

Downregulation of SETD7 promotes migration and invasion of lung cancer cells via JAK2/STAT3 pathway

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Abstract. [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7 (SETD7) is a protein lysine methyltransferase that methylates both histone H3K4 and non-histone proteins, such as transcription factors. The methylation on proteins alters their activity and affects a series of biological processes. Recent studies have demonstrated that SETD7 contributes to tumor progression and may play different roles in tumor development. However, the effect of SETD7 on lung cancer cell migration and invasion has not been fully elucidated. The present study demonstrated that the expression of SETD7 was significantly downregulated in lung cancer tissues in comparison with that in matched non-cancer tissues, and lung cancer cell lines also exhibited lower SETD7 levels compared with normal human bronchial epithelial cells. Overexpression of SETD7 inhibited the migration and invasion of lung cancer cells, whereas decreased SETD7 expression promoted cell migration and invasion. Further study revealed that SETD7 regulated the expression of the metastasis-related genes metalloproteinase 2, Twist1 and vascular endothelial growth factor. Furthermore, SETD7 knock-down activated the Janus kinase 2/signal transducer and activator of transcription 3 (STAT3) signaling pathway and enhanced lung cancer cell migration, whereas the STAT3-specific inhibitor

Stat3 abrogated the effect of SETD7 on cell migration. Taken together, these data indicated that SETD7 acts as a tumor suppressor, and the reduced expression of SETD7 may contribute to lung cancer progression. The findings of the present study suggest that SETD7 may be a novel candidate for the treatment of metastatic lung cancer.

Introduction

Lung cancer is one of the most aggressive types of cancer and the primary cause of cancer-related mortality, with >2.0 million new cases diagnosed and 1.7 million deaths annually worldwide (1). Non-small-cell lung cancer (NSCLC) cases comprise 80-85% of total lung cancer cases. NSCLC includes adenocarcinoma, squamous cell carcinoma, large-cell carcinoma and other poorly differentiated subtypes (2). Despite significant advances in lung cancer diagnosis and treatment, the 5-year survival rate is currently <15% (3). One of the most common causes of treatment failure is metastasis, but the exact underlying mechanism remains unclear (4).

Recent studies have demonstrated that post-translational histone modifications are implicated in various diseases. These histone modifications include acetylation, methylation and phosphorylation on N-terminal histone tails. Histone tail methylation is key to the regulation of gene transcription and protein function (5). [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7 (SETD7), also known as SET7, SET9, SET7/9 or KMT7, belongs to the protein lysine methyltransferase family (6). SETD7 was initially shown to catalyze histone monomethylation of H3K4 (7). However, recent studies have demonstrated that SETD7 also post-translationally modifies non-histone proteins, including p53, Msx2-interacting protein, and signal transducer and activator of transcription 3 (STAT3) (7-9).

It was previously indicated that SETD7 plays an important role in several types of cancer. SETD7 expression was found to be lost or reduced in gastric cancer, and low SETD7 expression was associated with poor prognosis (10). The expression of SETD7 was also decreased in breast cancer, and knockdown of SETD7 enhanced cell proliferation, migration, invasion and tumor growth *in vivo* (11). Liu *et al* demonstrated that resveratrol inhibited colorectal cancer cell growth via upregulating the

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expression of SETD7, suggesting that SETD7 is implicated in colorectal cancer cell growth (12). However, the role of SETD7 in lung cancer metastasis has not been fully elucidated.

The aim of the present study was to assess the expression of SETD7 in lung cancer tissues and cell lines, investigate its effect on lung cancer metastatic potential, and determine whether the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathway is involved in this process.

Materials and methods

Reagents and antibodies. The STAT3 inhibitor Stattic was purchased from Sigma-Aldrich; Merck KGaA. Antibodies against SETD7 (cat. no. ab14820) and H3K4me2 (cat. no. ab7766) were purchased from Abcam; antibodies against p-JAK2 (cat. no. 3771), p-STAT3 (cat. no. 9145), STAT3 (cat. no. 9132), N-cadherin (cat. no. 13116) and vimentin (cat. no. 5741) were purchased from Cell Signaling Technology, Inc.; antibody against H3 (cat. no. BMS-33042m) was purchased from Bioss; antibody against E-cadherin (cat. no. AF748) was purchased from R&D Systems; and antibody against β -actin was purchased from Sigma-Aldrich: Merck KGaA (cat. no. F3022). All antibodies were diluted at 1:1,000, except β -actin, which was diluted at 1:5,000.

Cell culture. The human lung cancer cell lines A549, H1299 and H661, and the human lung bronchial epithelial cell line BEAS-2B, were purchased from American Type Culture Collection. A549 and BEAS-2B cells were grown in DMEM, and H1299 and H661 cells were grown in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.), at 37°C with 5% CO₂. The medium was supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin (Gibco; Thermo Fisher Scientific, Inc.).

The lung cancer specimens were obtained from patients treated at the Tianjin Medical University General Hospital (TMUGH; Tianjin, China). None of the patients underwent chemotherapy or targeted therapy prior to tissue collection (Table I). The study protocol was approved by the Institutional Review Board of TMUGH.

Cell viability. Cells (5×10^3) were placed in 96-well plates. After treatment, cell viability was assessed using the Cell Counting Kit-8 assay (Dojindo Molecular Technologies, Inc.), according to the manufacturer's instructions.

Transfection and RNA interference. SETD7 was cloned into the pCMV-tag2B vector (Agilent Technologies, Inc.), according to the manufacturer's instructions. The primers were as follows: 5'-CGCGGATCCATGGATAGCGACGACGAGA-3' (forward); 5'-CCGCTCGAGTCACTTTTGCTGGGTGGCC-3' (reverse). The forward primer included a *Bam*HI restriction site (underlined), and the reverse primer included a *Xho*I restriction site (underlined) (Takara Biotechnology Co., Ltd.).

The siRNA duplex for SETD7 was purchased from Genepharma. The sequences of the siRNA duplex were as follows: Sense, 5'-CACUCCUUCACUCCAAACUTT-3', and antisense, 5'-AGUUUGGAGUGAAGGAGUGTT-3'. The sequences of the NC-siRNA duplex were as follows: Sense

5'-UUCUCCGAACGUGUCACGUTT-3', and antisense 5'-ACGUGACACGUUCGGAGAATT-3'.

Lung cancer cells were plated in 6-well plates at a density of 2.5×10^5 cells/well, and pSETD7 or si-SETD7 were transfected into the cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.).

Wound healing assay. Cell migration was assessed with the wound healing assay as previously described (13). Cells were cultured in 6-well plates at 37°C for 24 h until they reached 80% confluence. A sterile 200- μ l pipette tip was used to scratch the cell monolayer, and PBS was used to remove cell debris. Cells were then cultured at 37°C in DMEM or RPMI-1640 medium in the absence of FBS for 48 h, and cell migration was analyzed by evaluating wound closure over time using an inverted microscope (Nikon Corporation) at a magnification of x40. The closure rate was calculated as follows: Closure rate=(1-final wound area/initial wound area) x100%.

Cell invasion assay. Cell invasion ability was examined using the Transwell assay as previously described (14). Briefly, the upper chamber of the Transwell insert (Costar; Corning, Inc.) was coated with Matrigel (BD Biosciences, Inc.). SETD7-overexpressing or -silenced cells (1×10^5) were added to the upper chamber in serum-free medium. Complete medium with 10% FBS was added to the lower chamber. After 48 h, non-invading cells in the upper chamber were removed with a cotton swab. Invading cells in the lower chamber were stained with 0.1% crystal violet solution (Beyotime Institute of Biotechnology), and quantified by counting the cell number from 5 random fields under a light microscope (Nikon Corporation) at a magnification of x200.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. RT-qPCR was performed as previously described (15). Briefly, RNA was extracted from cells or tumor tissues using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.). RT was performed using a Takara kit following the manufacturer's instructions. The reaction protocol was 37°C for 15 min, and then 85°C for 5 sec. Gene expression was analyzed by qPCR using the Power SYBR-Green Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), on an ABI Prism 7500 Sequence Detector System. The reaction protocol was as follows: Stage 1 at 94°C for 3 min; stage 2 at 94°C for 30 sec and 60°C for 30 sec, for 40 cycles; and stage 3 at 95°C for 15 sec and 60°C for 1 min. GAPDH was used as an internal control. Primers were designed using Primer 5.0 (PREMIER Biosoft International) and synthesized by the Beijing Genomics Institute. The primer sequences are listed in Table II. The gene expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (16), by comparing the Cq values of the target genes to the Cq values of GAPDH.

Western blot analysis. Western blotting was conducted as previously described (17). Proteins were isolated from cells using RIPA buffer (Beyotime Institute of Biotechnology) containing protease inhibitor (Sigma-Aldrich; Merck KGaA). Protein was quantified using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Protein (20 μ g) was loaded and 12% SDS-PAGE was performed. The proteins were transferred to nitrocellulose membranes (EMD Millipore). The

Table I. Patient characteristics.

Number of patients	Sex	Age, years	Histology	Tumor differentiation
1	Male	59	Squamous cell carcinoma	I
2	Male	76	Squamous cell carcinoma	I
3	Female	66	Adenocarcinoma	I
4	Male	64	Adenocarcinoma	I
5	Male	80	Squamous cell carcinoma	I
6	Female	65	Mixed (65% squamous cell carcinoma and 35% adenocarcinoma)	I
7	Male	42	Mediastinal tumor	I
8	Male	70	Adenocarcinoma	I
9	Male	82	Adenocarcinoma	I
10	Male	50	Squamous cell carcinoma	I

Table II. PCR primer sequences.

Primers	Sequence (5'-3')	Length of amplicons (bp)
<i>MMP2</i>		
Forward	GCGGCGGTCACAGCTACTT	76
Reverse	CACGCTCTTCAGACTTTGGTTCT	
<i>Twist</i>		
Forward	GCCAATCAGCCACTGAAAGG	83
Reverse	TGTTCTTATAGTTCCTCTGATTGTTACCA	
<i>VEGF</i>		
Forward	AGGAGGAGGGCAGAATCATCA	76
Reverse	CTCGATTGGATGGCAGTAGCT	
<i>SETD7</i>		
Forward	CACGGAGAAAAGAACGGACG	141
Reverse	GTCTACATACGTGCCCTGGA	
<i>GAPDH</i>		
Forward	TGCACCACCAACTGCTTAGC	87
Reverse	GGCATGGACTGTGGTCATGAG	

MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

membranes were blocked with 5% milk in 0.1% TBS-Tween for 1 h at room temperature. The membranes were then incubated with primary antibodies at 4°C overnight. After washing, the membranes were probed with the corresponding HRP-conjugated secondary antibodies. The protein bands were visualized using the enhanced chemiluminescence technique (EMD Millipore).

Statistical analysis. The data are presented as mean \pm standard deviation, and they were obtained from at least three independent experiments. Statistical analysis was performed using SPSS version 13.0 (SPSS, Inc.). The differences between two groups were analyzed using Student's t-test, and among multiple groups using one-way analysis of variance followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate statistically significant differences.

Results

SETD7 is downregulated in human lung cancer tissues and cell lines. Ten pairs of lung cancer specimens, including tumor tissues and matched non-tumor tissues, were collected to study the role of SETD7 in human lung tumorigenesis. The mRNA levels were first compared by qPCR. As shown in Fig. 1A and B, in the majority of the samples (8/10), SETD7 expression was lower in tumor tissues compared with that in non-tumor tissues, although SETD7 was expressed at higher levels in the tumors in two pairs of tissues (2/10). The characteristics of the two patients in whom the expression of SETD7 was higher in tumor tissues were further reviewed; however, no specific characteristics were observed. This may be due to individual differences, such as genetic background. Protein expression was then detected in three pairs of tissues

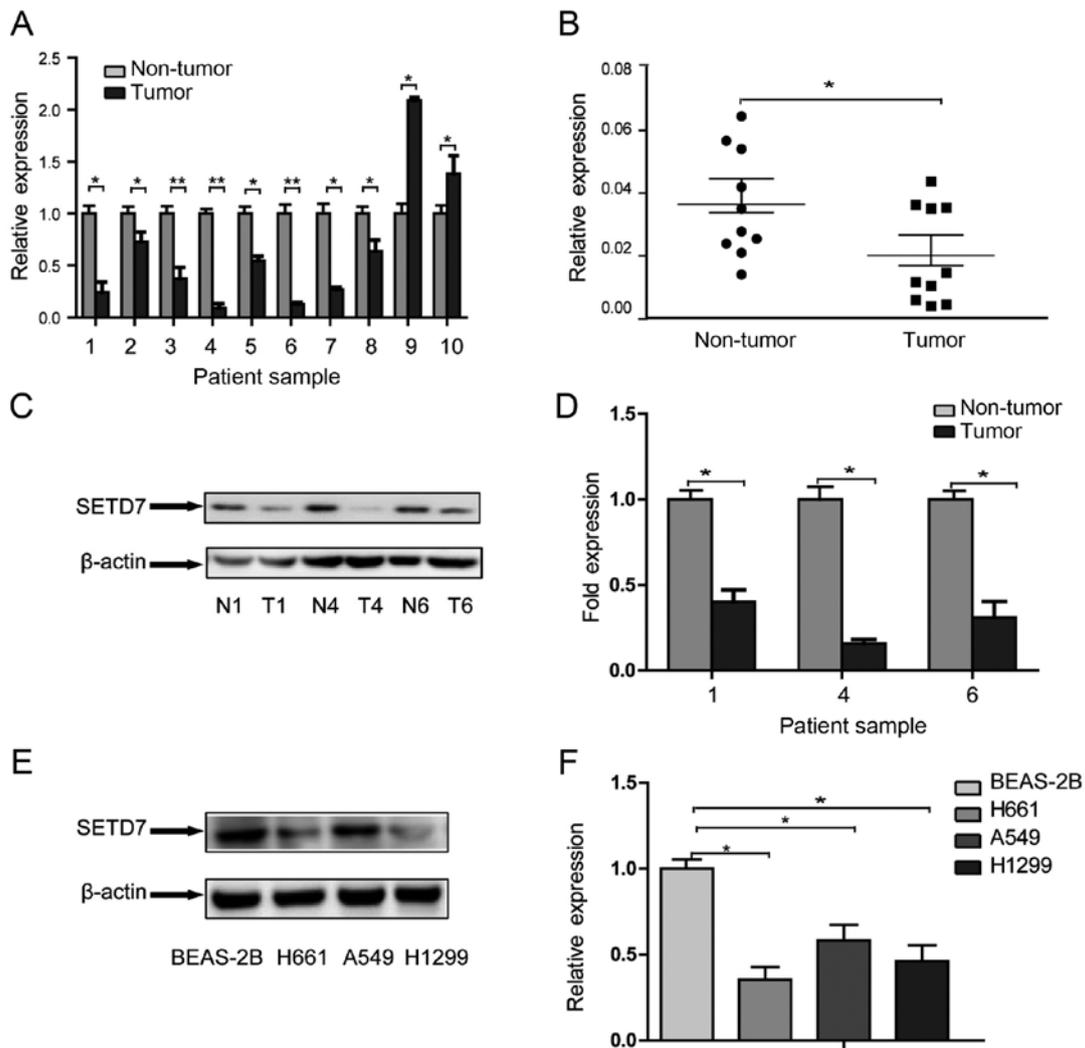


Figure 1. SETD7 is downregulated in tumor specimens and human lung cancer cell lines. (A and B) The mRNA expression of SETD7 in paired tumor and non-tumor specimens was detected by qPCR (n=10). (C and D) The protein expression of SETD7 in paired tumor and non-tumor specimens was detected by western blotting. (E and F) The protein expression of SETD7 in lung cancer and normal bronchial epithelial cell lines was detected by western blotting. *P<0.05, **P<0.01. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7; qPCR, quantitative PCR.

representing lung squamous cell carcinoma, adenocarcinoma, and mixed lung squamous cell carcinoma and adenocarcinoma. Western blot analysis also confirmed that the protein level of SETD7 in tumor tissues was reduced compared with that in non-tumor tissues (Fig. 1C and D).

We next compared the expression level of SETD7 in three human NSCLC cell lines, A549 cells (adenocarcinoma), H1299 cells (NSCLC) and H661 cells (large-cell carcinoma), to that in BEAS-2B cells (human normal bronchial epithelial cells) by western blotting (Fig. 1E and F). The expression of SETD7 was reduced by 35.3, 58.1 and 46.1% in H661, A549 and H1299 cells, respectively, compared with that in BEAS-2B cells. These findings indicated that SETD7 was markedly downregulated in lung cancer cells.

SETD7 exerts no effect on the viability of lung cancer cells. The role of SETD7 in lung cancer cell viability was investigated. As SETD7 is downregulated in lung cancer cells, SETD7 expression was evaluated in lung cancer cell lines. Lung cancer cells were transfected with pSETD7, and the overexpression of SETD7 was determined by western blotting (Fig. 2A). After

transfection, cell viability was assessed by the Cell Counting Kit-8 assay. As shown in Fig. 2B, SETD7 overexpression did not affect lung cancer cell viability in any of the three lung cancer cell lines.

To further confirm the role of SETD7, the expression of SETD7 was knocked down by RNAi technology. As shown in Fig. 2C, SETD7 expression was significantly reduced; however, SETD7 knockdown did not affect cell viability (Fig. 2D). These results demonstrated that SETD7 exerted no effect on lung cancer cell viability.

As SETD7 is a protein lysine methyltransferase that methylates histone H3K4, we also investigated the effect of SETD7 on histone methylation status. The histone methylation status can be assessed by western blotting (18,19). In particular, Chen *et al* reported that the expression of SETD7 is associated with the expression of H3K4me2 (18); therefore, the H3K4me2 level was measured in SETD7 overexpression and knockdown samples. The results demonstrated that SETD7 overexpression increased H3K4me2 expression, whereas SETD7 knockdown reduced H3K4me2 expression (Fig. 2E).

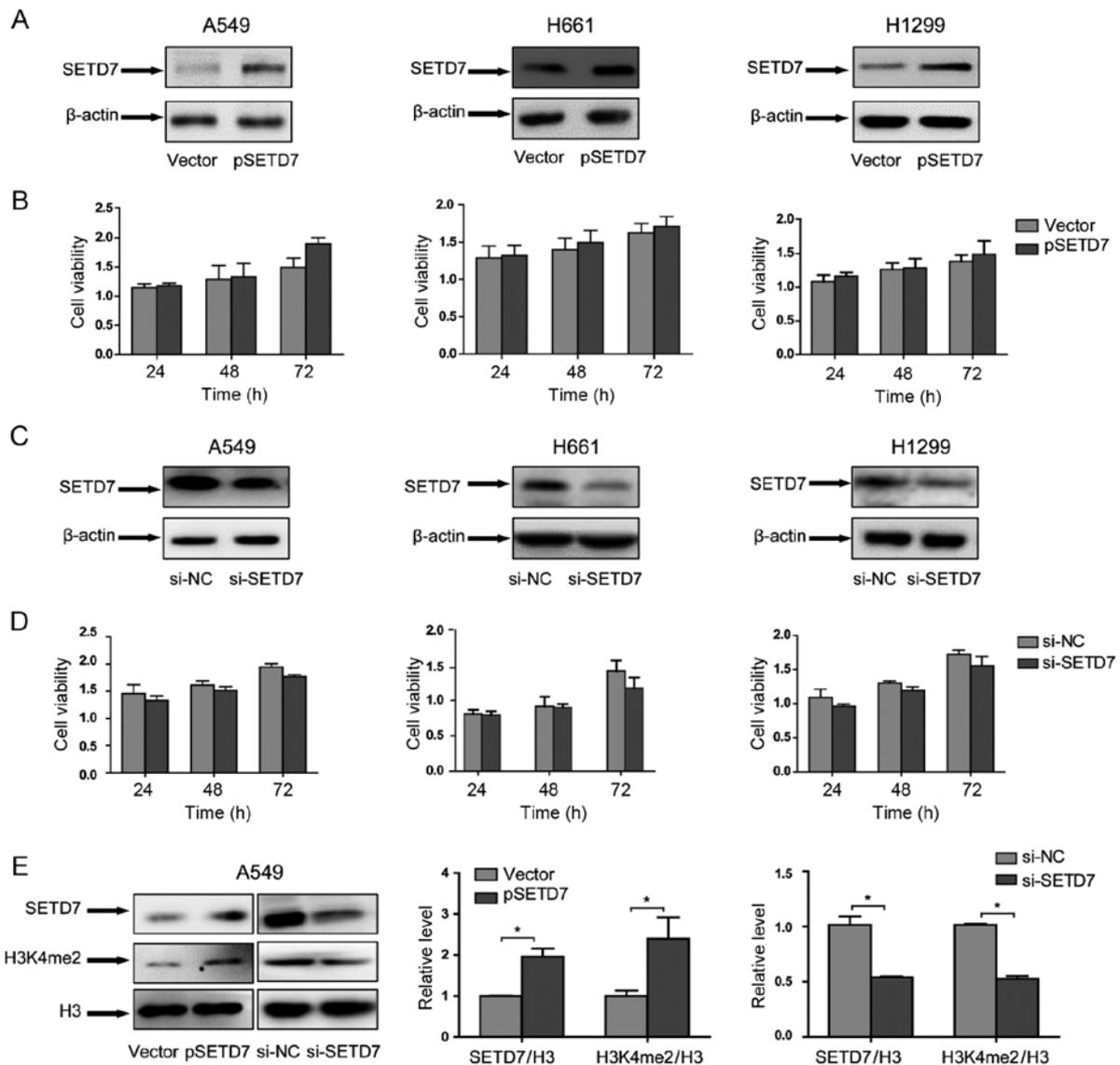


Figure 2. SETD7 exerted no effect on the viability of lung cancer cells. (A) SETD7 was overexpressed in lung cancer cells following transfection with pSETD7. (B) The effect of SETD7 overexpression on lung cancer cell viability was assessed by the CCK-8 assay at the indicated time points. (C) SETD7 expression was knocked down by RNAi. (D) The effect of SETD7 decrease on lung cancer cell viability was assessed by the CCK-8 assay at the indicated time points. Values represent the mean \pm standard deviation from three independent experiments. (E) SETD7 was overexpressed or knocked down in A549 cells, and the expression of H3K4me2 was detected by western blotting. Similar results were obtained from three independent experiments. * $P < 0.05$. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7; CCK-8, Cell Counting Kit-8.

SETD7 downregulation enhances the migration of lung cancer cells. The migration ability of cancer cells is responsible for tumor metastasis (20). Therefore, the effect of SETD7 on the migration of lung cancer cells was investigated by the wound healing assay. A549, H1299 and H661 cells were transfected with pSETD7, and cell migration was assessed at 48 h after transfection. As shown in Fig. 3A and B, the relative rates of wound closure at 48 h for A549, H661 and H1299 cells were 42.56, 55.09 and 59.12%, respectively. Interestingly, when SETD7 was overexpressed in lung cancer cells, the relative percentages of wound closure were reduced to 14.27% in A549 cells, 40.43% in H661 cells, and 31.72% in H1299 cells. The strongest inhibitory effect on migration was observed in A549 cells.

By contrast, when SETD7 expression was reduced by RNAi knockdown, the relative rates of wound closure were

increased in all three cell lines (Fig. 3C and D). These data indicated that SETD7 downregulation enhances lung cancer cell migration.

SETD7 downregulation enhances the invasion of lung cancer cells. Cell invasion is also an important step in tumor metastasis (20). The effect of SETD7 on cell invasion was examined by the Transwell assay. Lung cancer cells were transfected with pSETD7, and the invading lung cancer cells were stained and counted at 48 h after transfection. A marked suppression of cell invasion ability was observed, as the invading cell numbers were decreased to 0.53-fold in A549 cells, 0.60-fold in H661 cells, and 0.57-fold in H1299 cells (Fig. 4A and B).

Next, the effect of the reduction of SETD7 on cell invasion was investigated. As shown in Fig. 4C and D,

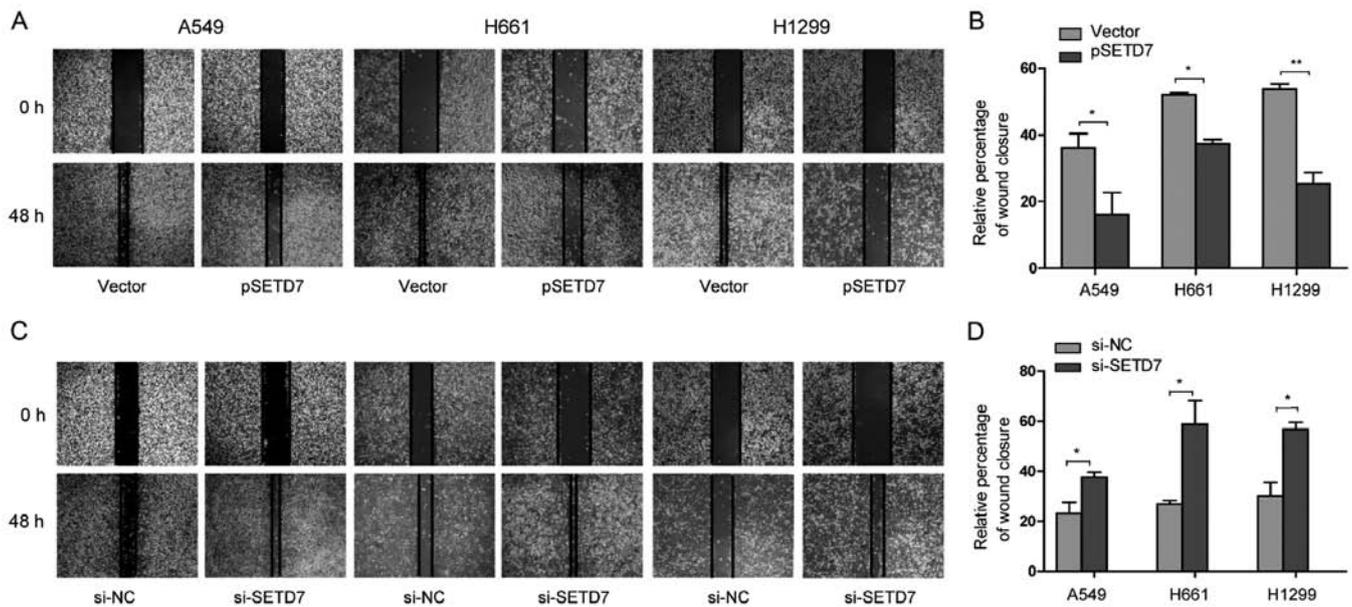


Figure 3. SETD7 inhibited the migration of lung cancer cells. (A and B) Lung cancer cells were transfected with pSETD7. Cell migration was assessed by the wound healing assay after 48 h. (C and D) Lung cancer cells were transfected with si-SETD7. Cell migration was assessed by wound healing assay after 48 h. Representative photographs of cells migration were presented (x40 magnification). Values represent the mean \pm standard deviation from three independent measurements. * $P < 0.05$, ** $P < 0.01$. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7.

suppression of SETD7 expression enhanced lung cancer cell invasion. Taken together, these results demonstrated that SETD7 downregulation enhances the metastatic potential of lung cancer cells.

SETD7 regulates metastasis-related gene expression. Given the observed inhibitory effect of SETD7 on the migration and invasion of lung cancer cells, its effect on the expression of metastasis-related genes was further investigated. Numerous studies have demonstrated that matrix metalloproteinase (MMP)2, Twist and vascular endothelial growth factor (VEGF) play important roles in tumor metastasis.

qPCR was used to compare the mRNA expressions of these three genes in tumor and non-tumor tissues. As shown in Fig. 5A, tumor tissues expressed higher levels of MMP2, Twist and VEGF compared with non-tumor tissues.

In order to determine the role of SETD7 in the expression of metastasis-related genes, SETD7 was first overexpressed in lung cancer cells. It was observed that the expression of these genes was markedly downregulated by SETD7. By contrast, when SETD7 expression was knocked down, the expression of these genes was upregulated (Fig. 5B). These results suggested that SETD7 suppresses the metastatic potential of lung cancer cells through modulating metastasis-related genes.

As epithelial-to-mesenchymal transition (EMT) is crucial for cancer cell invasion and metastasis, the role of SETD7 in EMT was further assessed. The expression of EMT markers was examined following overexpression or knockdown of SETD7 in A549 cells. It was observed that, after SETD7 knockdown, the expression of the epithelial phenotype marker E-cadherin was decreased, whereas the expression of the mesenchymal phenotype markers N-cadherin and vimentin was increased. These results indicate that SETD7 knockdown promotes EMT. SETD7 overexpression exerted no effect on EMT (Fig. 5C).

The JAK2/STAT3 signaling pathway mediates the effects of SETD7. To further elucidate the mechanism underlying the inhibitory role of SETD7 in lung cancer metastasis, metastasis-related signaling pathways were investigated. It was previously demonstrated that the JAK/STAT3 pathway plays a crucial role in cell proliferation, survival, differentiation and, in particular, tumor invasion and metastasis (21). This prompted us to explore the effect of SETD7 on the JAK/STAT3 pathway.

By transfecting pSETD7 in lung cancer cells, it was observed that SETD7 effectively suppressed the phosphorylation level of JAK2 and STAT3, while the total STAT3 level remained unchanged. As expected, when SETD7 was downregulated, the phosphorylation levels of both JAK2 and STAT3 were increased (Fig. 6A and B).

Furthermore, the role of the JAK/STAT3 pathway on cell migration was determined by introducing the STAT3-specific inhibitor Stattic. SETD7 expression was knocked down in lung cancer cells and the cells were treated with Stattic. The wound healing assay demonstrated that Stattic effectively attenuated the effect of SETD7 on cell migration (Fig. 6C and D). Taken together, these results indicate that the JAK/STAT3 pathway may be responsible for the effect of SETD7 on metastatic potential.

As it was demonstrated that SETD7 overexpression increased H3K4me2 expression, whereas SETD7 knockdown reduced H3K4me2 expression (Fig. 2E), these results suggest that histone methylation may be implicated in the effects of SETD7 on metastatic potential.

Discussion

SETD7 is a protein lysine monomethylase that methylates not only histone H3K4, but also non-histone proteins (22,23). The methylation on proteins alters their activity and affects a series of biological processes. The role of SETD7 in cancer

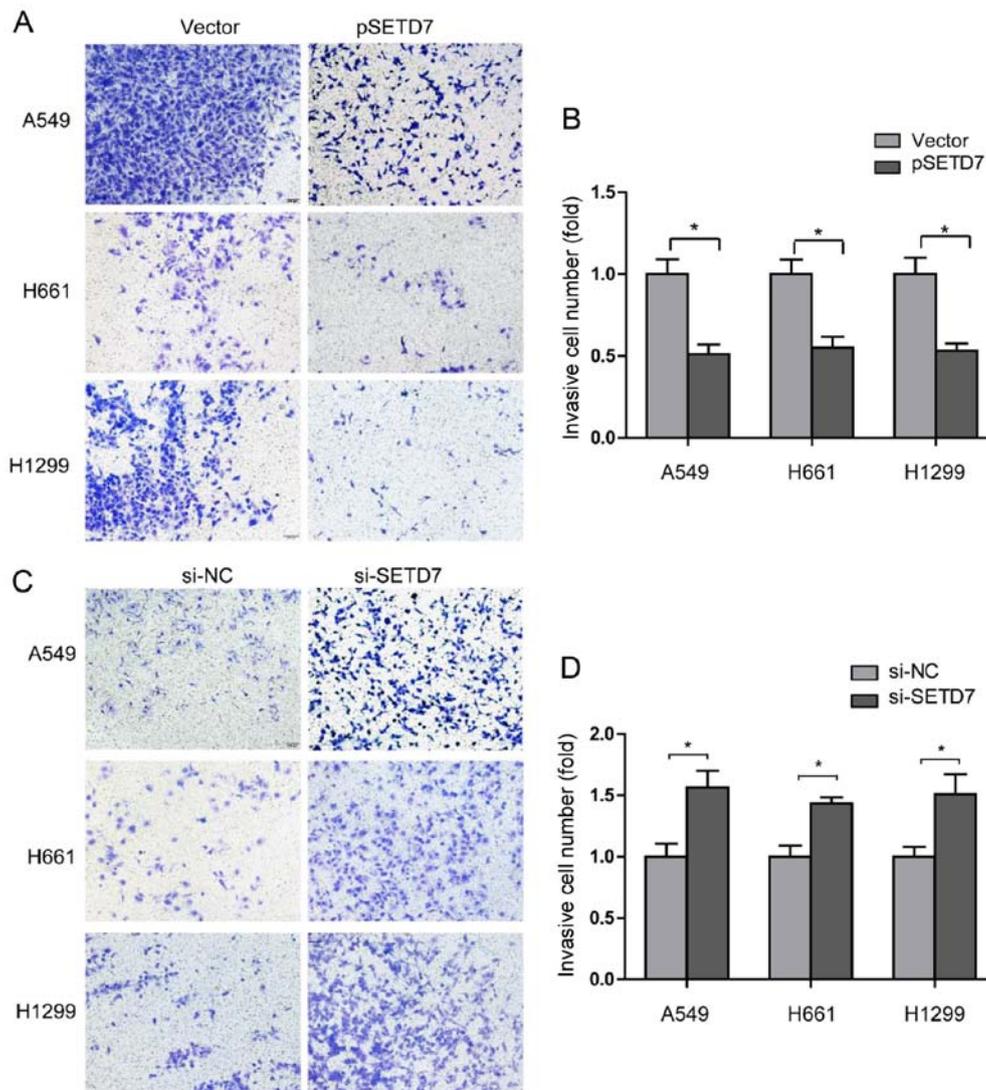


Figure 4. SETD7 inhibited the invasion of lung cancer cells. (A and B) Lung cancer cells were transfected with pSETD7. Cell invasion was assessed by Transwell assay after 48 h. (C and D) Lung cancer cells were transfected with si-SETD7. Cell invasion was assessed by Transwell assay after 48 h. Representative photographs of cell invasion are presented (magnification, x100). Values represent the mean \pm standard deviation from three independent measurements. * $P < 0.05$. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7.

development has recently attracted attention. In the present study, SETD7 was found to be downregulated in both lung cancer tissues and lung cancer cell lines. The expression of SETD7 was assessed by western blotting and qPCR analysis. Due to the limitations regarding the amount of tissue available, we were unable to perform immunohistochemistry assay, which can reveal the distribution of SETD7 expression across the tumor macrostructure. As the number of tissue samples was relatively small, more lung tumor tissues must be assessed in order to establish the correlation of SETD7 expression with tumor size and pathological stage.

The effect of SETD7 on cancer cell growth is controversial. SETD7 inhibits cell growth in colorectal cancer (12), acute myeloid leukemia (24), gastric cancer (10), cervical cancer and colon cancer (25). On the contrary, SETD7 promotes hepatocellular carcinoma cell growth (26). Interestingly, in breast cancer, Song *et al* demonstrated that SETD7 inhibited cell growth through regulation of Gli-1 expression (11), whereas Zhang *et al* reported that SETD7 promoted tumor growth

by interacting with the transcription factor GATA1 (27). The opposite roles of SETD7 in the same cancer may be due to the physiological subtype and stage of the tumor. In the present study, SETD7 did not affect lung cancer cell viability.

Cancer metastasis is the primary cause of treatment failure, and 90% of lung cancer patients eventually succumb to metastatic disease (20). Tumor metastasis is a complex process. Cell migration and invasion are crucial steps during metastasis. Therefore, the role of SETD7 in cell migration and invasion was investigated. The results demonstrated that SETD7 downregulation enhanced both cell migration and invasion. The role of SETD7 in migration and invasion is also supported by other studies. Akiyama *et al* demonstrated that SETD7 downregulation promoted gastric cancer cell migration and invasion (10), and Gu *et al* reported that SETD7 downregulation significantly decreased cell migration and invasion in hepatocellular carcinoma (26).

As our results demonstrated that SETD7 exerted no effect on cell viability, it was inferred that the role of SETD7 in

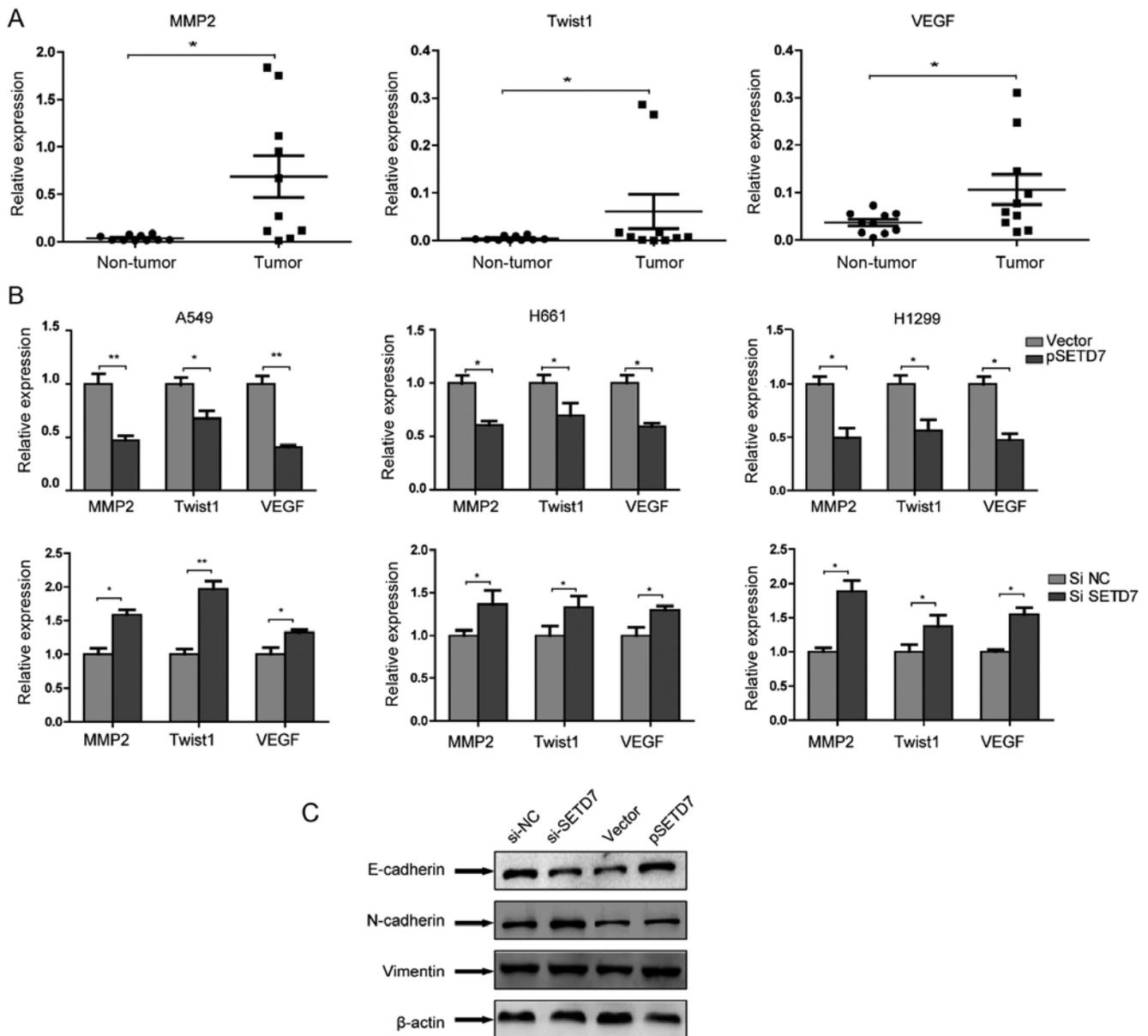


Figure 5. SETD7 regulated metastasis-related gene expression. (A) Gene expressions in tumor and non-tumor specimens were detected by qPCR. (B) Lung cancer cells were transfected with pSETD7 or siRNA. Gene expressions were detected by qPCR. (C) A549 cells were transfected with pSETD7 or siRNA. Protein expressions were detected by western blotting. Values represent the mean \pm standard deviation from three independent measurements. * $P < 0.05$, ** $P < 0.01$. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7; qPCR, quantitative PCR; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

metastasis relies on its regulation of metastasis-related genes, rather than the stimulation of cell proliferation. A number of genes have been demonstrated to be involved in metastasis. MMP2 mediates the promoting effect of chorionic gonadotropin on epithelial ovarian cancer metastasis (28). lncRNA TP73-AS1 stimulates ovarian cancer cell metastasis via regulation of MMP2 and MMP9 (29). In NSCLC, miR-149 inhibits cell growth and metastasis by targeting MMP2 (30). Furthermore, the transcription factor Twist1 is also involved in metastasis by regulating gene transcription.

Twist1 induces EMT and promotes metastasis (31); Twist1 is also responsible for hypoxia-induced angiogenesis and metastasis (32). In NSCLC, Li *et al* reported that promoting protein family member 3 enhances cell invasion and tumor metastasis via the STAT3/Twist1 pathway (33). VEGF is

another common metastasis-related gene. VEGF binds to the VEGF receptor, induces angiogenesis and promotes metastasis and tumor progression (34). Zhang *et al* demonstrated that a novel oncogene, KIF26B, promotes gastric cancer metastasis via activating the VEGF pathway (35). Zhou *et al* demonstrated that oxymatrine inhibits lung cancer cell migration by regulating miR-520/VEGF (36). In the present study, it was observed that lung cancer tissues expressed higher levels of MMP2, Twist1 and VEGF, and lower levels of SETD7, compared with non-cancerous tissue. Overexpression of SETD7 in lung cancer cells downregulated MMP2, Twist1 and VEGF, indicating that SETD7 modulates the expression of metastasis-related genes.

To further elucidate the mechanism underlying the role of SETD7 in metastasis, we investigated signaling pathways

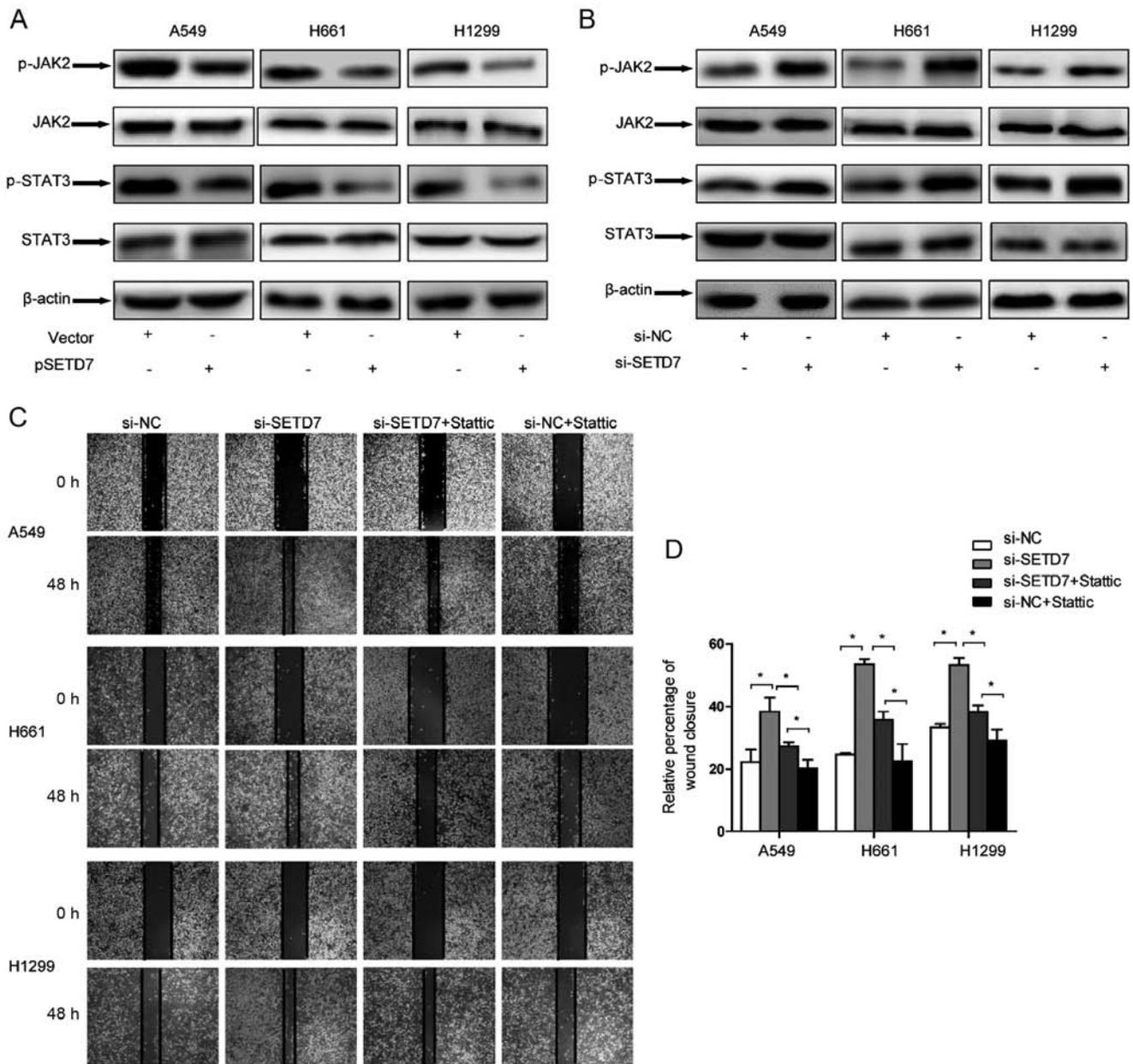


Figure 6. The effect of SETD7 is mediated by the JAK2/STAT3 signaling pathway. (A and B) Lung cancer cells were transfected with pSETD7 or si-SETD7. The levels of p-JAK2, STAT3 and p-STAT3 were examined by western blotting. (C and D) Lung cancer cells were transfected with si-SETD7 and treated with the STAT3 inhibitor Stattic (5 μ M). Cell migration was assessed by the wound healing assay after 48 h. Values represent the mean \pm standard deviation from three independent measurements. * $P < 0.05$. SETD7, [Su(var)3-9, enhancer of zeste, Trithorax] domain-containing protein 7; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3.

involved in cell invasion and metastasis, among which the JAK/STAT3 pathway attracted our attention. Indeed, it was demonstrated that the JAK2/STAT3 signaling pathway mediated the effect of SETD7. An *et al* reported that STAT3 induces long non-coding RNA LINC00668 expression to promote migration and invasion of NSCLC cells (37). Jin *et al* demonstrated that TRIM14 enhances colorectal cancer cell invasion through the STAT3 pathway (38). He *et al* reported that mesenchymal stem cells inhibit breast cancer progression by suppressing the STAT3 signaling pathway (39). Therefore, STAT has emerged as a promising target for cancer treatment (40,41).

To the best of our knowledge, the direct regulation of SETD7 expression has not been reported to date, and the

mechanism of SETD7 regulation remains unclear. miRNAs regulate their target genes, thereby regulating tumor development and progression. SETD7 may also be regulated by miRNAs, particularly those that have been found to be dysregulated in tumors. Further investigation on these miRNAs may help elucidate the SETD7 network in tumors.

In summary, the present study demonstrated that SETD7 is downregulated in lung cancer, and SETD7 downregulation enhanced the migration and invasion of lung cancer cells. It was further demonstrated that downregulation of SETD7 promoted the metastatic potential of lung cancer cells via the JAK2/STAT3 pathway. These findings may provide useful information regarding the potential of SETD7 as a novel therapeutic candidate for metastatic lung cancer treatment.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

LC, YR, XG, LW, QZ and XL performed the experiments. XW contributed to the data analysis. ZM contributed to the study design and data analysis. KX contributed to the study design, data analysis and wrote the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Tianjin Medical University General Hospital. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that there are no competing interests.

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