Original Article

# Overexpression of TWF1 promotes lung adenocarcinoma progression and is associated with poor prognosis in cancer patients through the MMP1 signaling pathway

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**Background:** It has been reported that twinfilin actin binding protein 1 (TWF1) is associated with the progression of breast and pancreatic cancers. However, the roles and mechanisms of TWF1 in lung adenocarcinoma (LUAD) have not been reported.

**Methods:** The expression levels of TWF1 in LUAD and normal tissues were analyzed using The Cancer Genome Atlas (TCGA) database, and validation was carried out with 12 clinical samples. The relationship between TWF1 expression and LUAD patients' clinical indices and immunity was investigated. Cell Counting Kit-8 (CCK-8) and migration and invasion assays were employed to explore the effects of downregulated TWF1 on LUAD cell proliferation and metastasis.

**Results:** TWF1 was upregulated in LUAD tissues, and upregulated TWF1 was correlated with the tumor (T) stage, node (N) stage, clinical classification, overall survival (OS), and progression-free interval (PFI) of LUAD patients. Moreover, the Cox regression analysis showed that TWF1 overexpression was an independent risk factor for the poor prognosis of LUAD patients. TWF1 expression was associated with tumor immune infiltration (such as dendritic cells resting, eosinophils, macrophages M0, and others), drug sensitivity (such as A-770041, Bleomycin, and BEZ235), tumor mutation burden (TMB), and sensitivity to immunotherapy. In the cell model, TWF1 expression interference significantly prohibited LUAD cell proliferation, migration, and invasion, which might be relevant to aberrant MMP1 protein downregulation.

**Conclusions:** TWF1 overexpression was correlated with poor prognoses and immune status of LUAD patients. Inhibited TWF1 expression delayed the growth and migration of cancer cells by downregulating MMP protein, implying that TWF1 is a promising biomarker for the prognoses of LUAD patients.

**Keywords:** Twinfilin actin binding protein 1 (TWF1); lung cancer; prognosis; lung adenocarcinoma (LUAD); bioinformatics analysis

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## Introduction

For nearly 60 years, lung cancer has been one of the most common cancers worldwide and a major cause of cancer-related death (1). In 2020, lung cancer had the second-highest incidence globally, and was one of the most invasive cancers (2). At present, lung cancer is broadly divided into small cell lung cancer and non-small cell lung cancer (NSCLC). As the primary subtype, NSCLC represents approximately 85% of all lung cancers, comprising lung adenocarcinoma (LUAD) (32–40%) (3), lung squamous cell carcinoma (25–30%), and large cell carcinoma (8–16%) (3,4). Due to the insidious onset, LUAD patients are commonly diagnosed at an advanced stage, resulting in loss of the opportunity for surgical treatment. The introduction of targeted therapy and immunotherapy has greatly improved the survival outcomes of patients with advanced LUAD (5,6). Hence, searching for more biomarkers of LUAD is of great significance. It has been reported that the matrix metalloproteases (MMPs) family can degrade basement membrane and extracellular matrix, and participate in many important physiological and pathological processes of lung cancer and inflammation. A large number of studies have reported that the expression of MMPs plays a key role in the occurrence, invasion and poor prognosis of various tumors including lung cancer (7). MMP1 belongs to collagenase, which is related to the ability of tumor cells to cross the basement membrane of vascular endothelial cells and epithelial cells. Overexpression of MMP1 is related to poor prognosis and more aggressiveness of tumors (8).

Twinfilin actin binding protein 1 (TWF1) is a relatively conserved actin-binding protein. Studies have shown that TWF1 plays a significant role in cancer progression (9). For instance, a study showed that TWF1 advanced breast cancer progression by modulating the expression of IL-11 and cyclins D1 and c-Myc (10). MiR-142-3p could inhibit the proliferation, invasion and migration of pancreatic cancer cells resistant to gemcitabine and promote apoptosis by competitively inhibiting the expression of SBF2-AS1 or TWF1 (11). These findings have preliminarily suggested that TWF1 is a potential pro-oncogene that advances cancer initiation and development, but no research has reported TWF1's role in LUAD. Therefore, in this study, we investigated TWF1's role in LUAD progression by using The Cancer Genome Atlas (TCGA) database and clinical samples and conducted cell experiments to appraise TWF1's significance in prognosis prediction of LUAD patients. We present this article in accordance with the MDAR and TRIPOD reporting checklists (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-395/rc).

### Highlight box

**Key findings**

- TWF1 could be a prognostic marker in lung adenocarcinoma.

**What is known and what is new?**

- The expression of TWF1 is related to the stage of LUAD, and can affect the immune infiltration of LUAD. Prior to this, it was known that TWF1 was significantly overexpressed in LUAD.
- We initially studied the significance of the increased expression of TWF1 in LUAD and the impact of high expression of TWF1 on LUAD cells.

**What is the implication, and what should change now?**

- In our study, we found that TWF1 was significantly overexpressed in lung adenocarcinoma, and highly expressed TWF1 could increase the proliferation, invasion and migration of lung adenocarcinoma cells.

## Methods

### *Data acquisition and processing*

The raw data (expression profile) and clinical information of LUAD cases were downloaded from TCGA (https://portal.gdc.cancer.gov/). The RNA-seq data (HTSeq and HTSeq-FPKM) of 594 LUAD samples were retrieved from TCGA database (Normal, n=59; Tumor, n=535). The dataset was filtered using the 'rma' function R (4.2.1; http://www.rproject.org/), and entries with missing data and duplicate entries were excluded, followed by transformation by log2 (TPM +1). The basic information of patients (age, gender, and clinical classification) was retrieved from the TCGA website, as well as tumor mutation burden (TMB). TMB was confirmed by calculating the number of insertion and deletion events in repeated genetic sequences. The clinical samples (n=12) were obtained from patients who were

diagnosed with LUAD in the Thoracic Surgery Department of the Affiliated Hospital of Zunyi Medical University from 1 June 2022 to 30 June 2022. Each sample included both tumor tissues and adjacent normal tissues. This study was approved by the Ethics Committee of the Affiliated Hospital of Zunyi Medical University [ethics approval number: (2021) 1-098] and conducted in accordance with the Declaration of Helsinki (as revised in 2013), and each patient signed informed consent.

### *TWF1 expression in LUAD*

TWF1 expression in LUAD and normal tissue samples were analyzed using the data downloaded from TCGA. Additionally, TWF1 expression was compared in paired samples (n=57). TWF1 expression in the clinical samples (tumor tissues *vs.* normal tissues) from 12 patients was analyzed using immunohistochemistry (IHC) and Western blotting.

### *Prognostic analysis of TWF1*

The predictive accuracy of each factor was assessed using the area under the curve (AUC) in the receiver operating characteristic (ROC) curves. TWF1's effects on the overall survival (OS) and progression-free survival (PFS) of LUAD patients were evaluated using Kaplan-Meier (KM) analysis. Besides, the association of TWF1 expression with the OS and disease-free interval (DFI) of LUAD patients was analyzed using the Cox regression analysis and TCGA database in the R environment. The survival curves of groups with high and low TWF1 expression were plotted using the KM method. The OS analysis was conducted by using ROC curves and the R package "survival" (rdocumentation.org/packages/survival). Differences between survival curves were evaluated with the log-rank test, and $P<0.05$ was considered statistically significant. The nomogram was constructed using a dataset of 594 LUAD patients from the TCGA database, which included RNAseq data in level 3 HTSeq-FPKM format in the LUAD lung adenocarcinoma project in TCGA, and RNAseq data in FPKM format that was transformed using log2. The purpose of the nomogram was to predict the 1, 3, and 5-year survival rates of patients based on five clinical variables: T stage, M stage, N stage, Pathologic stage, and TWF1 expression (grouped into high expression and low expression based on the median expression of TWF1). We used univariate Cox regression analysis to determine important prognostic factors and included clinically significant variables in the final model. Next, we converted the prediction equations into a graphical nomogram using the R package "rms". The nomogram was designed to allow for easy calculation of 1, 3, and 5-year survival probabilities by drawing a straight line from each variable to the corresponding point on the survival axis, and the sum of these points yielded the overall survival probability. Finally, we evaluated the performance of the nomogram using internal validation methods such as the concordance index (C-index) and calibration plot analysis. These analyses demonstrated that the nomogram had good discrimination and calibration capabilities, indicating its usefulness in predicting the survival outcomes of patients in the high and low expression groups of TWF1 in LUAD patients.

### *Correlation between TWF1 and tumor immune cell infiltration*

Tumor IMmune Estimation Resource (TIMER) is a web application driven by the database and is mainly applied to calculate the immune infiltration scores of 22 major immune cells (B cell, CD4+ T cell, CD8+ T cell, macrophage, neutrophil, and dendritic cell). The immune infiltration score of LUAD was obtained by using the data from the TCGA database with TIMER. Based on the data above, the association between TWF1 expression and immune infiltration was investigated.

### *Correlation between TWF1 and TMB, drug sensitivity, and sensitivity to immunotherapy*

In this study, TMB was defined as the number of somatic synonymous mutations per megabase (Mb) in each tumor sample without silent mutations. The TMB values of the mutant and nonmutant groups were compared using a Wilcoxon matched-pairs signed rank test. Genomics of Drug Sensitivity in Cancer (GDSC; https://www.ancerrxgene.org/) is a public database for tumor molecular markers and mutations (12). The gene mutation data of cell lines and half maximal inhibitory concentrations (IC50) of anticancer drugs were downloaded using the R package "pRophetic" to analyze the association between TWF1 expression level and patients' sensitivity to different anticancer drugs. The LUAD-associated immune score file was obtained from The Cancer Imaging Archive (TCIA) database (https://tcia.at/), and the correlation between TWF1 expression and LUAD-associated immune score

(PD1 and CTLA4) was analyzed.

### *Cell culture and model establishment*

A549 and H1299 cell lines were cultured in 1,640 culture solutions containing 10% fetal bovine serum (FBS), and normal alveolar epithelial cells Beas 2b were incubated in Dulbecco's modified Eagle medium (DMEM) culture solutions containing 10% FBS. Both 1,640 and DMEM culture medium were supplemented with 1% penicillin/streptomycin. All cells were prepared in a 37 ℃ incubator with 5% $CO_2$. Subsequently, LUAD cells (A549 and H1299; item number: CL-0016, Procell Life Science and Technology Co., Ltd., Wuhan, China) in the logarithmic growth phase were inoculated into a non-antibiotic-containing growth medium. Transfection was performed with a Lipofectamine® 3000 Transfection Kit (Invitrogen, Waltham, MA, USA). Cells were immediately placed in a humidified incubator (37 ℃, 5% $CO_2$) and cultured for 48 hours, followed by cell collection. The interfered cell model was detected with Western blotting after 48 hours of transfection. The TWF1 interference sequence (Sangon Biotech, Shanghai, China) was as follows: 5'-CGUCUGCUAGAAAUUGUAGAATT-3', 3'-UUCUACAAUUUCUAGCAGACGTT-5'.

### *Western blotting*

Following 48 hours of TWF1 expression interference, the total protein was extracted from cells with radioimmunoprecipitation assay (RIPA) lysis. Ground clinical tissue samples were treated with RIPA lysis buffer to extract the total protein. Protein quantification was performed using a bicinchoninic acid (BCA) assay. Proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to the polyvinylidene fluoride (PVDF) membrane (Immobilon-p 0.22 μm). Primary antibody reaction using MMP1 and TWF1 (Proteintech Group, Inc.; Cat No. 10371-2-AP; 11732-1-AP) was realized at 4 ℃ overnight, followed by incubation with secondary antibodies (1:10,000) for 1 hour at room temperature. The membrane was washed after the incubation. The grey values of enhanced chemiluminescence (ECL)-exposed films were quantized using Image Lab software (Bio-Rad, Hercules, CA, USA) and analyzed statistically using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

### *CCK-8*

The proliferation of A549 cells intervened with small interfering RNA targeting TWF1 (si-TWF1) and small interfering RNA negative control (si-NC) was determined with CCK-8 assay in accordance with the manufacturer's instructions. Cells were inoculated into a 96-well plate at 2,000 cells/well and cultured for another 12, 24, 48, and 72 hours, respectively. Subsequently, to detect cell vitality, 10 μL CCK-8 solution was added to each well and incubated for 4 hours with the optical density (OD) value read at 450 nm.

### *Scratch assay*

Si-TWF1 and si-NC model cells were plated in a 6-well plate. A scratch was made with a 100 μL sterile pipette tip when cell confluency reached 80–90%. The medium was replaced with serum-free culture solution after 3 washes in phosphate-buffered saline (PBS). The scratch gap was viewed under an inverted microscope and photographed at 0, 24, 48, and 72 hours to observe cell migration. The migration distance was quantified using the formula: scratch area 0–24/48/72 h scratch area/0 h scratch area ×100%.

### *Transwell experiment*

The cell suspensions in si-TWF1and si-NC models were adjusted to appropriate concentrations and added to the upper chamber of the transwell at about 10,000 cells/chamber with 200 μL serum-free medium supplemented. Then, 600 μL complete culture medium was added to the lower chamber of the transwell. The transwell plate was incubated at 37 ℃ with 5% $CO_2$ for 24 hours, followed by rinsing in PBS twice. Non-migrated/invaded cells on the upper surface of the membrane were removed with a cotton swab. Subsequently, cells were fixed with paraformaldehyde (PFA) and stained with crystal violet before counting.

### *IHC*

Tumor tissues and paracancerous tissues were fixed with PFA, dehydrated in graded ethanol (85%, 95%, 100%) followed by xylene, embedded in paraffin, sectioned, and processed for antigen repair. Primary antibodies (TWF1, 1:50 dilution) were applied and incubated overnight after blocking. The next day, samples were cultured with secondary antibodies, followed by chromogen

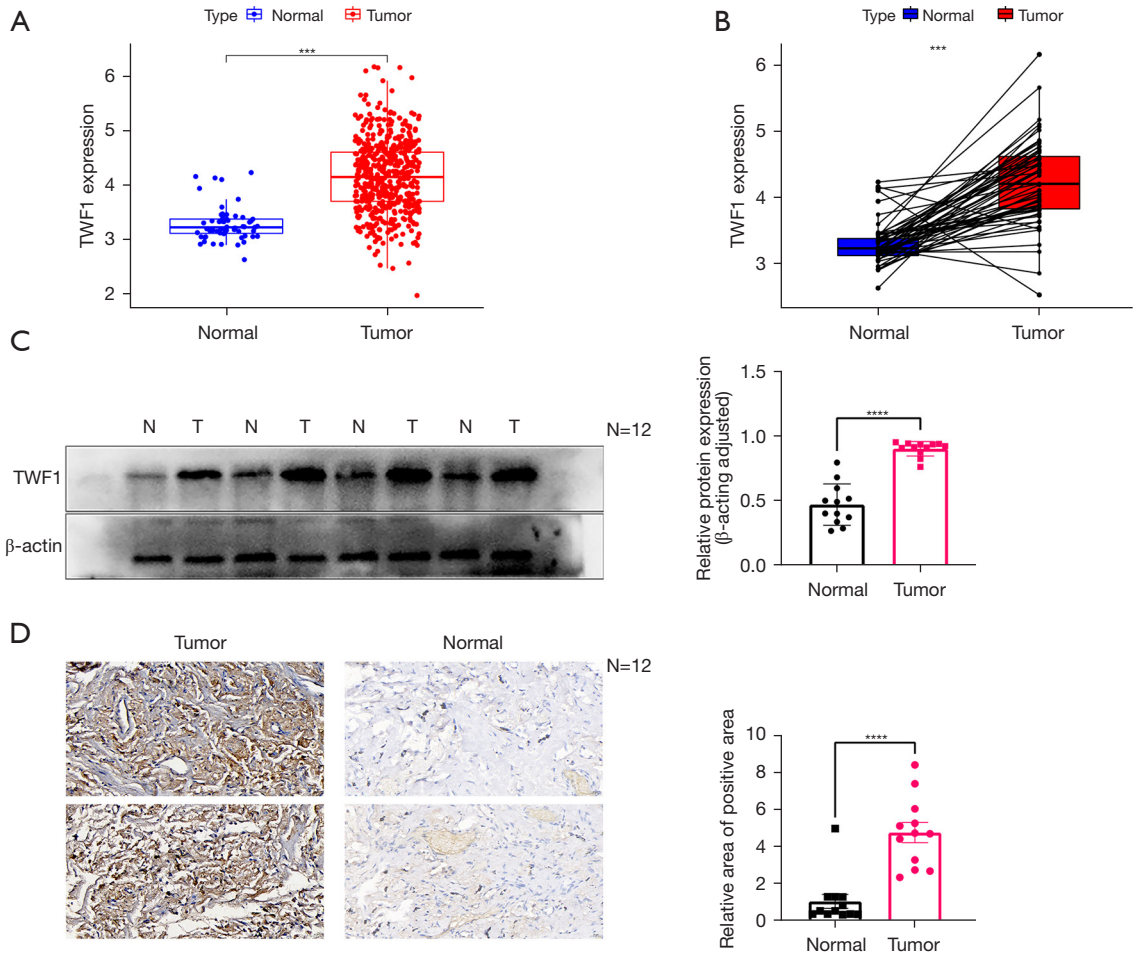


**Figure 1** Differential expression of TWF1. (A,B) TWF1 was differentially expressed in LUAD tissue in TCGA database; (C,D) differential expression of TWF1 in lung cancer tissues and normal tissues of 12 LUAD patients (stained with DAB immunohistochemical chromogenic reagent, 20× magnification). ***P<0.001, ****P<0.0001 by Student's *t*-test. TWF1, twinfilin actin binding protein 1; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; DAB, 3,3'-diaminobenzidine; TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; T, tumor; N, normal.

3,3'-diaminobenzidine (DAB) staining and hematoxylin counterstaining, and sections were sealed and photographed.

### *Statistical analysis*

The TWF1 expression in TCGA pan-cancer tissues was analyzed via the Wilcoxon rank sum test. The correlation between TWF1 expression and LUAD patients' prognoses was explored with Cox regression analysis and survival analysis. The between-group difference in functional analysis was evaluated using a *t*-test. We performed all statistical analyses using R version 4.2.1 (R Core Team, Vienna, Austria) and GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). We utilized R for complex statistical modeling and data manipulation, while GraphPad Prism was employed for generating graphs and conducting additional statistical tests. We used two-sided P values for hypothesis testing, with a significance level set at P<0.05. Each experiment was repeated 3 times.

## Results

### *Overexpression of TWF1 in LUAD tissues*

TWF1 was upregulated both in unpaired and paired LUAD tissues (both P<0.05) (*Figure 1A*,*1B*). In the LUAD samples

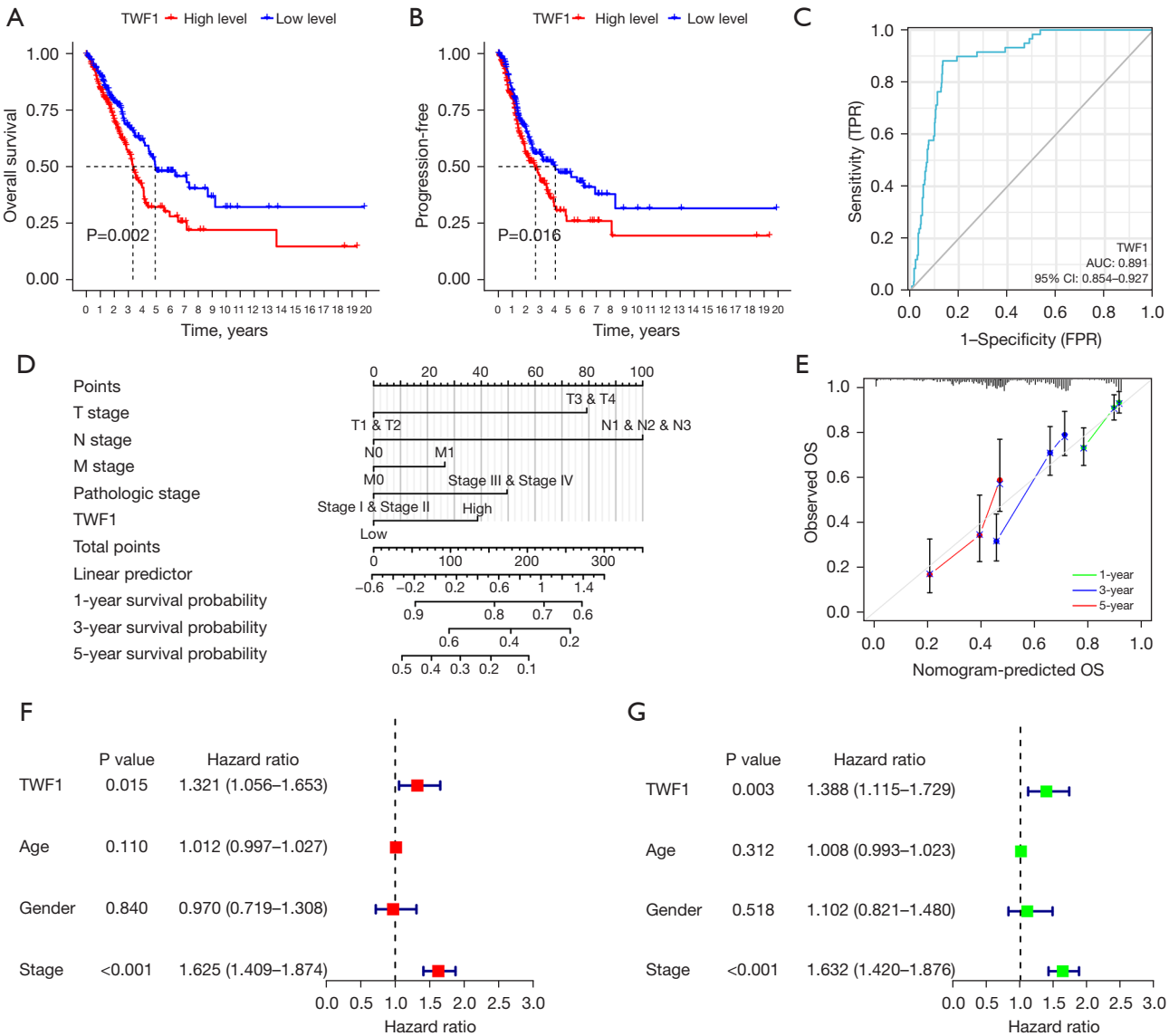


**Figure 2** The expression of TWF1 was associated with the prognosis of LUAD patients, (A,B) TWF1 overexpression was associated with OS and PFI in LUAD using KM survival analysis; (C) diagnostic value of TWF1 expression levels in LUADs determined using ROC analysis; (D) Nomogram for prediction of OS in LUAD based on TWF1 and clinical characteristic factors; (E) calibration of nomogram for predicting OS in LUAD based on TWF1 and clinical characteristic factors; (F) multivariate Cox regression analysis; (G) univariate Cox regression analysis. TWF1, twinfilin actin binding protein 1; LUAD, lung adenocarcinoma; OS, overall survival; PFI, progression-free interval; KM, Kaplan-Meier; ROC, receiver operating characteristic; TPR, true positive rate; FPR, false positive rate.

from our hospital, TWF1 was significantly upregulated in 11 cases (91.67%), which is in agreement with the results of database analysis (*Figure 1C,1D*).

### *TWF1 is an independent prognostic factor for LUAD*

The group with downregulated TWF1 had significantly better OS versus the group with upregulated TWF1 (*Figure 2A*). A Uniform pattern was shown in PFS that patients with low TWF1 expression had longer PFS (*Figure 2B*). In addition, based on the diagnostic ROC curves, TWF1 expression could accurately differentiate tumors from normal tissues (AUC=0.891) (*Figure 2C*). A nomogram was plotted combining clinicopathological

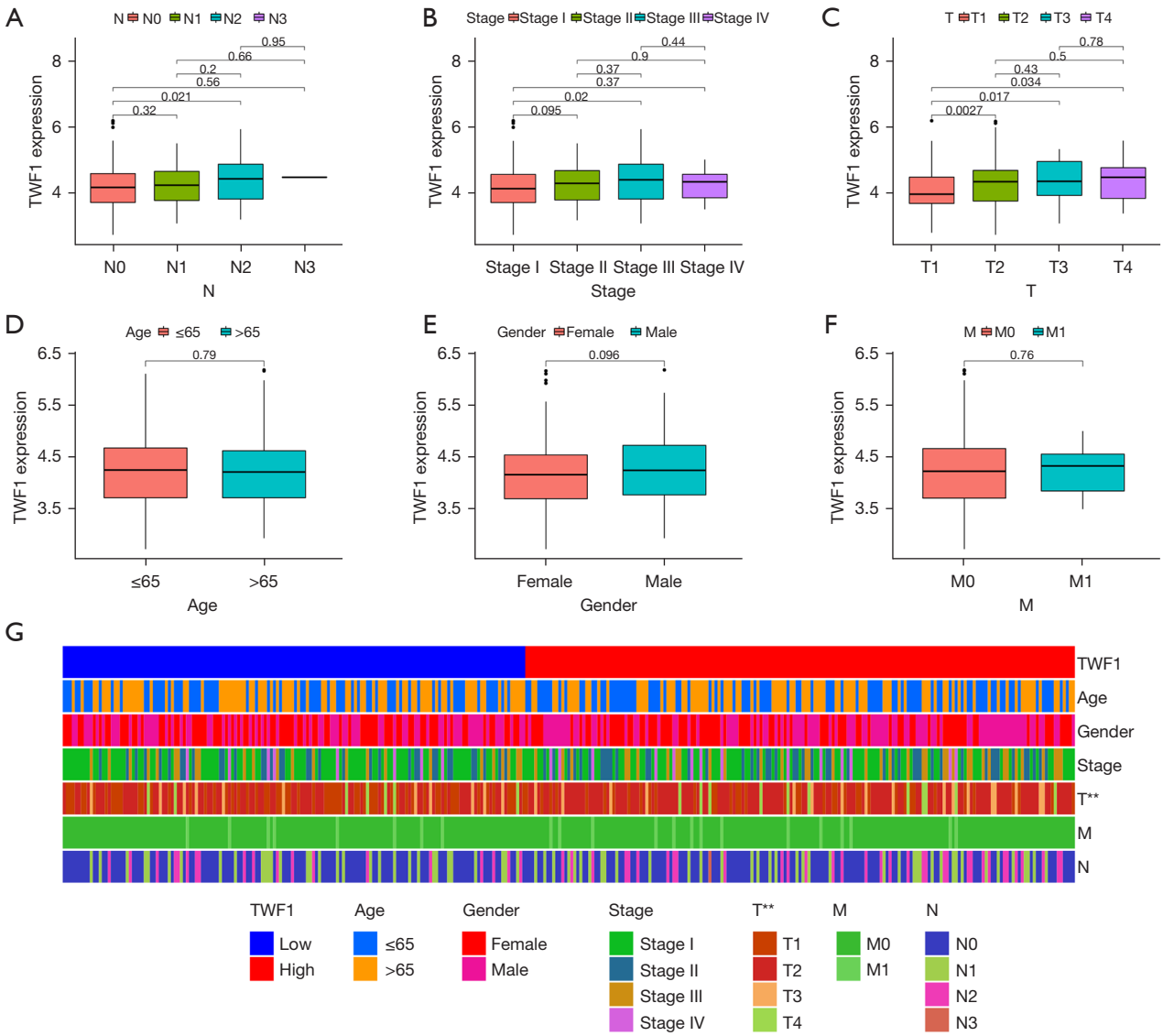


**Figure 3** TWF1 was differentially expressed in different clinical features, (A-F) N stage, stage, T stage, age, gender, M stage; (G) heatmap of TWF1 expression in LUAD tissues based on clinicopathological features. TWF1, twinfilin actin binding protein 1; N, node, T, tumor; M, metastasis; LUAD, lung adenocarcinoma. **P<0.01 by Student's *t*-test.

factors [tumor (T), node (N), metastasis (M) stage] and TWF1 expression in order to predict the 1-, 3-, and 5-year survival probabilities (*Figure 2D,2E*). TWF1 expression exhibited significance both in univariate and multivariate Cox regression analyses, implying that TWF1 might be an independent prognostic factor of LUAD (*Figure 2F,2G*). Moreover, by comparing TWF1 expression in subgroups of clinicopathological factors (age, gender, T, N, M classification, stage), a possible association between TWF1 expressions and LUAD progression was identified (*Figure 3*).

### *TWF1 expression is correlated with LUAD immune infiltration*

By comparing immune cell concentrations in groups with high and low TWF1 expression, significant differentiation in multiple immune cells and immunocompetence was demonstrated between the 2 groups. TWF1 expression was related to the infiltration abundance of B cells memory, dendritic cells resting, eosinophils, macrophages M0, macrophages M1, mast cells activated, mast cells resting,

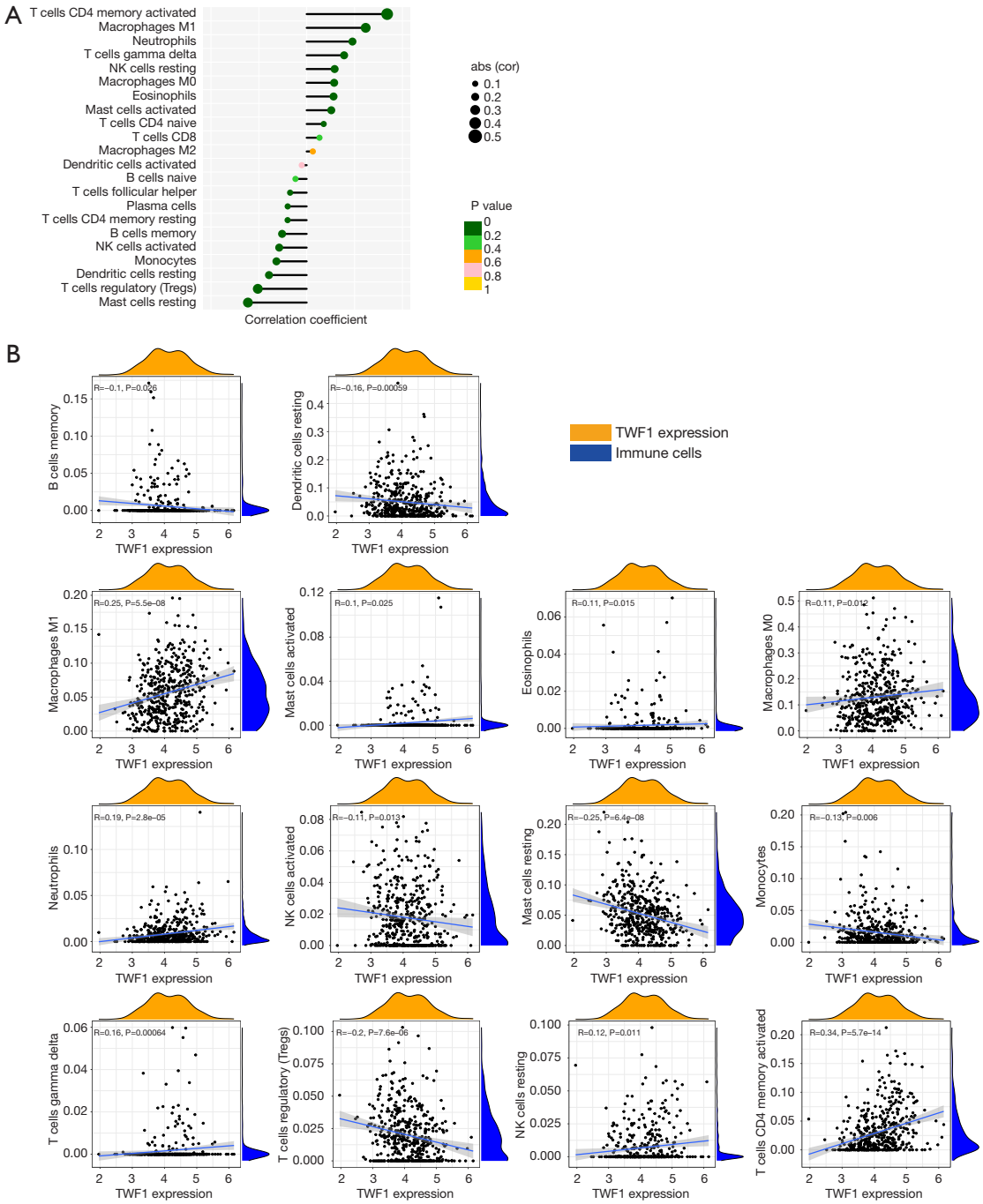


**Figure 4** TWF1 expression was associated with tumor immune infiltration. (A) Expression of TWF1 was associated with various immune cell infiltrations; (B) the relationship between TWF1 expression and 14 types of immune cells. TWF1, twinfilin actin binding protein 1.

monocytes, neutrophils, natural killer (NK) cells activated, NK cells resting, T cells CD4 memory activated, T cells gamma delta, and T cells regulatory (Tregs) (*Figure 4*).

### *TWF1 is associated with immune checkpoint, TMB, drug sensitivity, and immunotherapy sensitivity*

TWF1 expression was negatively correlated with TMB of

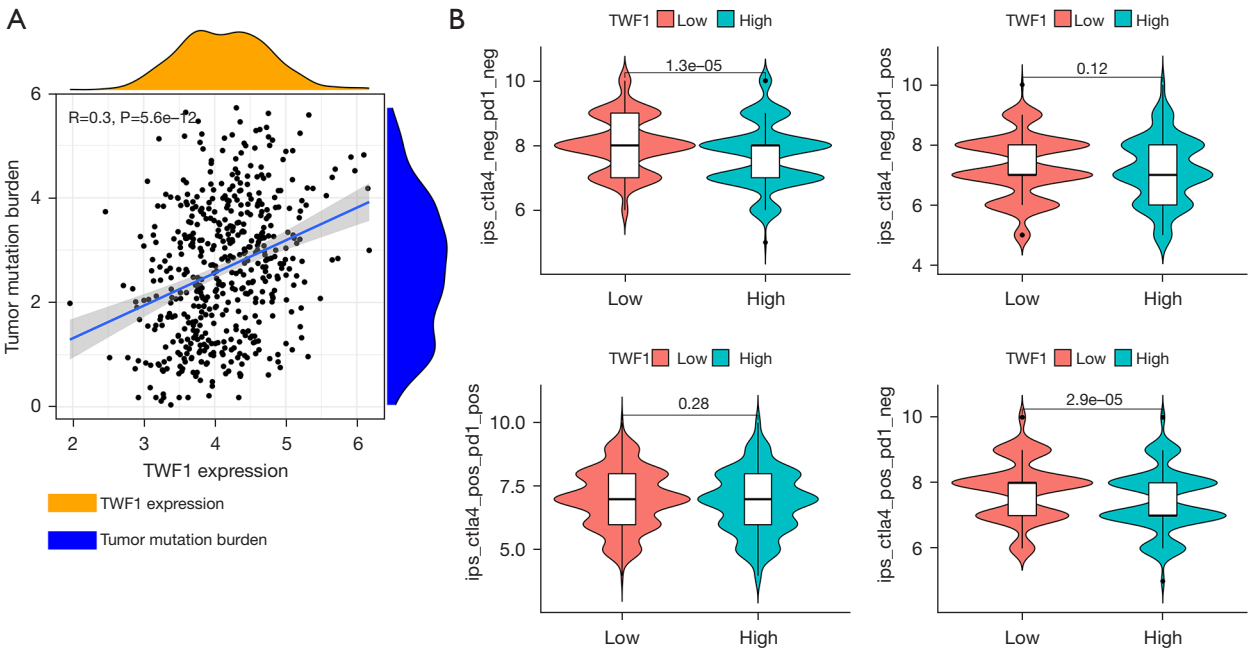


**Figure 5** The expression of TWF1 was related to TMB and immunotherapy sensitivity of LUAD. (A) TMB; (B) the relationship between TWF1 expression and immunotherapy sensitivity of LUAD. TWF1, twinfilin actin binding protein 1; TMB, tumor mutation burden; LUAD, lung adenocarcinoma.

LUAD (*Figure 5A*). Furthermore, the TWF1 expression was relevant to the immune score in immunotherapy. In CTLA4– PD1– and CTLA4+ PD1– patients, the immune scores were significantly higher in patients with lowly expressed TWF1 than those in patients with highly expressed TWF1 (*Figure 5B*). The association between TWF1 expression and chemotherapeutic drug sensitivity was also explored, in which significantly higher IC50s (A-770041, A-443654, Bleomycin, and BEZ235) were observed in the low TWF1 expression group compared to the high TWF1 expression group (*Figure 6*).

### *Interfered TWF1 expression inhibits LUAD progression via MMP1*

Via Western blotting, higher TWF1 expression was identified in LUAD A549 and H1299 cells versus in normal alveolar epithelial cells (*Figure 7A*). *Figure 7B* shows the cell model successfully established by inhibiting TWF1 expression with specific small interfering RNA (siRNA). According to the Transwell experiment (*Figure 7C*), relationship between TWF1 and MMP1 expression (this preliminarily indicated that TWF1 modulated LUAD cell invasion via regulating MMP1 expression) (*Figure 7D*), scratch assay (*Figure 8A*), and CCK-8 assay (*Figure 8B*), interfered TWF1 expression suppressed the proliferation, metastasis, and invasion of A549 cancerous cells.

## Discussion

Lung cancer is the leading cause of cancer-related death globally, and LUAD is the most common subtype (13). The popularization of spiral chest computed tomography (CT) has facilitated the diagnosis of patients with early-stage lung cancer (5,14). Nevertheless, because of the insidious onset of lung cancer, many patients already have mid-/late-stage cancers when they are diagnosed, thereby missing the optimal surgical timing, or requiring persistent anti-tumor therapy postoperatively. The promotion of non-surgical treatment such as targeted therapy has saved many patients with mid-/late-stage cancers. Epidermal growth factor receptor (EGFR) mutation is one of the driver mutations for LUAD (13,15). Multiple EGFR-targeted tyrosine kinase inhibitors (TKIs; e.g., gefitinib, erlotinib, afatinib, dacomitinib, and osimertinib) have been determined as standard initial treatment for patients harboring EGFR mutations, which has saved numerous patients with mid-/late-stage cancers (15,16). However, EGFR mutations can

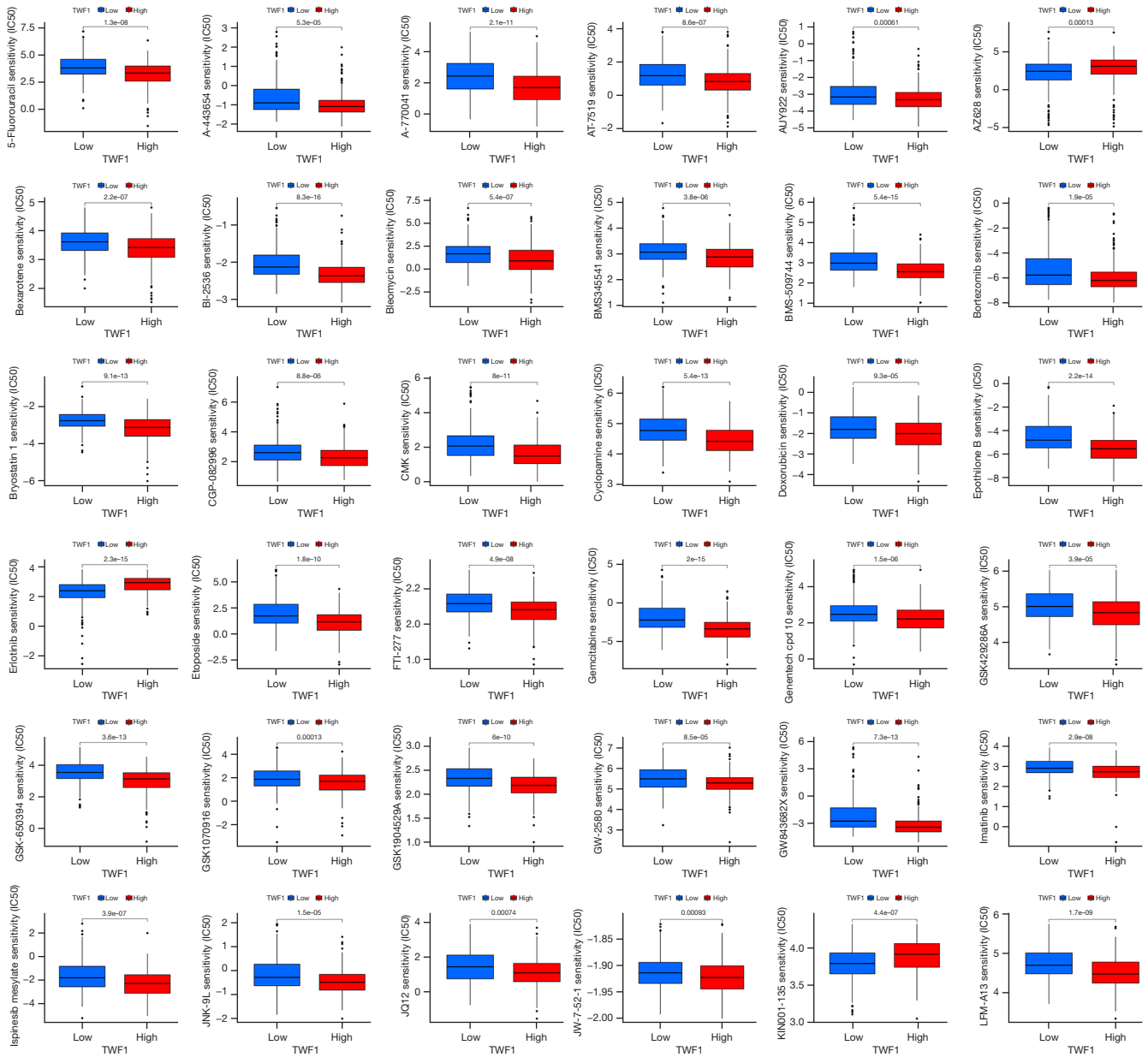


**Figure 6** The relationship between TWF1 expression and drug sensitivity. TWF1, twinfilin actin binding protein 1.

only be detected in about 50% of Asian and 10% of Eastern patients (16). Therefore, searching for more effective targets for LUAD treatment is of great significance.

Multiple genes are involved in cancer progression and are relevant to patients' prognoses and cancer development. For example, a study found that SPDL1 was overexpressed in esophageal carcinoma tissues, and upregulated SPDL1 predicted poor prognoses of esophageal cancer patients. Silencing SPDL1 at the cellular level suppressed the proliferation, migration, and invasion of esophageal cancer cells (17). Another study showed that APOC1 was upregulated in M2 and TAMs of macrophages, and it promoted macrophage M2 polarization by interacting with CD163 and CD206. Moreover, macrophages with high APOC1 expression promoted renal carcinoma metastasis by secreting CCL5 (18). It has been shown that GPR37 binding with CDK6 could induce cell cycle arrest in the G1 phase, thereby advancing LUAD tumor

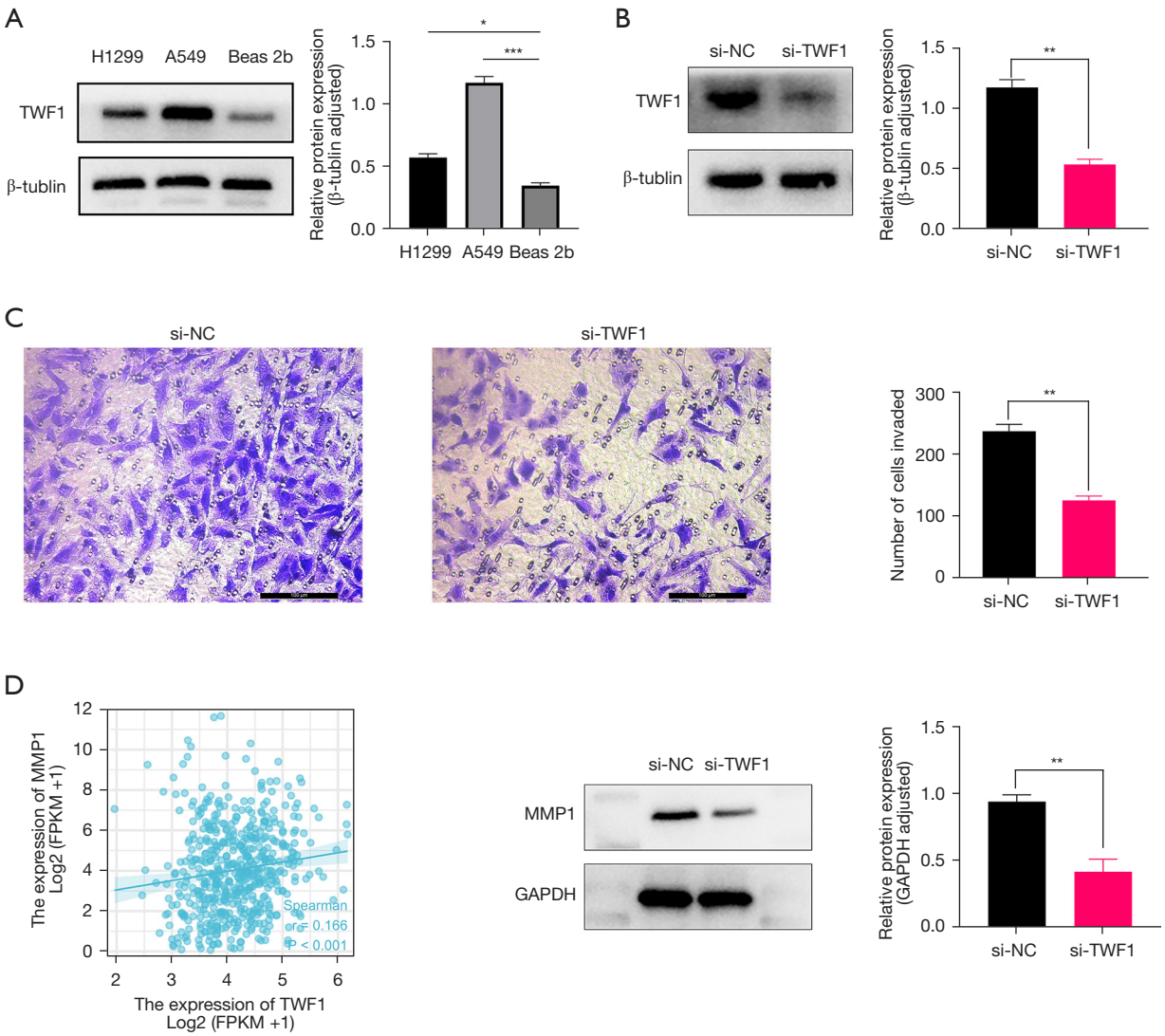


**Figure 7** Interfering with TWF1 expression inhibited the invasiveness of A549 cells. (A) Differential expression of TWF1 in H1299, A549, and Beas 2b cells; (B) interference with TWF1 expression in A549 cells; (C) transwell assay (stained with 0.1% crystal violet, 20× magnification); (D) the relationship between TWF1 and MMP1 was analyzed by TCGA database and validation of the relationship between TWF1 and MMP1. *P<0.05, **P<0.01, ***P<0.001 by Student's *t*-test. TWF1, twinfilin actin binding protein 1; si-NC, small interfering RNA negative control; si-TWF1, small interfering RNA targeting TWF1; FPKM, fragments per kilobase of transcript per million mapped reads.

progression (19). We found that TWF1 was significantly upregulated in LUAD tissues and cells. Upregulated TWF1 predicted poor prognoses of LUAD patients and served as an independent risk factor. Additionally, LUAD cells with TWF1 knockdown exhibited reduced proliferation, invasion, and migration capacities with suppressed MMP1 protein expression. Research has shown that MMP1 can promote the progression and invasion of lung, esophageal, breast, colorectal, and ovarian cancers (20-24). Taken together, it is preliminarily indicated that TWF1 prohibited LUAD progression via regulating MMP1 expression.

The tumor microenvironment (TME) contains various immune cells, some of which are formed prior to tumor initiation, and some of which are hematogenous cells recruited to the tumor site (25), all of which collectively participate in tumor development. Different cellular

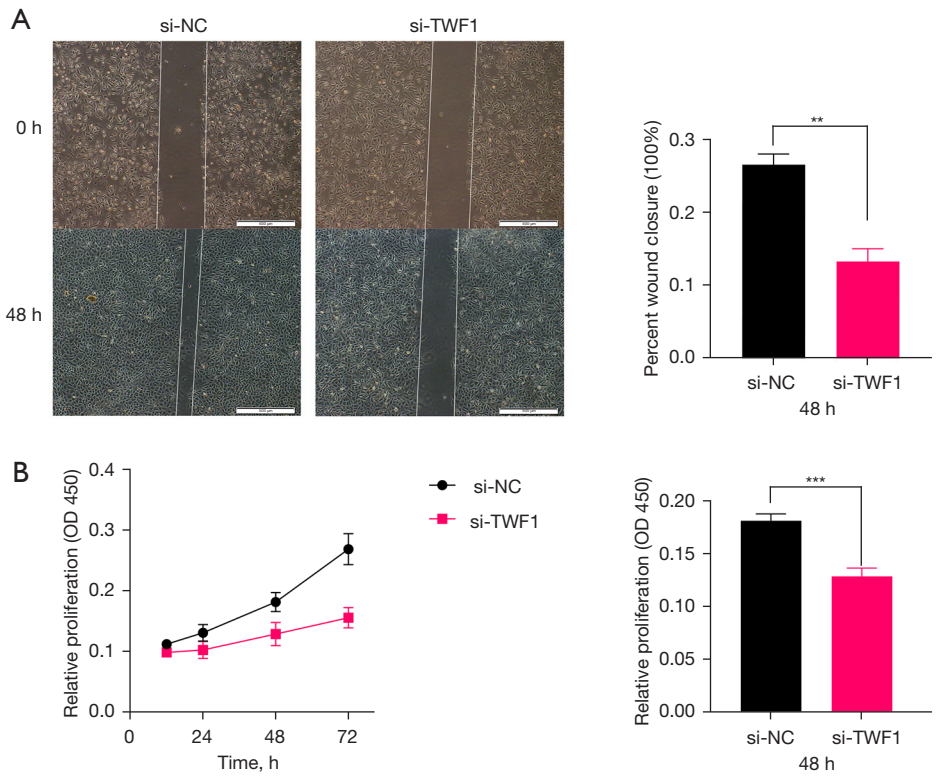


**Figure 8** Interfering with TWF1 expression inhibited the growth and migration of A549 cells. (A) Scratch assay (10× magnification); (B) CCK-8. **P<0.01, ***P<0.001 by Student's *t*-test. TWF1, twinfilin actin binding protein 1; si-NC, small interfering RNA negative control; si-TWF1, small interfering RNA targeting TWF1; OD, optical density; CCK-8, cell counting kit-8.

components in the TME lead to varying immune surveillance and immune response in tumors, which brings about distinct outcomes of immunotherapy, for instance, macrophage plays a dominant role in pancreatic cancer. Plentiful myeloid-derived suppressor cells (MDSC) reside in the local lesion of pancreatic ductal carcinoma, which can suppress the activity of infiltrating T lymphocytes, leading to resistance to immunotherapy (26,27). Currently, immunotherapy is one of the major therapies for cancer that can improve the prognoses of cancer patients (28). TWF1 expression has been shown to have an impact on tumor immune infiltration and drug response. One study found that high TWF1 expression in cancer cells is associated with reduced immune cell infiltration in the tumor microenvironment, leading to impaired anti-tumor immunity. Inhibition of TWF1 was found to promote T cell infiltration in the tumor microenvironment and enhance anti-tumor immune response (29). TWF1 can affect immune cell infiltration in lung adenocarcinoma. It may achieve this by modulating the phenotype of tumor cells to influence immune cell function, playing a role in intercellular communication (for example, by regulating the production of cytokines and chemokines, thus affecting immune cell recruitment), or by impacting immune-related signaling pathways. Further experiments are required to validate these potential mechanisms. TWF1 expression has also been implicated in cancer cell resistance to various anti-cancer drugs. In breast cancer cells, high TWF1 expression was associated with resistance to the chemotherapy drug paclitaxel, and its inhibition sensitized the cells to the drug. This suggests that targeting TWF1 may represent a promising approach for overcoming drug resistance (30). Our analysis revealed that TWF1 was correlated with immune cell infiltration in the tumor stroma. The relationship between TWF1 and immune checkpoints PD1 and CTLA4 and sensitivity to immunotherapy was further analyzed, illustrating that in CTLA4– PD1– and CTLA4+ PD1– patients, the immune scores were significantly higher in patients with lowly expressed TWF1 than those in patients with highly expressed TWF1. This provided support for

TWF1's significance in LUAD immunity. Furthermore, TWF1 expression affected the sensitivity of LUAD patients to chemotherapeutic drugs such as A-770041, Bleomycin, and BEZ235.

## Conclusions

Given the limitations of currently known targeted therapies, it is crucial to conduct research aimed at discovering additional potential therapeutic targets. This study investigated the functions of TWF1 in LUAD via bioinformatics and basic experiments and proposed some potential roles of TWF1 in LUAD. However, more basic experiments are required to validate these findings, such as the association of TWF1 with drug sensitivity and tumor immune cell infiltration.

TWF1 is an independent risk factor for poor prognosis of LUAD patients with diagnostic values for LUAD and is correlated with tumor immunity. Additionally, interfered TWF1 expression can inhibit the growth, migration, and invasion of A549 cells. Taken together, TWF1 might serve as a diagnostic and prognostic biomarker for LUAD patients.

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## Footnote

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