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MARVELD3 inhibits the epithelial-mesenchymal transition and cell migration by suppressing the Wnt/β-catenin signaling pathway in non–small cell lung cancer cells

Shirong Li[1] | Saiping Qi[2] | Yanmeng Li[2] | Chunpan Zhang[2] | Lan Sun[3] | Chunquan Liu[4] | Haoyan Wang[5]

[1]Department of Infectious Disease, Beijing Friendship Hospital, Capital Medical University, Beijing, China

[2]Beijing Institute of Clinical medicine Research, Beijing Friendship Hospital, Capital Medical University, Beijing, China

[3]Department of Pathology, Beijing Friendship Hospital, Capital Medical University, Beijing, China

[4]Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University, Beijing, China

[5]Department of Respiratory Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing, China

Correspondence
Lan Sun, Department of Pathology, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China.
Email: sunlan83@163.com

Chunquan Liu, Department of Thoracic Surgery, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China.
Email: liuchunquan1990@ccmu.edu.cn

Haoyan Wang, Department of Respiratory Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing, 100050, China.
Email: haoyanw@ccmu.edu.cn

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Abstract

Background: The epithelial-mesenchymal transition (EMT), featured by abatement of cell–cell contact, is related to exacerbating non–small cell lung cancer (NSCLC) by inducing metastasis. MAL and relevant proteins for vesicle trafficking and membrane link domain 3 (MARVELD3) is a novel tight junction protein participated in the EMT. There is limited information available about the mechanism of MARVELD3 in NSCLC. In this trial, the inhibition effect of MARVELD3 on human NSCLC cells will be discussed.

Methods: MARVELD3 expression was measured in NSCLC tissues and para-carcinoma tissues. The expression of MARVELD3 and EMT-related genes were examined in transforming growth factor (TGF)-β1-induced NSCLC cells. NSCLC cells with MARVELD3-knockdown and overexpressed were established to analyze the relationship between MARVELD3 and EMT and cell migration. The Wnt/β-catenin pathway expression was also analyzed in NSCLC cell models and clinic species.

Results: Lower protein levels of MARVELD3 were observed in NSCLC samples than para-carcinoma specimens, and the decreased expression of MARVELD3 in NSCLC specimens was associated with tumor metastasis. E-Cadherin and MARVELD3 expression was reduced in TGF-β1 treated NSCLC cells, whereas increased Vimentin expression was detected. MARVELD3 changed the EMT-related genes and induced cell migration. In addition, Wnt/β-catenin pathway and target genes, MYC and CCND1, expressions were inhibited in MARVELD3 overexpressed NSCLC cells.

Conclusions: TGF-β1 induced EMT in human NSCLC cells can be suppressed by MARVELD3 through Wnt/β-catenin signaling pathway. These results indicate that MARVELD3 might be a potential therapeutic modality useful in the treatment of NSCLC.

KEYWORDS
EMT, MARVELD3, NSCLC, TGF-β1, Wnt/β-catenin

INTRODUCTION

Lung cancer is one of the major causes of cancer relevant deaths in the world, with ∼85% of lung cancers being non–small cell lung cancer (NSCLC).[1–3] Despite improvements in the diagnosis and treatment of lung cancer, the 5-year

Shirong Li, Saiping Qi, and Yanmeng Li have contributed equally to this work and should be considered co-first authors.

survival rate for NSCLC remains lower than 20%.[4] This outcome is predominantly because of tumor metastasis, which is the main cause of death in ~90% of malignancy patients.[5] Therefore, elucidating the mechanisms of NSCLC occurrence and metastasis is becoming a top priority of current research.

In recent years, the epithelial mesenchymal transition (EMT) is known as a classical theory in tumor metastasis.[6] EMT is known as a process causing epithelial cells to lose their cell–cell adhesions and apical-basal polarity, changing cytoskeleton composition to obtain mesenchymal cell features, then increasing migration ability and invasiveness,[7,8] which is characterized by protein expression changes such as E-Cadherin, N-Cadherin, and Vimentin.[9] Transforming growth factor β (TGF-β) is considered to be a critical mediator in the process of EMT and the pathogenesis of NSCLC cell invasion.[10,11] In addition, the EMT can modulate the activity of junction proteins, such as Occludin and Claudins by the Snail family.[12]

The Wnt/β-catenin signaling pathway is known as the canonical pathway of Wnt widely involved in process of EMT. It could be stimulated by various factors, then promote the β-catenin nucleus translocation and accumulation to enhance the abnormal expression of the downstream genes, such as MYC and CCND1.[13] Next, this pathway is involved in the initiation and tumor evolution of quite a few cancers, such as oral carcinoma, liver carcinoma, and lung cancer.[14,15] Recent studies showed that activation of Wnt/β-catenin signaling pathway is probably critical for the EMT in NSCLC.[13,16]

Tight junctions (TJs) is intercellular adhesion complex locating at the uppermost domain of cell–cell contacts, which acts as multifunctional molecular gates and plays a very important role in maintaining the epithelial cells.[17–19] TJs consist of vital membrane proteins, for instance claudins, occludin, and junctional adhesion molecules (JAMs).[20] As one of tight junction-associated marvel proteins (TAMPs), MARVELD3 is a major integral component of bicellular domain similar to occludin and tricellulin. Its functions in regulating cell behavior and survival is being gradually explored.[21–23] Despite that, there is still a lot of mystery surrounding how MARVELD3 is regulated in NSCLC.

In this study, we found the lower expression of MARVELD3 in NSCLC tissues and human NSCLC cells during EMT process by means of TGF-β intervention. Furthermore, we explored the impact of MARVELD3 on regulation of pulmonary EMT process and the possible potential mechanisms.

MATERIALS AND METHODS

Ethics statement

This study was approved by the ethical board of Beijing Friendship Hospital (no. 2022-P2-192-01, Beijing, China) and the study subjects provided written consent to participate Funding support for key clinical projects in Beijing.

Patients

Fourteen paired NSCLC and para-carcinoma lung tissues samples with different degrees of metastasis obtained from 14 patients who underwent surgery at Beijing Friendship Hospital of Capital Medical University diagnosed with NSCLC that relied on histopathological examination. The stage of patients was in accordance with the eighth edition of TNM staging, the standard for lung cancer of International Association for the Study of Lung Cancer.[24] All tissue samples were instantly snap-frozen within liquid nitrogen and long-term preserved at −80°C until required.

Cell culture and TGF-β treatment

Two NSCLC adenocarcinoma cell lines (PC9 and HCC827 cells) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences. They were cultured according to the instructions and maintained under standard condition in a humidified atmosphere of 5% CO_2 at 37°C. Recombinant human TGF-β was purchased from PeproTech, stored as a 10 μg/mL stock solution at −20°C, and diluted 10 ng/mL with culture medium when needed.

Small interfering RNA and plasmid transfection

MARVELD3-small interfering RNA (siRNA) (ID stB0014 718A) and negative control siRNA (ID siN0000001-1-5) applied in this study were purchased from RiboBio. The *MARVELD3* gene fragment was cloned into the PCDH-CMV-MCS-EF1-copGFP-T2A-Puro plasmid vector between EcoR1 and Not1 sites. Transfection was operated adopting LTX reagent (Invitrogen) as the product specification. The medium was replaced 12 h after transfection. The cells were harvested after 24 h for use in various assays.

Cell viability analysis

NSCLC cells were initially plated at a density of 3000 cells/100 μL per well in a 96-well culture plate and incubated. The assay was performed in triplicate for each set of conditions. A total of 10 μL of MTS reagent (Promega) mixed with 100 μL of culture medium was added to the cells at 37°C for 2 h according to the manufacturer's instructions. 100 μL of cell culture supernatant was transferred to a new 96-well plate with flat-bottomed. The absorbance was read at an OD of 450 nm with a 96-well plate reader.

Tumor cell wound healing assay

NSCLC cells transfected after *MARVELD3* siRNA and negative control siRNA transfection were seeded into plates and cultured through the night. Three linear wounds were

established on cell monolayers that had reached 90% or greater using 200-μL pipette tip next day. The cells were washed using ice-cold phosphate-buffered saline (PBS) then cultured in RPMI-1640 containing 10% FBS in a water-saturated atmosphere of 5% CO_2 at 37°C for 24 h. Photographs of the wounded zone were taken after generating the scratch at 0 h and 48 h respectively to examine the cells wound-healing ability. The experiments were operated in triplicate and repeated once at the least.

Tumor cell transwell migration assay

Transwell migratory apparatus (Costar, Corning Costar) was applied to assess tumor cell migration capacity. *MARVELD3*-siRNA or expression plasmid transfected cells at a density of 1×10^5 per well were seeded into the chamber at the top in serum-free medium, meanwhile 10% fetal bovine serum (FBS) medium was added to the beneath chamber. It was cultured for 24 to 8 h, the cells were fixed for 30 min in 4% paraformaldehyde, and then stained for 30 min in 0.1% crystal violet. Cells on the underside were calculated using microscope.

RNA extraction and quantitative polymerase chain reaction assays

Total RNA was isolated with TRIzol reagent (Sigma) according to the manufacturer's instructions. Total RNA (2000 ng) was reverse transcribed adopting random primers by routine settings with a PrimeScript RT reagent kit (Roche). SYBR Premix was used to identify gene expression levels according to the instructions of the manufacturer. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was used to normalize the results. The primers information applied are presented in Table S1. ABI 7500 was used to conduct the quantitative polymerase chain reaction (qPCR) assays collect data. Our qPCR results were analyzed and reported as fold changes in relation to threshold cycle (CT) values.

Western blotting analysis

Lung tissue samples and cells were homogenized in RIPA protein extraction reagent complemented with protease inhibitor and phosphatase inhibitor (Roche). BCA Protein Assay was used to protein quantified and subsequent the total protein added with loading buffer and boiled at 99°C for 5 min. The nucleoprotein is extracted mainly by the nuclear and cytoplasmic protein extraction kit(Beyotime). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to polyvinylidene fluoride membranes (GE) and blocked with 10% milk in tris-buffered saline plus tween 20 for 2 h at room temperature then incubated with specific antibodies. Immunoreactivity was visualized by ECL chromogenic substrate (Bio-Rad). Antibodies (1:1000) against E-Cadherin (3195), Vimentin (5741), and β-catenin (8480) were purchased from Cell Signaling Technology. Antibodies against MARVELD3 (ab118916), c-Myc (ab32072), cyclin D1 (ab16663), Histone H3(ab1791), and GAPDH (ab6276) were purchased from Abcam (1:1000 dilution).

Immunofluorescence staining

The cells were cultured in 24-well slides for 24 h after transfected with *MARVELD3*-siRNA. Next, the cells were permeabilized with 0.3% Triton X-100 for 20 min at room temperature after fixed with 4% paraformaldehyde for 15 min, then blocked with goat serum for 1 h. Anti-Vimentin and anti-E-Cadherin (1:200, Cell Signaling Technology) antibodies were incubated with cells at 4°C all night. Alexa Fluor 488 conjugated secondary antibodies (Thermo) were used to stain the cells for 2 h. 4′,6-Diamidino-2-phenylindole was used to identify the cell nuclei (Molecular Probes). Confocal microscope (FV300, Olympus) was used to measure and photograph.

Immunohistochemistry

Immunohistochemical (IHC) staining of 5-μm partially paired paraffin-embedded NSCLC specimens was performed in the Pathology Department (Beijing Friendship Hospital) using primary antibodies as standard protocols for standard diagnostic specimens. The staining intensities were determined by measuring the integrated optical density (IOD) by light microscopy using Image-Pro v6.0.

Statistical analysis

Statistical analyses were carried out by GraphPad Prism version 7.0 software. All data were presented as mean ± standard deviation. One-way ANOVA or Student's *t*-tests were used for performing statistical analyses as appropriate. Kruskal-Wallis tests or Mann–Whitney tests were applied for nonparametric analyses. P value <0.05 is considered statistically significant.

RESULTS

Decreased MARVELD3 expression in NSCLC specimens correlates with the variation of EMT markers compared with that in para-carcinoma normal specimens

MARVELD3 expression in nine NSCLC cases and the para-carcinoma normal specimens were measured by western blotting. Table S2 displays the patients' initial features. The

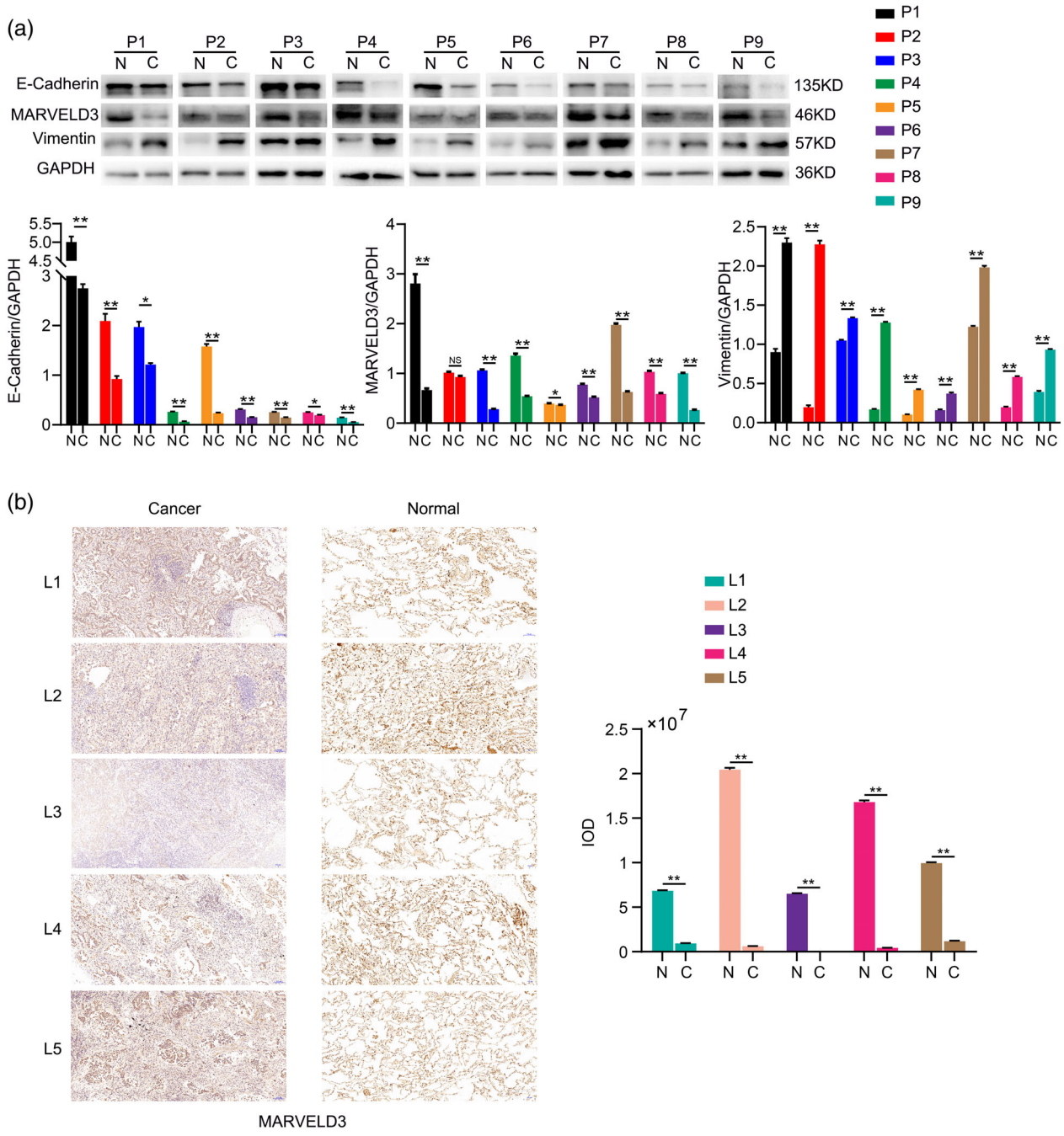

FIGURE 1 Association of decreased MARVELD3 expression with variation epithelial-mesenchymal transition markers in non–small cell lung cancer (NSCLC) tissues. (a) Western blots were used to evaluate the expression of E-Cadherin, MARVELD3, and Vimentin in normal lung tissues (N) and NSCLC tissues (C). The histogram represents relatively quantitative expression analysis. (b) Immunohistochemistry was performed to analyze MARVELD3 levels in NSCLC tissue sections (C) and normal lung tissues (N). Scale bars, 100 μm. The representative images and quantified levels are displayed.

patients had different degrees of tumor metastasis. Our results showed that the expressions of MARVELD3 and E-Cadherin protein were remarkably lower in NSCLC samples (without regard for tumor metastasis) than in normal tissues. In contrast, Vimentin protein expressions were higher in NSCLC samples (Figure 1(a)). The IHC results confirmed that the MARVELD3 expression was reduced in tumor tissues of five NSCLC patients (Table S3) compared to adjacent normal tissues (Figure 1(b)).

The decreased expression of MARVELD3 in NSCLC specimens was associated with tumor metastasis

For patients with NSCLC, clinical staging remains a crucial factor in the therapeutic effect and prognostication. In this study, the western blotting result indicated that MARVELD3 and E-Cadherin expressions were lower in stage IV NSCLC specimens with pleural metastases than that in stage I to III

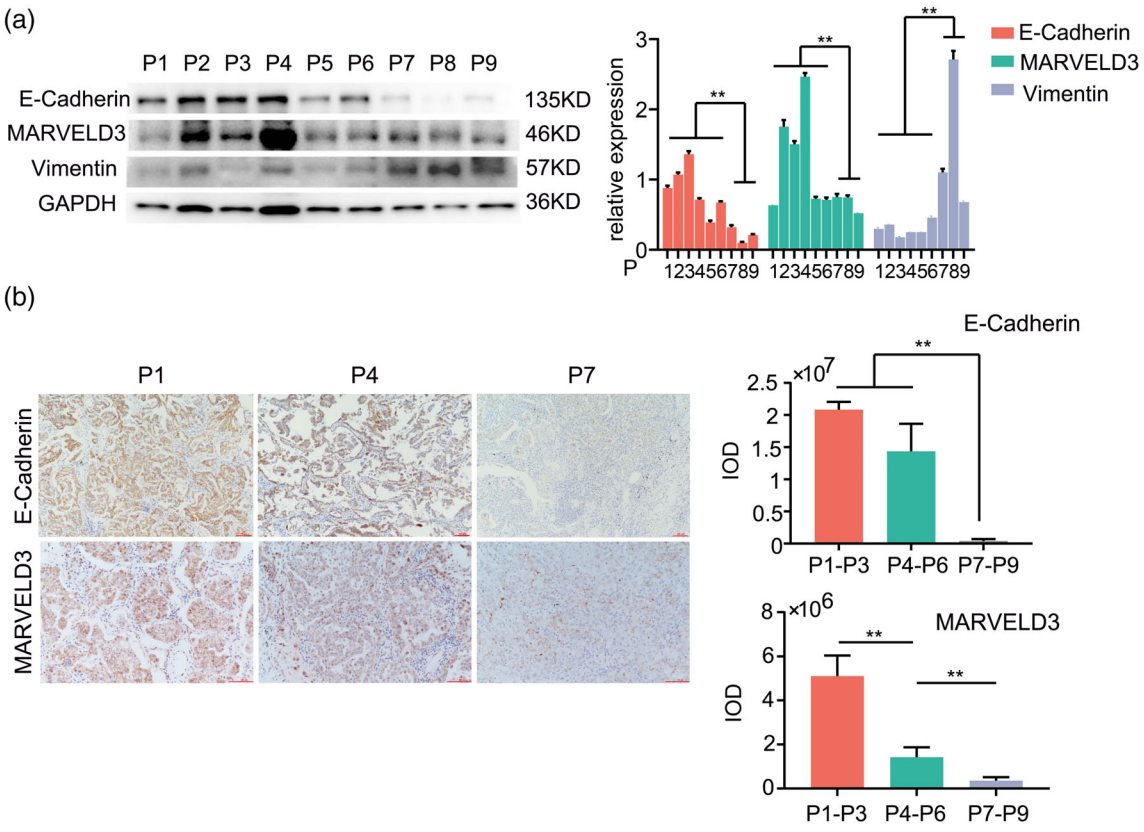

FIGURE 2 Association of decreased MARVELD3 expression with variation epithelial-mesenchymal transition markers in non–small cell lung cancer (NSCLC) tissues with different degrees of metastasis. (a) Western blot analysis showed the relative expression of MARVELD3, E-Cadherin and Vimentin in the NSCLC tissue of each patient with different degrees of metastasis. The histogram represents relatively quantitative expression analysis. (b) Immunohistochemistry data confirmed lower MARVELD3 and E-Cadherin expression in NSCLC tissues with pleural metastases than in NSCLC tissues with and without lymph node metastasis (scale bars, 100 μm). The representative images and quantified levels are displayed.

NSCLC specimens with or without lymph node metastasis; however, the expression of Vimentin showed the opposite trend (Figure 2(a)). The IHC results confirmed lower MARVELD3 and E-Cadherin expression in stage IV NSCLC specimens with pleural metastases than those in stage I to III NSCLC specimens with or without lymph node metastasis, and reduced MARVELD3 level was observed in NSCLC specimens with lymph node metastasis compared with those without lymph node metastasis (Figure 2(b)).

Downregulated expression of MARVELD3 in TGF-β1-induced EMT process of NSCLC cells

In tumor microenvironment, TGF-β regulates lung cancer cells migration by inducing the EMT.[25] To investigate the changes in MARVELD3 expression during TGF-β1-induced the EMT, NSCLC cell lines were treated with TGF-β1, then the changes of cell morpholog and the expressions of EMT markers and MARVELD3 were observed. A significant spindle shape change, in which cells are relatively scattered phenomenon, was observed (Figure 3(a)). Cell morphological alterations during the EMT are characterized by changes in spindle form and a lack of cell–cell interaction. Therefore, we compared the expression of the EMT markers and MARVELD3. In western blotting, a decrease in MARVELD3 and E-Cadherin was observed on days 4 and 6 after TGF-β1 treatment, and Vimentin expression was upregulated over time (Figure 3(b)). According to the reverse transcription (RT)-PCR assay, mRNA expression levels of *CDH1* and *MARVELD3* were reduced on days 2 or 4 after TGF-β1 treatment, whereas *VIM* mRNA expression was increased (Figure 3(c)).

MARVELD3 knockdown's effects on the expression of EMT genes in vitro

EMT could be induced by many transcription factors as ZEB1, SNAIL, and Twist with different protein size, structure, and biological functions. The magic is that they all stimulate mesenchymal markers (i.e., Vimentin, N-Cadherin) and suppress of E-Cadherin.[26] E-Cadherin and Vimentin are regarded as EMT markers.[13] To further

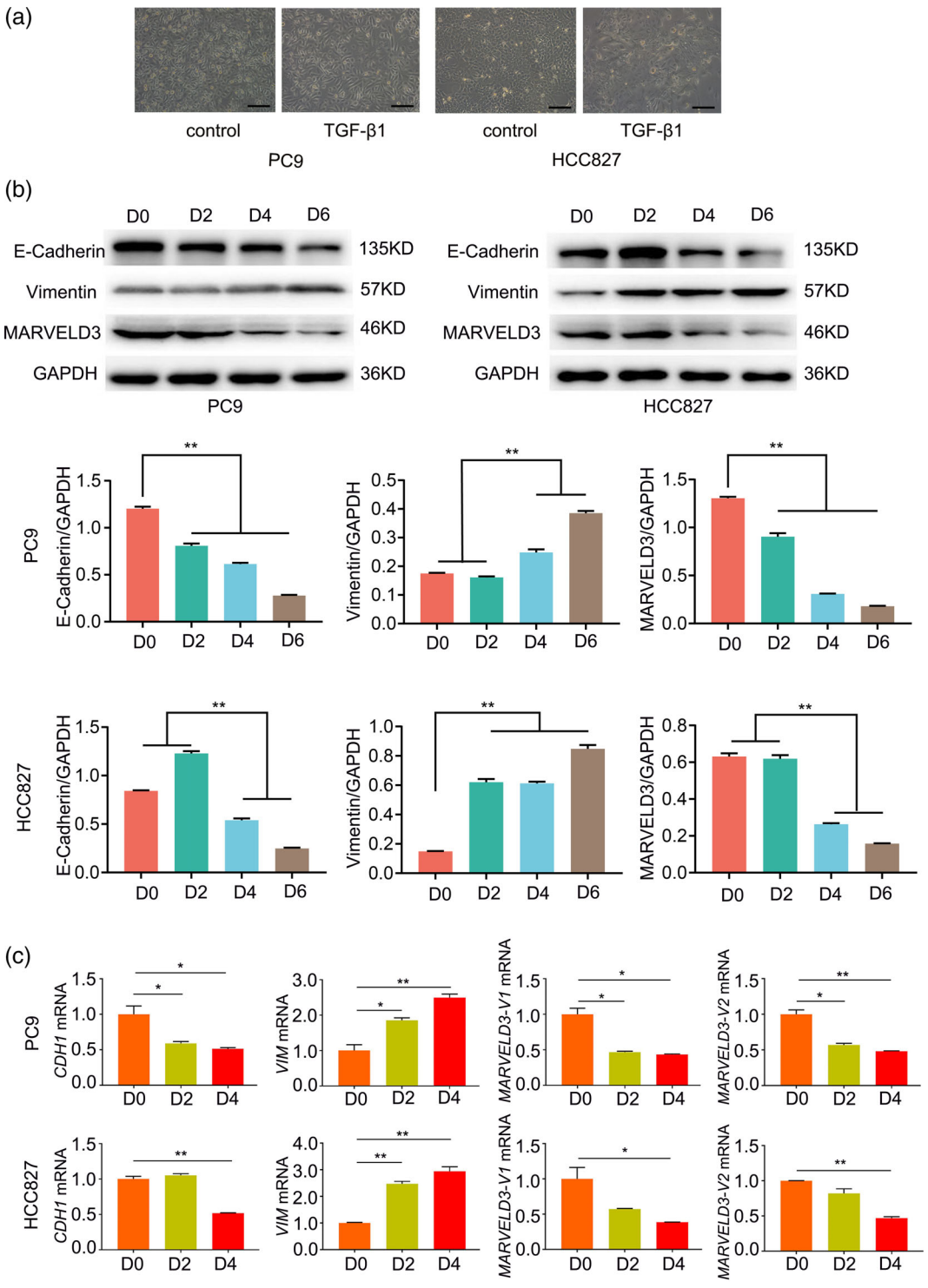

FIGURE 3 Downregulated expression of MARVELD3 in transforming growth factor (TGF)-β1-induced epithelial-mesenchymal transition process of non–small cell lung cancer cells. (a) The cell morphology of PC9 and HCC827 cell lines was changed after stimulation with TGF-β1 for 48 h (original magnification, ×10; scale bars, 200 μm). (b) Western blotting was used to assess the expression of MARVELD3, E-Cadherin, and Vimentin in PC9 and HCC827 cell lines treated with TGF-β1 after 0, 2, 4, and 6 days. The histogram represents relatively quantitative expression analysis. (c) Reverse transcription polymerase chain reaction was used to assess the expression of *CDH1*, *MARVELD3*, and *VIM* in PC9 and HCC827 cell lines treated with TGF-β1 after 0, 2, and 4 days. $^{*}p < 0.05$, $^{**}p < 0.01$.

investigate the effect of MARVELD3 in the NSCLC, we next established *MARVELD3*-siRNA NSCLC cell lines for the following study. Real-time quantitative reverse transcription (q-RT-PCR) was used to analyze E-Cadherin in MARVELD3-knockdown NSCLC cells. The results showed that MARVELD3 knockdown induced a diminution in

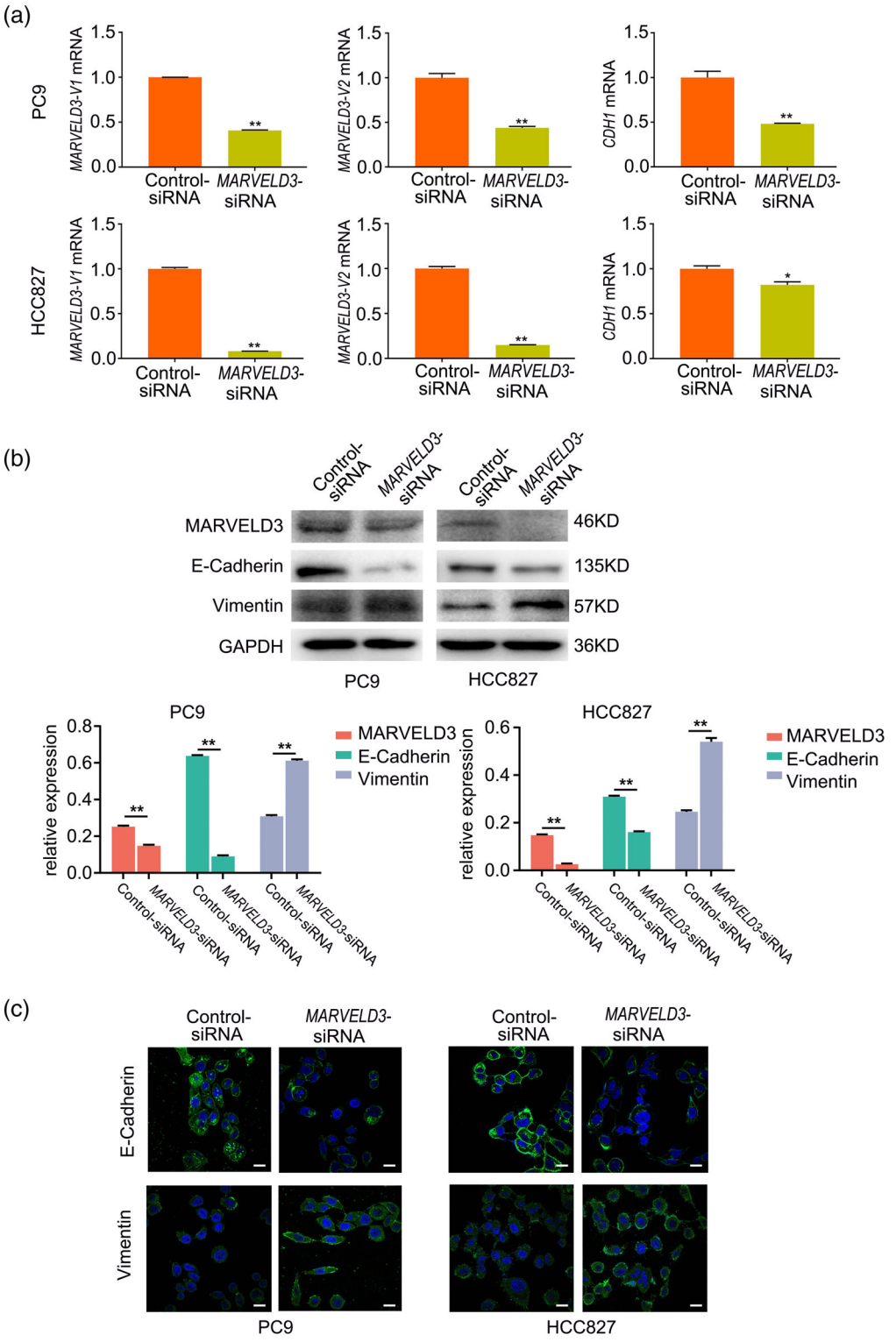

FIGURE 4 MARVELD3 knockdown's effects on the expression of epithelial-mesenchymal transition genes in vitro. (a) Real-time quantitative reverse transcription polymerase chain reaction and (b) western blotting were used to analyze the expression of MARVELD3, E-Cadherin, and Vimentin in control and MARVELD3-knockdown PC9 and HCC827 cell lines, respectively. The histogram represents relatively quantitative expression analysis. (c) Immunofluorescence staining was used to detect MARVELD3, E-Cadherin and Vimentin in control and MARVELD3-knockdown non–small cell lung cancer cells (original magnification, ×60; scale bars, 50 μm). $^{*}p < 0.05$, $^{**}p < 0.01$.

E-Cadherin (Figure 4(a)). Additionally, MARVELD3-knockdown cells showed a downregulation of protein expression in E-Cadherin and an upregulation of protein expression in Vimentin examined by western blotting (Figure 4(b)). Immunofluorescent staining showed that E-Cadherin expression was markedly decreased and Vimentin expression was

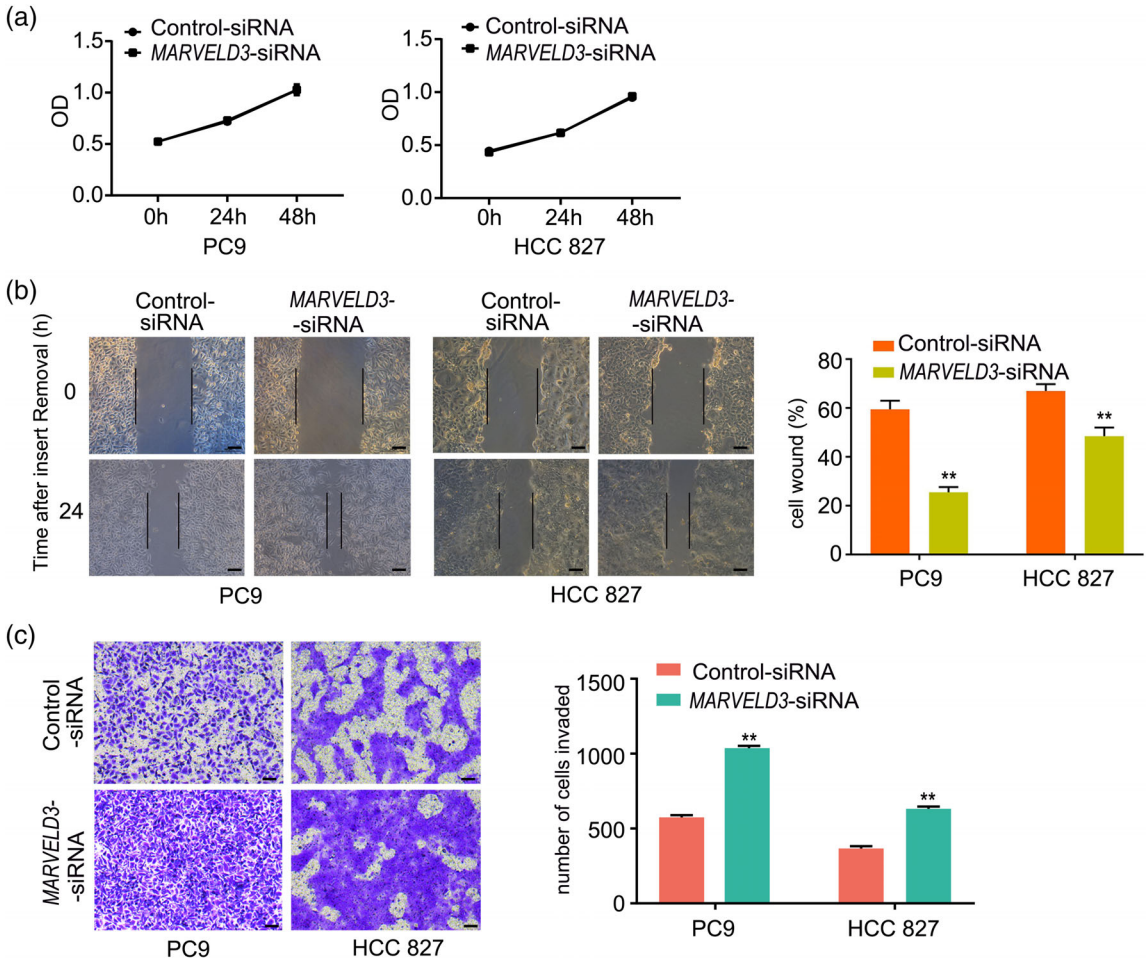

FIGURE 5 Effects of MARVELD3 knockdown on the promotion of non–small cell lung cancer (NSCLC) cell migration in vitro. (a) Cell viability analysis showed that MARVELD3-knockdown had no effect on the proliferation of PC9 and HCC827 cell lines. (b) Tumor cell wound healing assays showed that PC9/HCC827-MARVELD3-knockdown cells had a stronger migration ability than PC9/HCC827-control cells (original magnification, ×10; scale bars, 50 μm). (c) Transwell migration assays were used to test the migration ability of MARVELD3-knockdown NSCLC cells and control cells (original magnification, ×10; scale bars, 50 μm). $^{*}p < 0.05$, $^{**}p < 0.01$.

significantly increased in the MARVELD3-knockdown NSCLC cells in contrast with control cells (Figure 4(c)). The findings demonstrate that MARVELD3 can suppress the EMT process of the NSCLC cells in vitro.

MARVELD3 knockdown's effects on the migration of NSCLC cells in vitro

Cell migration was well known as one of the major biological process responsible of cancer metastasis. To determine the effect of MARVELD3 expression on the proliferation and migration of NSCLC cells, cell viability analysis, transwell migration assays, and wound healing examines were performed with tumor cell in vitro. Our results showed that MARVELD3-knockdown had no effect on the proliferation of PC9 and HCC827 cell lines (Figure 5(a)). However, MARVELD3-knockdown NSCLC cells had a notable wound-healing (Figure 5(b)) and transfer capacity (Figure 5(c)) compared with control cells, suggesting that MARVELD3-knockdown PC9/HCC827 cells have a stronger migration ability than control PC9/HCC827 cells. All of these results indicated that MARVELD3 could inhibit metastasis of NSCLC cells.

MARVELD3 overexpressed effect on the expression of EMT genes and the migration of NSCLC cells in vitro

To further confirm the role of MARVELD3 in NSCLC metastasis, we constructed NSCLC cell models of MARVELD3 overexpression (MARVELD3-OE). The results showed that MARVELD3-overexpression upregulated the expression of E-Cadherin of PC9 and HCC827 cell lines and downregulated the expression of Vimentin (Figure 6(a)). In addition, we found that overexpression of MARVELD3 had no influence on the proliferation of NSCLC

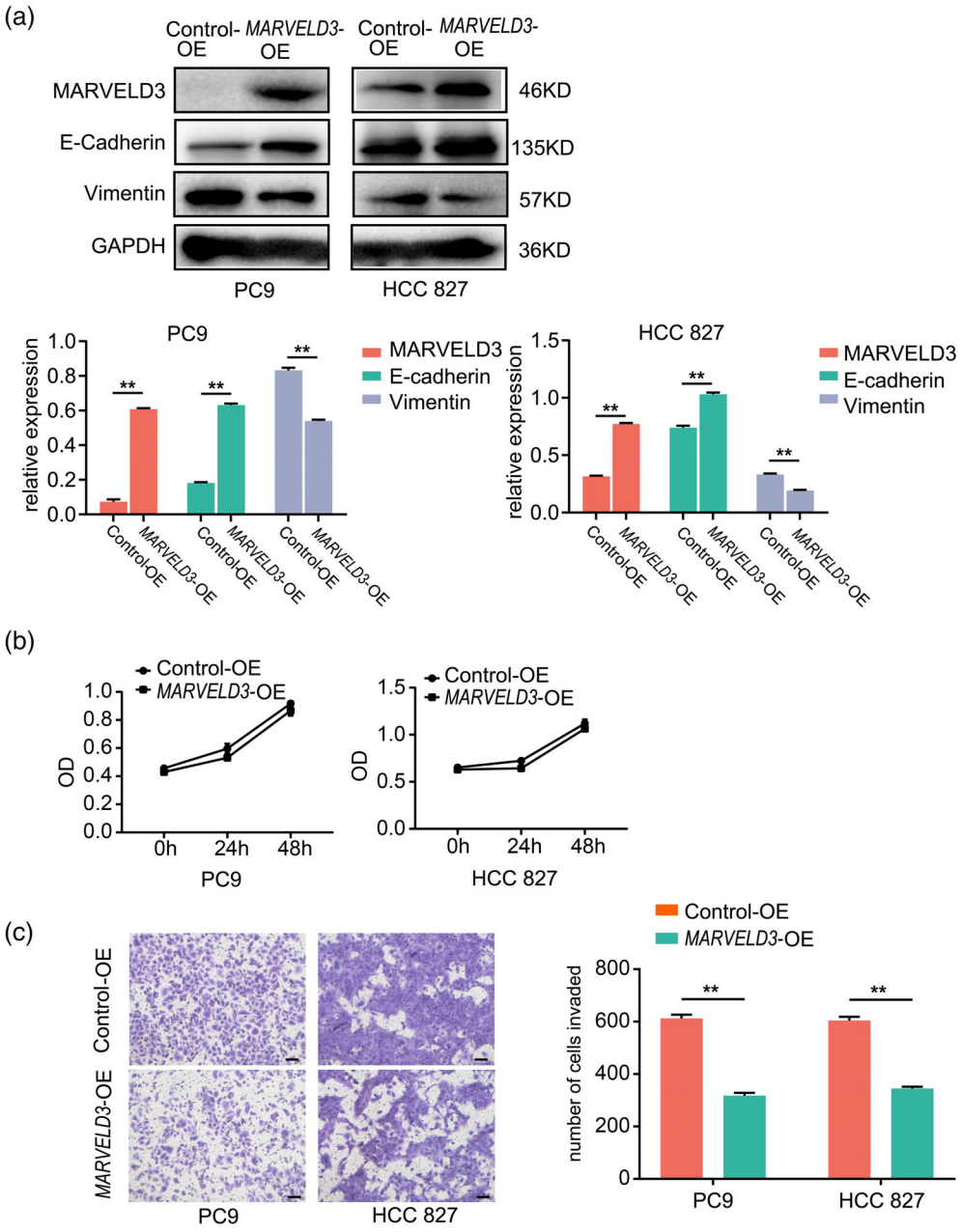

FIGURE 6 Effects MARVELD3 overexpressed on the expression of epithelial-mesenchymal transition genes and the migration of non–small cell lung cancer (NSCLC) cells in vitro. (a) Western blotting was used to analyze the expression of MARVELD3, E-Cadherin, and Vimentin in control and MARVELD3-overexpressed PC9 and HCC827 cell lines, respectively. The histogram represents relatively quantitative expression analysis. (b) Cell viability analysis showed that MARVELD3-overexpressed had no effect on the proliferation of PC9 and HCC827 cell lines. (c) Transwell migration assays were used to test the migration ability of MARVELD3-overexpressed NSCLC cells and control cells (original magnification, ×10; scale bars, 50 μm). $^{*}p < 0.05$, $^{**}p < 0.01$.

cells within 48 h (Figure 6(b)), but reduced cell migration (Figure 6(c)).

Effects of MARVELD3 on the inactivation of the Wnt/β-catenin signaling pathway

To verify the mechanism of MARVELD3-inhibited the EMT process in NSCLC, we considered that many pathways might be concerned. The Wnt/β-catenin pathway is known as a definitive theory that promotes tumorigenesis and EMT process.[27] The nucleus translocation and accumulation of β-catenin is the hallmark of the activation of Wnt/β-catenin signal pathway. Therefore, we examined related vital genes of the Wnt/β-catenin pathway in MARVELD3-knockdown and overexpression NSCLC cell lines. We found that knocking down MARVELD3 upregulated c-Myc, cyclin D1, and nucleus β-catenin expression and in turn downregulated cytoplasmic β-catenin expression (Figure 7(a),(c)). Although MARVELD3 overexpressed in PC9 and HCC 827 cell lines reversed the changes (Figure 7(b),(d)). This finding suggested that MARVELD3 restrained Wnt/β-catenin pathway by inhibiting β-catenin translocated from cytoplasm to nucleus and then downregulated downstream target genes expression in NSCLC cells. We further found that lower expression of β-catenin in NSCLC tissues was correlated with worse disease progression (Figure 7(e)).

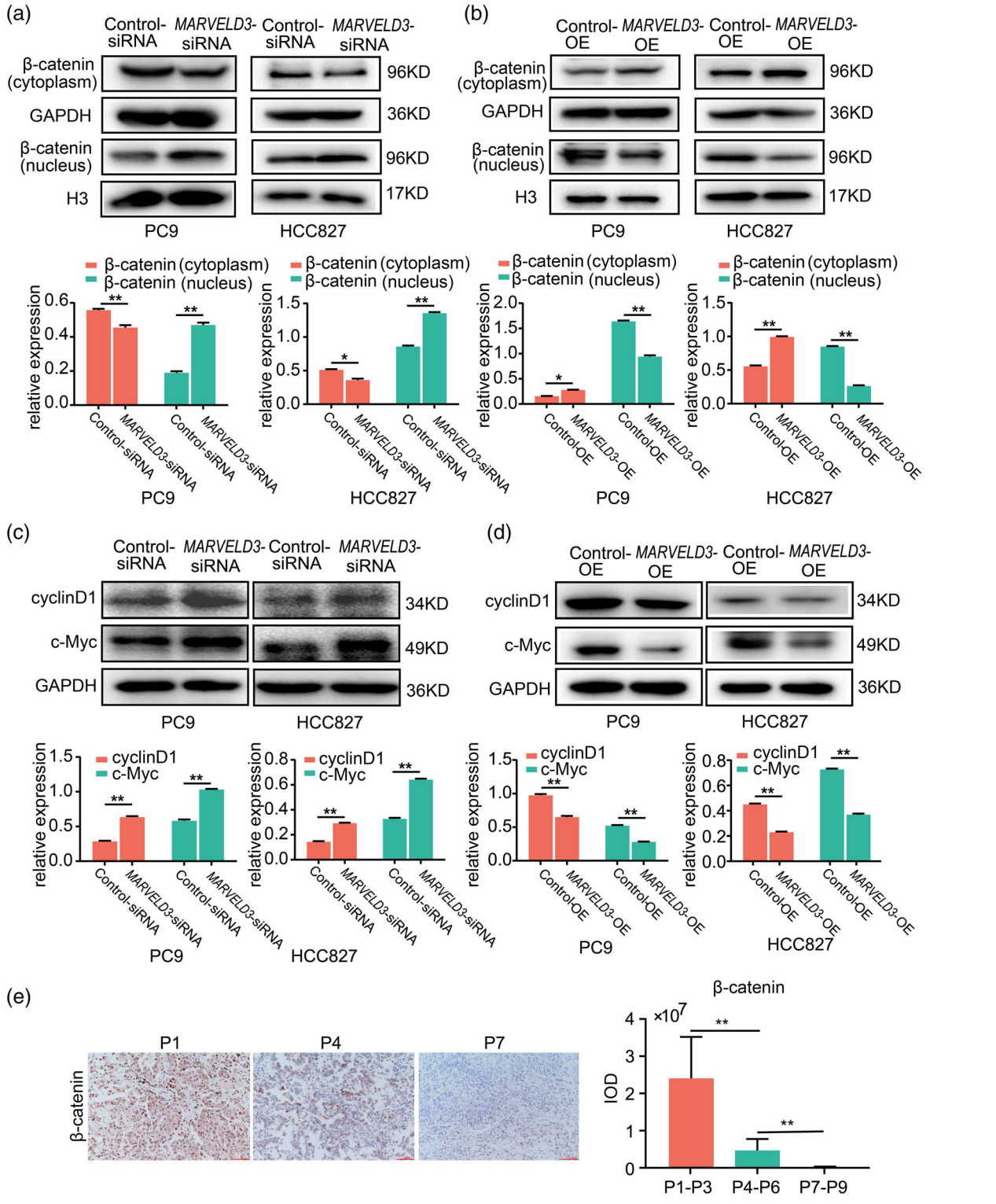

FIGURE 7 Effects of MARVELD3 on inactivation of the Wnt/β-catenin signaling pathway. (a), (b) Western blotting were used to examine the expression of nuclear β-catenin and cytoplasmic β-catenin. The histogram represents relatively quantitative expression analysis. (c), (d) Western blotting were used to examine the expression of key genes (c-MYC and CCND11) related to the Wnt/β-catenin pathway in control, MARVELD3-knockdown and MARVELD3-overexpressed PC9 and HCC827 cell lines. The histogram represents relatively quantitative expression analysis. (e) Immunohistochemistry was used to verify the lower expression of β-catenin in non–small cell lung cancer tissues with pleural metastases (original magnification, ×10; scale bars, 100 μm). The representative images and quantified levels are displayed.

DISCUSSION

In the current research, the expression of MARVELD3 and EMT biomarker were detected in NSCLC tissues and normal lung tissue, and then the correlation of MARVELD3 and NSCLC metastasis was evaluated. In vitro studies showed that MARVELD3 changed EMT-related biomarkers and inhibited transwell transfer capacity in NSCLC cells. Furthermore, at the molecular level, we found that MARVELD3 could inhibited Wnt/β-catenin pathway, which prevented the EMT process of tumor sequentially.

Identified as the third member of occludin family TAMPs MARVELD3 was critical for epithelial paracellular permeability.[17] Dysexpression of these MARVEL domain junctional proteins can occur in particular malignancies or cancer cell lines.[28–30] However, it is still unclear how these changes make the pathological impact. Aberrant claudin-3 expression intimately related to the tumorigenesis of human malignancies. MARVELD3 was reported to be transcriptionally downregulated in colon adenocarcinoma cells, poorly differentiated pancreatic cancer cells and hepatocellular carcinoma.[23,29,31] We first investigated the reason for the lower expression of MARVELD3 in NSCLC tissue than in normal lung tissue (Figure 1), which suggested that MARVELD3 may be a tumor suppressor gene in NSCLC. Moreover, the expression of MARVELD3 is lower in NSCLC with a more-distant metastasis (Figure 2).

Recent studies reported that TGF-β1, overexpressed in various tumor tissues, promotes tumor metastasis and is associated with the robust induction of the EMT in lung cancer cells.[32,33] EMT is a notable biological process associated with the invasion, metastasis, and prognosis of many malignant tumors. The loss of E-Cadherin-mediated cell-to-cell junctions is a hallmark of the EMT[32] and is usually related to the increased invasion and the formation of metastasis. It has been reported NSCLC is linked to abnormal E-Cadherin and Vimentin expression.[34,35] Our studies also revealed reduction expression of E-Cadherin as well as the increased expression of Vimentin in NSCLC tissue, which was associated with MARVELD3 expression (Figure 1), and the expression of MARVELD3 and EMT genes may be related to metastasis of NSCLC (Figure 2). Furthermore, it has been confirmed that MARVELD3 was decreased during EMT process induced by Snail in human pancreatic cancer cells.[29] Using NSCLC cells, we also found that TGF-β1 repressed E-Cadherin expression, induced Vimentin expression, and inhibited MARVELD3 expression (Figure 3). Subsequently, we constructed MARVELD3-knockdown and overexpressed NSCLC cells and revealed that decreased MARVELD3 expression was linked to abatement of E-Cadherin expression at the same time negative correlated with the expression of Vimentin (Figure 4), and overexpressed MARVELD3 did the opposite effect (Figure 6). These demonstrated that MARVELD3 can regulate the EMT in NSCLC cells.

As a newly discovered TAMP members, MARVELD3 regulates epithelial cells proliferation, migration, survive and distribute widely in various tissues.[23] Steed et al.[31] verified that MARVELD3 regulated Caco-2 cell (a human intestinal cell line) migration and proliferation. In fact, other studies found that MARVELD3 expression was significantly decreased in many tumor cell lines, such as pancreatic, hepatocellular carcinoma, and prostate tumors.[23] In this study, we found the migration capacity of NSCLC cells was promoted induced by MARVELD3 knockdown (Figure 5), while suppressed by MARVELD3 overexpression (Figure 6). These data are consistent with previous research and demonstrate that loss of MARVELD3 expression is associated with NSCLC development and progression.

A wide range of signaling pathways involved in EMT process in NSCLC. In this study, we clarified the downstream signaling molecules accountable for MARVELD3 function in suppressing NSCLC. Moreover, we discovered that MARVELD3-inhibited tumor metastasis could attribute to the inactivation of Wnt/β-catenin signaling pathway by blocking nucleus translocation of β-catenin at least in part (Figure 7), which is crucial for the tumorigenesis and development of NSCLC.[36] The Wnt/β-catenin signaling pathway engaged in a variety of biological processes, including proliferation, differentiation, migration, invasion, and tissue maintenance and repair.[37–39] It has been confirmed that abnormal Wnt/β-catenin signaling pathway plays a role in the genesis and development of tumors.[40] β-catenin is the critical gene of the Wnt/β-catenin pathway, which is degraded by ubiquitination or phosphorylation.[40,41] Nuclear accumulation and transcription of β-catenin is a symbol of Wnt/β-catenin signal pathway activation.[41] Our results showed the varied expression of β-catenin, cyclin D1, and c-Myc in MARVELD3-knockdown and overexpression NSCLC cells. Notably, we found that MARVELD3 resulted in an increase in cytoplasmic β-catenin expression and a decrease in intranuclear β-catenin in NSCLC cells (Figure 7). These data suggested that MARVELD3 inhibits the Wnt/β-catenin signaling pathway and downregulates the expression of Wnt pathway target genes.

For another, β-catenin in the cytoplasm is a crucial component of adherence junctions by combining with E-Cadherin.[42] The decreased of β-catenin and E-Cadherin in the cytoplasm might weakened the β-catenin/E-Cadherin dependent adherens junction, caused EMT, and accelerated cell migration of NSCLC cells, therefore, the same trends are often observed clinically.[13,16] Our data found that reduced expression of β-catenin was observed to be related to more-distant metastasis (Figure 7). This was not consistent with the results obtained from NSCLC cell lines. The contradiction between cell line results and clinical research has also been reported, but it has not been fully understood up to now and needs further research.[43] These results suggest that MARVELD3 can prevent NSCLC cell EMT progression and transferred via suppressing Wnt/β-catenin pathway activation.

In summary, MARVELD3 is an effective inhibitor of the EMT and metastatic responses induced by TGF-β1 in human NSCLC cells and is mediated by the Wnt/β-catenin signaling pathway. As a consequence, MARVELD3 can be a potential agent for treating human non-small cell lung cancer.

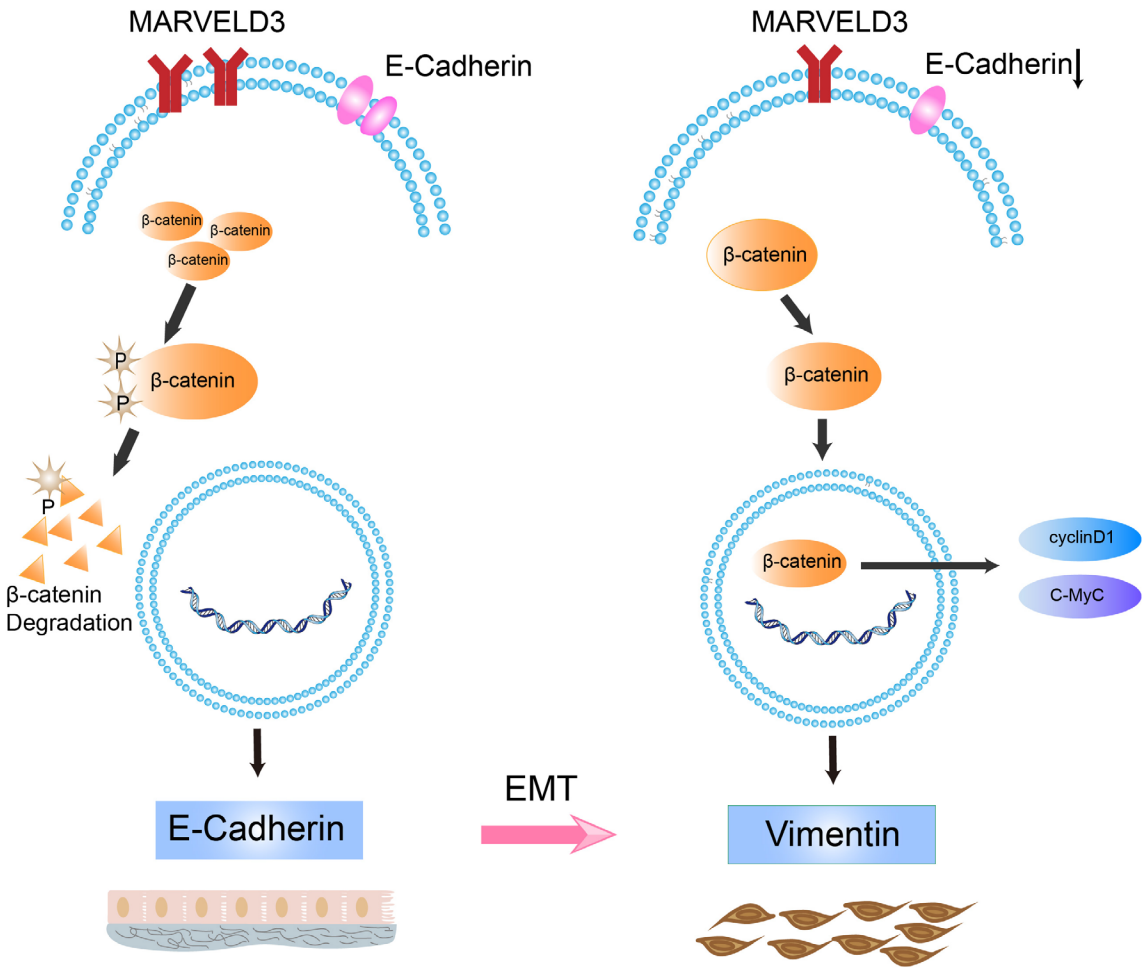

FIGURE 8 A schematic model illustrating our findings on novel role of MARVELD3 in non–small cell lung cancer was shown.

AUTHOR CONTRIBUTIONS

Haoyan Wand contributed to project design and supervision. Chunquan Liu collected clinical data and applied for compliance with medical ethics. Lan Sun was responsible for pathological diagnosis, immunofluorescence, and immunohistochemistry. Shirong Li and Saiping Qi performed the most experiments. Chunpan Zhang statistically analyzed the data. Shirong Li and Yanmeng Li wrote the manuscript and performed the revisions. The manuscript has been read through and approved by each author to ensure the authenticity or integrity of this study is adequately investigated and resolved.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The datasets used during the current study are obtainable from the corresponding author on rational requirement.

ETHICS APPROVAL

The protocol of this study was approved by the Research Ethics Committees of Beijing Friendship Hospital (no. 2022-P2-192-01, Beijing, China).

ORCID

Shirong Li https://orcid.org/0000-0003-0227-901x
Saiping Qi https://orcid.org/0000-0002-8168-842X
Yanmeng Li https://orcid.org/0000-0003-3151-7099
Chunpan Zhang https://orcid.org/0000-0002-4750-2564
Lan Sun https://orcid.org/0000-0003-2623-9645
Chunquan Liu https://orcid.org/0000-0002-7075-3732
Haoyan Wang https://orcid.org/0000-0002-2534-3590

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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