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ORIGINAL RESEARCH

Ebp1 p48 Promotes Oncogenic Properties in Non-Small Cell Lung Cancer Through PI3K/Akt Signaling Pathways

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Purpose: Lung cancer, particularly non-small cell lung cancer (NSCLC), is highly deadly globally. The potential carcinogenic role of p48, a long isoform of ErbB3-binding protein 1 (Ebp1), is well established in other cancers, but its impact on NSCLC remains unconfirmed.

Patients and Methods: Several databases were utilized to compare Ebp1 expression in normal lung and NSCLC. Immunohistochemical staining was employed to identify Ebp1 expression in both types of tissue. The TCGA database assessed Ebp1 expression in NSCLC and its impact on overall survival. Ebp1 expression was knocked down in A549 and PC9 cells, and the impact of Ebp1 on the cell growth was tested by CCK-8, plate clone colony, soft agar colony generation assay, and cell cycle assays. Scratch, transwell, and in vivo were also used to confirm the effects of Ebp1 on Lung cancer cells migration, invasion. Western blot detection of EMT and signal pathway-related proteins.

Results: This study revealed that NSCLC had significantly higher levels of Ebp1 p48 expression. We discovered a correlation between Ebp1 p48 expression and pathological grade, lymph node metastasis, clinical stage, and overall survival (OS) using NSCLC tissue microarrays. In vitro and in vivo tumor cell growth, migration, invasion, the epithelial-mesenchymal transition (EMT) process, and cell proliferation are all markedly suppressed when Ebp1 p48 is knocked down in NSCLC cells. Moreover, PI3K and Akt phosphorylation levels were decreased by Ebp1 p48 knockdown.

Conclusion: According to these findings, Ebp1 p48 stimulated the PI3K/Akt signaling pathway in NSCLC, which in turn facilitated invasion, migration, and proliferation. As a result, in NSCLC, Ebp1 p48 may be a prospective therapeutic target as well as a predictive biomarker.

Keywords: malignant tumor, proliferation, invasion, epithelial mesenchymal transformation

Introduction

Globally, lung cancer remains the most lethal kind of malignant tumor. It is a progressive disease and profound threat to human health and quality of life,[1] with a five-year survival rate of 23%.[2] Non-small cell lung cancer (NSCLC) is the most prevalent pathological type of lung cancer. Current treatment strategies for NSCLC have changed dramatically with the introduction of molecular targeted drugs. Immunomodulatory drugs such as anti-PD1 are also used in cancer treatment. However, the overall efficacy is still not satisfactory. Therefore, in order to develop a new, trustworthy method for diagnosing and treating non-small cell lung cancer (NSCLC), it is essential to investigate the molecular mechanisms behind the development and progression of NSCLC as well as the tumor microenvironment.

Ebp1, a multifaceted eukaryotic protein that is widely expressed and highly conserved, contains 394 amino acid residues, is the receptor-binding protein for ErbB3. Two isoforms of ebp1 were produced by alternative splicing: the full-length p48

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form and the shortened p42 form. p48 is mainly distributed in cytoplasm and nucleus. It can inhibit cell apoptosis and is a tumor promoter. But as a tumor suppressor, p42 is mostly found in the cytoplasm and encourages cell differentiation.[3] Prior research has confirmed that human gliomas have significant expression of the oncoprotein p48 Ebp1. It down-regulates the expression of p53 protein and promotes the occurrence of tumors, leading to poor clinical treatment effect.[4] In addition, breast cancer patients with high expression of Ebp1 p48 had a poor prognosis[5] and Ebp1 p48 expression increased with the progression of prostate cancer.[6] According to these investigations, Ebp1 p48 may promote the invasion and growth of malignant cells. According to these findings, a number of malignant tumor cells' biological processes depend heavily on Ebp1 p48. Studies have demonstrated that Ebp1 p42 has a tumor suppressor function in non-small cell lung cancer,[7] however, the mechanisms by which Ebp1 contributes to NSCLC pathogenesis have not been documented.

This study uncovers the pro-tumor effect of Ebp1 p48 in non-small cell lung cancer (NSCLC) and its molecular mechanism. Clinical analysis showed upregulation of Ebp1 p48 in NSCLC tissues, linked to poor prognosis, suggesting its role in tumor progression. Gene knockdown experiments confirmed that inhibiting Ebp1 p48 reduced cell proliferation, invasion, and metastasis in vitro and in vivo, highlighting it as a key regulator of NSCLC malignancy. Mechanistically, Ebp1 p48 promotes tumor progression via activation of the PI3K/Akt pathway, enhancing cell proliferation and apoptosis resistance. Additionally, Ebp1 p48 induces epithelial-mesenchymal transition (EMT), contributing to metastasis. These findings provide new insights into NSCLC metastasis and suggest Ebp1 p48 as a potential target for early diagnosis and therapy.

Materials and Methods

Tissue Acquisition

NSCLC tissue chip (HLugA180Su06) was purchased from Shanghai Outdo Biotech Co., Itd. Among them were 86 matched neighboring non-tumor tissues and 94 NSCLC samples. Ages, genders, tumor site, tumor size, pathological grade, clinical stage, survival, and lymph node metastases are among the data contained in NSCLC tissue chips.

Cell Culture

The American Type Culture Collection (ATCC) provided a range of cell lines, including A549, H522, PC9, H650, and 16HBE. Above cell lines in the experiments were validated and were negative for mycoplasma. The ATCC suggested that cells be grown in DMEM medium, kept at 37°C in a humidified environment with 5% CO2, and supplemented with 10% FBS, 1% streptomycin, and penicillin.

Reagents and Antibodies

DMEM medium and FBS were purchased from Gibco (Carlsbad, CA, USA). Marker: Thermo Fisher 26616; ECM kit: Boster ek1002.

The Antibodies we use in Western blotting are as follows:

Ebp1 (N-terminus): Millipore SAB1402863; E-cadherin: Cell Signaling #3195; N-cadherin: Cell Signaling #13116; Vimentin: Cell Signaling #5741; Snail: Cell Signaling #3879; Slug: Cell Signaling #9585; Akt: Santa Cruz sc-5298; p-Akt (Thr 308): Santa Cruz sc-271966; p-Akt (Ser 473): Santa Cruz sc-293125; PI3K: Abcam ab247253; p-PI3K: MCE HY-P81211; β-actin: Affinity Biosciences #AF7018;

Cell Viability Assay

Two cell lines, A549 and PC9, were injected into 96-well plates, with 5×10^3 cells per well. Both the experimental group (sh-p48 Ebp1) and the control group (sh-NC) were established. Cell proliferation activity was respectively measured once per day for 6 days. Using the CCK8 kit (CCK8, Dojindo, Japan), cell viability was evaluated using the guidelines provided by the manufacturer. The absorbance at 450 nm was measured using a microplate reader.

Immunohistochemical Staining and Hematoxylin-Eosin Staining

Immunohistochemistry (IHC) staining was implemented on tissue microarray samples and nude mouse tumors. As directed by the manufacturer (DAKO, Glostrup, Denmark), anti-Ebp1 p48 (1:100), anti-Ki67 (1:100), and anti-cyclinD1

(1:100) were examined using a streptavidin-horseradish peroxidase conjugate and a secondary biotinylated antibody. After that, haematoxylin and eosin (H&E) was used to stain the xenograft tumor.

Western Blot Analyses

The cells were lysed using RIPA, and the protein level was then determined using the BCA protein assay kit. The proteins were separated using 10% SDS-PAGE and then transferred onto polyvinylidene difluoride membranes using the transfer buffer. After blocking the membranes with 5% skim milk for two hours at room temperature, they were vigorously shaken and incubated once more for the whole night at 4°C, using antibodies against Ebp1 p48, snail, slug, vitementin, N-cadherin, E-cadherin, and β-actin. Following this, following three thorough membrane washes using 1×Tris-Buffered Saline and Tween 20 (TBST), for one hour at room temperature, they were treated with the goat anti-rabbit/mouse IgG secondary antibody (1:5000) coupled to horseradish peroxidase. The ECL kit procedure was followed when performing the detection.

Lentivirus shRNA Transfection

The Ebp1 short hairpin (sh) RNA lentivirus and the standard lentivirus were procured from Shanghai Jikai Gene Chem Co., Ltd. The information of the primers was as follows: PA2G4: uptream:5'-CAGGAGCAAACTATCGCTGAG-3', downtream:5'-GGACCGAAGTACCCTGTTGG-3. Following the manufacturer's instructions (Sigma Aldrich), the A549 and PC9 cells were transfected using an Invitrogen Lipofectamine 3000 kit. The cells were arranged in a 6-well plate with 1.5×105 cells per well, incubated for 24 hours, and then exposed to infectious lentiviral supernatant and a medium containing 8 μg/mL of hexadimethrine bromide at a 1:1 ratio. Following a week of puromycin selection (at concentrations of 2μg/mL, after conducting a test that depended on the dosage of the substance, the cells were broken down and the technique of Western blotting was employed to show the inhibition of Ebp1.

Wound Healing Assay

In 24-well plates, cells were sown and left to adhere for the entire night. To eliminate the nonadherent cells, use a 200 μL pipette tip and scrape the area before washing it with PBS. Using an inverted microscope, cell migration to the wound area was seen at 0 and 48 hours.

Transwell Assays

The activity of cell invasion was assessed using the Transwell Invasion Assay. With free serum medium, cell lines were injected into the top chamber. After being added to the lower chamber, the medium (10% FBS, Invitrogen) served as a chemoattractant. For 48 hours, the cell lines were incubated at 37°C with 5% CO2 to induce invasion. Later, the cells that had successfully invaded the lower surface of the filter were temporarily immobilized for 30 minutes through exposure to 70% ethanol. After that, the cell lines were stained for ten minutes with crystal violet (0.1%). Five randomly selected visual regions were examined under an inverted microscope to count the cells that went to the bottom. There were three iterations of the experiment.

Colony Formation Assay

Six-well plates were loaded with 1×10^3 cells in each well, and then incubated at 37 °C for two weeks. For fixation, 4% paraformaldehyde was applied to the cells, and Giemsa stain was applied for a duration of 30 minutes. After a thorough rinse with tap water, colony count commenced, only including those with a cell count of more than 50.

Cell Cycle Analysis

Flow cytometry was used to examine the cell cycle of A549 and PC9 cell lines. Trypsin digestion was employed to prepare the cell suspension. The cells were washed twice with pre-chilled PBS, followed by centrifugation and removal of the supernatant. The cells were then resuspended by pipetting to achieve a fully suspended state. Subsequently, the A549 and PC9 cell suspensions were added to pre-chilled anhydrous ethanol and fixed at 4°C for 20 hours. After fixation, the cells were centrifuged to remove the ethanol, resuspended, and stained with PI solution. The mixture was gently

vortexed with a pipette and incubated in the dark for 30 minutes. After completion, the samples were analyzed using a flow cytometer, and the data were saved for subsequent processing.

In vivo Experiments

Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) provided ten 4-week-old female nude mice. The institutional guidelines for the treatment and use of laboratory animals at Yanbian University School of Medicine were adhered to, and all animals were housed in designated pathogen-free housing with enough of food and water. The Yanbian University Ethics Committee gave its approval to the protocols for the use and care of animals. 100 microliters of a solution, comprising 5 million PC9 cells (shNC; shEbp1) in suspension with PBS and matrigel, was implanted on the sides of female nude mice. The tumors were implanted in paraffin after being treated with 4% paraformaldehyde, and the mice were euthanized on day 56. After being anesthetized with an intravenous injection of sodium pentobarbital at a dosage of 70 mg/kg body weight, all mice were killed by cervical dislocation. The cessation of breathing and heartbeat for more than five minutes verified death. Every technique was used in compliance with the applicable rules and regulations. The ARRIVE criteria were followed in this investigation. The institutional review board gave its approval to the protocol and the informed consent statement. The Yanbian University Ethics Committee gave their approval to the trial.

Statistical Analysis

A correlation study was conducted using the Pearson chi-square test. The researchers utilized the Kaplan-Meier method to assess overall survival (OS) and employed the Log rank test to make comparisons. For the multivariate analysis, they utilized the Cox proportional hazards model. Analyze each factor's HR and 95% confidence intervals (CI). The data is presented using the mean ± standard deviation format. Group differences were examined using one-way analysis of variance (ANOVA). When $P < 0.05$, a difference was considered significant. To do the statistical analysis, SPSS 25.0 and GraphPad Prism 8.0 were utilized.

Results

Expression of Ebp1 p48 in NSCLC

Data obtained from the Oncomine database (included 58 normal lung tissues and 58 lung adenocarcinoma of Selamat data sets; included 30 normal lung tissues and 527 lung adenocarcinoma of Su data sets) showed that Ebp1 mRNA is overexpressed in NSCLC (Figure 1A and B). To further confirm, IHC staining was undertaken. Compared with Normal lung tissue, Ebp1 p48 showed strongly positive staining in NSCLC (Figure 1C). We then analyzed Ebp1 expression levels by Western blot in 16HBE of Bronchial epithelial cell and four different NSCLC cell lines, A549, H522, PC9, H650. Compared to 16HBE cells, four cancer cell lines had significantly greater Ebp1 p48 expression levels (Figure 1D). Together, these data implied that Ebp1 p48 is overexpressed in NSCLC tissues and NSCLC cell lines.

Ebp1 p48 Expression Is Related with Unfavorable Clinicopathological Features and Poor Prognosis in NSCLC

In order to investigate Ebp1's potential therapeutic use in non-small cell lung cancer, we conducted an analysis of Ebp1 expression via IHC labeling utilizing tissue microarray (TMA) in tissue samples obtained from 94 patients with primary human NSCLC. 64 samples from patients with non-small cell lung cancer tested positive for Ebp1 p48, as shown in Table 1. We discovered a correlation between Ebp1 p48 overexpression and lymph node metastases (P=0.001), histological grade (P=0.021), and clinical stage (P=0.016), as indicated in Table 2.

A worse prognosis for individuals with non-small cell lung cancer was associated with high expression of Ebp1 mRNA, according to an analysis of the survival curve in the TCGA database (Figure 1E). In order to determine if low prognosis in NSCLC patients was predicted by Ebp1 p48 expression, Kaplan–Meier analysis was employed. It was discovered that there was a strong correlation between low overall survival (OS; P=0.001) and Ebp1 p48 expression in NSCLC patients (Figure 1F). Furthermore, the following were shown to be significant poor prognostic variables for OS in patients with non-

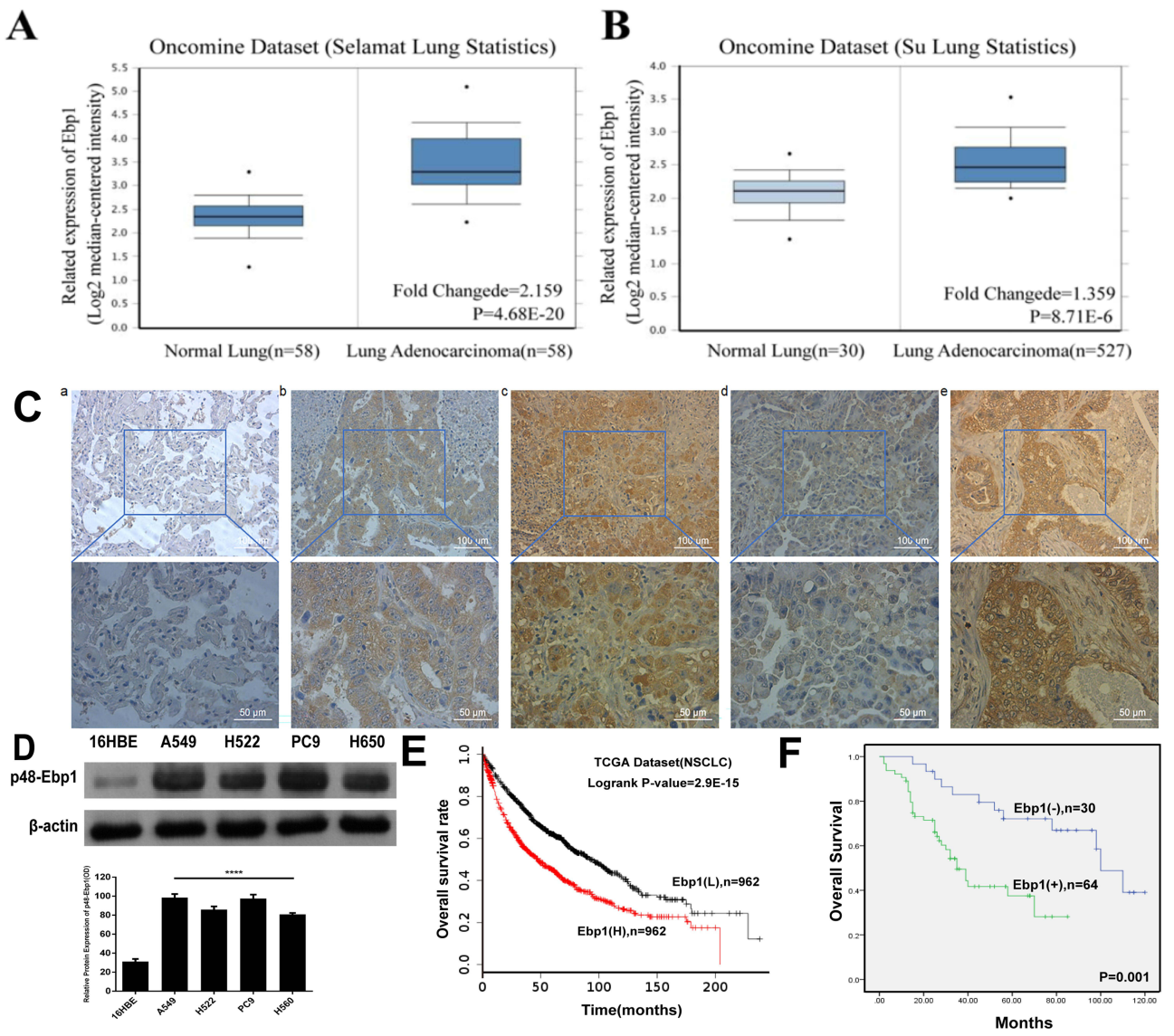

Figure 1 The expression of Ebp1 p48 is upregulated in NSCLC tissues and cells and closely related with patient prognoses. (**A**) Selamat data sets showed that Ebp1 mRNA is overexpressed in NSCLC. (**B**) Su data sets showed that Ebp1 mRNA is overexpressed in NSCLC. (**C**) Immunohistochemical staining of Ebp1 p48 expression in normal lung tissue and NSCLC tissues: a) adjacent cancer tissues; b) clinical stage I NSCLC tissues; c) clinical stage II–IV NSCLC tissues; d) without lymph node metastasis of NSCLC tissues; e) with lymph node metastasis of NSCLC tissues; (magnification 200×, scale bar=100μm; magnification 400×, scale bar=50μm).(**D**) Expression of p48 ebp1 in each cell line (****P<0.0001 vs A549, H522, PC9, H560 group, n=3). (**E**) TCGA database analyzed the relationship between the expression of Ebp1 in NSCLC and OS. (**F**) Kaplan-Meier analysis of the expression of Ebp1 p48 is closely related to the overall survival of NSCLC patients.

small cell lung cancer (NSCLC): histological grade (P=0.002 for OS), clinical stage (P=0.018 for OS), lymph node metastases (P=0.008 for OS), and Ebp1 p48 expression (P=0.000 for OS, Table 3). These findings were obtained using univariate Cox regression analysis. Histological grade (P=0.018) and Ebp1 p48 expression (P=0.010) were found to be

Table 1 Immunohistochemistry Analysis of Ebp1 Expression in NSCLC

		Ebp1 Expression (%)		
Group	**N**	**Low**	**High**	***P* value**
Normal	86	65(75.6%)	21(24.4%)	*P*<0.05
Cancer	94	30(31.9%)	64(68.1%)	

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Table 2 Comparison of Clinicopathologic Characteristics According to the Ebp1 Expression in Non-Small Cell Lung Cancer

Variable	n	Ebp1 (-) n(%)	Ebp1 (+) n(%)	X^2	*P*
Age(years)				0.047	0.828
<65	61	19(31.1)	42(68.9)		
≥65	33	11(33.3)	22(66.7)		
Sex				0.001	0.970
Male	53	17(32.1)	36(67.9)		
Female	41	13(31.7)	28(68.3)		
Tumor location				0.234	0.628
Left	41	12(29.3)	29(70.7)		
Right	53	18(34.0)	35(66.0)		
Tumor size				0.287	0.592
<3	35	10(28.6)	25(71.4)		
≥3	59	20(33.9)	39(66.1)		
Grade				5.304	0.021
G1-2	63	25(39.7)	38(60.3)		
G3	31	5(16.1)	26(83.9)		
Clinical stage				5.776	0.016
I	31	15(48.4)	16(51.6)		
II~IV	63	15(23.8)	48(76.2)		
Lymph node metastasis				10,445	0.001
Negative	43	21(48.8)	22(51.2)		
Positive	51	9(17.6)	42(82.4)		

Table 3 Cox Proportional Risk Regression Model Was Used to Analyze the Influencing Factors of Survival of 94 Patients with NSCLS

Variable	HR	95% CI	*P*
Gender	1.080	0.668–1.768	0.748
Age, Years	0.920	0.593–1.428	0.705
Tumor location	0.948	0.592–1.516	0.826
Tumor size(cm)	0.925	0.579–1.475	0.747
Histological grade	0.392	0.240–0.658	0.002*
TNM Clinical stage	0.541	0.330–0.894	0.018*
LN metastasis	0.520	0.328–0.838	0.008**
Ebp1	0.196	0.101–0.365	0.0004**
Multivariate			
Histological grade	0.495	0.272–0.893	0.018*
TNM Clinical stage	0.825	0.384–1.776	0.618
LN metastasis	0.913	0.405–2.052	0.825
Ebp1	0.335	0.142–0.785	0.010*

Notes: *P<0.05; ** P<0.01.
Abbreviation: HR, Hazard ratio.

independent predictors of poor OS by multivariate Cox regression analysis (Table 3). These findings imply that Ebp1 p48 expression may be a predictor of a poor outcome in patients with non-small cell lung cancer.

Ebp1 p48 Knockdown Suppresses Proliferation in NSCLC Cells

Ebp1 p48 expression was elevated in A549 and PC9 cells, as seen in Figure 1D. Thus, we used Western blotting to examine the transfection effectiveness after knocking down Ebp1 p48 in A549 and PC9 cells (Figure 2A). Next, we

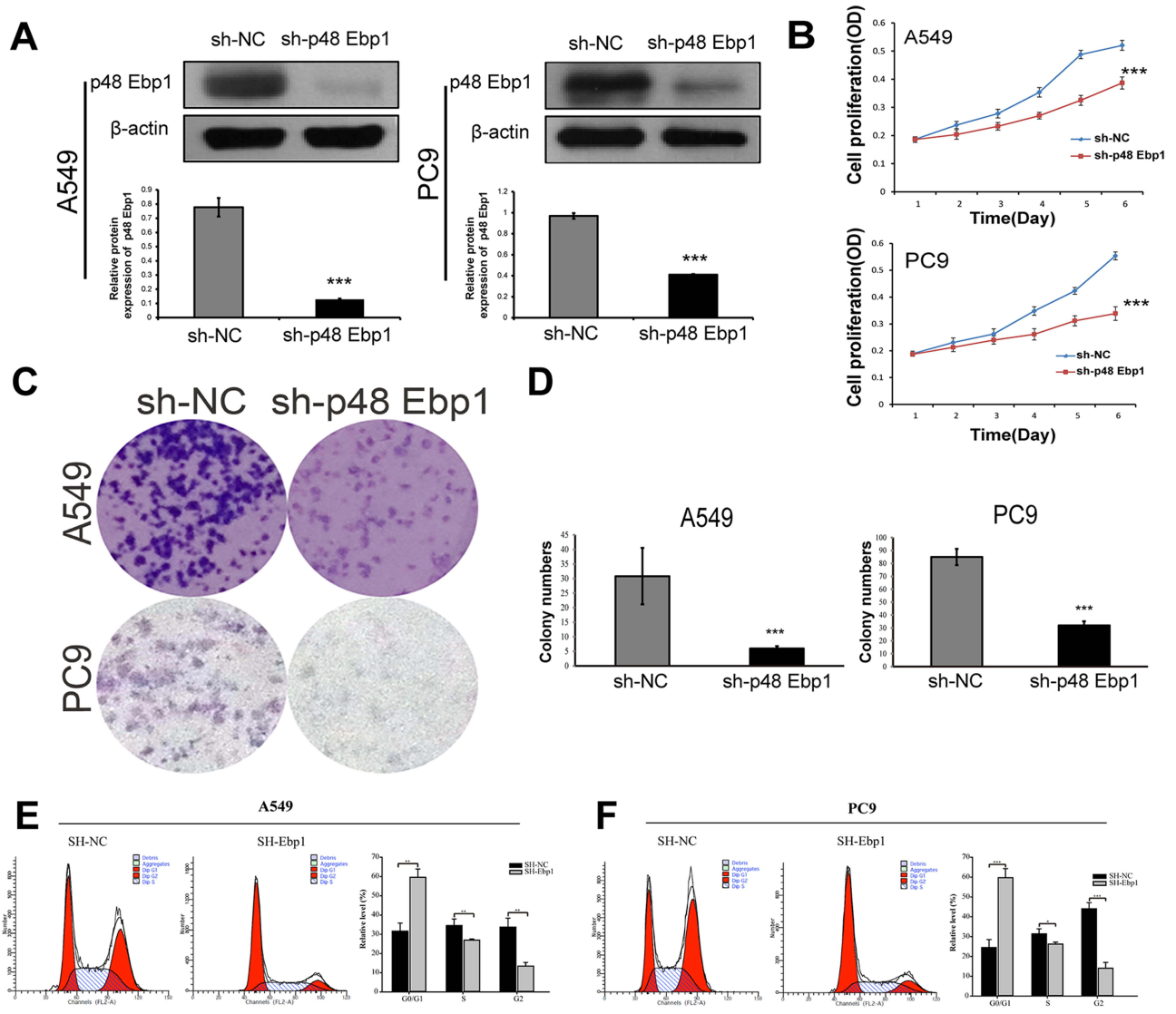

Figure 2 Knockdown of Ebp1 p48 suppresses the growth and proliferation of NSCLC cells. (**A**) The protein expression level of Ebp1 p48 in A549 and PC9 cells, which were infected with shRNA or negative control (NC) of Ebp1 p48. (**B**) CCK8 assay showed that the cell viability of A549 and PC9 were downregulated by sh-Ebp1 p48 (***P<0.001 vs shNC group, n=4). (**C** and **D**) Colony forming assays showed that silencing of Ebp1 p48 suppressed the colony forming ability of cells (***P<0.001 vs shNC group, n=3). (**E** and **F**) Cell cycle analysis was used to detect the cell cycle distribution after transfection (***P<0.001, **P<0.01, *P<0.05 vs shNC group, n=3).

investigated Ebp1 p48's function in NSCLC cell growth. We discovered that Ebp1 p48 knockdown can dramatically reduce A549 and PC9 cell proliferation using CCK-8 assay (Figure 2B). Additionally, it was shown that Ebp1 p48 knockdown significantly decreased NSCLC cells' capacity to form colonies through colony formation (Figure 2C and D). To further confirm this result, we performed a cell cycle analysis. The findings demonstrated that G0/G1 phase increased in A549 and PC9 cells knocked down by Ebp1 p48, although S phase and G2/M phase decreased (Figure 2E and F).

Knockout of Ebp1 p48 Can Inhibit the Migration and Invasion of NSCLC Cells and Change the Expression of EMT-Related Proteins

Through scratch studies, we discovered that Ebp1 p48 knockdown may dramatically decrease cell migratory capacity, which allows us to better understand the biological roles of Ebp1 p48 in NSCLC cell migration and invasion (Figure 3A and B). By using the transwell invasion assay, we discovered that following Ebp1 p48 knockdown, A549 and PC9 cells' capacity for invasion was dramatically decreased (Figure 3C and D). After validating the correlation between tumor invasion and epithelial mesenchymal transition (EMT), we proceeded to ascertain the levels of EMT-related proteins. On repressing Ebp1 p48

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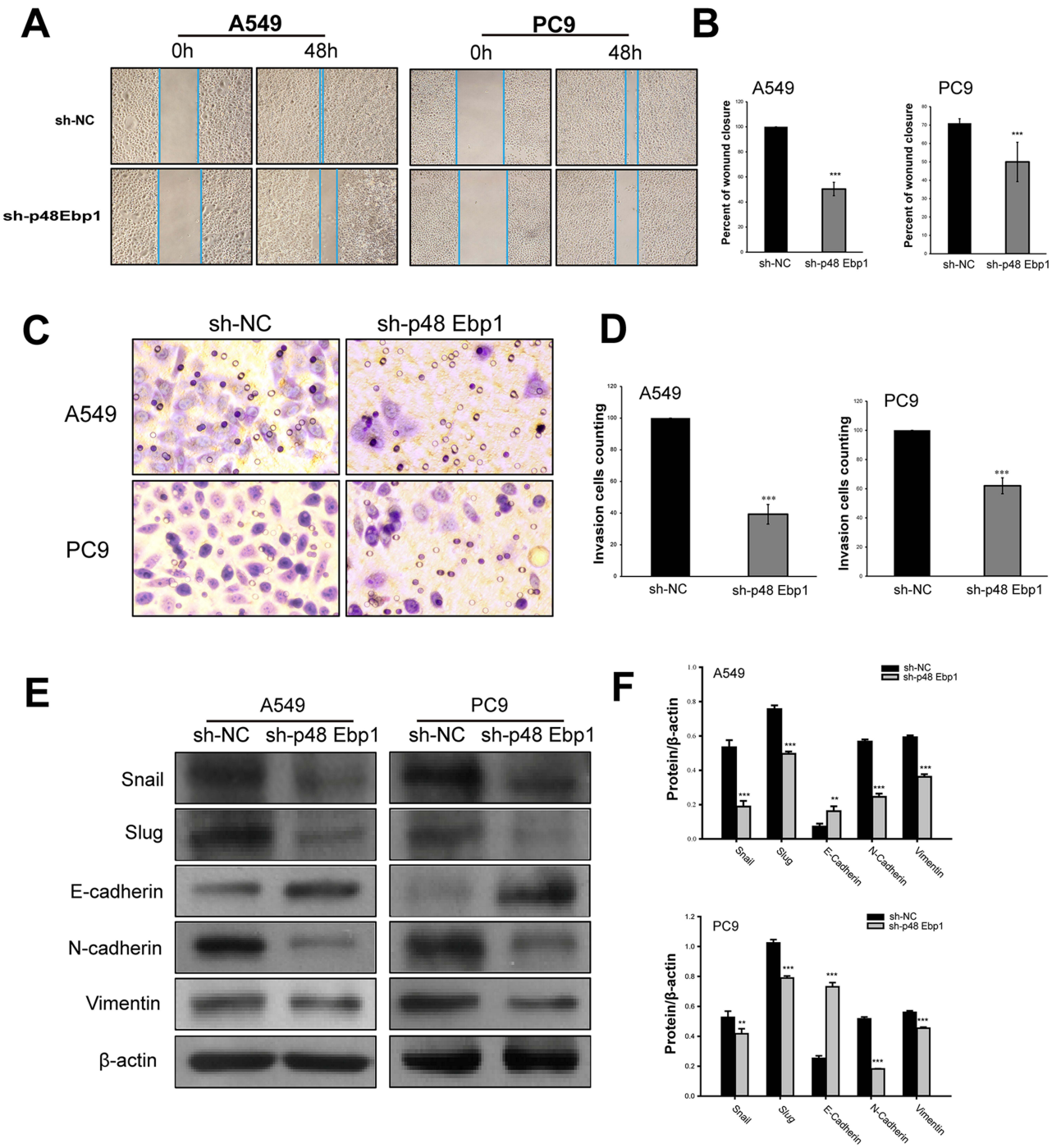

Figure 3 Knockdown of Ebp1 p48 suppresses the migration and invasion of NSCLC cells. (**A** and **B**) Knockdown of Ebp1 p48 decreased the healing ability of A549 and PC9 cells (***P<0.001 vs shNC). (**C** and **D**) Transwell assay tested the invasion ability of A549 and PC9 cells after Ebp1 p48 knockdown (***P<0.001 vs shNC). (**E** and **F**) Western blot analysis of Snail, Slug, E-cadherin, N-cadherin and Vimentin expression in NSCLC cells (***P<0.001 vs shNC, **P<0.01 vs shNC).

expression, the epithelial marker E-cadherin showed an increase in expression, whereas the mesenchymal markers Snail, Slug, N-cadherin, and Vimentin showed a substantial reduction (Figure 3E and F).

Ebp1 p48 Expression Activates the PI3K/Akt Signaling Pathway in NSCLC

We looked into the relationship between Ebp1 p48 expression and PI3K/Akt pathway activity in NSCLC to see if it is connected to the signaling pathway. We used shRNA to knock down p48-Ebp1 and perifosine (1.5μM) and LY294002

(2.5μM) to block the PI3K/Akt pathway in A549 and PC9 cells. Ebp1 p48 knockdown A549 and PC9 cells showed decreased levels of phosphorylated PI3K-p110 and Akt (Thr308 and Ser473). However, the results showed that, in comparison to the control group, there were no discernible differences in the expression of total PI3K and total Akt (Figure 4A and B). The findings imply that Ebp1 p48 controls the proteins involved in the PI3K/Akt pathway and that PI3K/Akt activation may be necessary for the development of tumors overexpressing p48-Ebp1.

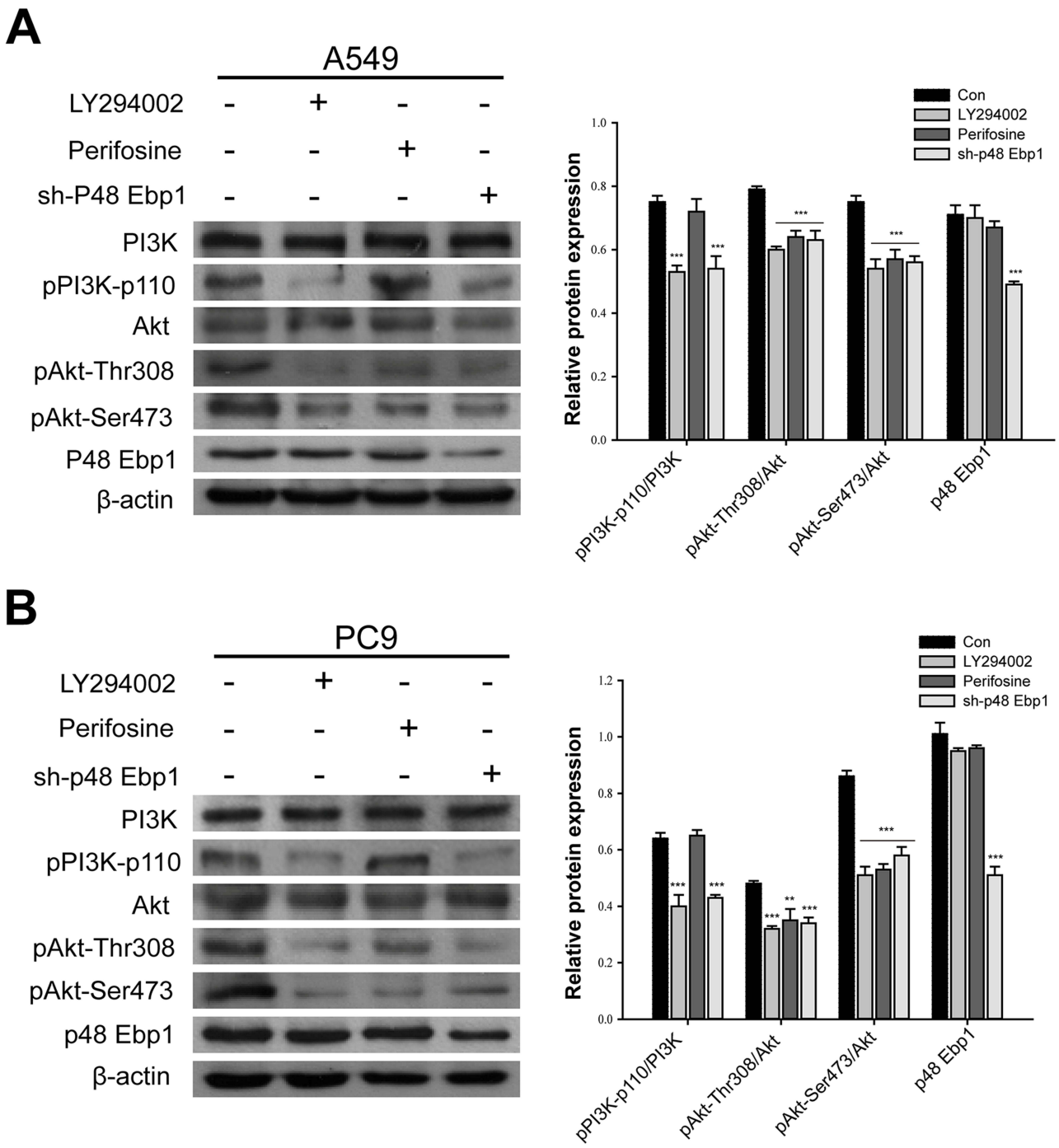

Figure 4 Ebp1 p48 knockdown reduced the phosphorylation level of PI3K/Akt in NSCLC cells. (**A** and **B**) Western blotting analysis of PI3K, pPI3K-p110, Akt, pAkt-Thr308, pAkt-Ser473, and Ebp1 p48 expression in NSCLC cells after treatment with PI3K inhibitor LY294002, AKT inhibitor Perifosine and transfected with p48-Ebp1sh RNA. Western blot data were normalized to β-actin. The expression as fold changes relative the control group (**P < 0.01, ***P < 0.001 vs control group).

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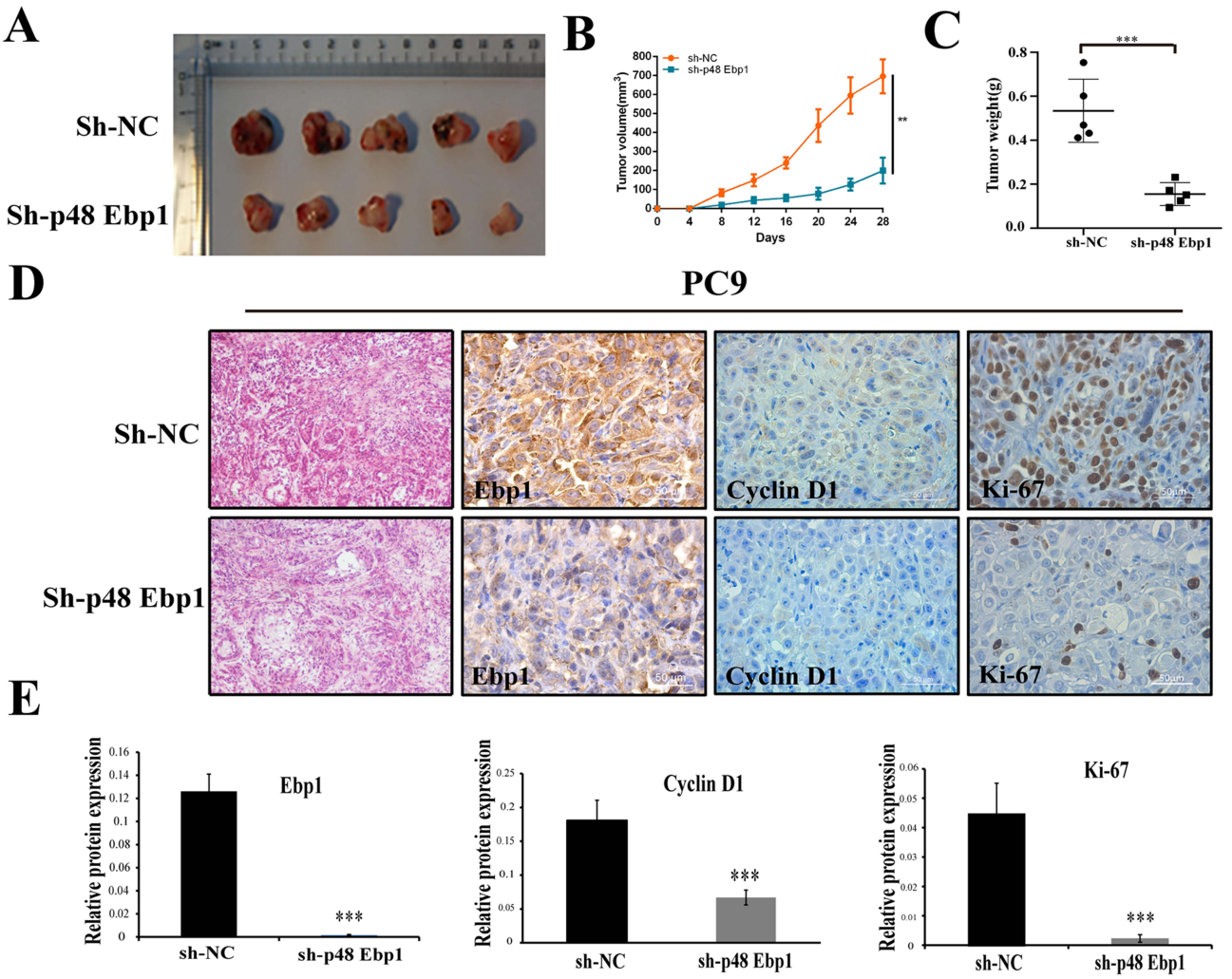

Figure 5 Ebp1 p48 knockdown suppresses PC9 cell tumorigenesis in vivo. (**A**) Formation of subcutaneous tumors in rodents. (**B**) Tumour volume was calculated at the indicated intervals (**P<0.01 vs shNC). (**C**) Comparison of tumor weight of nude mice in each group (***P<0.001 vs shNC). (**D** and **E**) The tumors of nude mice in the two groups were subjected to H&E staining and immunohistochemical (Ebp1 p48, CyclinD1 and Ki-67) (***P<0.001 vs shNC; original magnification 400 ×, scale bar =50 μm).

Knockdown of Ebp1 p48 Attenuates Tumor Growth in Vivo

To verify Ebp1 p48's role in in vivo carcinogenesis, we injected PC9 sh-NC and PC9 sh-ebp1 p48 cells subcutaneously into the shoulders of nude mice. After 28 days, the sh-NC group's tumors were noticeably bigger and more massive than those in the sh-EBP1 p48 group (Figure 5A–C). Furthermore, utilizing IHC analysis and H&E staining, the Ebp1 knockdown group's expression of the Ki-67, CyclinD1, and Ebp1 p48 proteins was noticeably lower than that of the control group (Figure 5D and E).

Discussion

The opposite effect of the two subtypes of Ebp1 in cancer has become an attractive subject of investigation in recent years.[8–10] The oncogenic role of Ebp1 p48 was identified in several types of cancers including hepatocellular carcinoma,[11] glioblastoma,[12] colon cancer.[13] Ebp1 p48's involvement in NSCLC has not been shown, nevertheless. Ebp1 p42 has a tumor suppressor function in NSCLC, according to Ahn et al.[7] Research indicates that the most common form of Ebp1 in mammalian cells is Ebp1 p48. As a result of ubiquitin-mediated degradation in a variety of human malignancies, Ebp1 p42 is very hard to find. This could be related to the instability of the protein structure, which is brought on by p42's lack of 54 amino acid residues.[14,15] Thus, we think that more research into the function of Ebp1 p48 in NSCLC is still required.

Through the examination of NSCLC tissue microarrays, we observed a strong correlation between poor patient prognosis and high expression of Ebp1 p48, indicating its significant role in NSCLC development. Our research demonstrated that inhibiting Ebp1 p48 notably decreased NSCLC cell migration, invasion, and proliferation. Further cell cycle analysis revealed that Ebp1 p48 enhances NSCLC cell proliferation by regulating the cell cycle. EMT has been implicated in tumor invasion and metastasis.[16] The downregulation of the EMT-related transcription factors and the epithelial marker E-cadherin is what sets it apart. Slug and Snail.[17] Our findings suggest that Ebp1 p48 may promote NSCLC cell invasion and migration by influencing EMT. Specifically, Ebp1 p48 knockdown led to upregulation of E-cadherin, a hallmark of epithelial cells, and downregulation of mesenchymal markers, including N-cadherin, Vimentin, Snail, and Slug. These changes reversed EMT and inhibited the migration and invasion of NSCLC cells. This research highlights the pivotal role of Ebp1 p48 in enhancing NSCLC cell proliferation and metastasis, primarily through regulating the EMT process and the cell cycle. Thus, targeting Ebp1 p48 may provide a promising strategy to suppress NSCLC progression, offering potential therapeutic benefits for improving patient prognosis. According to recent studies, the PI3K/Akt signaling system regulates growth, metastasis, angiogenesis, metabolism, and survival.[18,19] Additionally, it has been shown that lung cancer develops as a result of activation of the PI3K/Akt signaling pathway.[20,21] In this investigation, we discovered that knocking down Ebp1 p48 in A549 and PC9 cells led to a significant decrease in the expression levels of pPI3K-p110, p-Akt-Thr308, and p-Akt-Ser473. These proteins are key components of the PI3K/Akt signaling pathway, which is crucial for regulating various cellular processes, including cell growth, survival, motility, and metabolism. Our findings strongly suggest that Ebp1 p48 plays a critical role in the activation of the PI3K/Akt signaling pathway, thereby promoting NSCLC cell motility, invasion, and proliferation. This evidence supports the idea that Ebp1 p48, through the PI3K/Akt signaling pathway, enhances the invasive potential and proliferative capacity of NSCLC cells, highlighting its potential as a therapeutic target for limiting NSCLC metastasis and growth. Further studies will be needed to explore the exact mechanisms by which Ebp1 p48 regulates the PI3K/Akt pathway and how it could be leveraged in the development of targeted treatments for NSCLC.

In summary, we discovered that Ebp1 p48 was necessary for the invasion, migration, and proliferation of NSCLC cells. Ebp1 p48 may stimulate EMT and the PI3K/Akt signaling pathway to aid NSCLC cells in invasion, migration, and multiplication. Given the central role of Ebp1 p48 in the progression of NSCLC, our findings suggest that it holds significant promise as both a prognostic biomarker and a potential therapeutic target. Clinically, measuring Ebp1 p48 expression could help identify high-risk patients with aggressive forms of NSCLC, providing an opportunity for earlier and more personalized interventions. Additionally, targeting Ebp1 p48 directly or through small molecules or RNA-based therapies could offer new avenues for treating NSCLC, particularly in patients with advanced disease or those resistant to conventional therapies. Furthermore, clinical trials assessing the therapeutic potential of Ebp1 p48 inhibitors or modulators will be essential to evaluate their efficacy in real-world scenarios. The development of diagnostic tools based on Ebp1 p48 expression could also play a key role in the early detection and prognosis of NSCLC, ultimately guiding more effective and individualized treatment plans. Therefore, Ebp1 p48 stands out not only as a critical factor in NSCLC biology but also as a promising target for future therapeutic advancements, potentially transforming the landscape of NSCLC treatment and improving patient survival rates.

Conclusion

Ebp1 p48 plays a key role in the proliferation, migration, and invasion of NSCLC cells, likely through EMT and the PI3K/Akt pathway. It may serve as a prognostic biomarker and therapeutic target for NSCLC. Future studies will focus on its interactions with other tumor-driving factors and explore small molecule inhibitors or RNA therapies targeting Ebp1 p48 to curb metastasis and improve prognosis. Additionally, we will examine its role in the tumor microenvironment and immune regulation. Clinically, Ebp1 p48 could be a dual tool for early NSCLC diagnosis and treatment, identifying high-risk patients for early intervention and personalized strategies.

Abbreviations

NSCLC, non-small cell lung cancer; Ebp1, ErbB3-binding protein 1; EMT, epithelial-mesenchymal transition; OS, overall survival; IHC, Immunohistochemistry; HE, haematoxylin and eosin; CI, confidence intervals; TMA, tissue microarray.

Data Sharing Statement

The data generated in the present study may be requested from the corresponding author Xiangdan Li.

Ethics Approval and Consent to Participate

The study was done in accordance with Good Clinical Practice and the ethical principles originating in the Declaration of Helsinki. The protocol and informed consent document were approved by the institutional review board. The experiment was approved by the Ethics Committee of Yanbian University (YD20240926015).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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