Article

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NAT10 Promotes Malignant Progression of Lung Cancer via the NF-κB Signaling Pathway

Xia Liu[1,†], Xinyun Liu[2,†], Chaoshi Zhuo[1], Jiangfeng Shen[3], Kaijin Lu[3], Min Sha[4], Jun Ye[4], Junxing Huang[5], Hexu Han[6], Hong Yu[2,*]

[1]Department of Pathology, Xuzhou Central Hospital Affiliated to Nanjing University of Chinese Medicine, 221000 Xuzhou, Jiangsu, China
[2]Department of Pathology, The Affiliated Taizhou People's Hospital of Nanjing Medical University, 225300 Taizhou, Jiangsu, China
[3]Department of Cardiothoracic Surgeryy, The Affiliated Taizhou People's Hospital of Nanjing Medical University, 225300 Taizhou, Jiangsu, China
[4]Translational Medicine Center, The Affiliated Taizhou People's Hospital of Nanjing Medical University, 225300 Taizhou, Jiangsu, China
[5]Department of Oncology, The Affiliated Taizhou People's Hospital of Nanjing Medical University, Taizhou School of Clinical Medicine, Nanjing Medical University, 225300 Taizhou, Jiangsu, China
[6]Department of Gastroenterology, The Affiliated Taizhou People's Hospital of Nanjing Medical University, Taizhou School of Clinical Medicine, Nanjing Medical University, 225300 Taizhou, Jiangsu, China
*Correspondence: yuhong@njmu.edu.cn (Hong Yu)
[†]These authors contributed equally.
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Background: NAT10 (N-acetyltransferase 10) is a newly identified novel acetyltransferase. Abnormal expression of NAT10 is associated with several human disorders, including cancer, autoimmune diseases, and cardiovascular disease. This study aimed to investigate the role of NAT10 in promoting lung cancer malignant progression through the NF-κB (nuclear factor κB) signaling pathway.

Methods: Cells lines BEAS-2B, NCI-H524, A549, PC-9, NCI-H23, and NCI-H258 were cultured for identification. Western blotting and PCR assays determined gene expression within the sample cells. Cellular functionality was assayed using CCK8 (Cell Counting Kit-8), Dual-Luciferase Reporter, and Colony formating.

Results: The PCR assay and Western blotting showed a significant elevation of NAT10 levels within tumor tissues compared to paraneoplastic tissues ($p < 0.05$). Specifically, NAT10 only affected the expression and content of RelA/p65 in lung cancer. Analysis from the TCGA (The Cancer Genome Atlas) database indicated that elevated expression levels of NAT10 in tumors can be a good prognostic indicator for lung cancer patients. The CCK8 assay showed that the knockdown of NAT10 significantly suppressed the A549 cells' progression rate ($p < 0.05$). The colony formation assays further confirmed that the overexpression of NAT10 significantly increased the generation of clones in the NCI-H524 cells ($p < 0.05$). The proliferation rate influenced by the overexpression of NAT10 was inhibited by blocking the NF-κB signaling pathway ($p < 0.05$). Dual-luciferase reporter gene assay results revealed NAT10's potential in promoting the NF-κB signaling pathway's activity in lung cancer. Immunohistochemical staining underscored a strong link between NAT10 protein expression and the NF-κB signaling pathway in lung cancer tissues.

Conclusions: NAT10's expression is significantly upregulated in tumor tissues, supported by PCR results. NAT10 plays a role in the development and proliferation of lung cancer cells and can activate the NF-κB signaling pathway in lung cancer. Hence, NAT10's regulation of the NF-κB signaling pathway is critical in the malignant proliferation of lung cancer.

Keywords: NAT10; NF-κB signaling pathway; lung cancer

Introduction

Globally, lung cancer stands as a prevalent form of malignancy with high risk of pleural effusion [1]. World Health Organization (WHO) has estimated that over 2 million individuals receive a new diagnosis of lung cancer, with approximately 1.8 million deaths annually attributed to this disease [2]. Notably, our country ranks among those with the highest prevalence of lung cancer worldwide [3]. Enhancing public health awareness and fostering preventive measures against lung cancer is a collective imperative.

Among all cancers, lung cancer tops the charts in both prevalence and mortality rates globally. Factors such as long-term smoking [4], exposure to second-hand smoke, environmental pollution, and occupational hazards contribute significantly to its incidence [5]. Nevertheless, considerable advancements have been made in its prevention and treatment, such as developing lung cancer screening initiatives and applying targeted therapies and immunotherapy [6–8]. Confronting lung cancer is a collective responsibility, demanding focused attention and proactive measures.

The NF-κB (nuclear factor κB) signaling pathway is pivotal in regulating various cellular functions, including cell growth, apoptosis, and immune responses [9]. Its role is particularly important in lung cancer's onset and progression. Study underscores its significant influence on lung cancer cell proliferation, metastasis, immune response evasion, and targeted treatments [10]. Within the tumor microenvironment, certain, including chronic inflammation, viral infection, and specific drugs, can stimulate and activate the NF-κB pathway, further leading to the progression and metastasis of lung cancer [11]. Furthermore, recent observations suggest that the NF-κB signaling pathway can modulate lung cancer progression by inhibiting tumor cell apoptosis and promoting angiogenesis. The NF-κB signaling pathway regulation has been extensively studied in lung cancer therapy, and inhibiting it can amplify the effectiveness of conventional chemotherapy, radiotherapy, and other cancer treatments. Therapeutic strategies targeting the NF-κB pathway incorporate small-molecule inhibitors, bioactive molecules, and RNA interference [12]. To sum up, utilizing the NF-κB pathway is crucial for comprehending and suppressing lung cancer progression, offering promising therapeutic interventions and disease strategies.

N-acetyltransferase 10, commonly referred to as NAT10, is a novel acetyltransferase. The protein encoded by the NAT10 gene protein is a nuclear protein mainly involved in processing and modifying RNA precursor molecules [13]. Abnormal expression of NAT10 has been associated with various human disorders, including cancer, autoimmune disorders, and cardiovascular diseases. As a result, NAT10 is seen as a potential therapeutic target for cancer [14]. Concurrently, NAT10 plays a role in cell apoptosis, DNA damage repair, cell cycle regulation, and other vital biological processes [15].

In this study, we found that NAT10 expression was elevated in various lung cancer tissues, and there was a negative correlation between its expression level and patient prognosis. Further investigations have revealed that the overexpression of NAT10 in lung cancer can directly enhance the function of the NF-κB signaling pathway, thereby promoting tumor malignancy by increasing the protein content of p65. These insights offer a fresh perspective on understanding the molecular pathogenesis of lung cancer.

Materials and Methods

Cell Cultivation and Stable Cell Lines

Cell lines BEAS-2B (FH0319), NCI-H524 (FH0680), A549 (FH0045), PC-9 (FH0083), NCI-H23 (FH0616), and NCI-H358 (FH0046), sourced from Fuheng Biotechnology Co., Ltd. (Shanghai, China), were cultured in their respective medium. Each medium was supplemented with streptomycin (100 µg/mL), penicillin (100 U/mL), and 10% fetal bovine serum (FBS). All these cells were grown in a chamber set at 37 °C with 5% CO_2 and controlled humidity. All cells underwent STR (Short Tandem Repeats) identification and were regularly tested for mycoplasma presence.

Plasmid Construction

We engaged GENEWIZ Biotechnology Co., Ltd. (Suzhou, China), to construct NAT10 knockout and overexpression of plasmids, using lentiCRISPR-V2-puro and pcDNA3.1 - puro - C - 3 flag plasmid, respectively. Once verified by sequencing, they have employed with the following primers:

sg-NAT10: ATTGAGAATGGAGTAGCTGAG
ov-NAT10-F: ATGCATCGGAAAAAGGTGGA
ov-NAT10-R: TTTCTTCCGCTTCAGTTTCATATC

We engaged GENEWIZ Biotechnology Co., Ltd. (Suzhou, China) to construct a p65 knockout plasmid using pLKO.1-TRC-mCherry-T2A-BSD plasmid. After verification by sequencing, it was employed with the following primers:

sh-p65-1: CGGATTGAGGAGAAACGTAAA
sh-p65-2: CACCATCAACTATGATGAGTT

We successfully obtained the lentiCRISPR v2-NAT10 and pcDNA3.1-Puro-C-3Flag-NAT10 plasmids. These plasmids and the control plasmid were transferred into the cells using the lipo8000 transfection reagent (Beyotime, Shanghai, China). Stable cell lines were subsequently established by puromycin (1 ug/mL) selection. The detailed steps include:

(1) Into an enzyme-free EP tube, we added 2 ug of the previously sequenced-verified plasmids and 2 ug of their corresponding plasmids.

(2) Each EP tube was supplemented with 200 uL of Opti-MEM transfection medium. By lightly flicking the tube, plasmid, and Opti-MEM transfection medium were mixed thoroughly and then left to incubate for 10 minutes.

(3) To the aforementioned mixture, we added 3 uL lipo8000 transfection reagent. The contents of the EP tube were thoroughly mixed and allowed to stand for 10 minutes.

(4) The resulting mixture was then added dropwise into a culture medium containing cells (with a cell density of 70–80%) using a pipetting pistol. Six hours later, fresh medium was added.

(5) After 72 hours, a medium containing puromycin (2 ug/mL) was added. Cultivation continued until no dead cells were observed floating, indicating the successful establishment of stable cells.

In the case of constructing A549-sg-NAT10 cells, we initially obtained mixed clone cells. After equalizing cell counts and equal dilutions, a cell suspension (10 cells/mL) was prepared. 100 uL of this cell suspension was then seeded into each well of a 96-well plate for single-cell cloning. Following WESTER verification, we successfully established the NAT10 knockout A549 cell line.

Western Blotting Assay

Cellular extracts were meticulously prepared and subsequently analyzed through the western blotting, using appropriate antibodies, as detailed in the reference methodologies [16]. The primary antibodies used in our study included: anti-NAT10 (1:1000, 13365-1-AP, Proteintech, Wuhan, China), anti-RelA/p65 (1:1000, 8242, CST, Boston, MA, USA), anti-Flag-tag (F1804, Sigma, St. Louis, MO, USA), anti-IKba (1:1000, 4814, CST, Boston, MA, USA), anti-phospho IKba (Ser32/36) (1:1000, 9426, CST, Boston, MA, USA), anti-NF-κB p65 (Ser536) (1:1000, 3033, CST, Boston, MA, USA), anti-IKKB (1:1000, 15649–1-AP, Proteintech, Wuhan, China), anti-IKK-alpha (1:1000, GB11923, ServiceBio, Wuhan, China), and anti-β-actin (1:10,000, 66009-1-AP, Proteintech, Wuhan, China). The employed secondary antibodies were Horseradish peroxidase-labeled goat anti-rabbit IgG (H + L) (A0208, Beyotime, Shanghai, China) and Horseradish peroxidase-labeled goat anti-mouseIgG (H + L) (A0216, Beyotime, Shanghai, China).

For subsequent quantitative analysis, we used image J (V1.8.0.112, Rawak Software Inc., Stuttgart, Germany) software to assess the grayscale intensities of bands in each lane, with the grayscale value of β-actin as control. GraphPad Prism7 (GraphPad Software Inc., San Diego, CA, USA) facilitated our graphic representation statistical analysis.

Lung Cancer Tissue Samples

In adherence to the Declaration of Helsinki, lung cancer tissue samples were procured from three cohorts at The Affiliated Taizhou People's Hospital of Nanjing Medical University. The written informed consent was collected from the patients, and the experimental protocol was permitted by the institutional ethics review board of The Affiliated Taizhou People's Hospital of Nanjing Medical University (KY2021-008-01). The identity of each specimen was confirmed through pathologic analysis. Prior to the surgery, none of the patients underwent chemotherapy or radiation treatments.

PCR Assay

RNAs were extracted from fresh clinical tissues and cell samples utilizing an RNA-easy Isolation reagent (R701, Vazyme, Nanjing, China). After that, HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (R312, Vazyme, Nanjing, China) has been utilized in order to perform reverse transcription, and AceQ Universal SYBR qPCR Master Mix (Q511, Vazyme, Nanjing, China) was employed to detect corresponding genes [15] quantitatively. The $2^{-\Delta\Delta CT}$ method was used to analyze the differences between the experimental and control groups.

RT-NAT10-F: 5′-GCCTCTTGTAAGAAGTGTCTCG-3′

RT-NAT10-R: 5′-TCTTTTCAGAGATGCCCTCGAT-3′

RT-β-actin-F: 5′-TCCCTGGAGAAGAGCTACG-3′

RT-β-actin-R: 5′-GTAGTTTCGTGGATGCCACA-3′

RT-p65-F: 5′-ATGTGGAGATCATTGAGCAGC-3′

RT-p65-R: 5′-CCTGGTCCTGTGTAGCCATT-3′

Cell Function Related Experiments

CCK8 (Cell Counting Kit-8) Assay

Approximately 1×10^3 cells were inoculated into individual wells of a 96-well plate, which was then incubated for 2 hours. After this incubation, the optical density (OD) at 450 nm was measured by the Synergy HTX Multifunctional Microplate Detector (Synergy HTX, BioTek, VT, USA). This procedure was replicated on days 2, 3, and 4 to draw the proliferation curve. Concurrently, the Cell Counting Kit-8 Kit (A311, Vazyme, Nanjing, China) was used in accordance with its provided instructions for the corresponding experiments.

Colony Formation Assays

A total of 500 cells were evenly seeded into each well of a 6-well plate. These plates were then incubated at a consistent temperature of 37 °C for 3 weeks. Following incubation, the cell colonies were fixed using a 4% paraformaldehyde solution and stained with crystal violet. Once stained, the plates were photographed against a white background under natural light. The acquired images were sorted and analyzed using Image J software (V1.8.0.112, Rawak Software Inc., Stuttgart, Germany).

Dual-Luciferase Reporter Assay

We utilized the pGL4.10-hRluc reporter vector, which carries sea kidney and firefly fluorescence proteins. The vector underwent sequential enzymatic digestion and gelling recovery, first with KpnI (R3142, NEB, Ipswich, SD, USA) followed by BglII (R0144, NEB, Ipswich, SD, USA). A synthetic insert containing the p65 binding sequence "GGGAATTTCCGGGAATTTCCGGGAATTTCCGGGAATTTCC" was generated. After annealing, this fragment was ligated into a pGL4.10 - hRluc reporter vector using T4 ligase (M0202, NEB, Ipswich, SD, USA)—subsequent transformation into bacteria allowed for colony selection. The positive clones were confirmed via sequencing.

These constructed reporter plasmids were transfected into A549-sg-NAT10/H524-ov-NAT10 cell lines and their corresponding control cells. Forty-eight hours post-transfection, fluorescence was assessed using the Promega kit (E1910, Promega, Madison, WI, USA) with a Multifunctional Microplate Detector (Synergy HTX, BioTek, VT, USA).

Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was conducted as follows. Paraffin-embedded tissue sections were treated with 3% acetic acid and 0.1% trypsin solution at 37 °C for

15 minutes. The sections were incubated in 5% (v/v) normal goat serum for 2 hours at room temperature to block non-specific binding. The sections were treated with primary antibodies anti-NAT10 (1:100, 13365-1-AP, Proteintech, Wuhan, China) and anti-RelA/p65 (1:200, 8242, CST, Boston, MA, USA)-which were diluted in 5% (v/v) normal goat serum. The incubation was set overnight at 4 °C in a humidified chamber. HRP-labeled Goat Anti-Rabbit IgG(H+L) (A0208, Beyotime, Shanghai, China) were used per the manufacturer's recommendations. The antibody-antigen complex was visualized using 3,3-Diaminobenzidine (DAB) staining.

Statistical Methods

Data were analyzed using the GraphPad Prism7 software (GraphPad Software Inc., San Diego, CA, USA). Both one-way ANOVA and the student's *t*-test were utilized to assess the differences between groups. At the same time, for comparisons involving multiple factors like cell types, treatments, or time durations, a two-way ANOVA was employed. The relative intensity of NAT10 staining in comparison to NF-κB-p65 was determined utilizing Image-Pro Plus software (V6.0, Media Cybernetics Inc, San Diego, CA, USA). Levels of significance were denoted as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. The abbreviation "ns" indicates no statistical significance.

Results

Elevated Expression of NAT10 in Lung Cancer Tissues

In our initial investigation of fresh clinical lung cancer tissue samples, western blotting assays have revealed elevated NAT10 expression levels in tumor tissues compared to their corresponding para-cancer tissues (Fig. 1A). This finding was corroborated by qPCR (Real-time Quantitative PCR Detecting System) assays, which further confirmed the increased expression ($p < 0.05$) (Fig. 1B). Reinforcing our findings, an analysis of lung cancer data from the TCGA (The Cancer Genome Atlas) database (https://portal.gdc.cancer.gov) aligned with our conclusions, indicating that NAT10 is highly expressed in tumor tissues ($p < 0.05$). Additionally, the data showed a negative association between NAT10 expression and patient prognosis. The ROC curve, with an AUG = 0.855, further substantiated that NAT10 serves as a valuable prognostic marker for lung cancer (Fig. 1C).

NAT10 Enhances Proliferation of Lung Cancer Cells

To delve deeper into the function of NAT10 within lung cancer tissues, we analyzed its protein expression across human bronchial epithelial cells (Beas-2B cells) and several lung cancer cell lines. Our findings indicated that NAT10 was most prominently expressed in A549 cells and had the lowest expression in H524 cells (Fig. 2A). Based on these results, we engineered NAT10 knockout cell lines from A549 cells and NAT10 overexpressed cell lines from NCI-H524 cells (Fig. 2B). Utilizing these tailored cell lines, our CCK8 assays demonstrated a significant progression rate of A549 cells post NAT10 knockout ($p < 0.05$), whereas NCI-H524 cells showed a marked increase in proliferation upon NAT10 overexpression (Fig. 2C). The patterns are also consistent in our Colony formation assays (Fig. 2D).

NAT10 Promotes Lung Cancer through the Activation of NF-κB Signaling Pathway

Previous studies have suggested a significant involvement of the NF-κB signaling pathway in lung cancer progression [16,17]. Exploring NAT10's influence on the activation of the NF-κB signaling pathway, we used Dual-luciferase reporter gene assays on our previously established stable cell lines. The data showed a significant inhibition in the activity of NF-κB signaling pathway post-NAT10 suppression in A549 cells. Conversely, enhancing NAT10 in NCI-H524 cells enabled the NF-κB signaling pathway activity ($p < 0.05$), as shown in Fig. 3A. We then employed western blotting to assess the key components within the NF-κB signaling pathway. Among these, only RelA/p65 protein content exhibited significant alterations in tandem with its phosphorylated form (p-p65) (Fig. 3B and Fig. 3D). Complementing this, qPCR experiments revealed that NAT10 knockout led to a reduction in p65 mRNA levels in A549 cells, while its amplification in NCI-H524 resulted in elevated p65 mRNA levels ($p < 0.05$), as illustrated in Fig. 3C. Collectively, these results point to NAT10's potential in modulating the activity of the NF-κB signaling pathway through its influence on RelA/p65 in the context of lung cancer.

NAT10 Modulates Lung Cancer Development through the NF-κB Signaling Pathway

In order to further explore the function of the NF-κB signaling pathway and NAT10's role in the aggressive malignant proliferation of lung cancer, we administered the NF-κB signaling pathway inhibitors (Bay11-7082) and small hairpin RNA (shRNA) to the engineered H524-ov-NAT10 cells. This aimed to suppress the activation of the NF-κB signaling pathway prompted by NAT10 overexpression (Fig. 4A,B). Subsequent colony formation assays confirmed that suppressing the NF-κB signaling pathway could effectively negate the proliferation rate stimulated by NAT10 (Fig. 4C). Histochemical staining of previously procured paraffin-embedded lung cancer tissue specimens illustrated a marked association between NF-κB pathway activation (evidenced by p-p65) and NAT10 protein levels in lung cancer samples (Fig. 4D). The data compellingly suggested that NAT10 enhances lung cancer malignant progression via the NF-κB signaling pathway.

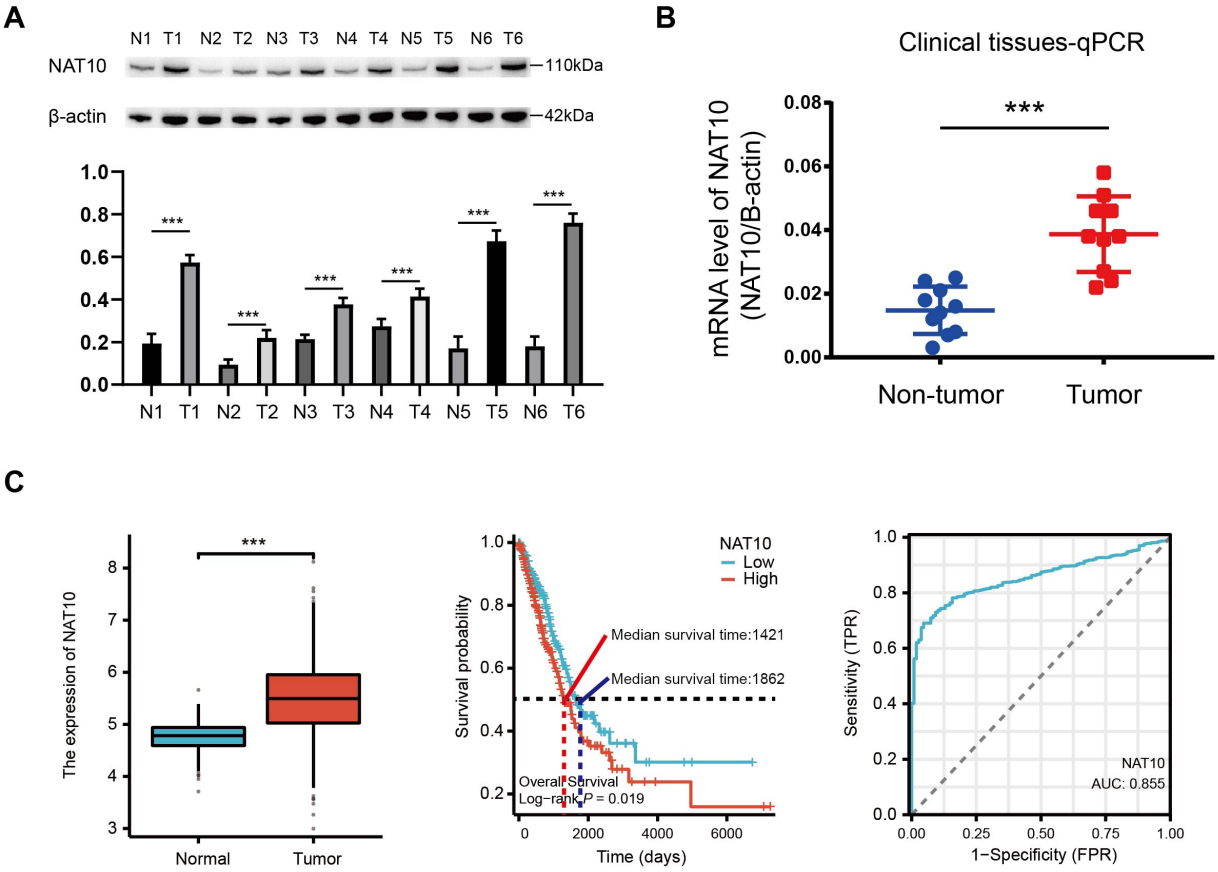

Fig. 1. NAT10 (N-acetyltransferase 10) exhibits overexpression in tumor tissues. (A) The results obtained from western blotting assays demonstrated a significant elevation in the different levels of NAT10 in tumor tissue compared to para-cancer tissue (β-actin as the loading control; N: non-tumor; T: Tumor). (B) qPCR assays revealed that the content of NAT10 within tumor tissue demonstrates significant elevation compared to para-cancer tissue (β-actin as the loading control). (C) TCGA (The Cancer Genome Atlas) database analysis revealed that the elevated expression of NAT10 within the tumor tissue can be a reliable predictive marker for lung cancer. ***$p < 0.001$.

Discussion

Lung cancer remains a health concern globally, primarily attributable to its elevated prevalence and death rates [17]. A prevalent challenge with lung cancer is its latent detection, often only identified in advanced stages [18]. Its clinical manifestations, such as persistent cough, chest pain, and shortness of breath, lack specificity and are attributed to other pulmonary disorders [19]. While low-dose computed tomography (LDCT) has shown promise in early detection among high-risk demographics, such as current and former smokers [20], the widespread adoption of these screening initiatives is curtailed by financial and operational constraints. Another challenge is the heterogeneity of lung cancer, with different subtypes having different treatment options and response rates. Non-small cell lung cancer (NSCLC), the predominant form, offers multiple therapeutic strategies like surgery, radiation therapy, chemotherapy, targeted, or immunotherapy, each contingent upon the cancer disease progression and specific genetic mutations [21]. Conversely, small cell lung cancer (SCLC) is rare but more virulent and chemotherapy and radiation therapies are primarily poor on it. Continued research focused on identifying biomarkers that could pave the way to develop personalized treatment approaches for lung cancer [22]. However, treatment of lung cancer poses significant challenges, including treatment-induced complications, development of resistance to therapies, and steep expenses of cutting-edge targeted and immunotherapy drugs. Consequently, there is a compelling need to deepen the understanding of lung cancer progression and treatment resistance roots and to innovate more potent and patient-friendly therapeutic strategies.

The NF-κB signaling pathway regulates several cellular processes, encompassing inflammation [23], cell proliferation [24], and differentiation [25]. Disruption in the regulation of this particular pathway has been implicated in the initiation and advancement of various cancers, notably lung cancer. Several studies have revealed the activation of

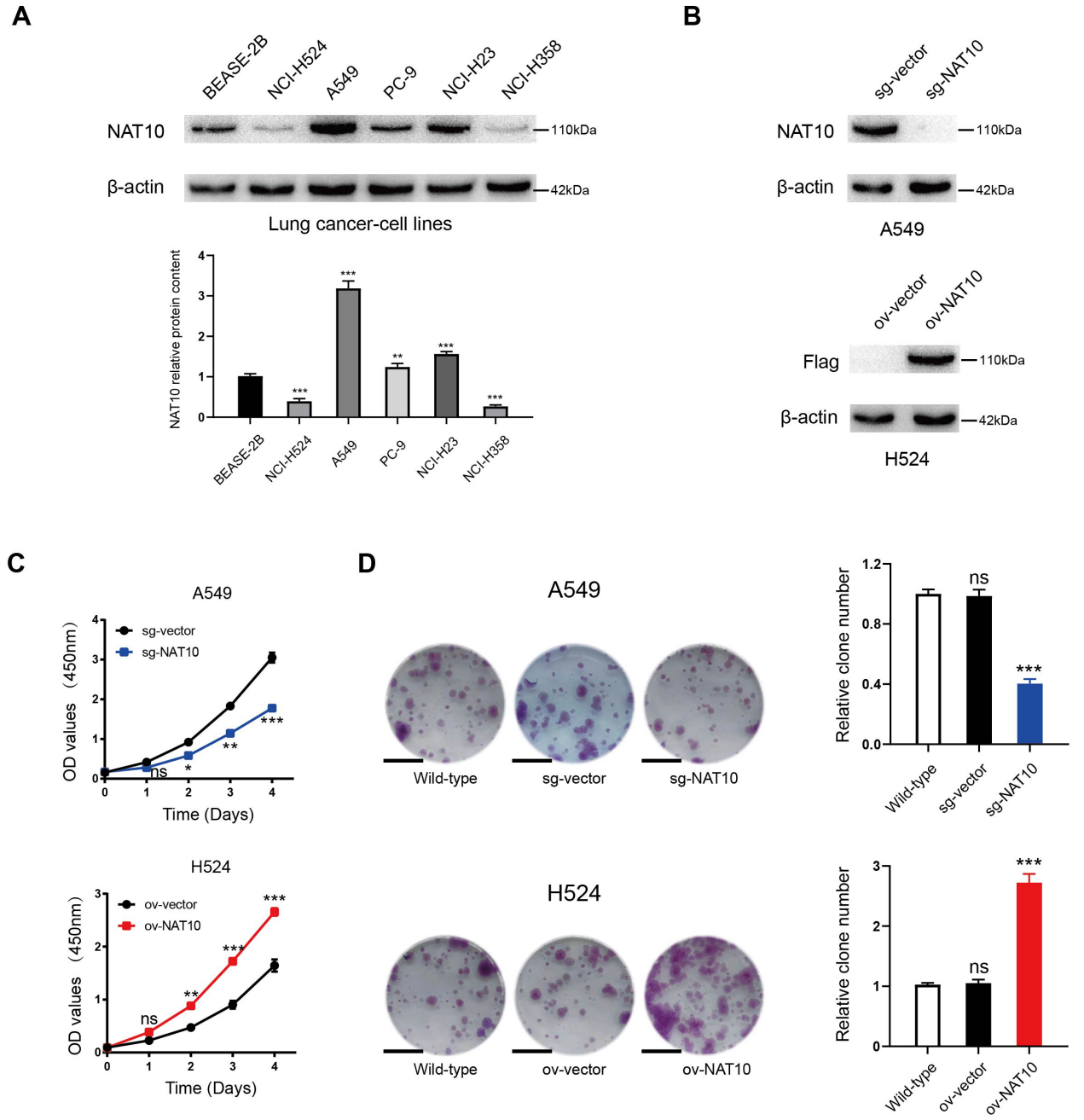

Fig. 2. NAT10 regulates the development and proliferation of lung cancer cells. (A) Western blotting analyzed the protein content of NAT10 in different cells. (B) A549 and NCI-H524 cells were used to construct knockout and overexpression cell lines, respectively. (C) In CCK8 (Cell Counting Kit-8) assays, the knockdown of NAT10 significantly inhibited the proliferation rate of A549, while its enhancement showed the opposite effect. (D) In Colony formation assays, overexpression of NAT10 significantly increased the number of clones formed in NCI-H524 cells, and vice versa, Scale bar, 1 cm. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; "ns" indicates no statistical significance.

the NF-κB pathway within lung cancer cells [26], spurring tumor growth [27] and enhancing cell survival [28]. The regulatory role of this pathway in modulating gene expression is implicated in cell cycle progression, angiogenesis, and anti-apoptotic activities, all of which amplify the aggressive nature of lung cancer.

Recent studies have drawn attention to the interplay between inflammation and lung cancer, with the NF-κB pathway at the nexus of this interaction [29,30]. The induction of Inflammatory cytokines and chemokines activates the pathway, sparking the release of reactive oxygen species (ROS) and sustained release of pro-inflammatory mediators, which potentially augment tumor growth and metas-

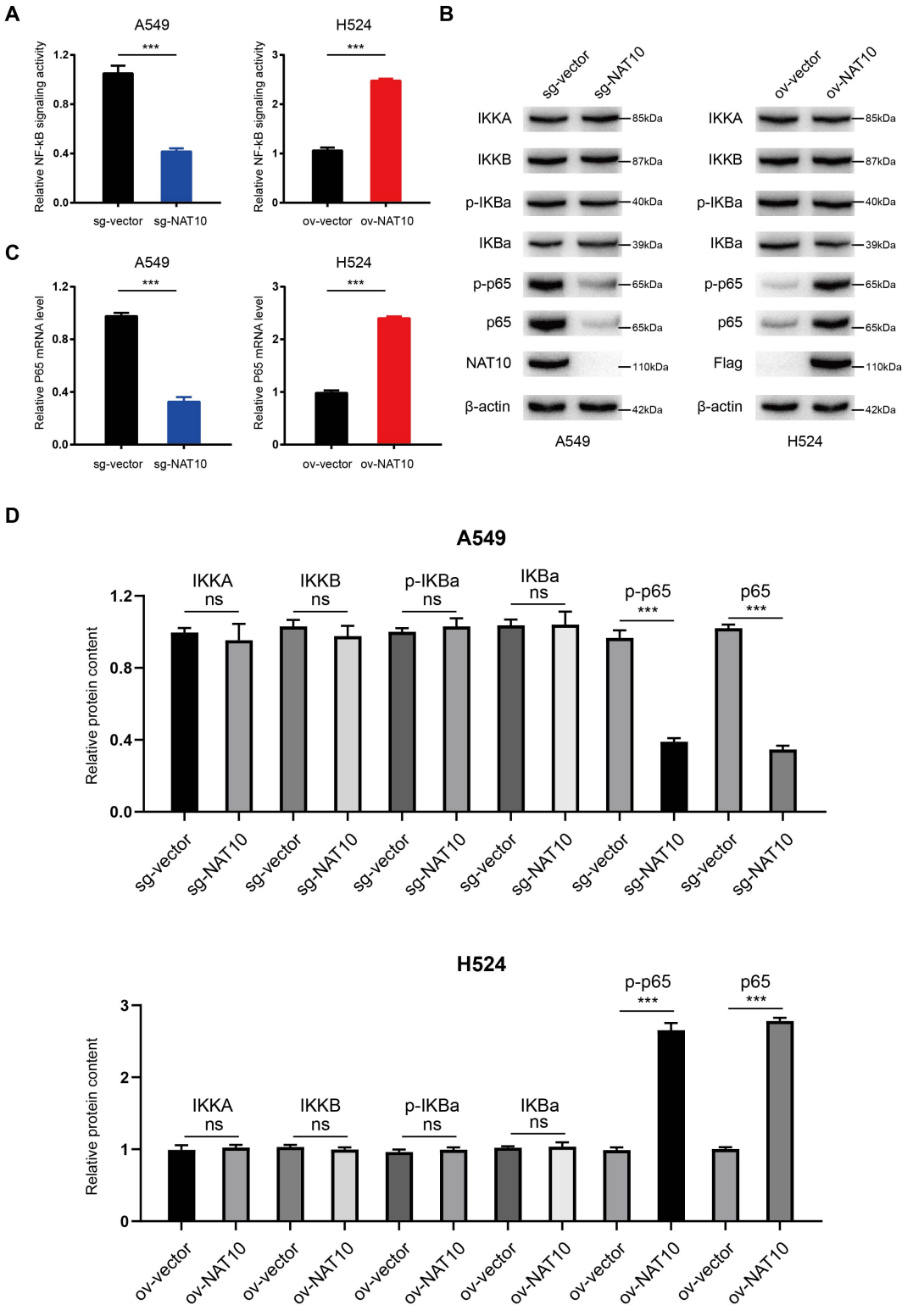

Fig. 3. NAT10 enhances the NF-κB (nuclear factor κB) signaling pathway in lung cancer. (A) The improvement of the NF-κB signaling pathway activity by NAT10 in the context of lung cancer was confirmed using dual luciferase reporter gene assays (***$p <$ 0.001). (B,D) Western blotting revealed that NAT10 only affected RelA/p65 differential expression in lung cancer. (C) qPCR assays revealed that NAT10 only affected RelA/p65 mRNA content in lung cancer. ***$p < 0.001$; "ns" indicates no statistical significance.

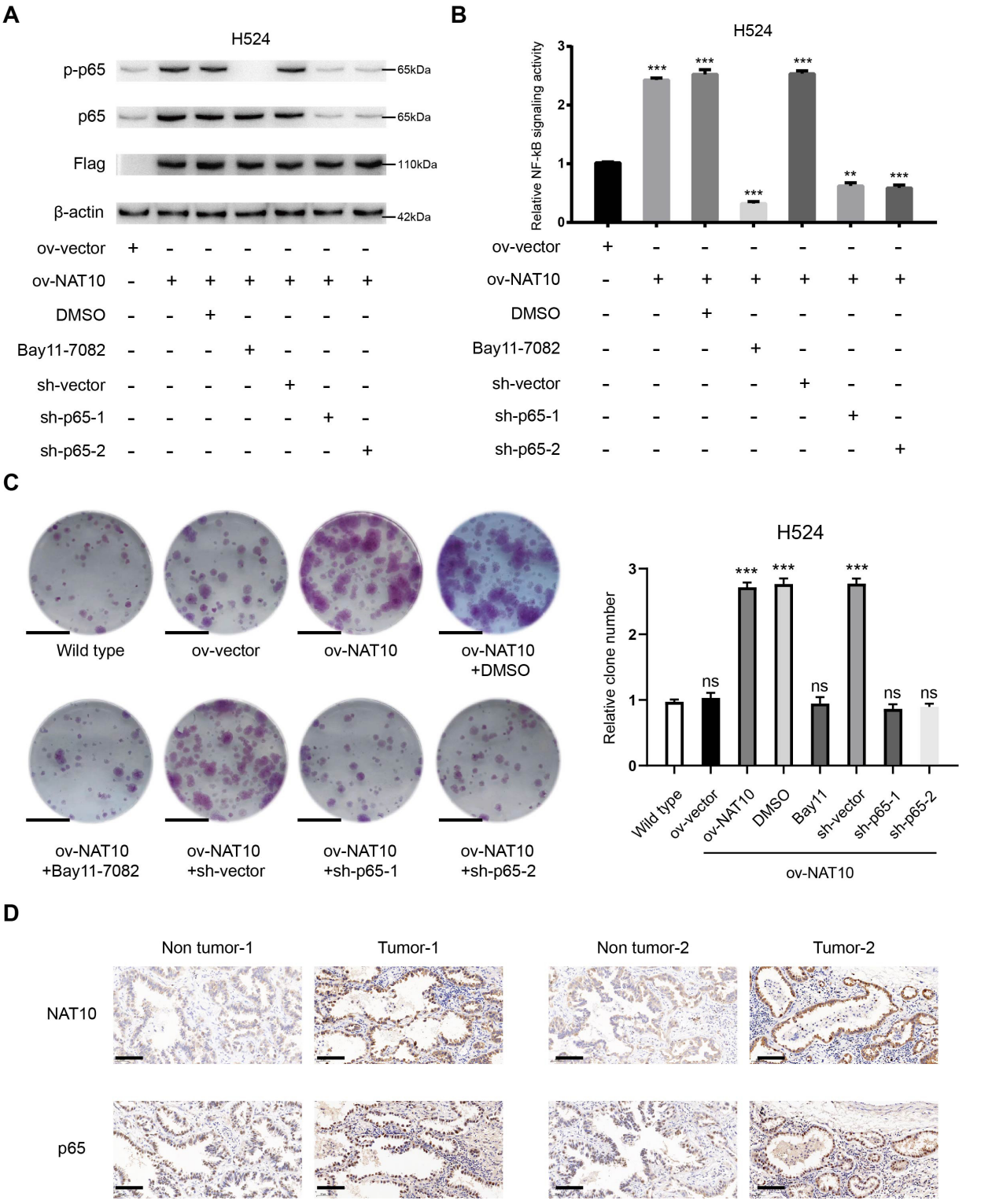

Fig. 4. NAT10 modulates the malignant progression of lung cancer via the NF-κB signaling pathway. (A) Western blotting were employed to test the impact of NF-κB signaling pathway inhibitor (Bay11-7082) and small hairpin RNA (shRNA) to inhibit the NF-κB signaling pathway. (B) The effect of an inhibitor (Bay11-7082) of the NF-κB signaling pathway was confirmed using dual luciferase reporter gene assays and small hairpin RNA (shRNA) to block the NF-κB signaling pathway. (C) Colony formation assays confirmed that suppression of NF-κB signaling pathway could block the increase of proliferation rate caused by overexpression of NAT10 , Scale bar, 1 cm. (D) Immunohistochemical staining showed the NAT10 protein expression in lung cancer tissues is indeed strongly associated with the NF-κB signaling pathway (demonstrated by p65), Scale bar, 100 μm. $^{**}p < 0.01$, $^{***}p < 0.001$; "ns" indicates no statistical significance.

tasis [31]. However, advancing our understanding of the research progress in the NF-κB pathway in lung cancer has encountered some challenges. A primary obstacle lies in the identification of specific targets within the pathway that could improve the effectiveness of the therapeutic interventions. While some inhibitors targeting the pathway are in the developmental stages, their clinical efficacy and safety profiles demand rigorous scrutiny.

Our study found an upregulation of NAT10 expression in lung cancer, which displayed an inverse relationship with the patient's prognosis. Delving deeper into the associated mechanisms, our findings suggest that NAT10 can directly potentiate the function of the NF-κB signaling pathway, primarily through elevating p65 levels. This study enriched our grasp of lung cancer's molecular pathology and serves as a solid foundation for further studies. Nevertheless, our study has certain limitations. For example, it's recognized that NAT10 acts as an executor protein for mRNA AC4C modifications [32]. Our study illustrated that NAT10 can activate the NF-κB signaling pathway by enhancing p65 mRNA content. However, the precise modus operandi still needs to be discovered. In particular, whether NAT10 exerts its corresponding function directly through the mRNA of p65 or other pathways requires additional studies. In summary, lung cancer stands as a formidable health adversary. There is an imperative need to improve early detection methods, better understand the complexity of the disease, and innovate more potent therapeutic interventions to optimize patient outcomes.

Conclusions

We identified that NAT10 was highly expressed in lung cancer and has an inverse correlation with patient prognosis. From a mechanistic perspective, NAT10 amplifies the malignant progression of lung cancer by enhancing the content of p65 within the tumor, subsequently activating the NF-κB signaling pathway. This is conducive to deepening our comprehension of lung cancer and establishes a solid theoretical foundation for future clinical applications and more comprehensive investigations.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

HXH and HY designed the study, analysed the data, and wrote the manuscript. XL and XYL performed all experiments, with the assistance from CSZ (Fig. 1), JFS and KJL (Fig. 2), MS (Fig. 3), JY and JXH. JY and JXH (Fig. 4) also helped with critical reading of the manuscript. HXH and HY supervised the entire study. All the authors fully participated in the project establishment, design, experiment (data acquisition and data analysis), and the writing and discussion of the paper. Professor HY provided the corresponding financial support for this paper. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

According to the Declaration of Helsinki, three cohorts of lung cancer tissue samples were collected at the Affiliated Taizhou People's Hospital of Nanjing Medical University. The written informed consent was collected from the patients, and the experimental protocol got permission from the institutional ethics review board of the Affiliated Taizhou People's Hospital of Nanjing Medical University (KY2021-008-01). Pathologic analysis validated the identity of all specimens. Prior to surgery, no patients had chemotherapy or radiation.

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Conflict of Interest

The authors declare no conflict of interest.

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