in lung cancer, but its reciprocal with microRNA-107/Forkhead box k1 (miR-107/FOXK1) is not disclosed clearly. Hence, the mechanism of NEAT1/miR-107/FOXK1 was delved out in lung cancer.

Methods: NEAT1, miR-107 and FOXK1 expression in clinical cancer tissues were determined, along with their interactions. The relation between the survival of lung cancer patients and NEAT1 expression was determined. NEAT1 and/or miR-107-related lentivirus or plasmid were transfected into A549 cells, after which cell biological functions were tested. Tumors were xenografted in mice to observe tumor formation and cell apoptosis.

Results: High NEAT1 and FOXK1 and low miR-107 levels were tested in lung cancer tissues. High NEAT1 was connected with low overall survival of lung cancer patients. Depleting NEAT1 or augmenting miR-107 inhibited the biological functions of lung cancer cells. Depleted NEAT1 suppressed tumor-forming ability of A549 cells *in vivo*. Inhibited miR-107 antagonized depleted NEAT1-mediated effects on A549 cells. NEAT1 regulated FOXK1 by competitively binding miR-107.

Conclusion: Silenced NEAT1 suppresses lung cancer development through elevating miR-107 and reducing FOXK1 expression, which supplies a plan to treat lung cancer.

Introduction

Lung cancer tops the malignancy with the highest global incidence and mortality and it is subgrouped to non small cell lung cancer (NSCLC) and small cell lung cancer [1]. Lung cancer causes deaths, mainly due to the late diagnosis and local or distant metastasis [2]. At present, surgical resection, radiotherapy and chemotherapy are introduced to treat lung cancer, but systemic toxicity, chemo-resistance and metastasis result in an unsatisfactory outcome [3]. Moreover, treatments of lung cancer are impelled to face challenges such as poor-understood pathogenesis, limitations of CT diagnosis due to high false positive rates and subjective error in pathological diagnosis [4]. Given that, the effective but applicable target agents are asked.

Long non-coding RNA nuclear enriched abundant transcript 1 (NEAT1) is indicative of tumor stage and lymph node metastasis (LNM) and of importance in mediating malignant phenotype of lung cancer [5]. During the biological progress of lung cancer, the network of NEAT1 with its downstream target microRNAs (miRNAs) has been discussed. Chen LM *et al*. have documented that NEAT1 could regulate the aggressiveness of NSCLC cells via modulation of has-microRNA (miR)-376b-3p [6]. In addition, Zhao L *et al*. have explained the repressive impacts of depleted NEAT1 on NSCLC cell activities through interacting with miR-153-3p [7]. Moreover, NEAT1 could affect the proliferative and apoptotic activities of lung cancer cells by mediating miR-1224 [8]. As a component of miRNAs, the actions of miR-107 have been indicated in cancers including lung cancer. As reported, miR-107 is a modulator of cell proliferation

and apoptosis in lung cancer through modification of its target gene, TP53 regulated inhibitor of apoptosis 1 [9]. Besides, from another perspective, miR-107 serves to facilitate lung cancer cells to be sensitive to parthenolide through inhibiting cell proliferation and inducing apoptosis [10]. Also, low miR-107 advances the development of NSCLC [11]. Impressively, NEAT1 has been proved to be a decoy of miR-107 [12]. Given to the proved NEAT1-miR-107 network in cancers [13, 14], their interaction is speculated to function in lung cancer. Forkhead box k1 (FOXK1) pertains to FOX transcription factor family, which accounts for the aggressive behaviors of cancer cells [15]. Specifically, FOXK1 is considered as a promoting driver in lung cancer [16]. Mechanistically, FOXK1 is mediated by lncRNA-miRNA to modulate the proliferation and invasion capacities of lung cancer cells [17]. Based on the reports, this study was executed to explore the mechanism of NEAT1-miR-107-FOXK1 axis in the process of lung cancer.

Methods And Materials

Ethics statement

This study was conducted with the approval of the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The written informed consent of all participants was obtained. Animals were treated compatibly with "Guidelines for the Care and Use of Laboratory Animals" (National Academy of Sciences Press, revised in 2010).

Clinical sample collection

The matched cancer tissues and adjacent tissues from 96 lung cancer patients were obtained by surgical resection in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from July 2013 to March 2015, and frozen in liquid nitrogen. There were 61 males, aged 21–68 years with an average age of (53.18 ± 6.23) years and 35 females, aged 18–70 years with an average age of (52.01 ± 5.59) years. None of the patients had received radiotherapy or chemotherapy before surgery. Postoperative follow-up (at least 5 years) was carried out by telephone or outpatient until April 2020. The 5-year overall survival was recorded [17].

Cell culture

Lung cancer cell lines (A549, H1299, SPC-A-1) and human normal bronchial epithelial cells (16HBE) were provided by Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco's modified Eagle's medium which was added with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin [18].

Cell transfection

The lentivirus and plasmid were constructed by Sangon Biotech Co., Ltd. (Shanghai, China). A549 cells were transfected with NEAT1 shRNA negative control (NC) lentivirus, NEAT1 shRNA lentivirus, miR-107 mimic NC, miR-107 mimic, NEAT1 shRNA NC lentivirus and miR-107 inhibitor NC, NEAT1 shRNA lentivirus

and miR-107 inhibitor NC, or NEAT1 shRNA lentivirus and miR-107 inhibitor. The cells at passage 3 were trypsinized, seeded to 24-well plates (2.5×10^5 cells/well) and grew to a monolayer. Cell transfection was conducted by Lipofectamine 2000 (11668-019, Invitrogen, CA, CA, USA) [19].

Before the formal experiment, virus titer was adjusted by PBS. A549 cells in 96-well plates were infected with the virus. After 24 h, the fluorescence intensity of GFP with different titer was observed under a fluorescence microscope. The most appropriate titer was used for formal infection. Cells (5×10^4 cells/well) in logarithmic growth phase were added virus solution and 10 ug/mL Polybrene (H8761, Solarbio) to promote infection efficiency. The solution was changed after 16–24 h. After 72 h, 1 ug/mL puromycin (A1113803, Invitrogen, USA) was added in cells, and cells were detected by reverse transcription quantitative polymerase chain reaction (RT-qPCR) [19].

Cell counting kit (CCK)-8 assay

After 48-h transfection, cells were trypsinized and fostered in 96-well plate at 5×10^3 cells/well. Three parallel wells were set in each group. After incubation for 24, 48, and 72 h, respectively, cells were joined with 20 μL CCK-8 solution and incubated for another 2 h. Optical density values at 490 nm were measured by a microplate reader.

Colony formation assay

A549 cells were cultured for 11–14 d in 6-well plates (800–1000 cells/well). The formed colonies (> 50 cells/colony) were fixed by methanol, stained with crystal violet solution, and counted under a microscope. Colony formation rate = the number of cells producing colonies/the number of seeded cells.

Transwell assay

The diluted Matrigel (100 μL, 1 mg/mL) was spread evenly on the membrane of the Transwell chamber for 4–5 h. The trypsinized cells were suspended in serum-free medium, and the cell suspension (150 μL, 1×10^5 cells/mL) was hatched in the upper chamber for 24 h with the lower chamber containing 600 μL culture medium (20% FBS). Subsequently, the cells were fixed in anhydrous methanol, stained with 0.1% crystal violet solution and counted under an inverted microscope in 5 fields of view.

Migration experiment: The procedures were the same as the invasion experiment, but matrigel coating was not required.

Flow cytometry

Cells were detached, adjusted to 1×10^5 cells/mL and spread in 6-well plates. Cells with 80% confluence were detached, centrifuged (3000 r/min for 5 min), and fixed by pre-cooled 70% ethanol (5 mL) overnight. Then, cells were harvested by centrifugation at 1000 r/min and then resuspended with 0.4 mL PBS. Subsequently, cells were mingled with RNase-A (about 3 μL) to reach 50 μg/mL, detached in water bath and added with propidium iodide (PI; 50 μL) to reach 65 μg/mL. Following ice bath without light

exposure, cells were filtered through a 300-mesh (pore size: 40–50 μm) nylon mesh and detected by a flow cytometer. Cell cycle distribution was determined and the ratio of cell phase was calculated.

After 48 h of transfection, cells were suspended in 1 × binding buffer (10^6 cells/mL). The cell suspension (100 μL) was stained with fluorescein isothiocyanate-Annexin V (5 μL) and PI (5 μL), and supplemented with 400 μL 1 × binding buffer. Cell apoptosis was analyzed in a FACS Calibur BD flow cytometer (BD Bioscience, NJ, USA) [20].

Dual luciferase reporter gene assay

The binding sites of miR-107 with NEAT1 and FOXK1 were predicted through the Starbase website. The NEAT1 or FOXK1 3'UTR sequence containing the miR-326 binding site was introduced to the psiCHECK-2 (Promega, WI, USA). NEAT1-WT (AUGCUGA) or FOXK1-WT (UGCUGC) sequences were generated. Using SiteDirected mutagenesis kit (TransGene, Beijing, China), the mutated sites were introduced into NEAT1 or FOXK1 3'UTR sequence and inserted into the psiCHECK-2vector to generate NEAT1-MUT (UACGACG) or FOXK1-MUT (ACGACG) sequence. The cells were seeded in a 24-well plate and transfected with WT or MUT sequence and miR-107 mimic or NC mimic. After 24 h, the luciferase activity was evaluated by dual luciferase assay system (E1910, Promega) [21].

Tumor xenografts in nude mice

Mice (3 groups, 5 mice/group) were injected with A549 cells (1×10^6 cells/0.2 mL) subcutaneously. The volume of the tumor was estimated by measuring the length and width with a vernier caliper every 4 d. Volume = (length × width^2)/2. All mice were euthanized after 3 w. The tumors were weighed and photographed.

RT-qPCR

Trizol reagent (15596026, Invitrogen) was adopted to extract total RNA from cells. For mRNA, First Strand cDNA Synthesis Kit (K1622, Fermentas, USA) was applied to reverse transcribe total RNA into cDNA; for miRNA, TaqManTM MicroRNA Reverse Transcription Kit (4366597, Applied Biosystems, USA) was implicated in reverse transcription into cDNA; for lncRNA, lnRsmart lncRNA qPCR kit (Olaibo, WHO125, Beijing) was used for reverse transcription. Finally, the Fast SYBR Green PCR kit (Applied biosystems) and the ABI PRISM 7300 RT-PCR system (Applied biosystems) were used to quantitatively analyze the RNA. U6 was an internal reference of miRNA, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was that of other genes. The $2^{-\Delta\Delta Ct}$ method was utilized data analysis. Table 1 showed the primer sequences (Sangon).

Table 1
Primer sequences

Genes	Forward (5′→3′)	Reverse (5′→3′)
NEAT1	TTACCAGCTTCCTCCTGGTG	TCTGCTGCGTATGCAAGTCT
miR-107	AGCAGCATTGTACAGGG	TCAACTGGTGTCGTG
FOXK1	ACACGTCTGGAGGAGACAGC	GAGAGGTTGTGCCGGATAGA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	CTCTGCTCCTCCTGTTCGAC	GCGCCCAATACGACCAAATC

Note: NEAT1, Long non-coding RNA nuclear enriched abundant transcript 1; miR-107, microRNA-107; FOXK1, Forkhead box k1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

Western blot assay

Radio-Immunoprecipitation assay lysis buffer containing 3% protease inhibitor was applied to extract total protein from tissues and cells. The protein lysate was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Invitrogen). After blockade with skim milk, the membrane was probed with the primary antibodies FOXK1 (1:1000, ab18196, Abcam, MA, USA) and GAPDH (1:1000, ab8245, Abcam) and re-probed with the secondary antibody conjugated with horseradish peroxidase. The signals were detected by an enhanced chemiluminescence system (Amersham Biosciences) [16].

Transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL) staining

The distribution of TUNEL-positive cells in tumors was evaluated by TUNEL analysis kit (Roche Diagnostics via Shanghai Ltd, Shanghai). The tumors were made into paraffin sections and baked at 60°C, followed by dewaxing in xylene and hydration with gradient ethanol. Next, the sections were treated with proteinase K working solution, rinsed with acid buffer and incubated with TUNEL reaction working solution (TdT: acid mixture = 1:9). Next, the sections were treated with 3% H_2O_2 methanol solution, reacted with peroxidase, and developed by diaminobenzidine. Then, the sections were counterstained with hematoxylin, followed by dehydration with gradient ethanol, clearance in xylene, and mounting with neutral gum. Under the light microscope, the percentage of TUNEL-positive cells was counted.

Statistical analysis

Data analysis was conducted by SPSS22.0 statistical software. Measurement data were expressed as mean ± standard deviation. Data were evaluated by independent sample t test (normal distributed data between two groups) or one-way analysis of variance (multiple groups) and Tukey's multiple comparisons test (pairwise comparison). Pearson correlation was indicated to analyze the correlation between NEAT1, miR-107 and FOXK1. Graphpad Prism 7, Adobe PhotoShop CS6 and Adobe Illustrator CC were used to draw figure. When the *P* value was less than 0.05, the data were statistically significant.

Results

NEAT1 is overexpressed in lung cancer

Overexpressed NEAT1 increased the proliferation, migration and invasion of papillary thyroid cancer cells [23]. Also, NEAT1 was a carcinogenic factor in liver cancer [24] and was overexpressed in lung cancer patients [22]. In this experiment, NEAT1 expression was detected in 96 pairs of matched lung cancer tissues and adjacent tissues by RT-qPCR, and the results determined that NEAT1 was up-regulated in lung cancer tissues (Fig. 1A).

To observe the impact of NEAT1 on the prognosis of lung cancer patients, NEAT1 expression in tumor tissues of patients of different ages, genders, lymph node metastasis (LNM) and TNM stages were detected. The results revealed that LNM and TNM staging (both $P < 0.05$) were correlated with NEAT1 expression while age and gender were not (both $P > 0.05$) (Table 2, Fig. 1B,C). Moreover, patients with low NEAT1 expression had increased overall survival than those with low NEAT1 expression ($P < 0.05$) (Fig. 1D).

Table 2
Correlation between NEAT1 expression and clinicopathological parameters in patients with lung cancer

Clinicopathological data	n	NEAT1 expression		*P*
		Low expression (n = 48)	High expression (n = 48)	
Age (years)				0.1939
≤ 65	64	35	29	
> 65	32	13	19	
Gender				0.289
Male	61	33	28	
Female	35	15	20	
Lymph node metastasis				0.0412
No	48	29	19	
Yes	48	19	29	
TNM stage				0.0006
I-II	34	25	9	
III-IV	62	23	39	

Human lung cancer cell lines (A549, H1299 and SPC-A-1), and normal bronchial epithelial cell line 16HBE were selected to identify the effect of NEAT1 on the biological functions of lung cancer cells. NEAT1

expression was tested by RT-qPCR and the finding indicated that NEAT1 expression was increased in lung cancer cell lines, among which A549 showed with the highest NEAT1 expression (Fig. 1E).

Depleting NEAT1 impedes lung cancer cell proliferation and induces apoptosis

The role of NEAT1 in the growth of lung cancer cells was discovered. A549 cells were transfected with NEAT1 shRNA lentivirus to knock down NEAT1 expression (Fig. 2A). CCK-8 assay, colony formation assay, flow cytometry and Transwell assay tested that A549 cells with low NEAT1 expression had suppressed proliferation, colony-forming, invasion and migration abilities and increased apoptosis rate, and arrested in G0/G1 phases (Fig. 2B-G).

Depleting NEAT1 disrupts invasion and migration and metastasis in lung cancer

To study whether NEAT1 regulates lung cancer cells *in vivo*, A549 cells transfected with NEAT1 shRNA lentivirus were injected subcutaneously into the dorsal side of nude mice. Observation of tumor formation and TUNEL staining demonstrated that NEAT1 shRNA lentivirus reduced tumor weight and volume in mice, and increased the apoptotic rate of tumors (Fig. 3A, B).

NEAT1 targets miR-107

miR-107 expression was reported to be down-regulated in patients with multiple myeloma [25]. In this study, miR-107 expression was confirmed to be down-regulated in lung cancer tissues and cells (Fig. 4A, B). Through Pearson analysis, it was disclosed that NEAT1 and miR-107 expression levels in lung cancer tissues (n = 96) were negatively correlated (Fig. 4C). Based on that, a targeting relationship between NEAT1 and miR-107 was speculated. The bioinformatics website (Starbase) detected the targeting sites of the two (Fig. 4D) and dual luciferase reporter gene assay further verified the interaction of NEAT1 and miR-107 (Fig. 4E). Also, down-regulating NEAT1 could raise miR-107 expression in A549 cells (Fig. 4F), proving the negative regulation of NEAT1 on miR-107.

Augmenting miR-107 retards lung cancer cell progression

With the aim to decode the mechanism of miR-107 in lung cancer cell progression, miR-107 mimic was transfected into A549 cells to up-regulate miR-107 expression (Fig. 5A). Analysis of various assay presented that the cell proliferation, invasion, migration and colony forming abilities were repressed, and G0/G1 phase cells and apoptosis rates were elevated by up-regulating miR-107 (Fig. 5B-G).

FOXK1 is mediated by miR-107

The above experiments indicated that miR-107 could be considered a target for the treatment of lung cancer. However, the molecular mechanism of miR-107 in the progression of lung cancer was unclear.

FOXK1 was reported to be overexpressed in lung cancer tissues, promoting lung cancer cell proliferation and invasion. [16]. The bioinformatics website searched out the binding sites of miR-107 and FOXK1 (Fig. 6A), and dual luciferase reporter gene assay further verified that miR-107 directly regulated FOXK1 in lung cancer (Fig. 6B). RT-qPCR and Western blot assay displayed that FOXK1 expression was decreased in A549 cells by up-regulating miR-107 (Fig. 6C,D). In clinical lung cancer tissues and cells, FOXK1 expression was highly expressed (Fig. 6E, F). FOXK1 expression was negatively connected with miR-107 expression in clinical tissues (Fig. 6G), hinting that miR-107 negatively regulated FOXK1 expression in lung cancer cells.

NEAT1 binds to miR-107 to regulate FOXK1 in lung cancer

To verify whether NEAT1 regulated lung cancer through the miR-107/FOXK1 axis, the effect of NEAT1 interference on FOXK1 was examined. It was concluded that silencing NEAT1 inhibited FOXK1 protein expression while inhibiting miR-107 could alleviate this effect on FOXK1 protein expression (Fig. 7A), indicating that NEAT1 regulated FOXK1 by competitively binding to miR-107. Functional assays delineated that the biological behaviors of A549 cells inhibited by NEAT1 depletion were reversed by miR-107 inhibition (Fig. 7B-G), concluding that NEAT1 regulated FOXK1 through miR-107 to affect lung cancer cell progression.

Discussion

Lung cancer, causing a large amount of deaths every year, is incurable with extremely low survival rate [26]. We have emphasized on the play of NEAT1 in lung cancer and finally examined that NEAT1 was highly expressed in lung cancer, which was substantially correlated with the inferior survival of lung cancer patients. Also, we stressed that depleted NEAT1 impeded lung cancer cell progression via competitively binding miR-107. Next, we further defined the role of miR-107 in lung cancer and discovered that restored miR-107 retarded lung cancer cell progression via targeting FOXK1. Eventually, it was concluded that NEAT1/miR-107/FOXK1 axis vitally played in the process of lung cancer.

Recently, up-regulated NEAT1 has been documented to involve in NSCLC, and NEAT1 depletion caused the inhibition of cell proliferative, migratory, invasive and anti-apoptotic activities of NSCLC cells by sponging has-miR-376b-3p to modulate sulfatase-1 [6]. Detected clinically, NEAT1 expression went toward an increment in lung cancer, which was tightly linked to tumor stage and LNM, while NEAT1 suppression could depress cell migration and invasion [5]. Of note, NEAT1 was also investigated to overexpress in NSCLC while knocking down NEAT1 limited the proliferation, invasion and migration properties of NSCLC cells [7]. Additionally, the up-regulated NEAT1 was measured in lung cancer and its down-regulation raised miR-1224 to suppress Krüppel-like factor 3, thereafter dramatically suppressing proliferation and anti-apoptosis of cancer cells [8]. An intriguing finding in a former research noticed that the heightened NEAT1 was present in NSCLC that established a liaison with TNM stage and metastasis, and NEAT1 silencing suppressed cancer cells to proliferate, migrate and invade by regulating miR-101-3p/SRY-box 9-mediated Wnt/β-catenin signaling pathway [27]. Besides, lung adenocarcinoma expressed

up-regulated NEAT1 who served to sponge miR-193a-3p to regulate USF1, thereby augmenting the cancer progression [28]. Moreover, in NSCLC, siRNA-induced knockdown of NEAT1 interfered cell proliferation, invasion and migration and induced cell apoptosis by acting as a sponge of let-7a to modify insulin-like growth factor 2 [29].

In terms of the cooperating network of NEAT1 and miR-107, it was evident that NEAT1 knockdown elevated miR-107 expression, so as to suppress colony-forming and anti-apoptotic capacities of glioma cells [30]. Actually, miR-107 expression demonstrated a decrease in NSCLC, and the augment of miR-107 oppressed the ferocious performance of cancer cells through the modification of its downstream actor cyclin-dependent kinase 6 [31]. An experimental exploration has detected that overexpressing miR-107 damaged the malignant phenotype of NSCLC cells partially by serine/threonine kinase 33-meidated extracellular regulated protein kinases pathway [32]. Amazingly, hsa-miR-107, once sponged by lncRNA FYVE, RhoGEF and PH domain containing 5 antisense RNA 1, indirectly offered a niche for NSCLC cells to proliferate [33]. Particularly, lowly expressed miR-107 was exhibited in serum specimens of NSCLC patients and miR-107 would mediate transforming growth factor β receptor 2 and then jeopardize the proliferation and migration of cancer cells [34]. Importantly, a decline was witnessed in miR-107 expression in NSCLC, however, miR-107 up-regulation would retard cell progression by down-regulating brain-derived neurotrophic factor to inactivate phosphoinositide 3-kinase/protein kinase B pathway [25].

FOXK1, is often found to be regulated by miRNAs to participate in cancer progression. Exactly, FOXK1, when mediated by up-regulated miR-195-5p, would repress cell proliferating and metastatic activities of lung cancer cells [16]. Performed in a similar way, down-regulated FOXK1, the modified actor of restored miR-365-3p, disrupted breast cancer cell proliferation, migration and invasion [15]. Mechanistically, FOXK1, if negatively regulated by augmented miR-1275, was impeded the proliferation and invasion of lung cancer cells [17].

To briefly summarize, NEAT1 positively regulated FOXK1 via miR-107, and stimulated lung cancer cell to grow with aggressiveness. To some extent, the discoveries in this work replenished the knowledge of the mechanism of NEAT1/miR-107/FOXK1 axis in lung cancer and probably supplied a potency in treating lung cancer. This study was limited to relative small research scale and the results obtained were supposed to verify in more researches.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figures

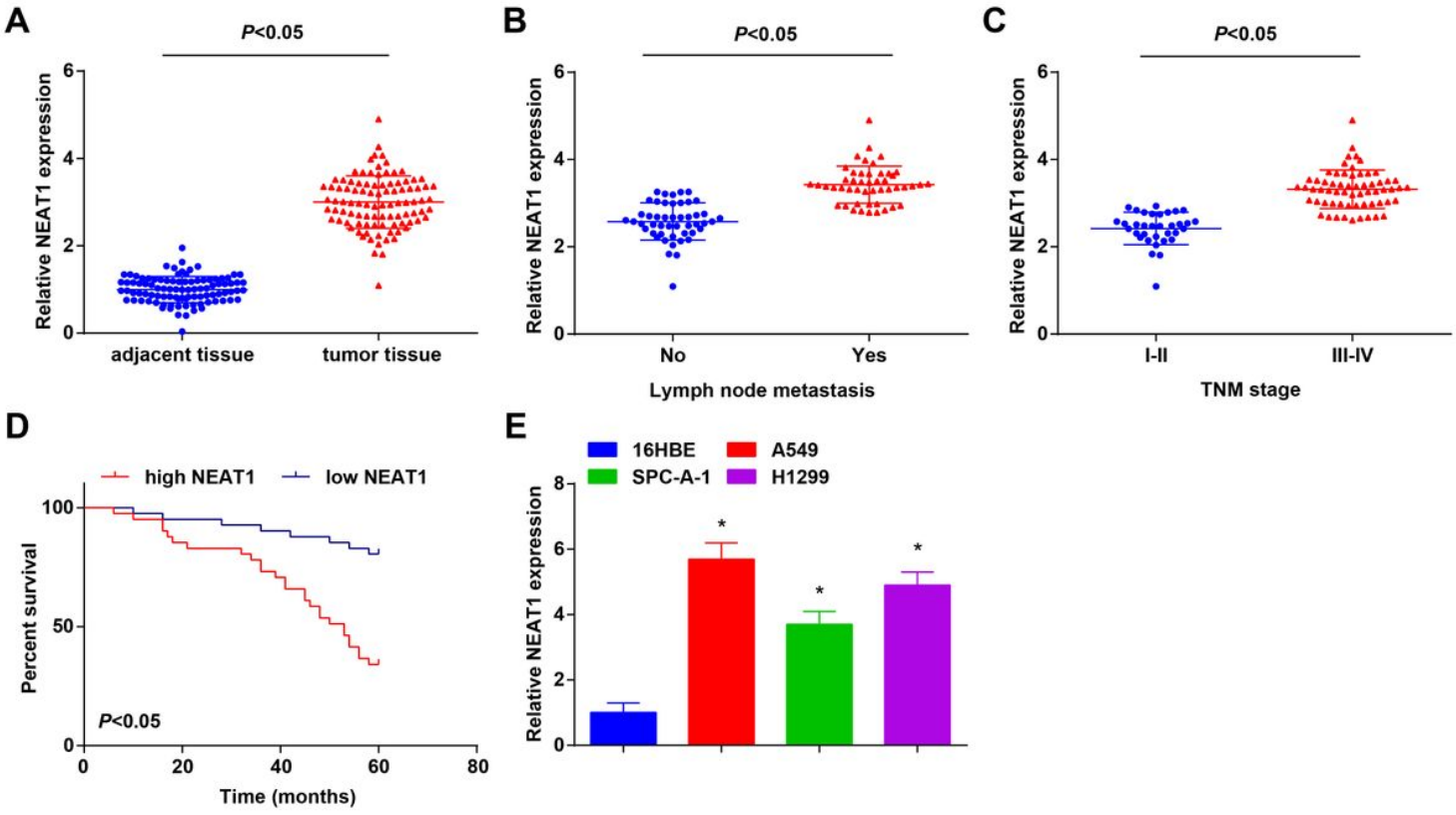

Figure 1

NEAT1 is overexpressed in lung cancer and predicts the inferior prognosis. A. RT-qPCR detected NEAT1 expression in lung cancer tissues and adjacent tissues (n = 96); B. RT-qPCR detected NEAT1 expression in lung cancer patients with or without lymph node metastasis; C. RT-qPCR detected NEAT1 expression in lung cancer patients at different TNM stages; D. The relationship between NEAT1 expression and survival of lung cancer patients; E. RT-qPCR detected NEAT1 expression in lung cancer cell lines and normal bronchial epithelial cells. The data were expressed as the mean ± standard deviation (N = 3). * P < 0.05 compared with 16HBE cells.

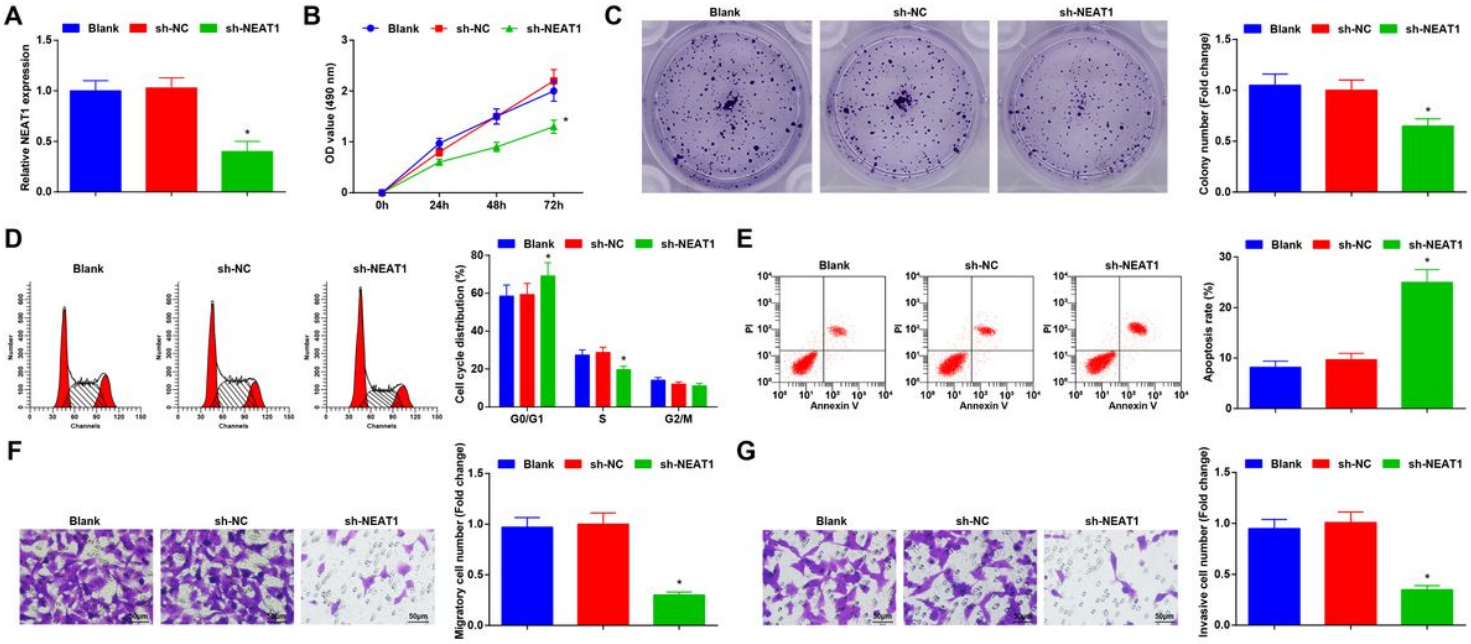

Figure 2

Depleting NEAT1 impedes lung cancer cell growth in vitro. A. RT-qPCR detected NEAT1 expression in A549 cells; B. CCK-8 assay detected the proliferation of A549 cells; C. Colony formation assay detected colony-forming ability of A549 cells; D. Flow cytometry detected the cell cycle distribution of A549 cells; E. Flow cytometry detected the apoptosis rate of A549 cells; F. Transwell assay detected the migration ability of cells; G. Transwell assay detected the invasion ability of cells; The data were expressed as the mean ± standard deviation (N = 3). * P < 0.05 compared with the sh-NC group.

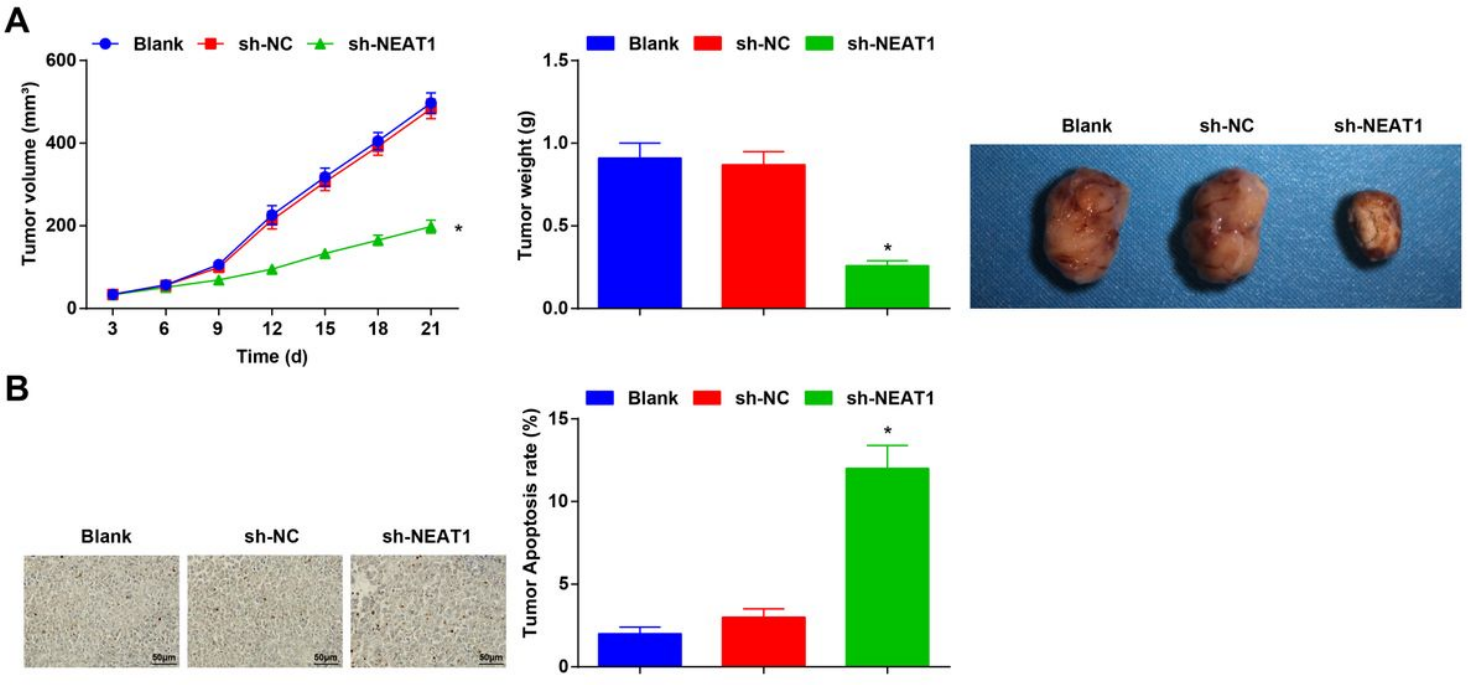

Figure 3

Depleting NEAT1 disrupts tumor growth in nude mice in lung cancer. A. Changes in tumor volume and weight in nude mice; B. TUNEL staining detected tumor cell apoptosis, n = 5/group. The data were expressed as the mean ± standard deviation (N = 3). * P < 0.05 compared with the sh-NC group.

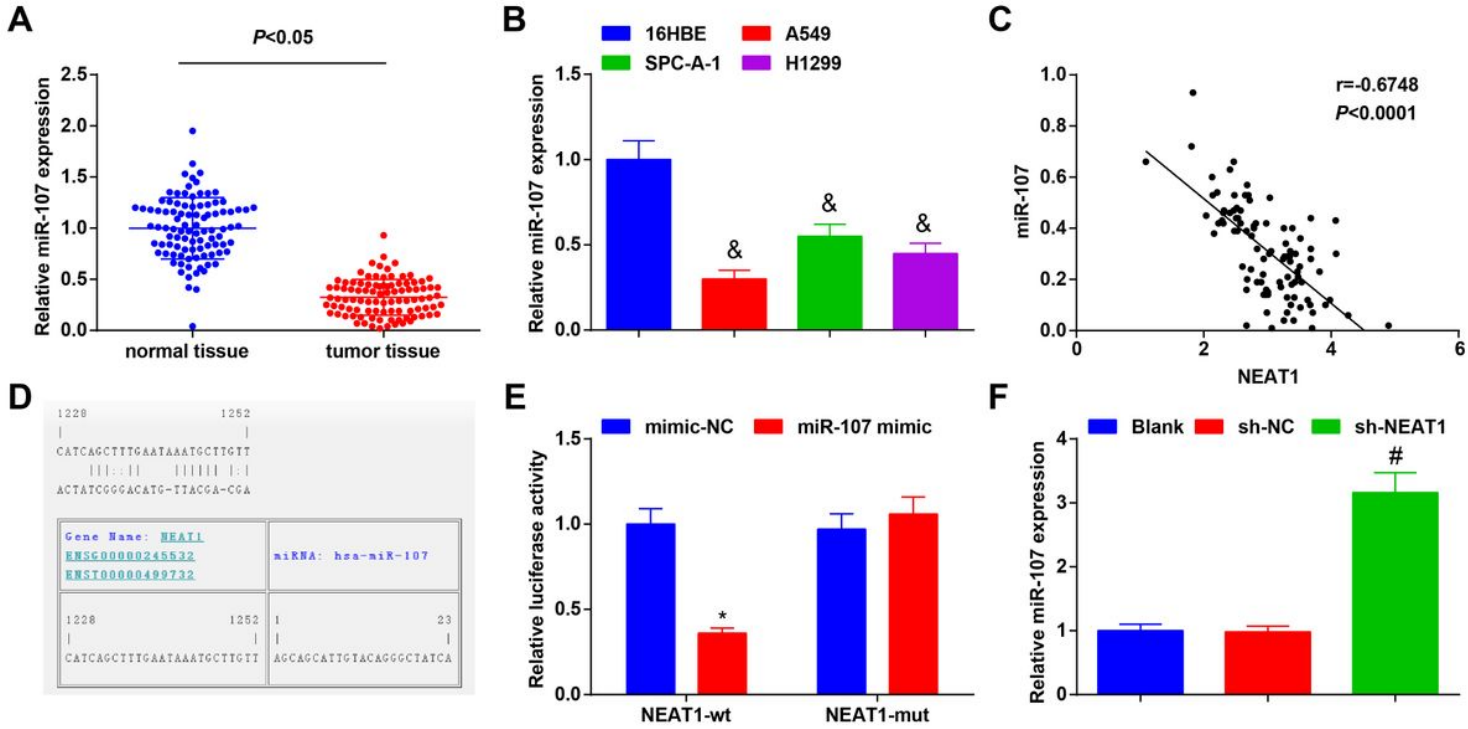

Figure 4

4 NEAT1 binds to miR-107. A. RT-qPCR detected miR-107 expression in adjacent tissues and lung cancer tissues (n = 96); B. RT-qPCR detected miR-107 expression in lung cancer cell lines and normal bronchial epithelial cell; C. Pearson analyzed the correlation between NEAT1 and miR-107 expression in lung cancer tissues (n = 96); D. Starbase website predicted the targeting sites of NEAT1 and miR-107; E. Dual luciferase reporter gene assay detected the targeting relationship between NEAT1 and miR-107; F. RT-qPCR detected miR-107 expression in A549 cells after down-regulation of NEAT1. The data were expressed as the mean ± standard deviation (N = 3). & P < 0.05 compared with 16HBE cells; * P < 0.05 compared with the mimic-NC group; # P < 0.05 compared with the sh-NC group.

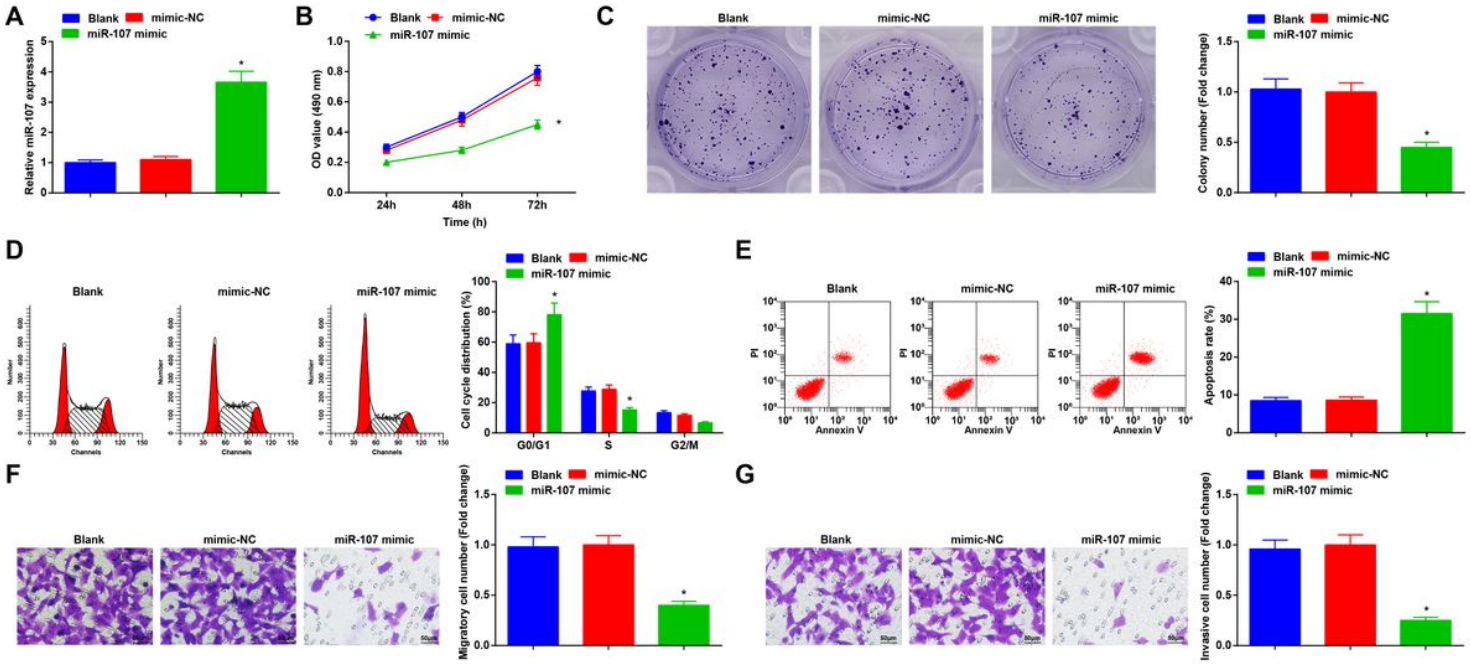

Figure 5

Augmenting miR-107 retards lung cancer cell progression. A. RT-qPCR detected miR-107 expression in A549 cells; B. CCK-8 assay detected the proliferation of A549 cells; C. Colony formation assay detected the colony-forming ability of A549 cells; D. Flow cytometry detected the cell cycle distribution of A549 cells; E. Flow cytometry detected the apoptosis rate of A549 cells; F. Transwell assay detected the migration ability of A549 cells; G. Transwell assay detected the invasion ability of A549 cells. The data were expressed as the mean ± standard deviation (N = 3). * P < 0.05 compared with the mimic-NC group.

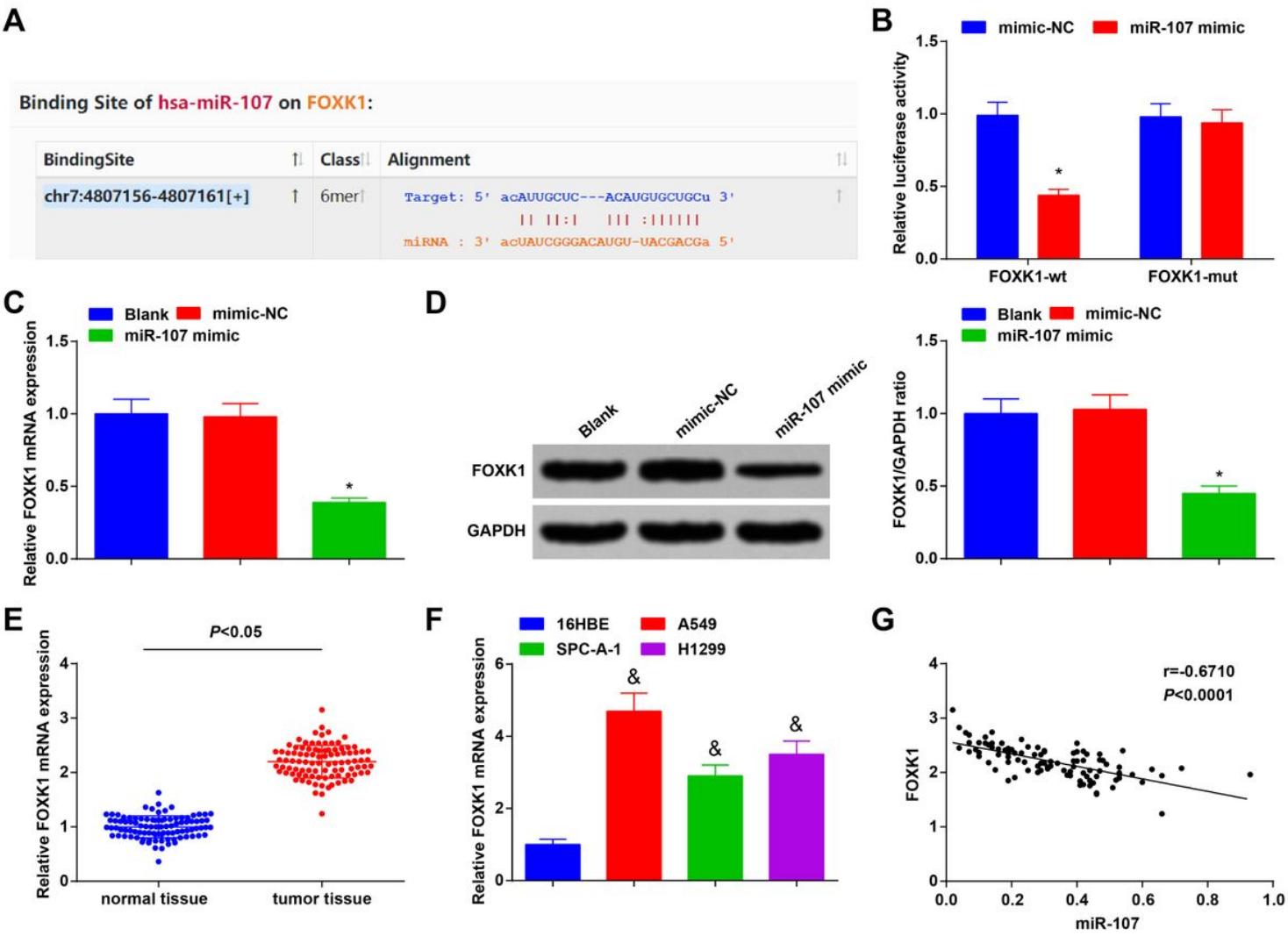

Figure 6

FOXK1 is mediated by miR-107. A. Bioinformatics website predicted the targeting site of miR-107 and FOXK1; B. Dual luciferase reporter gene assay detected the targeting relationship between miR-107 and FOXK1; C. RT-qPCR detected FOXK1 mRNA expression after miR-107 regulation; D. Western blot assay detected FOXK1 protein expression after miR-107 regulation; E. RT-qPCR detected FOXK1 expression in lung cancer tissues and adjacent tissues (n = 96); F. RT-qPCR detected FOXK1 expression in lung cancer cell lines and normal bronchial epithelial cells; G. Pearson evaluated the correlation of FOXK1 and miR-107 in lung cancer tissues (n = 96). The data were expressed as the mean ± standard deviation (N = 3). * and # P < 0.05 compared with the mimic-NC group; & P < 0.05 compared with 16HBE cells.

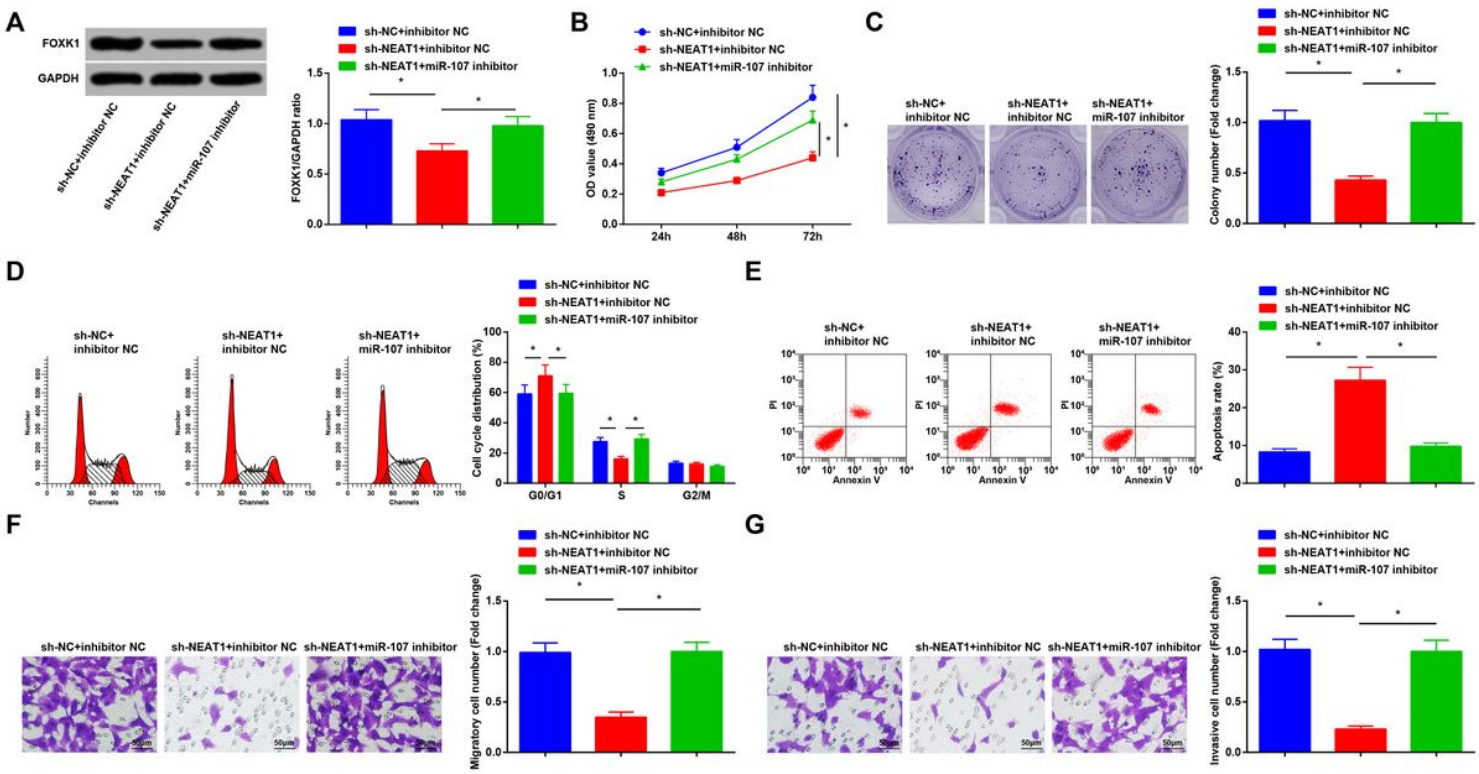

Figure 7

NEAT1 binds to miR-107 to regulate FOXK1 in lung cancer. A. Western blot assay detected FOXK1 expression of A549 cells; B. CCK-8 assay detected the proliferation ability of A549 cells; C. Colony formation assay detected colony-forming ability of A549 cells; D. Flow cytometry detected the cell cycle distribution of A549 cells; E. Flow cytometry detected the apoptosis rate of A549 cells; F. Transwell assay detected the migration of A549 cells; G. Transwell assay detected the invasion of A549 cells; The data were expressed as the mean ± standard deviation (N = 3). * represented $P < 0.05$.