

CEACAM5 stimulates the progression of non-small-cell lung cancer by promoting cell proliferation and migration

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Abstract

Objective: To detect the expression of CEA-related cell adhesion molecule 5 (CEACAM5) in non-small-cell lung cancer (NSCLC) and explore its function in the progression and development of NSCLC.

Methods: qRT-PCR and immunohistochemistry were performed to detect CEACAM5 expression in human NSCLC tissues and cell lines. The correlation between CEACAM5 expression and the clinicopathological features of patients with NSCLC was also investigated. MTT, colony formation, wound healing, and immunoblot assays were performed to detect the functions of CEACAM5 in NSCLC cells *in vitro*, and immunoblotting was used to detect the effects of CEACAM5 on p38–Smad2/3 signaling.

Results: CEACAM5 expression was elevated in human NSCLC tissues and cells. We further found that CEACAM5 expression was correlated with clinicopathological features including T division, lymph invasion, and histological grade in patients with NSCLC. The *in vitro* assays confirmed that CEACAM5 depletion inhibited the proliferation and migration of NSCLC cells by activating p38–Smad2/3 signaling. We verified the involvement of CEACAM5 in the suppression of NSCLC tumor growth in mice.

Conclusion: CEACAM5 stimulated the progression of NSCLC by promoting cell proliferation and migration *in vitro* and *in vivo*. CEACAM5 may serve as a potential therapeutic target for the treatment of NSCLC.

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Keywords

Non-small-cell lung cancer, CEA-related cell adhesion molecule 5, proliferation, migration, therapeutic target, p38–Smad2/3 signaling, tumorigenesis

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Introduction

Lung cancer is the leading cause of cancer-related mortality globally. Non-small-cell lung cancer (NSCLC), as a major type of lung cancer, can progress quickly, including with multiple metastases in its later stages. NSCLC caused an estimated 228,150 new cases and 142,670 deaths in the USA in 2018.¹ The 5-year survival rate of metastatic NSCLC is only 2%.¹ Currently, mortality caused by distant metastases remains the major obstacle in the treatment of NSCLC.² Thus, exploring the mechanisms of NSCLC progression is particularly urgent.

CEA-related cell adhesion molecule 5 (CEACAM5) belongs to the CEACAM family of highly glycosylated proteins with a typical N-terminal variable Ig-like domain followed by zero to six constant Ig-like domains, as well as a hydrophobic transmembrane domain with a cytoplasmic tail (CEACAM1 to CEACAM4) or a glycosylphosphatidylinositol lipid moiety (CEACAM5 to CEACAM8).^{3,4} CEACAM5 is involved in intercellular contact via both homophilic and heterophilic binding (with CEACAM1 or CEACAM6).⁵ In addition to its functions in cell adhesion and migration, CEACAM5 also inhibits anoikis.⁶ Because resistance to anoikis is a characteristic of cancer cells, the inhibitory effect of CEACAM5 on anoikis suggests its role in facilitating tumorigenesis

and metastasis. The tumorigenic functions of CEACAM5 have been depicted using 3D cultures and transgenic mice.^{7–9}

The members of the CEACAM family were reported to participate in cancerous growth and invasion by acting as either tumor suppressors or poor prognostic markers for the progression of malignancies.^{10–12} CEACAM5 is upregulated in approximately 90% of gastrointestinal, colorectal, and pancreatic cancers and 50% of breast cancers.¹³ CEACAM5 has been applied in the clinical detection of liver metastasis, colorectal cancer, and colon cancer relapse.¹⁴ Notably, CEACAM5 is commonly used as an accepted tumor biomarker and indicator of recurrence in patients with cancer, especially those with colorectal cancer.¹⁵ However, the utility of CEACAM5 in NSCLC has been less investigated.

In the present study, we investigated the role of CEACAM5 in NSCLC progression. We found that CEACAM5 was upregulated in NSCLC compared with its levels in adjacent non-tumor tissue. Notably, high CEACAM5 levels were closely related to the clinical features of patients with NSCLC. We also found that CEACAM5 depletion dramatically inhibited NSCLC cell proliferation and invasion by regulating p38–Smad2/3 signaling, which was also confirmed in mice. Therefore, CEACAM5 may serve as a potential therapeutic target for the treatment of NSCLC.

Materials and methods

Bioinformatic analysis

We performed bioinformatic analysis using GEPIA (<http://gepia.cancer-pku.cn/detail.php?gene=CEACAM5/>) to analyze The Cancer Genome Atlas data with thresholds of $P < 0.05$ and $\text{LogFC} > 1$ or < -1 for differentially expressed genes.

Clinical sample collection and preparation

The use of clinical specimens in this research was reviewed and approved by the Ethics Committee of Linyi Central Hospital on January 23, 2018, and the approval number was YKRL (26). NSCLC tissue specimens and adjacent normal tissue specimens were collected from patients at Linyi Central Hospital. This study was approved by the Ethics Committee and Scientific Medical Board of our hospital according to the Declaration of Helsinki of 2008. No patients had received radiotherapy or chemotherapy prior to tissue collection. Lung tissue was examined using qRT-PCR and immunohistochemical analysis. Written informed consent was obtained from all patients.

Immunohistochemistry (IHC)

Tumor samples were freshly isolated, fixed with paraformaldehyde (PFA), embedded in paraffin, and cut into 5- μm sections. Sections were deparaffinized with xylene, rehydrated via an ethanol gradient, and incubated with H_2O_2 for 10 minutes. Sections were blocked using 1% normal goat serum and treated with CEACAM5 antibody (1:500, Abcam, Cambridge, UK) for 2 hours at room temperature. Then, sections were incubated with secondary

antibody and counterstained with hematoxylin before coverslip mounting.

According to the results of IHC, CEACAM5 was mainly localized to the cytoplasm of NSCLC cells. The proportion of positively stained cells was graded as follows: 0, 0% positive staining; 1, 1% to 30% positive staining; 2, 31% to 80% positive staining; and 3, $\geq 81\%$ positive staining.

The staining intensity was evaluated on a three-point scale as follows: 0, no or weak staining; 1, moderate staining; and 2, intense staining. CEACAM5 expression was categorized using the staining index, which was calculated by summing the scores for positive staining and staining intensity. Staining indices of < 3 indicated low expression, whereas scores of ≥ 3 indicated high expression. For CEACAM5, the cutoff was defined as the median number of positive cells.

Cell culture and transfection

H1299, H358, HCC827, and A549 human NSCLC cells and HBE normal human bronchial epithelial cells were obtained from American Type Culture Collection (Manassas, VA, USA). H358, HCC827, H1299, and A549 cells were maintained and grown in RPMI 1640 medium (Gibco; Thermo Fisher Scientific, Waltham, MA, USA), and HBE cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (HyClone; GE Healthcare, Chicago, IL, USA) at 37°C in an atmosphere of 5% CO_2 in a humidified incubator.

Cells were seeded in six-well plates, incubated for 12 hours, then transfected with a plasmid encoding shRNA targeting CEACAM5 using Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific). The sequence for shCEACAM5 was

CCGGGCAGTATTCTTGGCGTATCAA CTCGAGTTGATACGCCAAGAATACT GCTTTTTG. In total, 5 μ L of transfection reagent and 1 μ g of the plasmid were mixed in 200 μ L of serum-free medium, incubated for 10 minutes, then mixed again. The mixture was left at room temperature for 15 minutes, and serum-starved cells were then incubated with the mixture for 6 hours. Stable depletion clones were screened via lentivirus infection in the presence of 5 mg/mL Polybrene (Sigma-Aldrich, St. Louis, MO, USA). Infected cells were selected for 14 days in the presence of 1 mg/mL puromycin (Sigma-Aldrich) and used for the animal assays.

qRT-PCR assays

Total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific) and reverse-transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Takara, Kusatsu, Japan). Gene expression was normalized to that of GAPDH. The mRNA levels of CEACAM5 were detected in tumor and normal tissues from patients. Power analyses in the study were conducted using G power. The number of required patients was 87 (97.8% [up 95% G-power]). The primers used in this assay were as follows: CEACAM5 forward, 5'-AGGCCAATAACTCAGCCAGT-3'; CEACAM5 reverse, 5'-GGGTTTGGAGT TGTTGCTGG-3'; β -actin forward, 5'-CTC CATCCTGGCCTCGCTGT-3'; and β -actin reverse, 5'-GCTGTCACCTTCA CCGTTCC-3'.

Immunoblotting

After extraction, proteins were separated via SDS-PAGE and transferred to polyvinylidene difluoride membranes (MilliporeSigma, Burlington, MA, USA). The membrane was blocked in 5% BSA and incubated with specific primary

antibodies against CEACAM5 (1:1000, 1:1000, 11077-R327, Sino Biological, Beijing, China Sino Biological, Beijing, China), anti-Ki67, anti-PCNA (1:1000, Cell Signaling Technology, Danvers, MA, USA), anti-MMP2 (1:1500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-MMP9 (1:1500, Santa Cruz Biotechnology), anti-cyclin D1 (1:1500 Santa Cruz Biotechnology), and β -actin (1:5000, ab8227, Abcam) at 4°C overnight. After washing, the membrane was incubated with HRP-conjugated secondary antibodies, and signals were detected using a Novex™ ECL Chemiluminescent Substrate Reagent kit (Thermo Fisher Scientific). The relative protein levels were quantified using ImageJ (US National Institutes of Health, Bethesda, MD, USA).

Cell viability and colony formation assays

For cell viability assays, cells were incubated in 96-well plates at a density of 2000 cells per well 1 day before the experiment. Then, 10 μ L of Cell Counting Kit 8 reagent (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) in 100 μ L of RPMI 1640 medium were added to each well, and cells were cultured for 2 hours at 37°C. The absorbance was measured at 450 nm. The experiment was replicated five times, including three independent replicates.

For colony formation assays, cells were seeded and incubated for 2 weeks to permit colony formation. To visualize colonies, cells were fixed in PFA for 10 minutes, stained with crystal violet, and photographed. For the control group, the shRNA targeting sequence did not target any intracellular RNAs. The numbers of colonies were counted manually.

Wound healing assay

To assess cell migration, the wound healing assay was performed. Briefly, cells

transfected with the indicated plasmids were wounded via scraping with a tip, followed by washing. Subsequently, the cells were cultured with complete culture medium to stimulate wound healing. Photographs were taken at immediately and 24 hours after wounding to evaluate the migration of cancer cells.

Animal experiments

The animal experiments performed in this research were reviewed and approved by the Ethics Committee of Medical Experimental Animals of Linyi Central Hospital on February 12, 2019, and the approval number was YKDL (52). Eight-week-old female BALB/c (nu/nu) nude mice were obtained from the Shanghai Experimental Animal Center (Shanghai, China).

To measure tumor growth *in vivo*, BALB/c (nu/nu) nude mice were divided into two groups randomly ($n=6$ for each group). A549 cells stably transfected with CEACAM5 shRNA or negative-control shRNA lentivirus cells were suspended in 50 μ L of 50% Matrigel in DMEM medium. Cells were subcutaneously injected into each mouse. After 14 days, tumors had formed, and we measured the tumor volume weekly. After 49 days, all tumors were isolated, and representative photographs were taken. All mice were given adequate food and water, and none died of natural causes. Mice were sacrificed via cervical dislocation before tumor tissue was removed, and their heartbeats were checked to confirm death. Adequate humanitarian care was given. The tumor volume ($\text{length} \times \text{width}^2 \times 0.5236$) was calculated every week.

Statistical analysis

Statistical analysis was performed using GraphPad (GraphPad, La Jolla, CA, USA) in this study. All data are presented as the mean \pm SEM for at least three

independent experiments. Statistically significant correlations between CEACAM5 expression and clinical features in patients with NSCLC were determined using the χ^2 test. A paired *t*-test was used to detect significant differences in CEACAM5 mRNA levels between tumor and normal tissues. Student's *t*-test was used for statistical analysis in the *in vitro* and *in vivo* assays. $P < 0.05$ indicated statistical significance.

Results

CEACAM5 is abnormal high expression in human NSCLC tissues

To assess the potential involvement of CEACAM5 in the development of lung cancer, we first conducted bioinformatic analysis of CEACAM5 expression in 483 human lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) tissues, as well as 347 normal tissues. CEACAM5 expression was abnormally high in LUAD and LUSC tumor tissues compared with that in normal tissues (<http://gepia.cancer-pku.cn/>, Figure 1a). To further confirm the result, we performed IHC to assess CEACAM5 expression in tumor tissues and corresponding normal tissues collected from 87 patients (53 men and 34 women; age range, 47 to 73 years) in our hospital (Figure 1b). Similarly, CEACAM5 levels were higher in tumor tissues than in adjacent non-tumor tissues (Figure 1c).

Subsequently, we detected the mRNA levels of CEACAM5 in H1299, H358, HCC827, A549, and HBE cells. CEACAM5 levels were dramatically enhanced in NSCLC cells compared with those in normal cells, consistent with the previous results (Figure 1d). Immunoblot assays also revealed the upregulation of CEACAM5 in NSCLC cells (Figure 1e). We therefore conclude that CEACAM5 is elevated in human NSCLC cells and tissues.

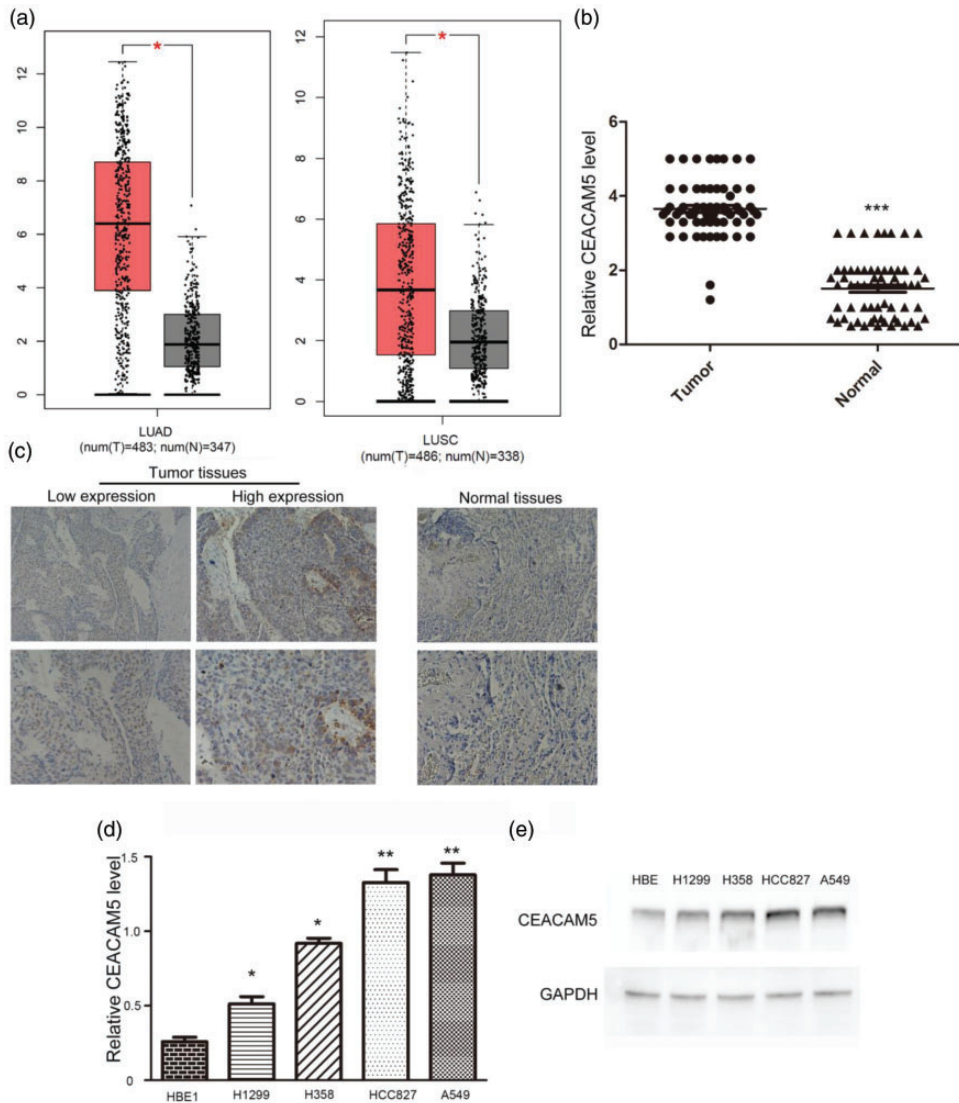


Figure 1. CEA-related cell adhesion molecule 5 (CEACAM5) expression was enhanced in human non-small-cell lung cancer (NSCLC) tissues and cells. (a) Bioinformatic analysis revealed enhanced CEACAM5 expression in patients with NSCLC compared with that in normal tissues according to The Cancer Gene Atlas database. (b) qRT-PCR assays depicted the mRNA levels of CEACAM5 in 87 pairs of NSCLC tissues and corresponding normal tissues (c) Immunohistochemical assays revealing higher CEACAM5 expression in NSCLC tissues than in the corresponding normal tissues. Representative photographs are presented ($\times 100$ and $\times 200$ magnification, respectively). (d) qRT-PCR and (e) immunoblot assays revealed the expression of CEACAM5 in HBE, H1299, H358, HCC827, and A549 cells.

Table 1. Relationship between CEACAM5 expression and clinical pathological characteristics of NSCLC patients (N = 87).

	N	CEACAM5		Chi-squared value	P
		Low expression	High expression		
Gender					
Female	34	13	21	0.165	0.685
Male	53	18	35		
Age (years)				1.333	0.248
<60 <60	35	15	20		
≥60 ≥60	52	16	36		
Smoking history				2.098	0.147
Non-smoker	28	13	15		
Smoker	59	18	41		
T stage				5.278	0.022
T1/2	48	12	36		
T3/4	39	19	20		
Lymph invasion				9.931	0.002
N0	45	9	36		
N1 or N2	42	22	20		
Histology				1.256	0.534
Squamous cancer	32	9	23		
Adenocarcinoma other	27 28	11 11	16 17		
Histological grade				12.916	0.002
Well	15	11	4		
Moderate Poor	41 31	14 6	27 25		

CEACAM5, CEA-related cell adhesion molecule 5.

CEACAM5 expression is correlated with the clinical pathological features of patients with NSCLC

To further explore the involvement of CEACAM5 in the progression of NSCLC, we assessed the correlations of CEACAM5 expression with the clinicopathological features of 87 patients with NSCLC. Patients were divided into two groups according to CEACAM5 expression. Fifty-six patients exhibited high CEACAM5 expression, and the remaining patients displayed low expression (Table 1). No obvious correlations were found between CEACAM5 expression and clinicopathological features including patient gender ($P=0.5263$), age

($P=0.1444$), smoking history ($P=0.3437$), and histology ($P=0.4001$, Table 1). Importantly, CEACAM5 expression was significantly correlated with T division ($P=0.0268$), lymph invasion ($P=0.0052$), and histological grade ($P=0.0144$, Table 1) in patients with NSCLC. We therefore demonstrated that CEACAM5 expression was correlated with the clinicopathological features of patients with NSCLC.

Ablation of CEACAM5 blocks NSCLC cell proliferation and migration in vitro

To further explore the role of CEACAM5 in the progression of NSCLC, we transfected A549 and HCC827 cells with

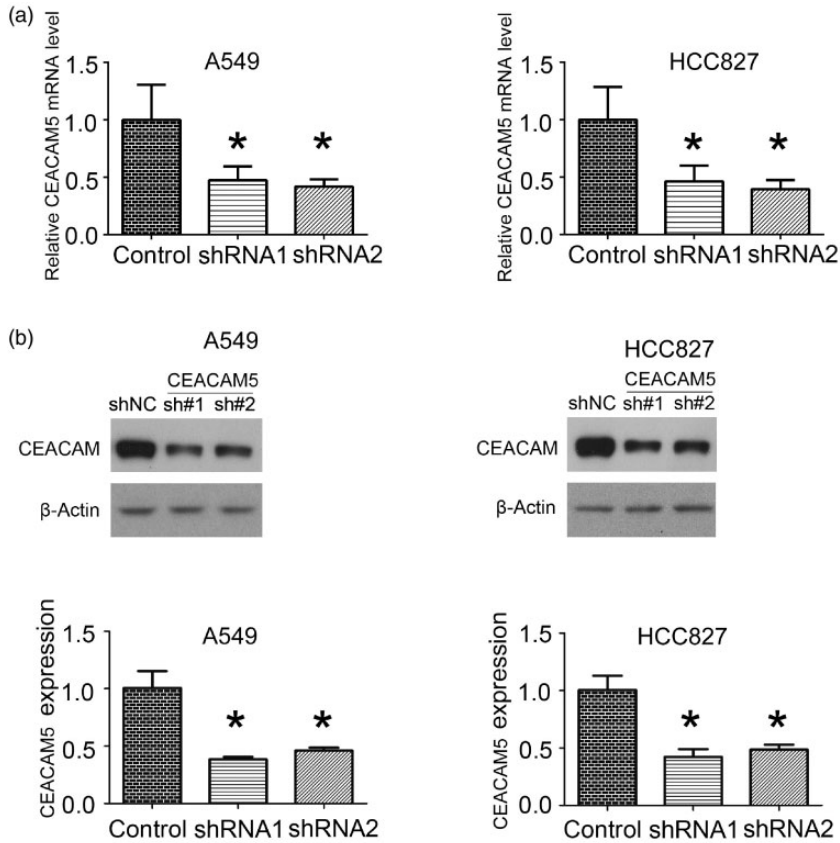


Figure 2. CEA-related cell adhesion molecule 5 (CEACAM5) expression was downregulated in A549 and HCC827 cells after CEACAM5 shRNA transfection. qRT-PCR (a) and immunoblot analyses (b) revealed the decrease in CEACAM5 levels in A549 and HCC827 cells transfected with CEACAM5 shRNA plasmids. β -actin was used as an internal control. * $P < 0.05$.

CEACAM5 shRNA plasmids to suppress CEACAM expression in these cells. As detected by qRT-PCR, CEACAM5 mRNA levels were obviously reduced after CEACAM5 shRNA transfection in A549 and HCC827 cells (Figure 2a). Similarly, immunoblot assays demonstrated that CEACAM5 expression was decreased after transfection of its shRNA plasmids (Figure 2b). Therefore, the efficiency of CEACAM5 shRNA plasmids was validated.

To assess cell proliferation after CEACAM5 depletion, we performed

colony formation assays. We observed reduced cell proliferation after CEACAM5 depletion (Figure 3a). We next performed wound healing assays to evaluate the effects of CEACAM5 on the migration of NSCLC cells and observed that CEACAM5 depletion dramatically inhibited wound closure in A549 and HCC827 cells (Figure 3b). Subsequently, we performed immunoblot assays to detect the expression of Ki67 and PCNA, two proliferation cell markers, in A549 and HCC827 cells. As expected, the ablation of CEACAM5 dramatically suppressed the expression of Ki67 and PCNA

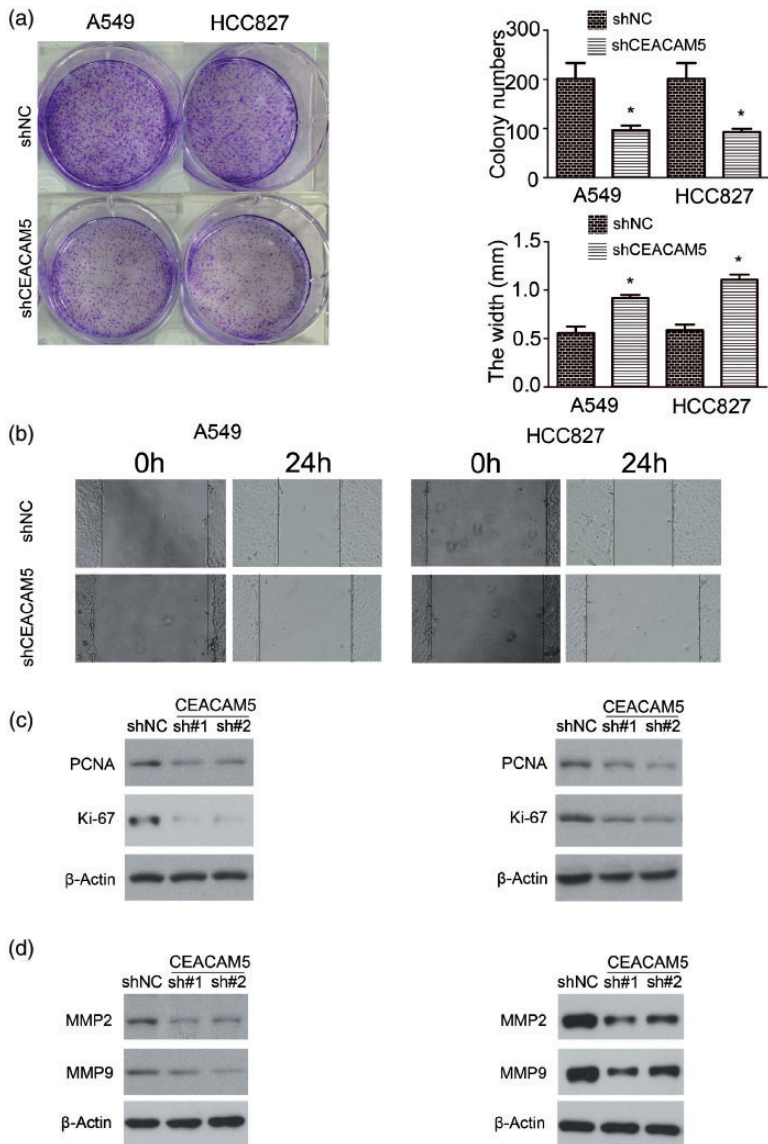


Figure 3. CEA-related cell adhesion molecule 5 (CEACAM5) promoted the proliferation and migration of non-small-cell lung cancer cells *in vitro*. (a) Colony formation assays revealed the inhibitory effect of CEACAM5 ablation on cell proliferation. (b) Wound healing assays were conducted to evaluate cell migration using A549 and HCC827 cells transfected with control or CEACAM5 shRNA plasmids. Representative photographs are presented. (c) Immunoblot assays revealed the reduced expression of PCNA and Ki67 in A549 and HCC827 cells after CEACAM5 ablation. (d) Immunoblot assays indicated the decreased expression of MMP2 and MMP9 in A549 and HCC827 cells following CEACAM5 ablation. Results are presented as the mean \pm SEM, * $P < 0.05$, ** $P < 0.01$.

(Figure 3c). Because MMP2 and MMP9 are necessary for tumor migration, we also examined their expression after CEACAM5 depletion. We observed decreased MMP2 and MMP9 expression after CEACAM5 depletion (Figure 3d). Collectively, our results indicated that CEACAM5 contributes to the proliferation and migration of NSCLC cells *in vitro*.

CEACAM5 promotes NSCLC cell proliferation and migration via the p38–Smad2/3 signaling pathway

The p38 kinases are involved in many complex biologic processes, such as cell proliferation, differentiation, death, migration, and invasion. Dysregulation of p38 mitogen-activated protein kinase (MAPK) levels are associated with advanced stages and short survival in patients with cancer. CEACAM5 has been reported to inhibit p38 activity and promote the growth of metastatic lesions.¹⁶ Thus, we examined the potential association of p38 activity with the CEACAM5-mediated enhancement of cell proliferation. Importantly, CEACAM5 depletion increased the TGF- β -mediated phosphorylation of Smads and p38 in A549 cells (Figure 4a). To explore the involvement of p38 in CEACAM5-mediated cell proliferation and migration, we depleted CEACAM5 and overexpressed p38 in A549 cells to examine for MMP2 and PCNA levels. We observed significant decreases in MMP2 and PCNA levels after CEACAM5 depletion ($P < 0.05$), and p38 overexpression blocked these effects (Figure 4b). Therefore, we deduced that p38 might be involved in CEACAM5-regulated proliferation and migration in NSCLC cells.

CEACAM5 induces NSCLC tumor growth via the p38/SMAD2/3 pathway *in vivo*

To further assess the function of CEACAM5 *in vivo*, we then evaluated

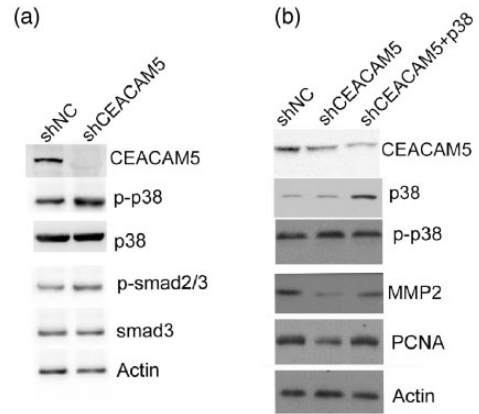


Figure 4. CEA-related cell adhesion molecule 5 (CEACAM5) promoted the proliferation and migration of non-small-cell lung cancer cells via TGF- β signaling. (a) Immunoblot assays revealed the levels of p38 and Smad3 and the phosphorylation status of p38 and Smad2/3 in A549 cells transfected with control or CEACAM5 shRNA plasmids and stimulated in the presence of 1 ng/mL TGF- β for 2 hours. (b) Immunoblot assays indicated the levels of MMP2 and PCNA and the phosphorylation of p38 in A549 cells transfected with CEACAM5 shRNA plasmids or both CEACAM5 shRNA and pcDNA3.1-p38 plasmids. Results are presented as the mean \pm SEM, * $P < 0.05$, ** $P < 0.01$.

whether CEACAM5 promoted NSCLC tumor growth in mice. The tumor volume and tumor growth curve are displayed in Figure 4a. Consistent with our expectation, the tumor volume in the CEACAM5 depletion group was significantly smaller than that in the control group ($P < 0.05$, Figure 5a, left). Similar results were denoted by the tumor growth curve (Figure 5a, right).

We further conducted IHC to detect CEACAM5 expression in tumor tissues from both groups. As expected, CEACAM5 expression was obviously downregulated in CEACAM5-depleted tumor tissues, confirming the effective depletion of CEACAM5 (Figure 5b). Similarly, we detected lower MMP2 and

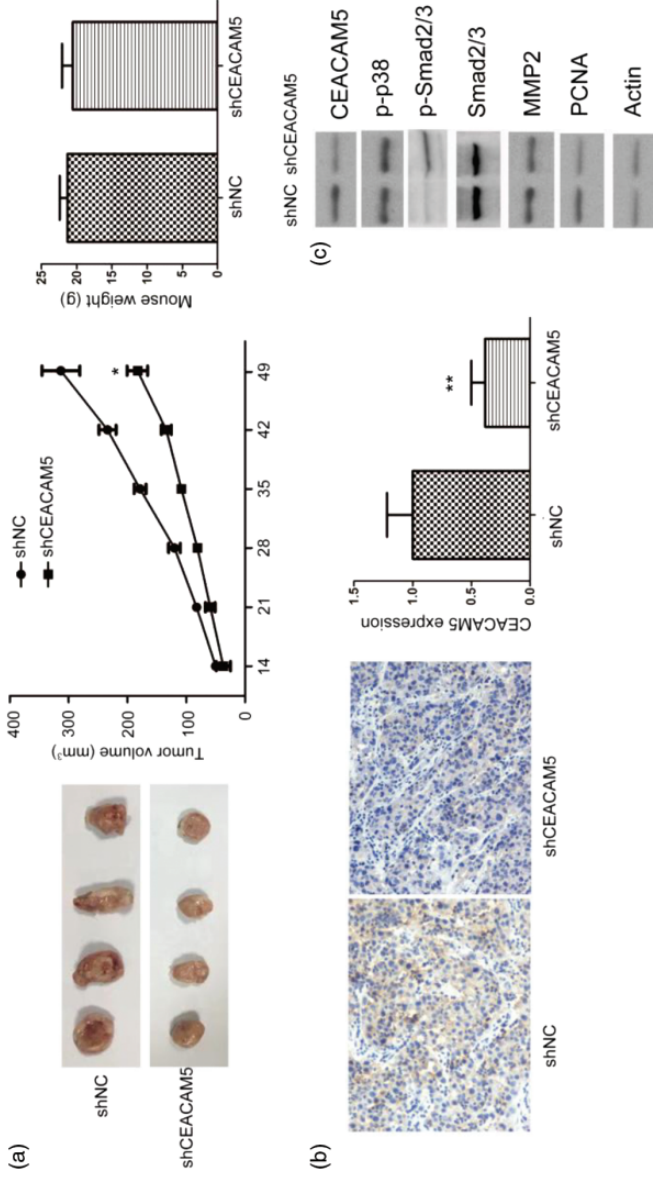


Figure 5. CEA-related cell adhesion molecule 5 (CEACAM5) depletion impaired non-small-cell lung cancer tumor growth *in vivo*. (a) A549 cells infected with CEACAM5 or control shRNA lentivirus were implanted into nude mice. The tumor volume was monitored every week from the third week. After 49 days, tumors were isolated (n = 6 for each group). Representative images of tumors are presented (left), and tumor growth curves and mouse weight were calculated (right). (b) Immunohistochemical assays revealed the expression of CEACAM5 in control or CEACAM5-depleted tumor tissues. (c) Immunoblot assays further confirmed the expression of CEACAM5, MMP2, and PCNA and the phosphorylation of p38 and SMAD2/3 in tumor tissues from control or CEACAM5-depleted mice. Results are presented as the mean \pm SEM, * $P < 0.05$, ** $P < 0.01$.

PCNA expression following CEACAM5 depletion as well as higher phosphorylated p38 and Smad2/3 expression (Figure 5c). We thus revealed that CEACAM5 might contribute to NSCLC tumor growth via the p38/SMAD2/3 pathway in mice.

Discussion

NSCLC is a primary type of lung cancer and one of the most common and deadly malignant tumors on a global scale.¹⁷ Because of the strong invasiveness of cancer, liposomal drug delivery systems have achieved good results,^{18,19} and many patients are diagnosed with advanced disease and distant metastasis.²⁰ Although great progresses have been made in past years, 5-year overall survival for NSCLC remains unsatisfactory.²¹ Identifying strategies for improving survival is thus necessary. Targeted therapy has been identified as an effective strategy with potential efficacy against lung cancer.²² Given the intratumoral heterogeneity in lung cancer, novel therapeutic targets remain urgently needed. In this study, we revealed that CEACAM5, a highly glycosylated protein, could serve as a novel molecular target for NSCLC.

In this study, we explored the possible involvement of CEACAM5 in NSCLC. Using qRT-PCR and IHC assays, we detected enhanced CEACAM5 expression in tumor tissues compared with that in normal tissues in patients with NSCLC, indicating the critical role of CEACAM5 in the pathogenesis of NSCLC. Concurrently, we observed high CEACAM5 expression in NSCLC cells compared with that in normal cells, which was consistent with the clinical data. Furthermore, we investigated the link between clinicopathological features and CEACAM5 expression in patients with NSCLC. We observed that clinical characteristics, including T division, lymph

invasion, and histological grade, were associated with CEACAM5 expression. Similar to our findings, high CEACAM5 levels have also been implicated with enhanced growth in malignancy.^{14,23–25} In breast tumors, high CEACAM5 expression is correlated with reduced patient survival.²⁴ CEACAM5 is also associated with the progression of colorectal and pancreatic cancers.^{26,27} These findings all indicated the important regulatory role of CEACAM5 in tumor progression.

CEACAM5 is overexpressed in multiple cancer types, including most gastrointestinal, colorectal, and pancreatic cancers.^{23–25} CEACAM5 could serve as a biomarker for these cancers.^{23–25} Recently, CEACAM5 expression has been detected using immunoassays and related techniques.²⁵ However, its effects on lung cancer were unclear. In this study, we confirmed the high expression of CEACAM5 in NSCLC cancers, further suggesting that CEACAM5 could act as a biomarker for NSCLC.

We further explored the role of CEACAM5 in the proliferation and migration of NSCLC cells using colony formation and wound healing assays. We demonstrated that CEACAM5 promotes the proliferation and invasion of NSCLC cells. Mechanically, CEACAM5 promotes cell proliferation and invasion via p38/Smad2/3 signaling in NSCLC, and we further verified this finding in an animal model. Consistently, overproduction of CEACAM5 delayed TGF- β -induced epithelial-mesenchymal transition (EMT). CEACAM5 inhibited p38 activity and promoted invasion and metastasis, in line with previous findings that the pharmacologic inhibition of p38 promoted the outgrowth of micrometastatic tumor cells.²⁸ Another study declared that CEACAM5 was also associated with the inhibition of cell differentiation, anoikis, and apoptosis in colon cells through its co-localization and activation of $\alpha 5\beta 1$ integrin signal transduction,

promoting PI3K/AKT activity.²⁹ These studies demonstrated that CEACAM5 plays a vital role in promoting tumor progression.

TGF- β kinase is activated when TGF- β binds to TGF- β RII, resulting in Smad2/3 phosphorylation. In addition, the stress-activated protein kinase p38 plays fundamental roles in TGF- β signaling in a variety of systems.³⁰ The p38–Smad2/3 pathway mediates multiple cellular processes.³¹ In this study, we noticed that CEACAM5 promoted the progression of NSCLC via this pathway and confirmed that the p38–Smad2/3 axis could serve as a therapeutic target for NSCLC treatment.

TGF- β signaling is considered a key driver of cancer cell proliferation and invasion through a variety of Smad-dependent and Smad-independent pathways, including the p38 MAPK pathway.³⁰ In progressive cancer tissues, TGF- β promotes tumor formation, and the upregulation of TGF- β is often correlated with cancer malignancy.³¹ TGF- β 1 induces EMT in pancreatic ductal adenocarcinoma cells along with the upregulation of various mesenchymal markers such as the small leucine-rich proteoglycan biglycan.³² These studies, together with our findings in this study, confirmed the key involvement of the TGF- β signaling pathway in cancer progression. Therefore, developing inhibitors targeting this pathway is imperative for developing anti-tumor drugs.

Conclusions

We observed the enhanced expression of CEACAM5 in human NSCLC tissues and cells and its correlations with the clinicopathological features of patients with NSCLC. We further verified the role of CEACAM5 in the progression of NSCLC *in vitro* and *in vivo* via the p38–Smad2/3 signaling pathway. Therefore, CEACAM5

could act as a potential therapeutic target for the treatment of NSCLC.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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