

Original Research

CD146 Promotes EMT-Mediated Migration and Invasion of NSCLC via PI3K/Akt Signaling Pathway

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Abstract

Background: Recurrence and metastasis are the main causes of non-small cell lung cancer (NSCLC)-related death. CD146 has been identified as a potential risk factor for poor prognosis, closely related to the distant metastasis and drug resistance in various cancers. However, the clinical significance of CD146 in NSCLC requires further investigation. **Materials and Methods:** This study explored the correlation between CD146 expression and clinical variables using tumor tissue samples collected from our hospital. CD146 expression levels in NSCLC cell lines and tissues were assessed and compared using immunohistochemistry, real-time polymerase chain reaction (RT-qPCR), flow cytometry, and western blot analysis. The invasion and migration capabilities of tumor cells were determined using transwell and wound healing assays. The levels of proteins related to epithelial-mesenchymal transition (EMT) as well as the underlying PI3K/Akt signaling pathway was measured by western blotting. **Results:** We discovered that CD146 expression is significantly associated with the EMT signaling pathway. High CD146 expression predicted lymph node metastasis, metastasis to distant organs, advanced Tumor, Node, Metastasis (TNM) staging, and poor survival in NSCLC patients. Wound healing and transwell assays showed that knocking down CD146 significantly suppressed cell migration along with cell invasion in NSCLC, whereas overexpressing CD146 notably enhanced these processes. Western blot analysis revealed significantly reduced levels of N-cadherin, vimentin, snail, twist, PI3K, and AKT phosphorylation in shCD146 H460 cells compared to vector control cells. Treatment with PI3K inhibitor PI3K-IN-1 increased E-cadherin expression levels but reduced N-cadherin, Twist, Vimentin, PI3K, and AKT phosphorylation levels in pcDNA3.1-CD146 A549 cells compared with the vector control cells. **Conclusions:** CD146 expression acts as a prognostic risk factor for adverse outcomes in NSCLC, promoting invasion and metastasis by activating the EMT through the PI3K/Akt signaling pathway. These findings underscore the potential therapeutic strategies targeting CD146, offering new treatment options for NSCLC patients, especially those at risk of metastasis.

Keywords: CD146; non-small cell lung cancer; epithelial mesenchymal transition; migration; invasion

1. Introduction

As the most common pathological type, non-small cell lung cancer (NSCLC) is the big clinical challenge all over the world and accounts for about 85% of lung cancer cases with a 5-year survival rate of only 21% [1,2], with early surgical resection still being the most effective treatment available [3]. Unfortunately, most lung cancer patients present with advanced disease and many also have distant metastases at the initial diagnosis, thus accounting for the low survival rate [4]. Consequently, there is an urgent to investigate the molecular mechanism of distant metastasis in this major cancer type [5].

CD146 was first discovered in the plasma membrane of human melanoma cells, and is therefore also called melanoma cell adhesion molecule (MCAM) [6,7]. Extensive research has revealed some of the characteristics and functions of CD146. It is a 113 kDa glycoprotein, a member of the transmembrane immunoglobulin superfamily, as well as a Ca^{2+} -independent adhesion protein. CD146 participates in a variety of biological processes [7], such as

progression of nervous system [8], kidney [9], retina [10], as well as angiogenesis [11] and lymph angiogenesis [12]. There is also evidence that CD146 is a potential therapeutic target in solid cancer, since it is closely related to tumor progression, metastasis and poor prognosis [13]. A meta-analysis of 12 studies involving 2694 patients found notable associations between the expression levels of CD146 and the overall survival (OS) (hazard ratio, HR = 2.5), and with disease-free survival (DFS) (HR = 2.45) [14]. We previously reported that CD146 protein expression was negatively related to the expression of the epithelial cell marker E-cadherin in NSCLC, and positively related to the expression of the interstitial cell marker vimentin [15]. Further investigation revealed that CD146 was significantly overexpressed in patients with NSCLC and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs)-targeted therapy resistance [16]. Moreover, cell migration and invasion were enhanced in lung cancer cells with CD146 overexpression. Tumor metastasis is known to be closely related to epithelial-mesenchymal transition (EMT)



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Table 1. Correlation between clinicopathological features and CD146 expression in patients with NSCLC.

Clinicopathological variables	Tumor CD146 expression		χ^2	<i>p</i> value
	Low (n=55)	High (n=95)		
Median age				
<65	20	46	2.055	0.152
≥65	35	49		
Gender				
Male	35	51	1.410	0.235
Female	20	44		
Pathological type				
Adenocarcinoma	30	51	0.01	0.919
Squamous carcinoma	25	44		
T stage				
T1-2	16	27	0.008	0.930
T3-4	39	68		
Lymph node metastasis				
Yes	35	80	8.242	0.004*
No	20	15		
Distant metastasis				
Yes	25	63	6.251	0.012*
No	20	38		
TNM stage				
I-II	17	14	5.557	0.018*
III-IV	38	81		

* *p* < 0.05. NSCLC, non-small cell lung cancer; T stage, Tumor stage; TNM, Tumor, Node, Metastasis.

[17,18]. During the initial phase of tumor metastasis, the EMT process is initiated either entirely or in part by tumor epithelial cells [19]. The previous studies have indicated that CD146 is involved in EMT, thus promoting tumor aggressiveness in various cancer types. Kristiansen *et al.* [20] showed that CD146 was expressed in a high proportion of NSCLC and was prognostic for worse survival in lung adenocarcinoma (LUAD) patients, consistent with the findings of Oka *et al.* [21]. In addition, Ma *et al.* [22] showed that CD146 promoted the switch of E-cadherin to N-cadherin during the process of TGF- β -induced EMT in ovarian cancer. Jiang *et al.* [23] found that CD146-induced EMT promotes invasion and metastasis and predicts the poor outcomes in hepatocellular carcinoma patients. However, the biological role of CD146 in NSCLC metastasis and the underlying mechanism has yet to be thoroughly explored.

The aim of this research was therefore to systematically investigate the expression of CD146 in NSCLC and its possible role in this tumor type. Our focus was on the relationship between CD146, tumor metastasis and EMT. The Cancer Genome Atlas (TCGA) database was first used to analyze and explore the signaling pathway by which CD146 might regulate NSCLC. Second, we analyzed association between CD146 expression and the clinical characteristics and outcome of NSCLC patients. Third, we established the models *in vitro* to explore the biologic significance of

CD146. Finally, we performed transcriptome sequencing to explore the potential mechanism by which CD146 promotes NSCLC metastasis.

2. Materials and Methods

2.1 Patients and Follow-up

LUAD samples with complete clinical information and gene expression data were obtained from The Cancer Genome Atlas Program (TCGA) database (<https://www.genome.gov/Funded-Programs-Projects/Cancer-Genome-Atlas>). To explore the underlying mechanisms related to CD146 expression, we conducted the functional enrichment analysis and signaling pathway enrichment analysis on the significant differentially expressed genes (DEGs) identified between high- and low-CD146 expression groups, as determined by the median CD146 expression value. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, gene set variation analysis (GSVA) as well as Gene Set Enrichment Analysis (GSEA) were all used to explore the underlying molecular mechanisms related to CD146 expression and the potential role of CD146 in NSCLC.

In addition, needle biopsy tissues were collected from 150 NSCLC patients treated at Zhengzhou University People's Hospital from 2017 to 2021. These patients were fol-

Table 2. Univariate and multivariate survival analysis on the clinical variables.

Clinicopathological variables	Univariate analysis			Multivariate analysis		
	HR	CI(95%)	p value	HR	CI(95%)	p value
Age (<65 vs. ≥65)	0.784	0.549–1.120	0.181	0.914	0.633–1.319	0.630
Gender (Male vs. Female)	1.020	0.712–1.462	0.914	0.822	0.483–1.398	0.469
Pathological type (LUAD vs. LUSC)	1.033	0.724–1.474	0.857	1.027	0.617–1.710	0.918
T stage (T1-2 vs. T3-4)	1.574	1.040–2.381	0.032*	1.506	0.958–2.366	0.076
Lymph node metastasis (Yes vs. No)	4.761	2.735–8.288	0.000*	2.015	1.062–3.825	0.032*
Distant metastasis (Yes vs. No)	3.245	2.170–4.853	0.000*	1.687	1.018–2.796	0.042*
TNM stage (I-II vs. III-IV)	5.325	2.837–9.988	0.000*	2.316	1.033–5.193	0.042*
CD146 expression (High vs. Low)	2.838	1.892–4.257	0.000*	2.439	1.583–3.758	0.000*

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; T stage, Tumor stage; TNM, Tumor, Node, Metastasis; HR, hazard ratio; CI, confidence interval. * p < 0.05.

lowed up for five years after diagnosis. The CD146 expression level of each sample was evaluated by immunohistochemistry, with samples then assigned to either the high- or low-CD146 expression groups by the median value. Our study was evaluated and approved by the Ethics Review Committee of Zhengzhou University People's Hospital and adhered to all relevant ethical and legal standards (Approval No 2021-27). The included NSCLC patients signed the informed consent for all the images, other personal and clinical data in this study.

2.2 Cell Lines and Establishment of Stable Cell Clones

The human lung cancer cell lines H460, A549, H1975, H1299, H661, SK-MES-1, H226 and PC9 were acquired from the cell bank of the Chinese Academy of Science (Beijing, China). They were cultured in the Dulbecco's Modified Eagle Medium (DMEM)/RPMI 1640 medium containing 10% fetal bovine serum (Gibco, Waltham, MA, USA). Lentiviruses for CD146 overexpression (Lv-CD146) or CD146 silencing (Lv-shCD146) were acquired from Genechem Co. Ltd (Shanghai, China). NSCLC cells were infected with Lentivirus in the range of 5 to 20 multiplicity of infection (MOI) in polybrene (10 µg/mL). The pcDNA3-CD146 plasmid was utilized to overexpress CD146 in A549 cell lines. The H460 cell line expresses high levels of CD146 and was infected with Lv-shCD146 to produce H460-shRNA cells. After 72 hours of infection, cells were acquired for the subsequent experiments by growing in the presence of puromycin (5 µg/mL) for 1 week. All cell lines were authenticated using short-tandem repeat (STR) DNA fingerprinting (Baseclear, Leiden, The Netherlands) and routinely tested for mycoplasma by polymerase chain reaction (PCR). All cell lines were mycoplasma-free.

2.3 Cytometric Analysis of Cellular Protein Expression

The CD146 expression level in H460, A549, H1975, H1299, H661, SK-MS-1, H226 and PC9 cells was analyzed using flow cytometry. Cells in the phase of logarithmic growth were collected, washed and resuspended in PBS. PE-IgG and PE-CD146 antibodies were added to the cell

suspension (1×10^6 cells/20 µL) and incubated on ice for 40 minutes in the dark. Then, the cells were collected by centrifuge and washed twice with phosphate buffered solution (PBS). They were then filtered with 200-mesh nylon and analyzed by FACS cytometry (BD Biosciences, New York, NY, USA). FlowJo software (v10.6.2, BD Biosciences) was used to determine the mean fluorescence intensity (MFI) of CD146 expression.

2.4 CD146 Detected by Immunofluorescence

H460 and A549 cells in the phase of logarithmic growth were seeded into 24-well plates at a density of 1×10^5 cells/well and grown overnight. Subsequently, the samples were gathered and rinsed using PBS, stabilized in 4% paraformaldehyde for a duration of 10 minutes, made permeable with 0.1% Triton X-100 for 10 minutes, obstructed using 1% Bovine Serum Albumin (BSA) for one hour, and thereafter subjected to incubation with rabbit primary antibody against CD146 (ab75769, Abcam, Cambridge, MA, USA, at a dilution of 1:250) for three hours at ambient temperature. The cells washed twice by PBS were incubated with red fluorescence second antibody (A-21245, Invitrogen, Carlsbad, CA, USA) and then stained with DAPI. Coverslips underwent preparation followed by examination with a laser scanning confocal microscope (Carl Zeiss Meditec AG, Oberkochen, Germany), which was outfitted with an Olympus IX81 digital camera (Olympus Corporation, Tokyo, Japan) and utilized a 20/0.75 numerical aperture objective for imaging.

2.5 Wound Healing and Transwell Assays

Wound healing assay analysis was performed to evaluate the ability of tumor cell migration. The cells were grown in culture plates and a straight scratch was made using a 10 µL pipette tip. Photographs were captured at multiple intervals (0 hour, 12 hours, 24 hours, and 48 hours) after the wound creation. The width of the wound was measured using an ocular ruler to ensure uniformity across all wounds at the initial time point.

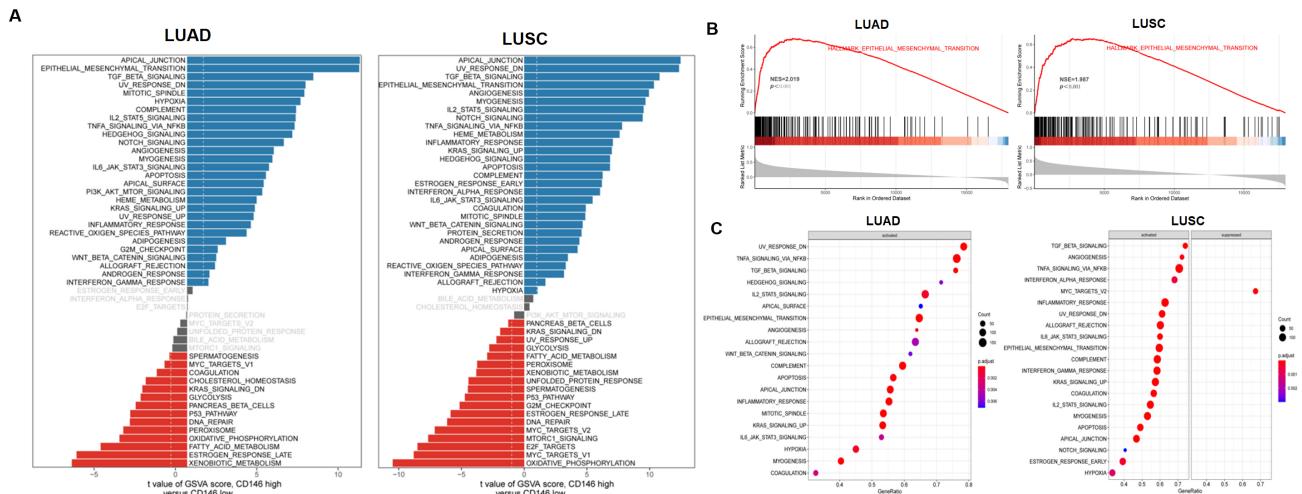


Fig. 1. Potential CD146-regulated signaling pathways. (A) Gene set variation analysis (GSVA), and (B) Gene Set Enrichment Analysis (GSEA) of correlations between CD146 expression and epithelial-mesenchymal transition (EMT)-related gene signatures in The Cancer Genome Atlas (TCGA) non-small cell lung cancer (NSCLC) dataset. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of CD146-related pathways in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) datasets from TCGA database.

For evaluating cell invasion, Transwell cell culture inserts were utilized in a 24-well plate. A total of 2×10^4 cells were incubated in the upper chamber with 200 μ L of serum-free medium, while, in the lower chamber, 800 μ L of medium containing 20% FBS was placed. Following a 24 h incubation period, cells on the underside of the insert were fixed using 100% methanol and subsequently stained with 0.5% crystal violet.

2.6 Western Blotting

Cells grown on a 10 cm dish were thoroughly washed once with PBS and whole-cell lysates were then extracted using RIPA lysis buffer (Solabio, Beijing, China). Following centrifugation, the resulting supernatants containing protein lysates were collected and the protein concentration assessed using a bicinchoninic acid (BCA) Protein Measurement Kit (Beyotime, Shanghai, China). Equal quantities of the proteins from the whole-cell lysate were first subjected to the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride membranes. The membranes underwent a blocking process for 1 hour in milk (0.1 g/mL), then were incubated in the refrigerator with the primary antibody at 4 °C overnight. Subsequently, they were rinsed thrice with Tris-buffered saline (TBS)-Tween and further subjected to a 1-hour incubation with the secondary antibody. An Electrochemiluminescence (ECL) detection reagent (Solabio, Beijing, China) was used to visualize the signal. The primary antibodies used here are listed in **Supplementary Table 1**.

2.7 Immunohistochemistry

Diaminobenzidine detection kit (Maixin-Bio Co. Ltd., Fuzhou, China) was utilized to conduct immunohistochem-

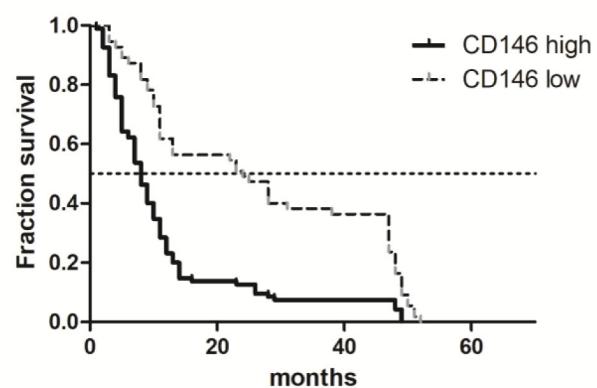


Fig. 2. The prognostic value of CD146 expression in NSCLC patients. Kaplan-Meier survival analysis showing that patients with NSCLC and high CD146 expression had a significantly shorter survival time.

ical staining based on the manufacturers' instructions. In brief, NSCLC tissues were subjected to standard histological processing of dewaxing and rehydration. To restore antigenicity, the sections were heated for two minutes in a pressure cooker. Next, endogenous peroxidase activity was blocked by treating the sections with 3% H₂O₂ for ten minutes, followed by incubation with the goat serum for ten minutes to prevent non-specific antibody binding. The section was then incubated in the primary antibody for one hour at 37 °C following by incubation with biotin-labeled secondary antibody for 10 minutes and streptavidin-peroxidase conjugate for an additional 10 minutes. To visualize peroxidase activity, a 0.02% solution of diaminobenzidine was used as the chromogen. Subsequently, the section

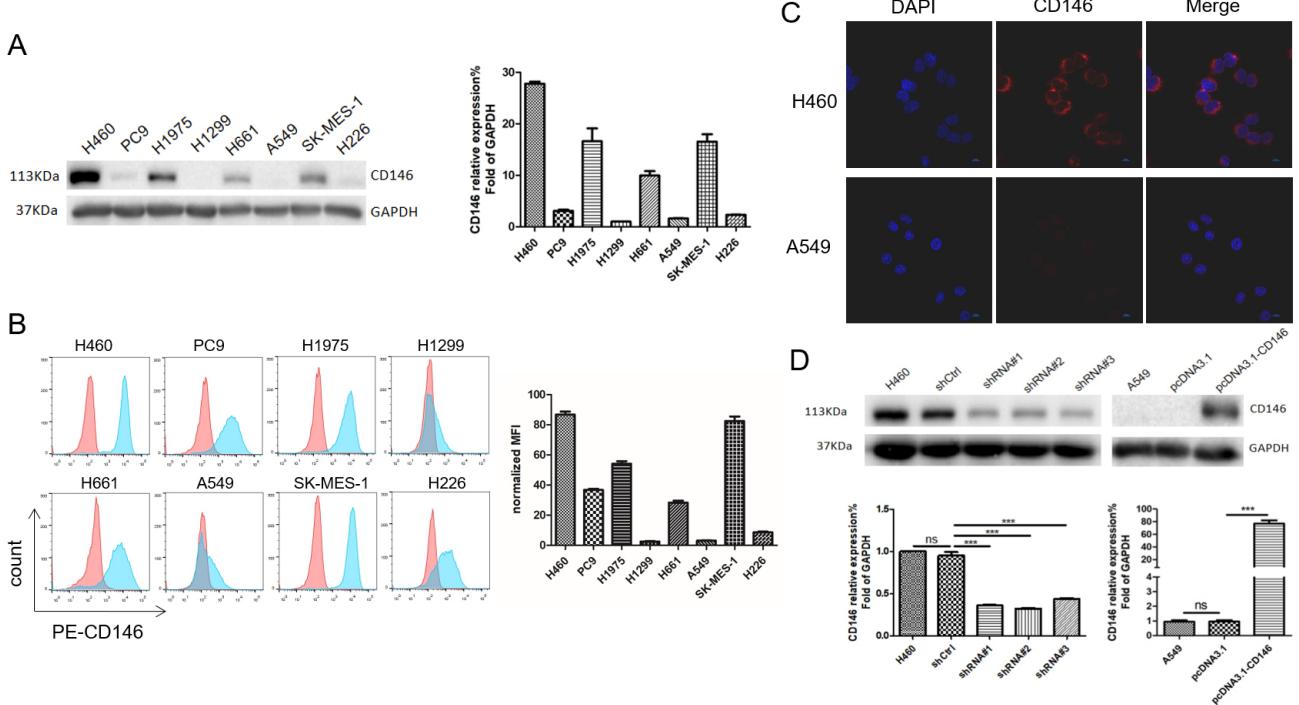


Fig. 3. Construction of NSCLC cell lines with stable CD146 overexpression or silencing. (A) Western Blot, and (B) flow cytometry analysis of CD146 expression in NSCLC cell lines. (C) Immunofluorescence analysis of CD146 expression levels in H460 and A549 cells, and cellular localization. (D) Construction of cell lines with high or low CD146 expression. Gray scale analysis of CD146/GAPDH. ns, not significant, *** $p < 0.001$.

was lightly counterstained with the hematoxylin, mounted using Permount, and examined under light microscope.

2.8 Statistical Analysis

All the statistical analyses in our study were carried out using SPSS 18.0 software (IBM Corp., Chicago, IL, USA). The results were shown as the mean \pm standard error of mean. Quantitative data and categorical data were analyzed by two-tailed Student's *t*-test as well as the χ^2 test, respectively. Kaplan-Meier with log-rank analysis were used to determine the survival between the two subgroups. A *p*-value ≤ 0.05 was considered to represent statistical significance.

3. Result

3.1 CD146 is Associated with EMT in NSCLC

To explore the potential molecular mechanism involving CD146, the RNA-seq profiles and clinical data of LUAD and lung squamous cell carcinoma (LUSC) samples were obtained from TCGA database. GSVA and GSEA analyses were then performed on the DEGs found between the high- and low-CD146 expression groups using R studio in R software (version 4.1.3, RStudio, Inc., Boston, MA, USA). In LUAD, 28 signaling pathways were found to be positively associated with CD146 expression, and 14 pathways were negatively associated with CD146 expression. In LUSC, 29 positively related-pathways and 18 neg-

atively related-pathways were found to be associated with CD146 expression (Fig. 1A). In both LUAD and LUSC, CD146 expression was involved in signaling regulation of the EMT pathway. GSEA analysis showed a positive association between the CD146 expression and EMT in NSCLC (Fig. 1B). This result was consistent with those obtained from KEGG analysis showing that CD146 expression was related to TGF- β , EMT, cell connection and other pathways, all of which are closely associated with tumor metastasis (Fig. 1C).

3.2 CD146 Expression Predicts Poor Outcomes of NSCLC Patients

A total of 150 NSCLC tissue samples confirmed by pathologic diagnosis were collected. Immunohistochemical staining was conducted to assess the expression level of CD146. Samples were assigned to high- ($n = 95$) or low-CD146 ($n = 55$) expression groups separated by the median expression value. The associations between CD146 expression and the clinical features of NSCLC and patient demographics were shown in Table 1. High CD146 expression was notably related to advanced TNM stages, including lymph node metastasis, and distant metastasis ($p < 0.05$), but no significant correlations were observed with age, gender, histopathological type, or tumor size.

We next analyzed whether CD146 expression was the risk factor for OS. Multivariate analysis revealed that high

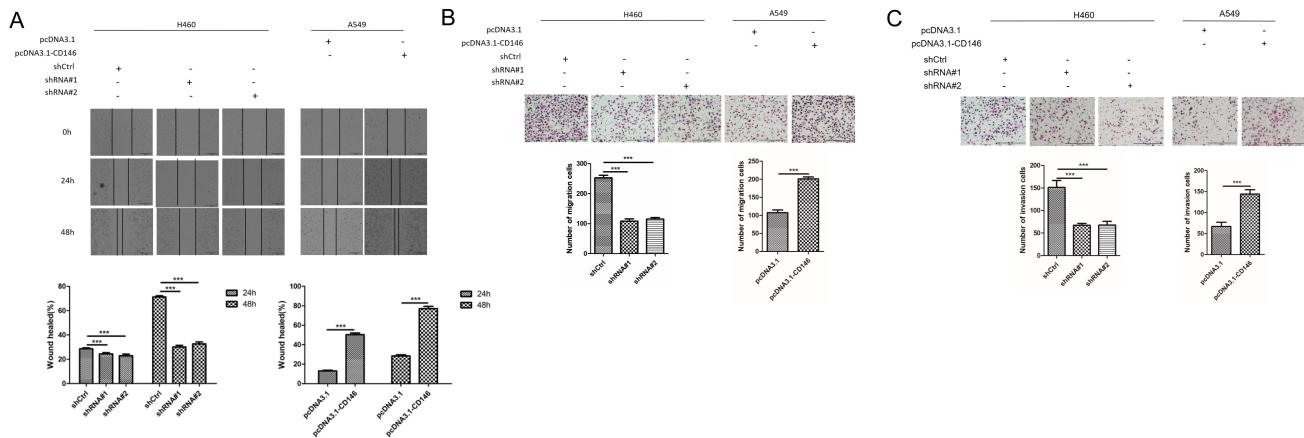


Fig. 4. *In vitro* migration and invasion assays. (A) Wound healing assays showed that inhibition of CD146 expression significantly reduced H460 cell migration, whereas CD146 overexpression increased the migration of A549 cells ($n = 3$, *** $p < 0.001$, Scale bars: 500 μm). (B) Transwell assay showed that CD146 silencing decreased the invasion of H460 cells, and CD146 overexpression increased invasion of A549 cells. *** $p < 0.001$. Scale bars: 500 μm . (C) Transwell assay showed that CD146 silencing decreased the migration of H460 cells, and CD146 overexpression increased migration of A549 cells. *** $p < 0.001$. Scale bars: 500 μm .

CD146 expression as well as TNM stage was the risk factor for worse survival of NSCLC patients (Table 2). Kaplan-Meier survival analysis indicated that patients with NSCLC and high CD146 expression had shorter OS (Fig. 2).

3.3 CD146 Promotes Tumor Cells Migration and Invasion in NSCLC

To assess its biological function in NSCLC, the expression level of CD146 in the eight NSCLC cell lines was first evaluated by Western blot and flow cytometry (Fig. 2). H460 cells were used in subsequent studies due to their high expression of CD146 compared to the other cell lines, while A549 cells were also used due to their low expression (Fig. 3A,B). Next, the siCD146 sequence (5'-GGAACTACTGGTGAACATATGT-3') was cloned into puromycin-resistant lentivirus vector (GenePharma, Shanghai, China) and cultured in medium with puromycin (10 $\mu\text{g}/\text{mL}$) for 48 h. The CD146 overexpression lentivirus (GenePharma) was also constructed and transfected. Transfection efficiency was confirmed by the knockdown and overexpression effects, as observed by multiplex immunohistochemistry and Western blot (Fig. 3C,D).

The role of CD146 in cell invasion and migration were tested by wound healing and transwell assays. These showed that CD146 knockdown significantly inhibited the migration and invasion of H460 cells compared with the negative control. In addition, CD146 overexpression significantly enhanced the cell migration and invasion of A549 cells compared with negative control (Fig. 4).

3.4 CD146 Promotes EMT by Activating PI3K/Akt

Tumor metastasis is a complex process regulated by a variety of factors, including changes in the microenvironment, interaction between cells and the cell matrix, loss

of cell adhesion, the migration as well as invasion of tumor cells. A prerequisite and necessary condition for tumor cell metastasis is EMT. The results presented above suggest that CD146 can promote the migration and invasion of NSCLC. Subsequently, we further explored the molecular pathways mediated by CD146 in promoting the migration as well as invasion of NSCLC. Moreover, western blot demonstrated that CD146 silencing reduced the expression of mesenchymal-associated marker proteins and transcription factors, such as N-cadherin, vimentin, snail and twist. Conversely, CD146 overexpression resulted in the upregulation of these proteins. Meanwhile, the expression of E-cadherin was decreased (Fig. 5).

To study the molecular mechanism by which CD146 promotes NSCLC invasion and metastasis, transcriptome profiling via RNA sequencing was performed on the NSCLC cell line H460 and its CD146-knockdown cell line H460-shRNA. Differential expression analysis identified 324 DEGs (174 upregulated and 150 downregulated) at false discovery rate (FDR) <0.05 , $\log_2 |\text{fold change}| >1$ after CD146 knockdown (Fig. 6A,B).

GO and KEGG analyses were performed to explore CD146-related downstream signaling pathways based on the identified DEGs. Cell adhesion and cell junction, both of which are closely related to tumor metastasis, were found to be associated with CD146 (Fig. 6C,D). GSEA confirmed the significant positive regulation of cell migration, focal adhesion pathways, the PI3K/Akt pathway, and the WNT pathway in CD146-high expression cells (Fig. 6E-H).

We next evaluated whether CD146 can activate the PI3K/Akt pathway. The phosphorylation levels of Akt and PI3K in H460 cells decreased following knockdown of CD146 expression, whereas CD146 overexpression considerably increased activation of the PI3K/Akt pathway

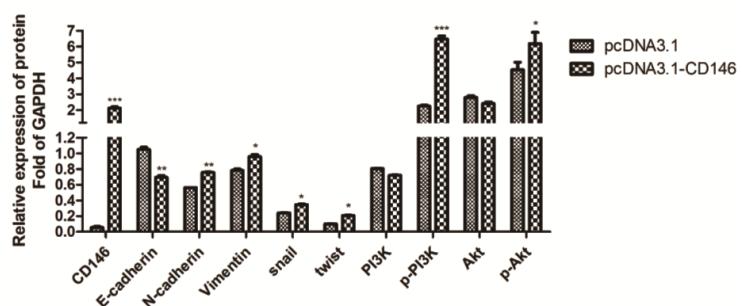
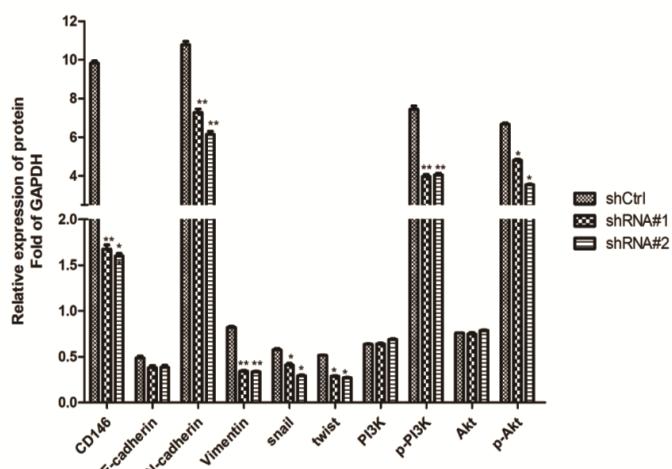
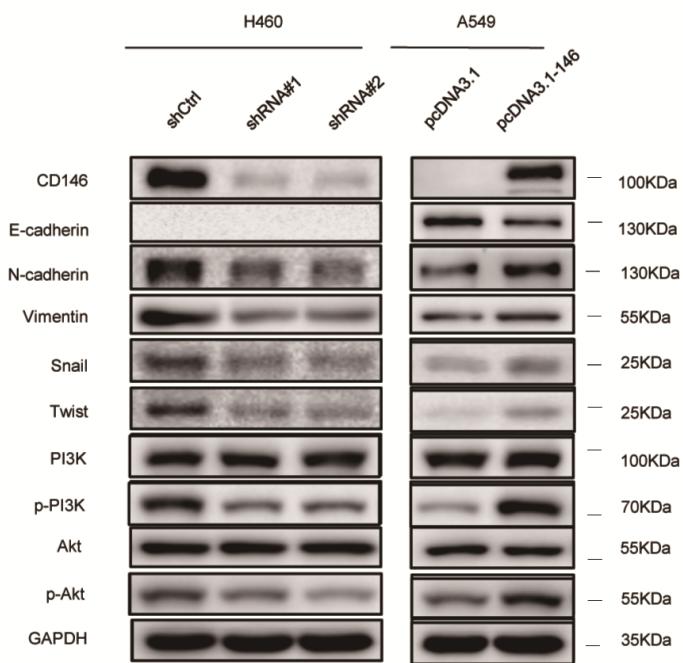


Fig. 5. CD146 induces epithelial-mesenchymal transition (EMT) in NSCLC. Western blot analysis of shCD146 H460 cells showed significant downregulation of N-cadherin, vimentin, snail and twist compared with vector control cells. Conversely, pcDNA3.1-CD146 A549 cells showed significant upregulation of N-cadherin, vimentin, snail and twist compared with vector control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

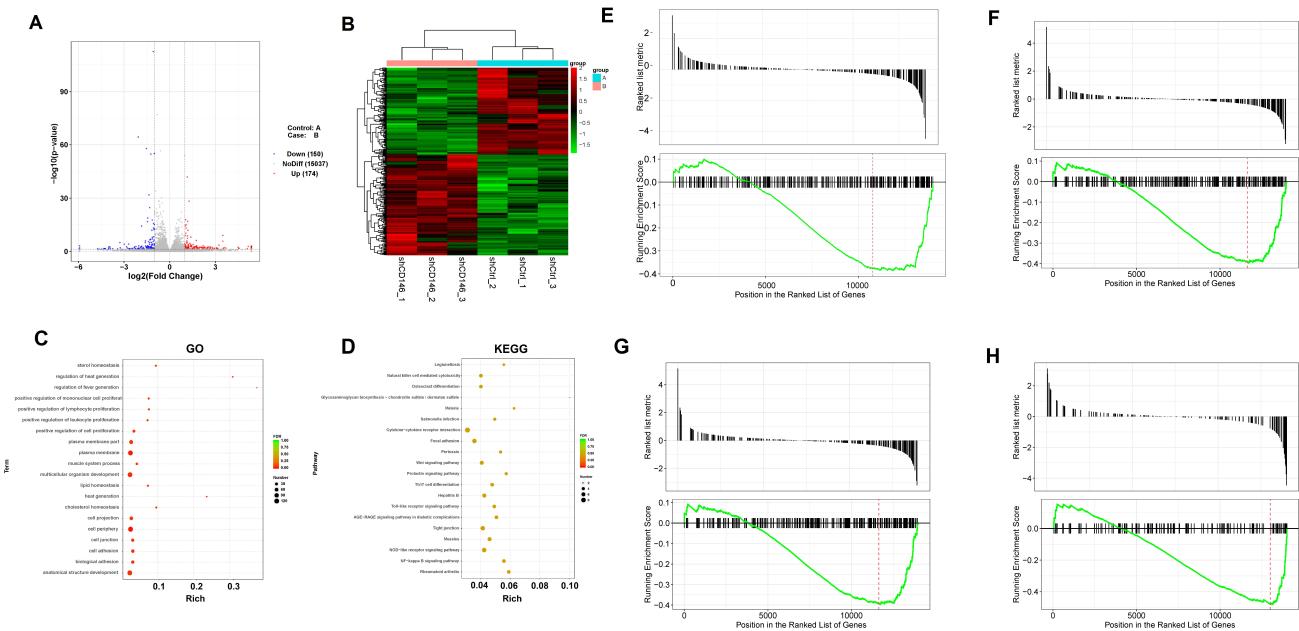


Fig. 6. Identification of differentially expressed genes (DEGs) between H460 cells and H460 cells following transfection with shCD146 vector, and functional enrichment analysis of the DEGs. Volcano plot (A) and heatmap (B) of DEGs showing the upregulated and downregulated genes. Bubble plots of the most enriched GO terms (C) and KEGG pathways (D) for all DEGs. GSEA analysis suggested that CD146 expression was significantly associated with positive regulation of cell migration (E), focal adhesion pathways (F), the PI3K/Akt pathway (G), and the WNT pathway (H).

based on phosphoprotein levels (Fig. 7). Moreover, inhibition of PI3K activity using PI3K-IN-1 markedly decreased N-cadherin, vimentin, snail and twist expression, and increased E-cadherin expression. In contrast, the activation of PI3K using 740YP markedly increased N-cadherin, vimentin, snail and twist expression. The above results suggest that CD146 mediates EMT in NSCLC by activating the PI3K/Akt pathway, thus facilitating the migration and invasion of NSCLC.

4. Discussion

Lung cancer remains the main cause of cancer-related deaths all over the world [24,25]. Despite significant improvements in patient survival due to various new treatments, the 5-year survival rate for late-stage patients with distant metastasis is still low [26]. Therefore, the identification of key molecules associated with the metastatic process is crucial for improving the outcomes of lung cancer patients [27]. CD146 is a calcium-independent adhesion molecule involved in angiogenesis, embryonic development, and neurotube formation [28–30]. It is also known as MCAM, which has been implicated in cancer development, progression, and metastasis [22,31,32]. Research has revealed a significant role for CD146 in tumor progression and metastasis, with several studies showing that it is upregulated in various cancer types and is related to increased aggressiveness, metastasis and poor prognosis [33–35]. CD146 also promotes tumor angiogenesis and facil-

tates the formation of new blood vessels that are crucial for tumor growth [36]. In addition, CD146 promotes tumor cell invasion and metastasis by favoring cellular mobility and interactions with the extracellular matrix [37]. CD146 has thus emerged as a novel biomarker for predicting tumor progression and patient outcome. It is also being explored as a therapeutic target in the development of new anti-cancer strategies [38,39]. However, the biological role of CD146 in the progression of NSCLC remains unclear.

The present research indicates that CD146 promotes EMT-mediated migration and invasion of NSCLC cells via the PI3K/AKT pathway. Through analysis of TCGA database, we found a positive association between CD146 expression and EMT activation in NSCLC. By analyzing NSCLC tissue samples, we also found positive associations between CD146 expression and TNM staging. Further analysis suggested that patients in the high CD146 expression group had shorter overall survival. Based on these findings, we next explored the specific role of CD146 in NSCLC and the underlying mechanisms by conducting several biological experiments. The results showed that silencing of CD146 induced by specific shRNA significantly reduced CD146 expression and inhibited EMT-induced invasion and migration of NSCLC cells. Conversely, CD146 overexpression enhanced EMT-induced migration and invasion of lung cancer cells. Hence, CD146 may be a novel therapeutic target for the intervention and prevention of NSCLC metastasis.

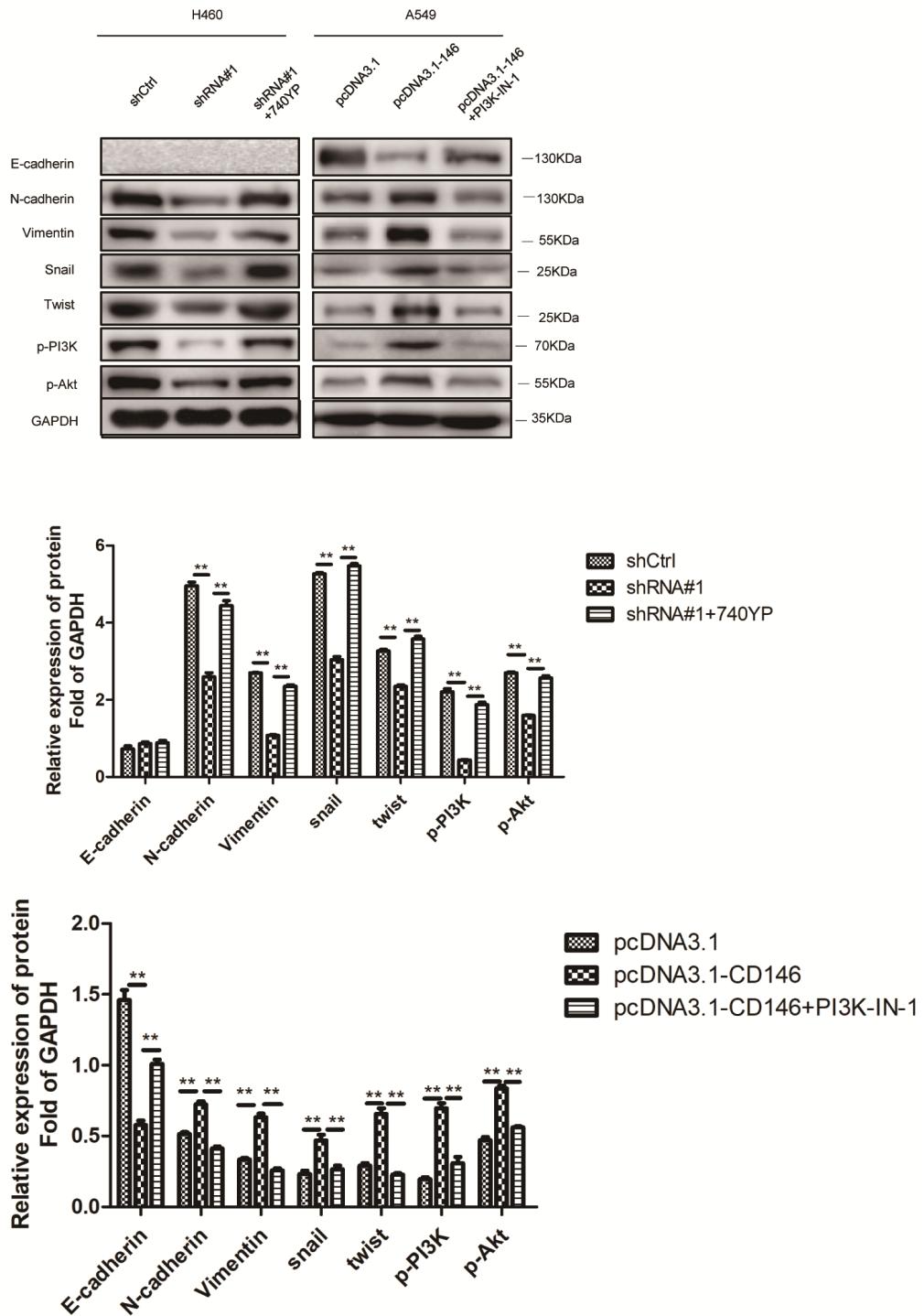


Fig. 7. CD146 induces the epithelial-mesenchymal transition (EMT) in NSCLC by activating the PI3K/Akt/Snail pathway. Western blot analysis showing that CD146 silencing in H460 cells elevated E-cadherin expression and reduced N-cadherin, vimentin, snail, and twist expression. Treatment of sh-CD146 H460 cells with 740YP decreased E-cadherin expression, but elevated N-cadherin, vimentin, snail, twist expression, as well as PI3K and AKT phosphorylation compared to vector control cells. CD146 overexpression in H460 cells resulted in inhibition of E-cadherin and elevation of N-cadherin, vimentin, snail, and twist expression. Treatment of pcDNA3.1-CD146 A549 cells with PI3K-IN-1 increased the expression of E-cadherin, but reduced N-cadherin, vimentin, snail and twist expression, as well as PI3K and AKT phosphorylation compared with vector control cells. ** $p < 0.01$.

We conducted further studies to investigate the potential mechanisms of CD146-induced lung cancer cell inva-

sion and migration. Through GSEA, we discovered a positive correlation between CD146 expression and the acti-

vation of PI3K/AKT signaling pathway. Further validation through Western blotting showed decreased levels of p-PI3K and p-AKT after knockdown of CD146. Furthermore, the administration of a p-PI3K activator increased the expression levels of epithelial markers and upregulated EMT-inducing transcription factors.

Greater knowledge of the EMT process should lead to a better understanding of tumor metastasis, which is currently the major barrier to improving the survival of cancer patients. Ma *et al.* [22] showed that CD146 was induced by TGF- β signaling, resulting in activation of the STAT3/Twist and ERK pathways. The transcriptional reaction triggered by the CD146/STAT3/Twist signaling pathway leads to a suppression of E-cadherin expression, while the CD146/ERK signaling pathway promotes an upregulation of N-cadherin expression. However, the current research showed that CD146 can also promote EMT through PI3K/AKT signaling pathways, thereby favoring NSCLC metastasis. Our study found that CD146 expression in NSCLC was closely associated with tumor aggressiveness, ability for distant metastasis, and overall patient survival. These results support the notion of CD146 as a potential biomarker for tumor progression and prognosis. Previous studies in various cancer types have reported that upregulation of CD146 was associated with increased aggressiveness and poor prognosis [34,40–42]. Our research also confirms that CD146 expression in NSCLC is positively correlated with the activation of EMT and TNM staging.

Secondly, our study suggests that CD146 promotes EMT-mediated migration and invasion of NSCLC cells via the PI3K/AKT signaling pathway. The discovery of this mechanism provides a new insight on the role of CD146 in tumor biology. Through GSEA and Western blot validation, we observed a positive correlation between CD146 expression and activation of the PI3K/AKT signaling pathway. This key signaling pathway is known to regulate cell proliferation and cell migration. Our findings indicate that therapeutic targeting of CD146 could block tumor aggressiveness and metastatic ability by inhibiting the PI3K/AKT pathway.

The present results therefore highlight the potential of CD146 as a novel therapeutic target. Given that NSCLC patients with high CD146 expression have worse survival, our work suggests that CD146 could serve not only as a prognostic biomarker, but also as a novel target for therapeutic intervention. However, before CD146 can be widely adopted as a biomarker for clinical treatment, further validation and clinical trials are required to assess the safety as well as the efficacy of therapeutic strategies targeting this protein.

Finally, although this study provides significant insights into the role of CD146 in NSCLC, careful consideration must be given prior to routine testing of this biomarker. Testing for CD146 may have potential value for identifying high-risk NSCLC patients, guiding treatment choices, and

monitoring treatment efficacy. However, the decision to adopt this test as a routine item should be based on broader clinical data and after an analysis of its cost-effectiveness.

5. Conclusions

In summary, we have provided strong evidence that CD146 induces EMT and promotes tumor cell migration and invasion of NSCLC through modulation of the PI3K/AKT pathway. CD146 could therefore be a potential therapeutic target to help block metastasis in NSCLC.

Availability of Data and Materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

NW and XJZ designed and performed the experiments, analyzed the data. NW wrote the initial draft and completed all subsequent edits. YY, KC, XRZ, XW and HHZ contributed to gathering the data and extracted tissue samples. XJZ designed the study and supervised and reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Zhengzhou University People's Hospital according to the ethical and legal standards (Approval No 2021-27). Written informed consents were obtained from all patients included in this study.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2904140>.

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