

Research Article

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ABI3BP can inhibit the proliferation, invasion, and epithelial–mesenchymal transition of non-small-cell lung cancer cells

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Abstract: Lung cancer, especially non-small-cell lung cancer (NSCLC), has a poor 5-year survival rate below 20%, with factors like smoking, air pollution, and genetic mutations contributing to its development. ABI3BP, an extracellular matrix protein, inhibits NSCLC progression by regulating key signaling pathways; however, its exact mechanisms remain elusive. This study aimed to explore ABI3BP's role in NSCLC and its impact on these pathways. We found that ABI3BP expression was significantly reduced in NSCLC cells compared to normal controls. Overexpression of ABI3BP in NSCLC cells resulted in a substantial reduction in cell growth and motility and induced cell cycle arrest. Furthermore, its overexpression suppressed the epithelial–mesenchymal transition (EMT) process in NSCLC cells. In addition, ABI3BP overexpression inhibited the MAPK/ERK pathway in NSCLC cells. Collectively, ABI3BP functions as a tumor suppressor in NSCLC by targeting the MAPK/ERK axis, thereby regulating cell proliferation, motility, and EMT. These findings suggest that ABI3BP represents a potential therapeutic target for NSCLC treatment.

Keywords: ABI3BP, non-small-cell lung cancer, MAPK/ERK pathway, epithelial–mesenchymal transition, tumor suppression

1 Introduction

Lung cancer remains one of the most prevalent malignancies worldwide, accounting for a significant proportion of cancer-related deaths in both men and women [1,2]. Non-small-cell lung cancer (NSCLC) represents approximately 85% of all lung cancer cases, with more than 60% of patients being diagnosed at advanced stages [3]. The 5-year survival rate for NSCLC remains dismal, at less than 20% [4]. Although smoking is a primary cause of lung cancer, a considerable number of cases occur in non-smokers due to factors such as air pollution, environmental exposure, genetic mutations, and single nucleotide polymorphisms [5]. These observations underscore the urgent need for novel diagnostic and therapeutic strategies that address both the etiology and progression of NSCLC.

The ABI family member 3 binding protein (ABI3BP) is a key extracellular matrix protein known to regulate cell proliferation, differentiation, and signal transduction in various tissues [6]. ABI3BP is integral to modulating key signaling pathways, particularly the MAPK/ERK pathway [7], which plays a vital role in cell proliferation, differentiation, and survival [8]. By inhibiting this pathway, ABI3BP can suppress uncontrolled cell growth, thereby impeding the progression of malignancies. Additionally, ABI3BP interacts with other critical pathways, including the PI3K/Akt and TGF- β cascades, both of which are involved in tumorigenesis and metastasis [6]. Through these interactions, ABI3BP exerts a wide range of biological effects, including cell cycle arrest, epithelial–mesenchymal transition (EMT) inhibition, and reduced invasive potential [6,9]. ABI3BP's activity is not limited to lung cancer; it has also demonstrated tumor-suppressive functions in other malignancies, such as esophageal and breast cancers, where it downregulates oncogenic signals, making it a versatile tumor suppressor across different cancer types [10,11]. Notably, ABI3BP interacts with oncogenic pathways, potentially serving as a multifaceted regulator of tumor biology [12]. Reduced expression of ABI3BP has been associated with tumor progression and metastasis, highlighting its pivotal role in tumor suppression.

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In NSCLC, however, the specific role of ABI3BP remains inadequately understood. This study investigates the therapeutic potential of ABI3BP in NSCLC, hypothesizing that it suppresses NSCLC progression through the regulation of key signaling pathways.

2 Materials and methods

2.1 Cell lines and cell culture

Human NSCLC cell lines HCC-827, A549, NCI-H460, and H1975, as well as normal bronchial epithelial cells (BEAS-2B), were purchased from ATCC. All cell lines were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco). Cells were maintained at 37°C in a humidified incubator with 5% CO₂.

2.2 Reagents and antibodies

Adenoviral vectors expressing ABI3BP (ad-ABI3BP) and control adenovirus (ad-NC) were purchased from Vigene Biosciences (China). Western blot analyses were performed using primary antibodies from Abcam, including anti-ABI3BP (ab154189, 1:1,000), anti-E-cadherin (ab40772, 1:1,000), anti-N-cadherin (ab18203, 1:1,000), anti-α-SMA (ab5694, 1:1,000), anti-AKT (ab8805, 1:1,000), anti-phospho-AKT (ab38449, 1:1000), anti-ERK1/2 (ab184699, 1:1,000), and anti-phospho-ERK1/2 (ab201015, 1:1,000). Horseradish peroxidase (HRP)-conjugated secondary antibodies were diluted at 1:5,000 (Abcam).

2.3 Adenovirus infection

Cells were infected with ad-ABI3BP or ad-NC adenoviruses at a multiplicity of infection of 100. The infection process was carried out for 24 h in serum-free medium, followed by incubation in complete medium for further experiments.

2.4 Immunoblot analysis

Samples were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride membranes (Millipore, USA). After blocking with 5% non-fat milk, membranes were incubated overnight at 4°C with the primary antibodies, followed by incubation with HRP-conjugated secondary antibodies for 1 h at room temperature. Protein bands were visualized using an

enhanced chemiluminescence detection kit (Beyotime), and band intensities were quantified using ImageJ software.

2.5 Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8, Beyotime) following the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader (Thermo Fisher, USA).

2.6 Migration and invasion assays

Cell migration and invasion were evaluated using Transwell assays (Corning, USA). For the migration assay, 1×10^5 cells in serum-free medium were placed in the upper chamber of a Transwell insert. The lower chamber was filled with RPMI-1640 medium containing 10% FBS. For the invasion assay, the upper chamber was pre-coated with Matrigel (Corning), and 2×10^5 cells were seeded. After 24 h, cells on the lower surface of the insert were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet. The number of migrated or invaded cells was counted in five randomly selected fields using a light microscope (Zeiss, Germany).

2.7 FCM assay

The cells were washed with PBS and fixed using 70% ethanol at -20°C for 2 h. Subsequently, the cells were stained with propidium iodide at 4°C. The stained cells were then analyzed using a flow cytometer (BD, USA).

2.8 Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A *p*-value of <0.05 was considered statistically significant.

3 Results

3.1 ABI3BP expression was significantly downregulated in NSCLC cells

To assess the expression of ABI3BP in NSCLC, we first measured its levels through the TCGA database. We observed

that ABI3BP had a lower TPM value in NSCLC tissues compared to normal tissues (Figure 1a). Subsequently, we detected the expression of ABI3BP in both normal bronchial epithelial cells (BEAS-2B) and NSCLC cell lines (HCC-827, A549, NCI-H460, and H1975). Immunoblot analysis revealed that ABI3BP expression was significantly lower in all NSCLC cell lines compared to normal bronchial epithelial cells (Figure 1b). Quantification of ABI3BP protein levels confirmed the marked reduction in its expression in NSCLC cells (Figure 1b). Thus, ABI3BP expression was significantly downregulated in NSCLC cells.

3.2 Overexpression of ABI3BP in NSCLC cells led to a marked reduction in cell growth as well as motility

Next, we evaluated the effect of ABI3BP overexpression on cell viability using the CCK8 assay. HCC-827 and NCI-H460 cells were transfected with either control (ad-NC) or ABI3BP-expressing adenovirus (ad-ABI3BP), and the overexpression efficiency was confirmed by Immunoblot (Figure 2a). Further, CCK-8 assays showed that overexpression of ABI3BP led to a significant decrease in cell viability in both NSCLC cell lines compared to the control and ad-NC groups (Figure 2b). However, its overexpression has modest effects on the viability of BEAS-2B

cells (Figure 2b). Colony formation assays further confirmed that the overexpression of ABI3BP suppressed the proliferation of HCC-827 and NCI-H460 cells (Figure 2c). Transwell migration and invasion assays were performed using HCC-827 and NCI-H460 cells. The results indicated that overexpression of ABI3BP significantly reduced the migratory capacity of both cell lines compared to the control groups (Figure 2d). In contrast, ABI3BP overexpression did not affect the migration of BEAS-2B cells, as confirmed by Transwell assays (Figure 2d). Additionally, the invasive ability of HCC-827 and NCI-H460 cells was also markedly suppressed following ABI3BP overexpression (Figure 2e). Further, the overexpression of ABI3BP led to the cell cycle arrest in HCC-827 and NCI-H460 cells (Figure 2f). Therefore, overexpression of ABI3BP in NSCLC cells resulted in a significant reduction in cell growth as well as motility.

3.3 ABI3BP overexpression suppressed the EMT process of NSCLC cells

Since EMT plays a crucial role in cancer metastasis, we examined whether ABI3BP influences EMT markers in NSCLC cells. Western Immunoblot analysis revealed that ABI3BP overexpression increased the expression of the epithelial marker E-cadherin and decreased the expression of mesenchymal markers N-cadherin and α -SMA in both

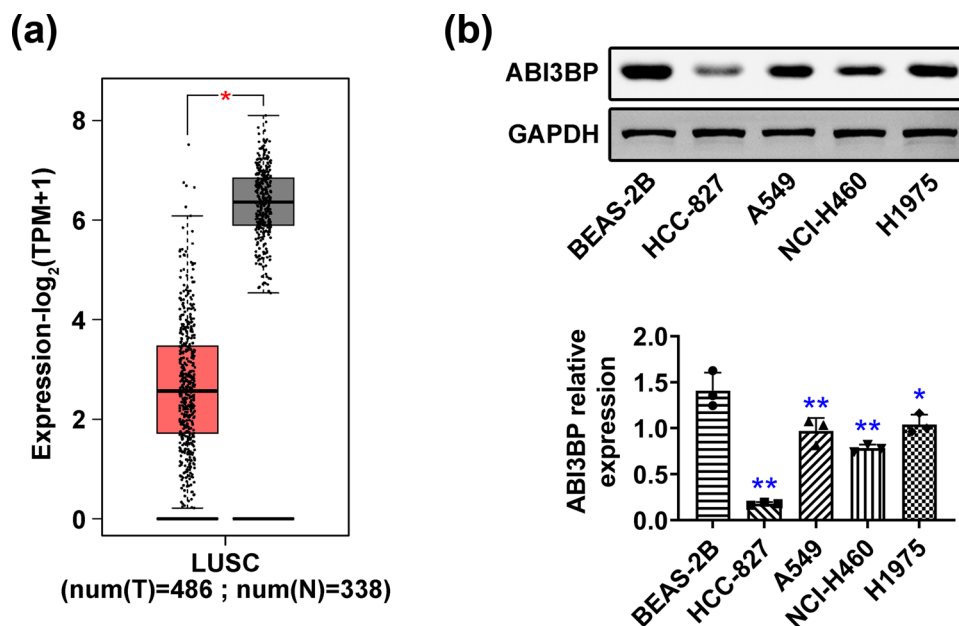


Figure 1: ABI3BP expression was significantly downregulated in NSCLC cells. (a) TCGA database showed the TPM (transcripts per million) value of ABI3BP in 338 normal tissues and 486 tumor tissues. (b) Immunoblot showing ABI3BP protein expression levels in normal bronchial epithelial cells (BEAS-2B) and NSCLC cell lines (HCC-827, A549, NCI-H460, H1975). Quantification of ABI3BP protein expression was shown. Data are represented as mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$. NSCLC, non-small-cell lung cancer.

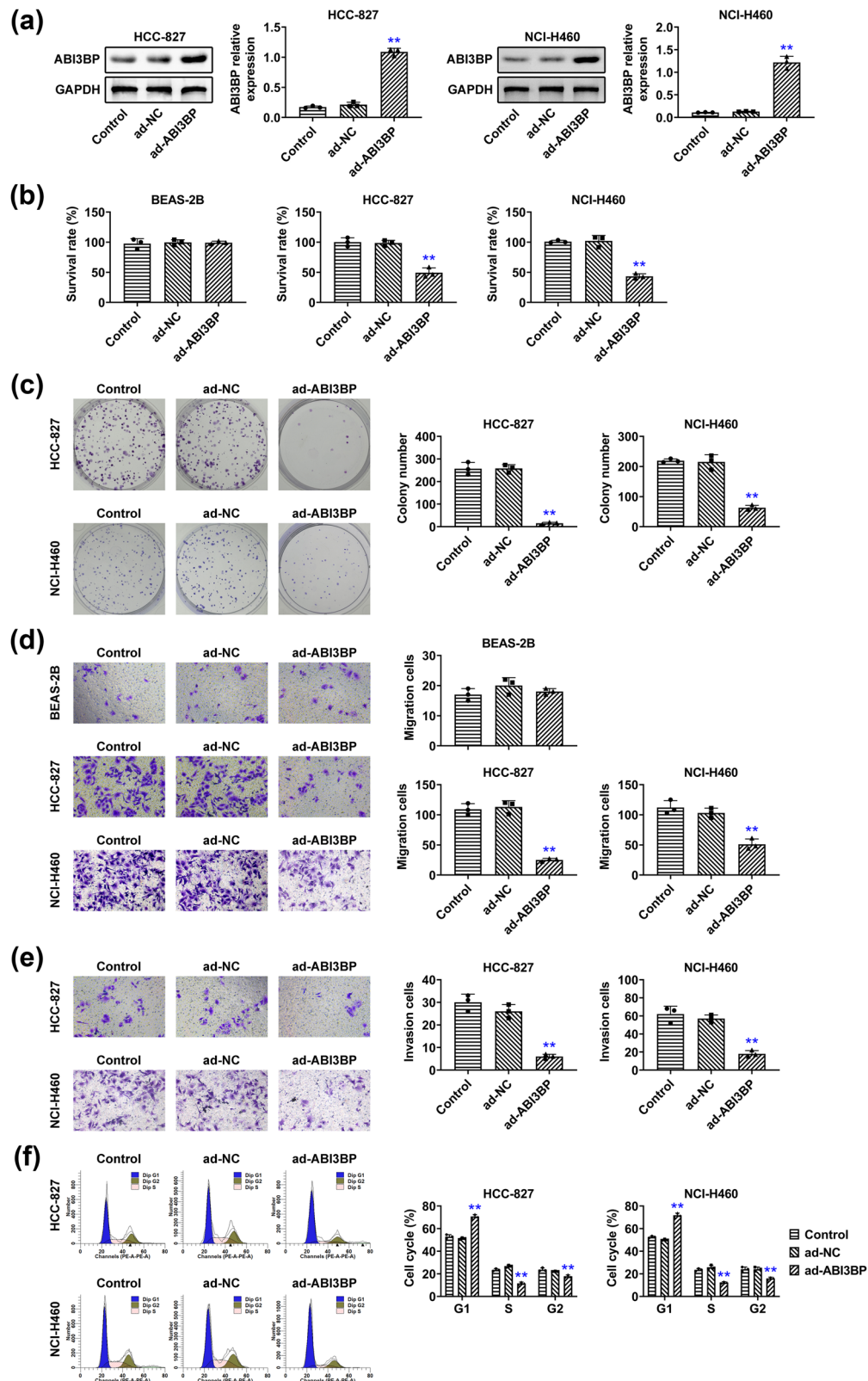


Figure 2: Overexpression of ABI3BP in NSCLC cells led to a marked reduction in cell growth as well as motility. (a) Immunoblot showing ABI3BP protein levels in HCC-827 and NCI-H460 cells upon the indicated infection. GAPDH was used as the loading control. Quantification of relative ABI3BP expression is shown on the right. (b) Cell viability assay (CCK8) results for BEAS-2B, HCC-827, and NCI-H460 cells following overexpression of ABI3BP. The survival rate (%) is shown for the control, ad-NC, and ad-ABI3BP groups. (c) Colony formation assays showed the effects of ABI3BP overexpression on the proliferation of HCC-827 and NCI-H460 cells. The colony numbers were counted. (d) Transwell migration assay of BEAS-2B, HCC-827, and NCI-H460 cells with images showing migrated cells in each group. Quantification of migrated cells is shown on the right. (e) Transwell invasion assay of HCC-827 and NCI-H460 cells with images showing invaded cells in each group. (f) FCM assays showed the effects on the cell cycle of HCC-827 and NCI-H460 cells. The cells at different phases were calculated. Quantification of invaded cells is shown on the right. Data are represented as mean $\pm$ SD. ** $p < 0.01$, ad-ABI3BP vs ad-NC. NSCLC, non-small-cell lung cancer. NC, negative control.

HCC-827 (Figure 3a) and NCI-H460 (Figure 3b) cells. These findings suggest that ABI3BP inhibits EMT, thereby reducing the invasive potential of NSCLC cells.

3.4 ABI3BP overexpression suppressed MAPK/ERK pathway in NSCLC cells

To explore the molecular mechanisms by which ABI3BP regulates NSCLC cell proliferation and migration, we investigated the MAPK/ERK pathway. Immunoblot analysis revealed that overexpression of ABI3BP reduced the phosphorylation of ERK and Akt in both HCC-827 (Figure 4a) and NCI-H460 (Figure 4b) cells, indicating that ABI3BP inhibits the MAPK/ERK pathway. These results suggest that ABI3BP suppresses NSCLC progression by modulating the MAPK/ERK pathway.

4 Discussion

NSCLC is the most prevalent form of lung cancer, comprising around 85% of all lung cancer diagnoses. It is highly aggressive, characterized by rapid cell proliferation, migration, and

EMT, which collectively drive tumor progression and metastasis [13–16]. These biological processes are regulated by several key signaling pathways and proteins that ensure proper cellular function. Disruption of these pathways often leads to poor clinical outcomes in NSCLC patients. In our study, we explored the role of ABI3BP in regulating cell proliferation, migration, and EMT, providing insights into its potential therapeutic relevance in NSCLC.

EMT plays a crucial role in advancing cancer by enhancing the metastatic ability of cancer cells [17]. Through this process, epithelial cells acquire mesenchymal traits, thereby increasing their capacity for migration and invasion. EMT is also implicated in resistance to cancer therapies, making it a critical target in NSCLC treatment strategies. Our findings revealed that ABI3BP overexpression led to a significant reduction in EMT in NSCLC cells, as evidenced by altered levels of EMT markers, such as E-cadherin and N-cadherin. These results underscore the importance of targeting EMT to limit the metastatic potential of NSCLC.

ABI3BP, a vital extracellular matrix protein, is involved in the regulation of various cellular functions such as cell growth, differentiation, and programmed cell death. Its role as a tumor suppressor has been established in multiple cancer types, including esophageal and gallbladder cancers, where it inhibits cellular proliferation and migration [10,11]. ABI3BP's interactions with key signaling pathways, including

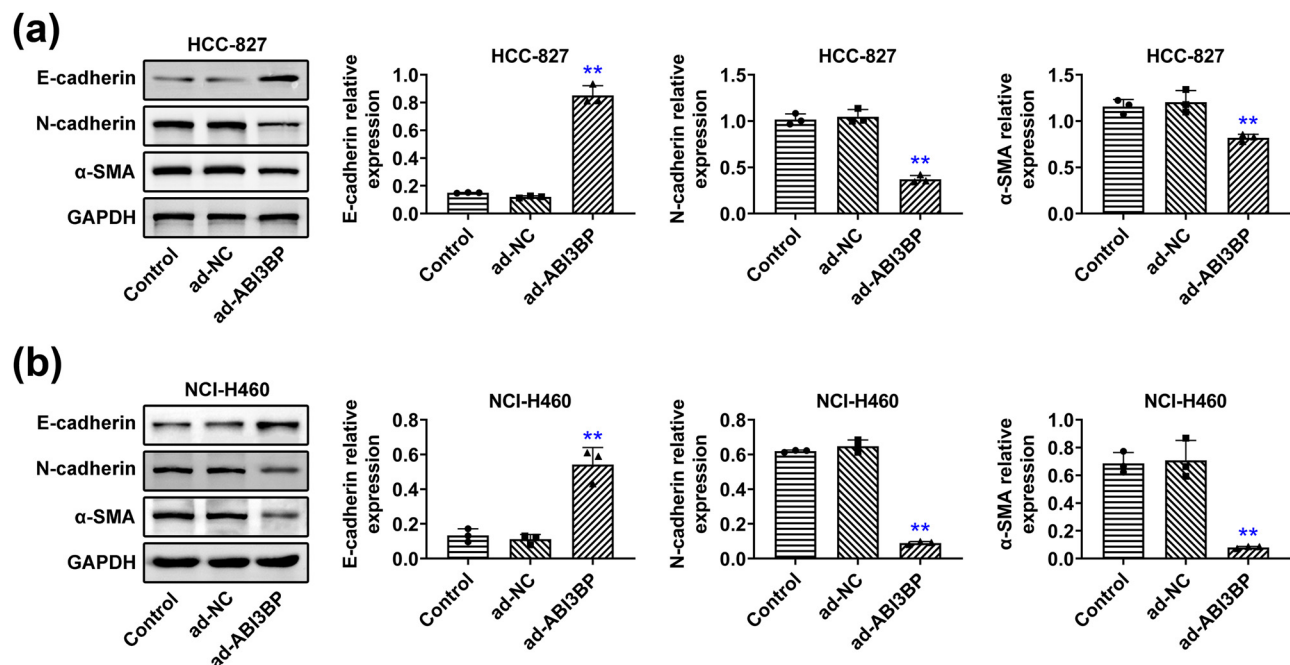


Figure 3: ABI3BP overexpression suppressed the EMT process of NSCLC cells. (a) and (b) Immunoblot showing E-cadherin, N-cadherin, and α-SMA protein levels in HCC-827 (a) and NCI-H460 (b) cells upon the indicated infection. GAPDH was used as the loading control. Quantification of relative E-cadherin, N-cadherin, and α-SMA expression is shown on the right. Data are represented as mean ± SD. ** $p < 0.01$, ad-ABI3BP vs ad-NC. NSCLC, non-small-cell lung cancer. NC, negative control.

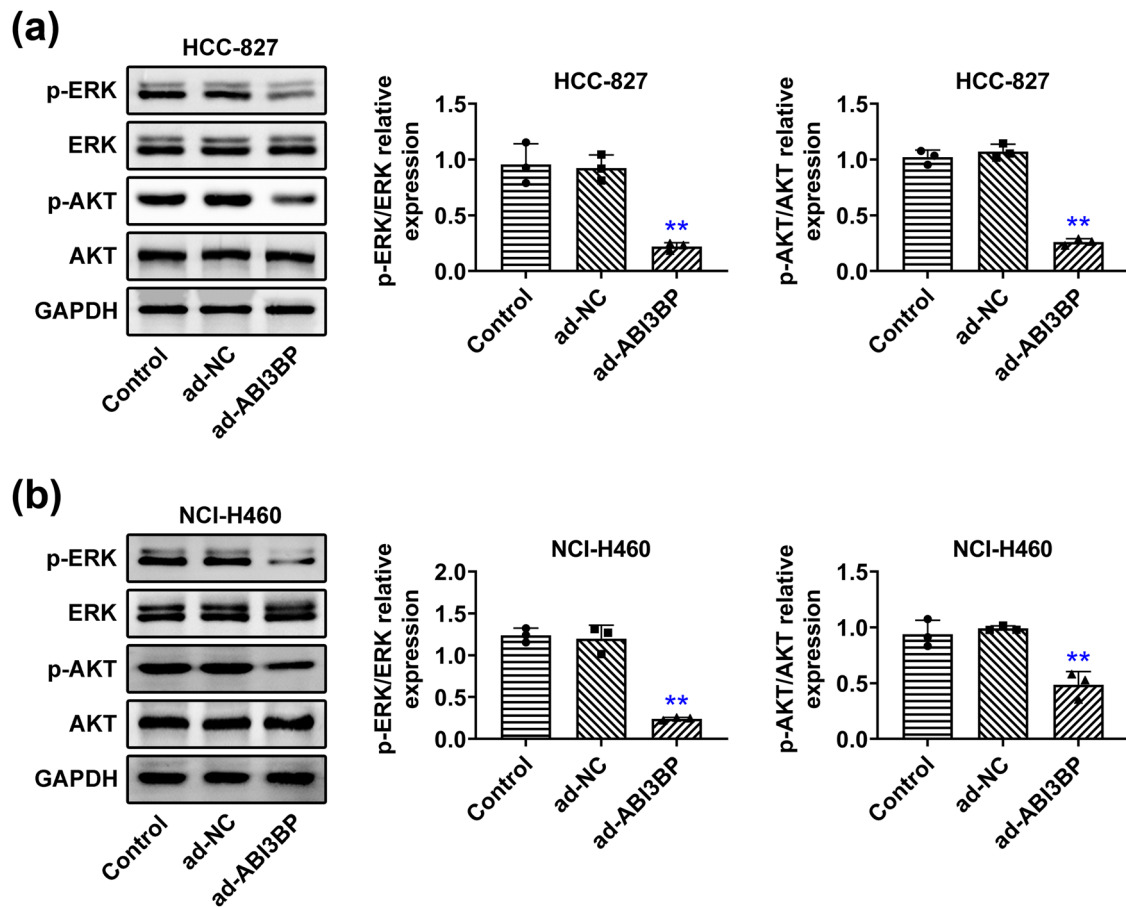


Figure 4: ABI3BP overexpression suppressed the MAPK/ERK pathway in NSCLC cells. (a) and (b) Immunoblot showing ERK and AKT protein levels and phosphorylation levels in HCC-827 (a) and NCI-H460 (b) cells upon the indicated infection. GAPDH was used as the loading control. Quantification of relative phosphorylation levels of ERK and AKT is shown on the right. Data are represented as mean $\pm$ SD. ** $p < 0.01$, ad-ABI3BP vs ad-NC. NSCLC, non-small-cell lung cancer. NC, negative control.

MAPK/ERK and PI3K/Akt, are essential for its tumor-suppressive activities. In this study, we observed that overexpression of ABI3BP in NSCLC cells led to reduced proliferation and invasion, consistent with its known role as a tumor suppressor.

Beyond these general cellular effects, ABI3BP exerts specific control over EMT, as well as cell proliferation and migration in NSCLC [18]. Our research demonstrated that ABI3BP overexpression decreased EMT-related markers, notably reducing N-cadherin levels while increasing E-cadherin expression. In addition, ABI3BP was shown to suppress NSCLC cell migration and invasion, suggesting that its inhibition of these processes may be a crucial factor in reducing tumor progression and metastasis. This highlights ABI3BP as a significant modulator of EMT and an inhibitor of NSCLC progression.

ABI3BP overexpression inhibited the MAPK/ERK pathway in NSCLC cells. Previous studies have reported that the MAPK/ERK pathway plays critical in mediating cancer cell proliferation,

and its alteration could affect the proliferation of cancer cells, including NSCLC cells [19,20]. The MAPK/ERK and PI3K/Akt pathways are pivotal in regulating cellular processes like proliferation, migration, and survival. Dysregulation of these pathways is common in cancer and is linked to enhanced tumor growth and metastasis [19,20]. The MAPK/ERK pathway is primarily activated in response to growth stimuli, facilitating cell proliferation and survival, while the PI3K/Akt pathway similarly drives cell growth and migration. Both pathways are intimately involved in regulating EMT, and their dysregulation accelerates NSCLC progression. In this study, we found that ABI3BP suppresses the activation of both ERK and Akt, thereby inhibiting NSCLC cell proliferation and EMT. These findings suggest that modulating these pathways through ABI3BP could be a promising approach to NSCLC therapy.

We propose that future research could include in vivo tumor formation experiments in nude mice to further validate the conclusions and clarify the downstream molecular mechanism through multi-omics analysis. Moreover, while

we focused on specific pathways, other signaling networks may also contribute to the tumor-suppressive effects of ABI3BP that have not yet been explored. Future research should aim to expand on these findings and investigate the clinical applications of ABI3BP as a therapeutic target.

In summary, our study highlights the tumor-suppressive role of ABI3BP in NSCLC, revealing its ability to regulate key signaling pathways involved in cell proliferation, migration, and EMT. ABI3BP presents a promising target for therapeutic strategies aimed at controlling NSCLC progression. Further research into its mechanistic interactions could lead to the development of novel treatments for NSCLC patients, potentially improving their prognosis.

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Conflict of interest: Authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- [1] Lovly CM. Expanding horizons for treatment of early-stage lung cancer. *N Engl J Med.* 2022;386(21):2050–1.
- [2] Yu JY, Chang H, Jung W, Heo S, Lee GT, Park JE, et al. Predicting mid-term survival of patients during emergency department triage for resuscitation decision. *Signa Vitae.* 2023;19(2).
- [3] Cooper A, Chaft JE, Bott MJ. Induction therapy for non-small cell lung cancer. *J Thorac Cardiovasc Surg.* 2024;168(2):411–6.
- [4] Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature.* 2018;553(7689):446–54.
- [5] Dowling CM, Zhang H, Chonghaile TN, Wong KK. Shining a light on metabolic vulnerabilities in non-small cell lung cancer. *Biochim Biophys Acta Rev Cancer.* 2021;1875(1):188462.
- [6] Hodgkinson CP, Gomez JA, Payne AJ, Zhang L, Wang X, Dal-Pra S, et al. Abi3bp regulates cardiac progenitor cell proliferation and differentiation. *Circ Res.* 2014;115(12):1007–16.
- [7] Delfin DA, DeAgüero JL, McKown EN. The extracellular matrix protein ABI3BP in cardiovascular health and disease. *Front Cardiovasc Med.* 2019;6:23.
- [8] Latini FR, Hemerly JP, Freitas BC, Oler G, Riggins GJ, Cerutti JM. ABI3 ectopic expression reduces in vitro and in vivo cell growth properties while inducing senescence. *BMC Cancer.* 2011;11:11.
- [9] Hodgkinson CP, Naidoo V, Patti KG, Gomez JA, Schmeckpeper J, Zhang Z, et al. Abi3bp is a multifunctional autocrine/paracrine factor that regulates mesenchymal stem cell biology. *Stem Cell.* 2013;31(8):1669–82.
- [10] Feng Y, Tao F, Qiao H, Tang H. A pan-cancer analysis of ABI3BP: A potential biomarker for prognosis and immunoinfiltration. *Front Oncol.* 2023;13:1159725.
- [11] Lin N, Yao Z, Xu M, Chen J, Lu Y, Yuan L, et al. Long noncoding RNA MALAT1 potentiates growth and inhibits senescence by antagonizing ABI3BP in gallbladder cancer cells. *J Exp Clin Cancer Res.* 2019;38(1):244.
- [12] Yang H, Chen W, Shi J, Huang D, Li J, Hu B, et al. Knockout of Abi3bp in mice does not affect their olfactory function, mental state and NNK-induced lung tumorigenesis. *Acta Biochim Biophys Sin (Shanghai).* 2016;48(9):820–6.
- [13] Le X, Nilsson M, Goldman J, Reck M, Nakagawa K, Kato T, et al. Dual EGFR-VEGF pathway inhibition: A promising strategy for patients with EGFR-mutant NSCLC. *J Thorac Oncol.* 2021;16(2):205–15.
- [14] Hong W, Xue M, Jiang J, Zhang Y, Gao X. Circular RNA circ-CPA4/let-7 miRNA/PD-L1 axis regulates cell growth, stemness, drug resistance and immune evasion in non-small cell lung cancer (NSCLC). *J Exp Clin Cancer Res.* 2020;39(1):149.
- [15] Liang G, Meng W, Huang X, Zhu W, Yin C, Wang C, et al. miR-196b-5p-mediated downregulation of TSPAN12 and GATA6 promotes tumor progression in non-small cell lung cancer. *Proc Natl Acad Sci U S A.* 2020;117(8):4347–57.
- [16] Ren K, Pei J, Guo Y, Jiao Y, Xing H, Xie Y, et al. Regulated necrosis pathways: A potential target for ischemic stroke. *Burn Trauma.* 2023;11:tkad016.
- [17] Tulchinsky E, Demidov O, Kriajevska M, Barlev NA, Imyaninov E. EMT: A mechanism for escape from EGFR-targeted therapy in lung cancer. *Biochim Biophys Acta Rev Cancer.* 2019;1871(1):29–39.
- [18] Terauchi K, Shimada J, Uekawa N, Yaoi T, Maruyama M, Fushiki S. Cancer-associated loss of TARSH gene expression in human primary lung cancer. *J Cancer Res Clin Oncol.* 2006;132(1):28–34.
- [19] Cui Y, Wu X, Jin J, Man W, Li J, Li X, et al. CircHERC1 promotes non-small cell lung cancer cell progression by sequestering FOXO1 in the cytoplasm and regulating the miR-142-3p-HMGB1 axis. *Mol Cancer.* 2023;22(1):179.
- [20] Liu C, Zheng S, Wang Z, Wang S, Wang X, Yang L, et al. KRAS-G12D mutation drives immune suppression and the primary resistance of anti-PD-1/PD-L1 immunotherapy in non-small cell lung cancer. *Cancer Commun (Lond).* 2022;42(9):828–47.