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TJP1, a Membrane-Expressed Protein, is a Potential Therapeutic and Prognostic Target for Lung Cancer

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Abstract

Objective: Lung cancer is a malignant tumor with the highest mortality rate in the world. It is necessary to develop effective biomarkers for diagnosis or prognostic treatment to improve the survival rate of patients. In this prospective study, we identified a membrane-expressed protein Tight Junction Protein 1 (TJP1), which is an ideal therapeutic target for lung cancer, and demonstrated its role in invasion, migration, and proliferation of lung cancer. **Methods:** High-throughput monoclonal antibody microarrays were used to screen for differential expression of monoclonal antibodies (mAbs) in lung cancer and normal lung tissue. Differentially expressed antibodies were used to immunoprecipitate their cellular targets to be identified by mass spectrometry. The identified target TJP1 was knocked down to observe the effect of reduced gene expression on lung cancer cell function. Immunohistochemistry on human tumor tissues and The Cancer Genome Atlas (TCGA) database was used to explore the relationship between TJP1 expression in multiple cancer types and patient prognosis. **Results:** The antibody CL007473 was overexpressed in tumor tissue and its target protein was identified by mass spectrometry and immunofluorescence as TJP1, a membrane-expressed protein. Knockdown of TJP1 in lung cancer cell lines showed that reduced expression of TJP1 could inhibit the invasion and migration of lung cancer cells and inhibit the proliferation of cancer cells, suggesting that membrane-expressed protein TJP1 may be used as a therapeutic target for lung cancer. TCGA database analysis showed that TJP1 was highly expressed in pancreatic cancer (PAAD) tissues compared with normal tissues, and low expression was more beneficial to the prognosis and survival of PAAD patients. **Conclusion:** Membrane-expressed protein TJP1 may be a good therapeutic and prognostic target for lung cancer and has the potential to be a prognostic biomarker in pancreatic cancer.

Keywords

lung cancer, monoclonal antibody microarray, TJP1, therapeutic targets, prognostic biomarker

Abbreviations

ADC, adenocarcinoma; ATCC, American Type Culture Collection; CNBr, cyanogen bromide; DBLC, diffuse large B-cell lymphoma; HRP, horseradish peroxidase; IP, immunoprecipitation; LGG, low-grade glioma of brain; LCLC, large cell lung cancer; mAbs, monoclonal antibodies; MAGUK, membrane-associated guanine kinase; PAAD, pancreatic cancer; PBST, PBS containing Tween-20; PETAL, Proteome Epitope Tag Antibody Library; siRNA, small interfering RNA; SCC, squamous cell carcinoma; THYM, thymoma; TJP1, Tight Junction Protein 1

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Introduction

Lung cancer is a malignant tumor with the highest mortality rate in the world and is divided into small cell lung cancer and non-small cell lung cancer according to their morphological characteristics and biological behavior. Non-small cell lung cancer

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accounts for more than 80% of lung cancer cases and has 3 major histological subtypes: lung squamous cell carcinoma (SCC), lung adenocarcinoma (ADC), and large cell lung cancer (LCLC).[1,2] About 85% of patients cannot be surgically resected after diagnosis. Due to the lack of effective treatment, the median survival time of patients is 13 months, and the 5-year survival rate is generally less than 20%.[3,4] Therefore, there is an urgent need to identify reliable biomarkers for early detection and personalized treatment of lung cancer patients.

Of all human proteins, there are about 6000 membrane proteins, most of which have not been directly explored as diagnostic or therapeutic targets in general and lung cancer in particular.[5,6] Monoclonal antibodies (mAbs) are powerful tools for discovering cell surface targets for tumors and are subsequently used as therapeutic agents for treatment. Yet, large-scale tumor surface antigen identification has been difficult due to a lack of mAbs and suitable screening platforms. Recently, a novel technology called Proteome Epitope Tag Antibody Library (PETAL) has been developed and used as a high-content screening tool for target and antibody discovery.[7,8] PETAL consists of more than 60 000 mAbs in an antibody array format for high-throughput, multiplexed protein profiling. Candidate targets for diagnosis and treatment have been identified through microarray screening of normal and tumor membrane proteomics.

In this study, we used PETAL to identify a monoclonal antibody overexpressed in lung cancer tissues. The cellular target of this antibody was identified to be Tight junction protein 1 (TJP1) which encodes a member of the membrane-associated guanine kinase (MAGUK) family of proteins that act as a tight junction adapter protein and regulate adhesion junctions. The expression and localization of TJP1 have been previously reported to be altered in pancreatic cancer (PAAD), colorectal cancer, and melanoma but its expression and function in a variety of cancers have not been fully analyzed.[9–11] Here we used the TJP1-binding PETAL antibody to probe tumor tissues by immunohistochemistry to establish TJP1 expression patterns in tumors. We further demonstrated that TJP1 expression level regulates migration, invasion, and proliferation of lung cancer. Finally, The Cancer Genome Atlas (TCGA) database mining together with our immunohistochemistry data revealed that TJP1 may act as a biomarker for diagnosis and prognosis in PAAD.

Materials and Methods

Cell Culture

The human cell lines were purchased from the American Type Culture Collection (ATCC) or stem cell bank, Chinese Academy of Sciences (Shanghai, China). Cell lines were identified by short tandem repeat analysis and mycoplasma was detected. According to the ATCC culture standards, SK-LU-1, BEAS-2B, SK-MES-1, and 293-T cells were kept in dulbecco's modified eagle medium (DMEM), supplemented with 10% fatal bovine serun (FBS). PC9, A549, NCI-2170, NCI-H69, NCI-H526, NCI-H520, NCI-H1975, NCI-H226, NCI-H460, NCI-H292, NCI-H23, and 1G2 cells were cultured in Roswell Park Memorial Institute (RPMI)1640 medium with 10%FBS. Calu-1 was cultured in McCoy's5A medium (Modified) with 10%FBS. All cell lines were maintained in a humidified incubator at 37 °C, 5% CO_2.

Screening PETAL Array With Patient Samples

This study is a prospective study, and all human studies follow the principles of the Declaration of Helsinki, with the consent of study participants and approval from local ethics committees and governments prior to commencement of the study. The study has obtained verbal consent from the participants, who are willing to participate in the study.

PETAL consists of 62 208 mAbs that target 15 199 peptides from different proteomes. Because of the polyspecificity of antibodies, an intrinsic antibody characteristic, PETAL provides a binding for a large No. of proteins in nature. Through MabArray screening, mAbs that can specifically target specific proteomes have been identified.

MabArray antibody chip is developed by Abmart (Shanghai) Co., Ltd, catalog number 705748. The clinical samples used for MabArray screening were from 3 lung cancer patients treated at the Shanghai Lung Hospital. The tumor tissue and adjacent normal tissue were cut into 0.5 mm sections and digested with 0.1% collagenase IV (17,104,019, Gibco) at 37 °C for 1 h. Cells were collected by centrifugation at 400 g for 15 min in a 70 μm cell filter (352,350,BD). The extracted proteins were partially labeled with Biotin using Sulfo-NHS-LC-biotin (21,336, Thermo Fisher). The 50 μg labeled protein was then hybridized with MabArray and incubated overnight. Antibody binding was detected by Cy3-Streptavidin (S6402, Sigma). Genepix software was used to analyze the fluorescence intensity. Antibodies with tumor/non-tumor tissue fluorescence ratio $\geq$1.5 times were selected as candidate antibody sets, and CL007473 (Abmart, TA5145) was selected for further validation.

IP and Mass spectrum Assays

Sepharose 4B (GE, No. 17-0430-02), activated by cyanogen bromide (CNBr), was used for immunoprecipitation (IP) determination. Please refer to the user manual. In simple terms, 200 g of purified PETAL monoclonal antibody is cross-linked with a 20 uL hydrolyzed CNBr bead to pull the target protein from a 1 mg sample of this PETAL cell membrane or nucleoprotein.

Basically, the target recognition of the imprinted successful monoclonal antibody for IP (producing a single band or a major single band) is done by comparing the silver staining results of the IP product with the imprinted data of the sample before and after the IP. Bands of desired size were selected (matched with silver staining and western blot) for mass spectrometry (MS) analysis. When analyzing MS results for this antibody, the list of identified proteins is prioritized using the total peptide number first. Most of the antibodies in this study selected unique proteins with the highest total identification peptide

number detected by silver staining and western blotting and matched protein size as candidate target proteins.

Immunofluorescence Validation

The green fluorescent TJP1 plasmid was transfected into 293-T cells. The cells were placed in phosphate buffer solution containing Tween-20 (PBST) and fixed with 4% paraformaldehyde for 15 min at room temperature. The fixed cells were incubated and sealed in 0.02% Triton X-100 solution for 15 min, followed by sealing in PBS with 2% FBS for 10 min at room temperature for nonspecific binding sites. CL007473 was diluted with 1×PBS (Gibico, C10010500BT) (diluted 1:50) and incubated at 4 °C overnight. After washing with PBS twice, the cells were incubated with sheep anti-rabbit FITC conjugated secondary antibody (Invitrogen Company) at room temperature for 1 h, and then washed with PBS twice more. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (1:10 000). Seal the tablet with an anti-fluorescence quenching agent. All photos were taken by GYS08-0115 fluorescence microscope, DP2 camera (Olympus), and DP2-BSW software.

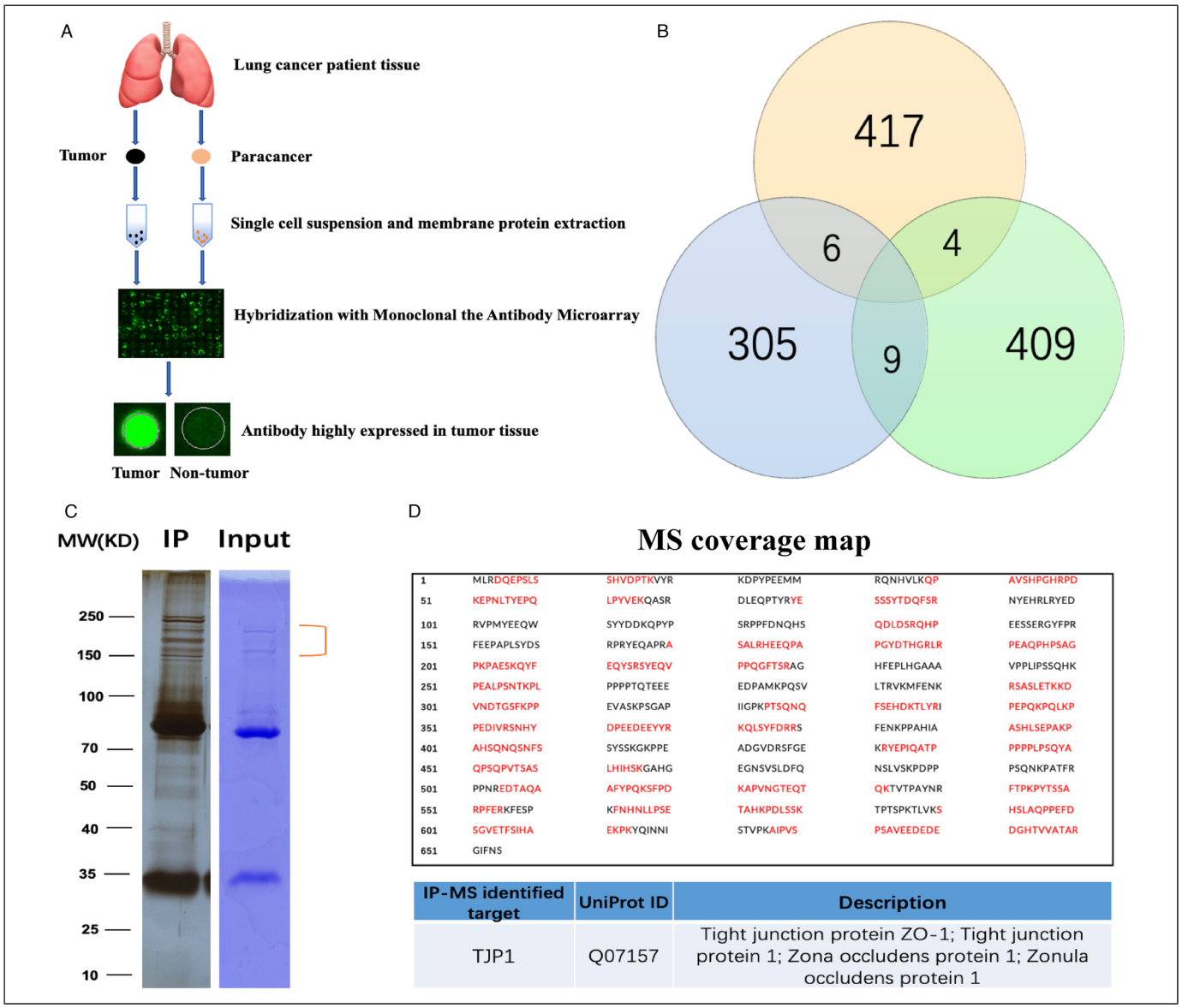

Figure 1. Screening of specific targets for lung cancer by Proteome Epitope Tag Antibody Library (PETAL). (A) MabArray technology was used to screen differentiated monoclonal antibodies (mAbs) from lung cancer and normal tissues. (B) Venn diagram shows the No. of highly expressed antibodies in lung cancer tissues of 3 patients compared with normal tissues, among which the antibody CL007473 was uniformly highly expressed in lung cancer tissues of 3 patients. The 3 colors represent the No. of antibodies differentially expressed in lung cancer tissue compared to normal tissue. (C) CL007473 was used for immunoprecipitation (IP) in SK-LU-1 cells. (D) IP targets were identified by mass spectrometry. The results suggest that CL007473 may target Tight Junction Protein 1 (TJP1). (The red sequence represents the polypeptide sequence detected by MS, and the black sequence represents the sequence not detected). The coverage rate of polypeptide sequence was 67%.

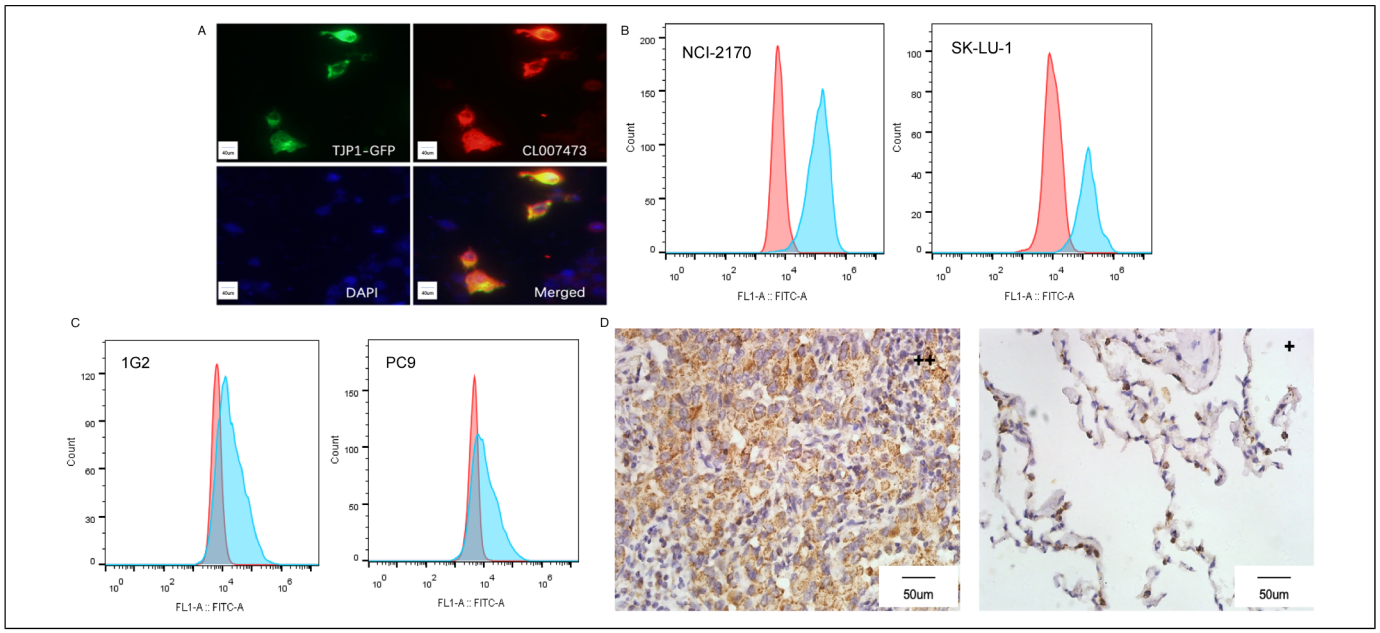

Figure 2. Immunofluorescence verification of target protein and screening of target protein expression in lung cancer multi-cell lines. (A) Immunofluorescence showed that CL007473 co-located with Tight Junction Protein 1 (TJP1), and TJP1 was expressed on the cell membrane. These results indicate that CL007473 specifically binds to TJP1, which is the target protein of CL007473. Scale: 40 μm. (B/C) Flow cytometry was used to detect the expression of TJP1 in lung cancer cells. Figure 2B shows that TJP1 is highly expressed in NCI-H2170 and SK-LU-1 cell lines and Figure 2C shows that TJP1 is low expressed in 1G2 and PC9 cell lines. Negative control IgG is shown in red and CL007473 in blue. (D) Immunohistochemical staining of lung adenocarcinoma (ADC) and adjacent tissues showed that TJP1 expression was significantly higher in lung ADC than in adjacent tissues. The immunohistochemistry of lung ADC is shown at the left and paracancer at the right. Scale: 50 μm.

Table 1. Flow Cytometry was Used to Detect the Expression of TJP1 in Lung Cancer Multi-cell Lines.

Cell Line	Relative MFI	Expression of TJP1 (H-M-L)
NCI-H69	24.9	H
NCI-H2170	23.8	H
NCI-H526	18	M
SK-LU-1	14.6	M
NCI-H520	13.6	M
Calu-1	12.4	M
NCI-H1975	5.8	L
A549	5.3	L
NCI-H226	5.2	L
BEAS-2B	5.1	L
SK-MES-1	5	L
NCI-H460	4.6	L
NCI-H292	3.2	L
NCI-H23	2.5	L
1G2	2.5	L
PC-9	2	L

Abbreviations: H, High expression; L, Low expression; M, Medium expression; TJP1, Tight Junction Protein 1.

Flow Cytometry

After digestion with ethylene diamine tetraacetic acid (EDTA), the cells were resuspended in a cold medium containing 10% goat serum, inoculated with 100 000 cells per well, and incubated for 30 min. Wash each well with 100 uL PBS 3 times. The cells were then suspended with 50 ug/mL CL007473 (Abmart, TA5145) and human IgG (Abmart, M22001) and incubated on ice for 1 h. Dilute CL007473 with 1 × PBS (Gibico, C10010500BT). After incubation, cells were washed with 100 uL frozen PBS for 3 times. Resuspended with FITC-labeled goat anti-human IgG medium (Jackson ImmunoResearch, No. 115-545-003) and incubated in darkness on ice for 1 h. Cell binding assay was performed by flow cytometry.

Immunohistochemistry

Paraffin embedding and tissue sectioning were performed by Avila Bio (Xi'an, China). The slides were baked at 60 °C for 30 min, washed 3 times in xylene for dewaxing, and alcohol gradient hydration was used. Antigen retrieval was performed with 1X Tris/EDTA buffer, pH 9.0 (Envision FLEX Target Retrieval Solution, high pH 9.0 (50X), DAKO, K800421-2CN). The antigen retrieval solution with high pH was carried out from 95 °C to 100 °C for 20 min. After cooling, blocking was realized using 10% H_2O_2 for 10 min and then incubated in blocking buffer (10% goat serum) for 30 min at room temperature. Slides were incubated 1 h at room temperature with primary antibody TJP1 (1:100, Abmart, TA51457). Slides were then washed 3 times in PBS + 0.05% Tween20 and incubated with anti-rabbit/mouse horseradish peroxidase (HRP)-conjugated secondary antibody (Dako REAL™ EnVision™ Kit, K5007) for

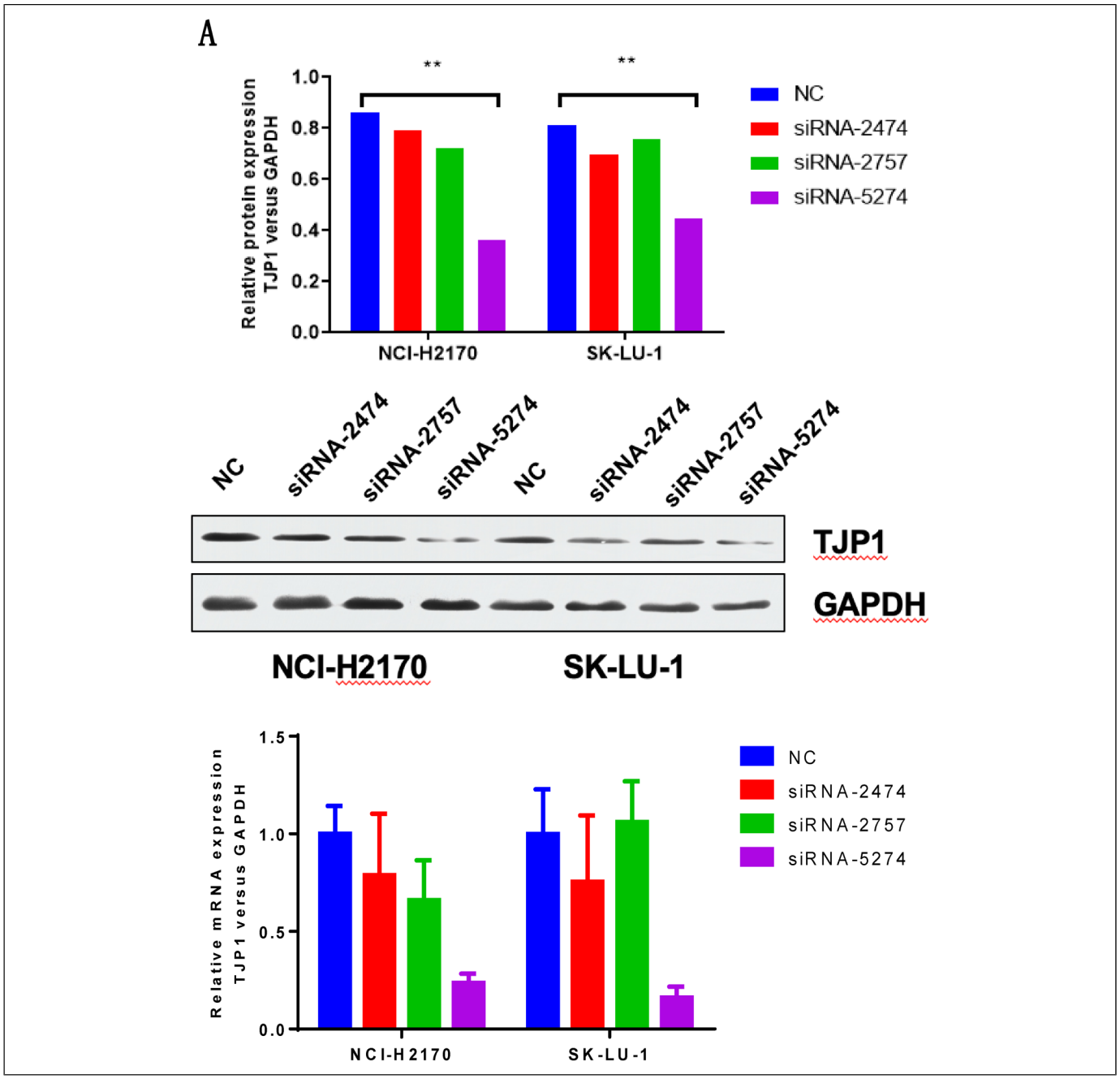

Figure 3. Knockdown of Tight Junction Protein 1 (TJP1) contributed to the inhibition of proliferation, invasion, and migration of lung cancer cells. (A) The knockdown efficiency of small interfering RNA (siRNA)-5274 on TJP1 fragment was the highest by qRT-PCR. TJP1 siRNA reduced WB signal of CL007473/TJP1 in NCI-H2170 and SK-LU-1 cells, and siRNA-5274 had the highest interference efficiency. Two-tailed Student's *t*-test, $P < .01$. (B) The gap distance of NC treated and siRNA-5274 treated cells was quantified at 0 h and 24 h, respectively. Three rectangular fields were randomly selected from each panel, and the gap distance was measured by ImageJ to draw a histogram. NC: a scrambled siRNA as the negative control. Scale: 250 μm. (C) The colony area of SK-LU-1 cells treated with NC group and siRNA-5274 group was quantified. Three rectangular fields were randomly selected in the panel, and the area was measured by ImageJ. The area of NC group was 1, and the relative colony area of the 2 groups was plotted. $P < .001$, Scale: 250 μm. (D) After NC and siRNA treatment, the relative proliferation ratios of SK-LU-1 and NCI-H2170 cells at 24 h, 48 h, and 72 h were detected, and the OD value of 0 h cells at 450 nm was calculated as 1.(continued)

30 min at room temperature. Slides were then counterstained using hematoxylin and bluing reagents. Imaging was done on an Olympus IX50 Inverted Fluorescence Microscope. Immunohistochemistry (IHC) staining intensity (H-score) was calculated according to the following formula: total H-Score = (% at 0) × 0 + (% at 1) × 1 + (% at 2) × 2 + (% at 3) × 3, with 0, no staining, 1, weak staining, 2, medium staining, and 3, strong staining.

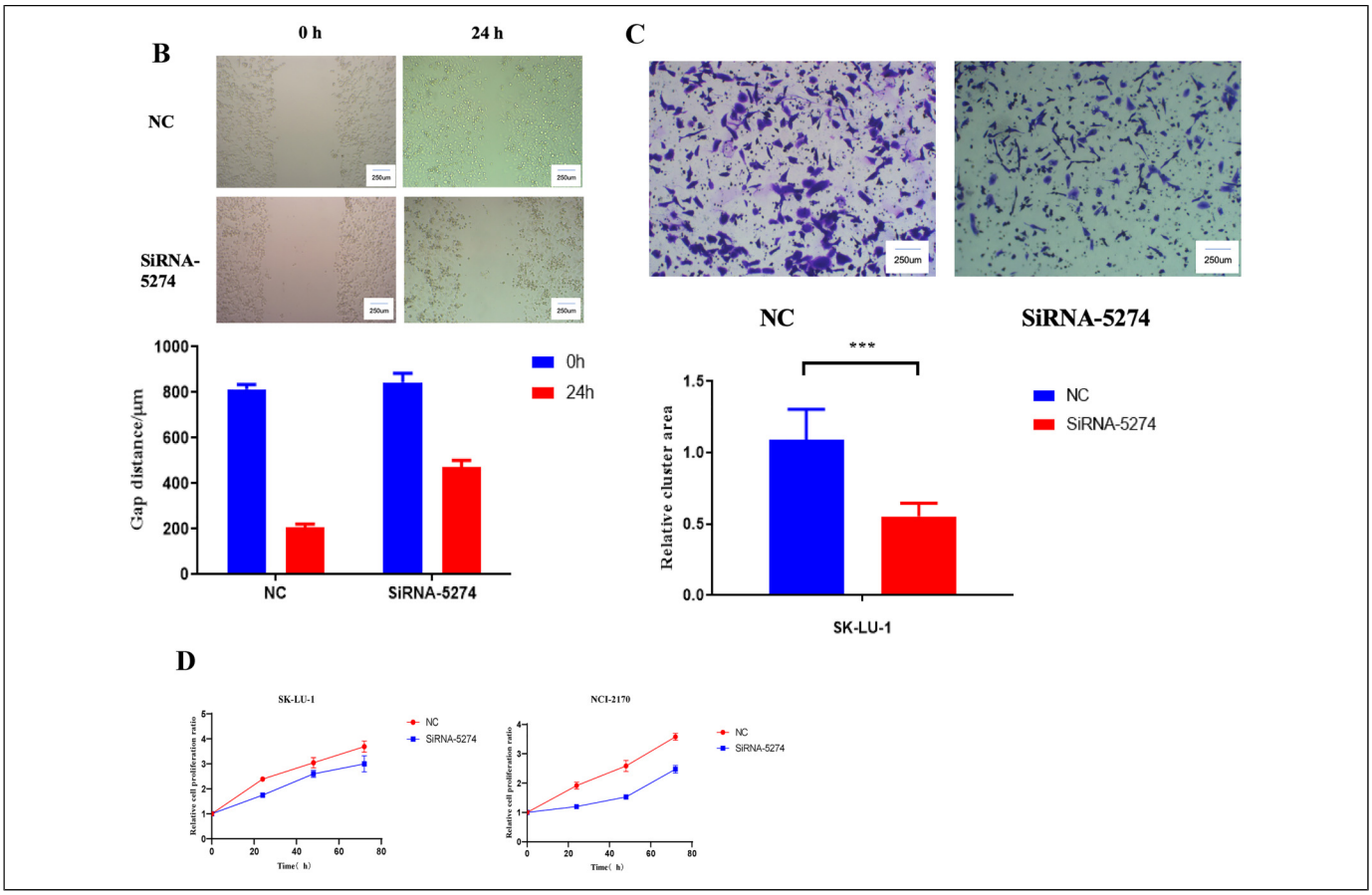

Figure 3. Continued.

Survival Analysis

TCGA database contains a large amount of sequencing data and related clinical data. To conduct data mining and study gene-related functions, we analyzed the survival of 239 patients with lung ADC with high and low TJP1 expression, and explored whether TJP1 expression affects the survival of lung cancer patients. In addition, we explored cancers with high TJP1 expression in the database and correlated the expression with patient prognosis.

SiRNA and Transfection

The small interfering RNA (siRNA) oligonucleotide for human TJP1 was synthesized by Sango Biotech. The 3 siRNA sequences synthesized were siRNA-2474

(Sense GCGAUCUCAUAAACUUCGUAATT, antisense UUACGAAGUUUAUGAGAUCGCTT), siRNA-2457(sense CAAGAGAAGAACCAGAUAUUUTT, antisense AAAUA UCUGGUUCUUCUCUUGTT), and siRNA-5274(sense CCAUGCAGAGAAGCCUAAAUATT, antisense UAUUU AGGCUUCUCUGCAUGGTT). According to the transfection instructions, siRNA was transfected into different cell lines using Lipofectamine 2000. Western Blotting and real-time fluorescent quantitative polymerase chain reaction (qRT-PCR) were used for interference efficiency detection, and siRNA with the highest interference efficiency was selected for subsequent experiments.

Scratch Assay

NCI-2170, SK-LU-1, NCI-1975, and A549 were inoculated on 6-well plates. When cell fusion is 70%-80%, scratch cells with a 200 μL sterile pipette. After the cell fragments were rinsed with PBS, the cells were preserved in a serum-free medium. Cells were photographed 24 h after the scratch. The gap distance was measured in the ImageJ (n = 10, randomly selected) and plotted with GraphPad.

Cell Invasion Assay

Cell migration was evaluated using a 24-well transwell Boyden chamber covered by a 8.0 mm aperture polycarbonate membrane (CorningV R CostarV R, Corning, NY). Cells were seeded at a density of 3×10^5/mL in the upper chamber, and 700 μL medium containing 15% serum was added to the lower chamber as a chemical attractant. The solution was incubated at 5% CO_2 at 37 °C for 24 h. The cells were fixed with paraformaldehyde for 30 min and stained with crystal violet dye at room temperature for 15 min. Migrating cells were counted in 3 random microscope fields and photographed with a light microscope.

Table 2. Expression of Tight Junction Protein 1 (TJP1) in Multicarcinoma Tissue Microarray.

Tissue type		+	++	+++	Total
Esophagus	Squamous cell carcinoma (SCC)	1/3	0/3	0/3	1/3 (33.33%)
	Paracancer	1/2	0/2	0/2	1/2 (50.00%)
Stomach	Adenocarcinoma (ADC)	1/2	0/2	1/2	2/2 (100.00%)
	Paracancer	2/3	0/3	1/3	3/3 (100.00%)
Colon	ADC	1/3	2/3	0/3	3/3 (100.00%)
	Paracancer	0/3	0/3	1/3	1/3 (33.33%)
Rectum	ADC	2/2	0/2	0/2	2/2 (100.00%)
	Paracancer	0/3	0/3	1/3	1/3 (33.33%)
Liver	Hepatocellular carcinoma	0/3	1/3	0/3	1/3 (33.33%)
	Paracancer	0/3	0/3	0/3	0/3
Lung	SCC and ADC	1/3	0/3	0/3	1/3 (33.33%)
	Paracancer	2/3	0/3	0/3	2/3 (66.67%)
Kidney	Clear cell carcinoma	0/3	1/3	0/3	1/3 (33.33%)
	Paracancer	0/3	2/3	1/3	3/3 (100.00%)
Breast	ADC	2/3	0/3	0/3	2/3 (66.67%)
	Paracancer	0/2	0/2	0/2	0/2
Cervix	SCC and ADC	0/3	1/3	0/3	1/3 (33.33%)
	Paracancer	1/3	0/3	0/3	1/3 (33.33%)
Ovary	SCC and ADC	0/3	0/3	2/3	2/3 (66.67%)
	Paracancer	0/3	0/3	0/3	0/3
Baldder	Urothelial carcinoma	0/3	0/3	1/3	1/3 (33.33%)
	Paracancer	2/3	1/3	0/3	3/3 (100.00%)
Lymph node	B-cell lymphoma	0/3	0/3	1/3	1/3 (33.33%)
	Paracancer	0/3	0/3	0/3	0/3
Skin	SCCs	0/3	0/3	0/3	0/3
	Paracancer	0/3	0/3	1/3	1/3 (33.33%)
Cerebrum	Astrocytoma	1/3	1/3	0/3	2/3 (66.67%)
	Paracancer	1/3	0/3	0/3	1/3 (33.33%)
Prostate	ADC	0/3	0/3	1/3	1/3 (33.33%)
	Paracancer	0/3	1/3	0/3	1/3 (33.33%)
Pancreas	ADC	1/2	0/2	0/2	1/3 (33.33%)
	Paracancer	0/2	0/2	0/2	0/2

Cell Proliferation Assay

SiNC and siRNA-5274 were transfected into NCI-2170 and SK-LU-1 and incubated together. Cell activity was detected at 0 h, 24 h, 48 h, and 72 h, respectively, and the cell proliferation ratio curve was plotted with GraphPad. Each group was repeated 3 times.

Statistical Analysis

In vitro experiments were conducted at least 3 times, and data were presented as mean +/−SD or single representative experiment. Statistical significance was assessed using Student's *t*-test and calculated using GraphPad Prism 9 software. *P*-values are represented as * ($P < .05$), ** ($P < .01$), and *** ($P < .001$), and "ns" stands for nonsignificant.

Results

Antibody Microarray Screening and Target Protein Identification

Membrane proteins are widely studied as diagnostic biomarkers and targets for novel therapies in the treatment of diseases, especially for cancer.[12] To screen for membrane proteins specifically expressed in lung cancer tissues, membrane proteins from lung cancer tissues and adjacent normal tissues were labeled and hybridized with PETAL antibody microarray. The discovery workflow is shown in Figure 1A. The fluorescence intensity changes of >1.5 times between tumor samples and normal samples were classified as "positive" or differentially expressed. Among 1110 positive antibodies from the lung membrane protein screening, 1 antibody (CL007473) was highly expressed in the lung cancer tissues (Figure 1B). To identify the cellular target of CL007473, we used this antibody to immunoprecipitate the cell membrane components of lung cancer cell line. Two specific immune bands compared to controls were obtained as shown in Figure 1C and were analyzed by mass spectrometry. Mass spectrometry data identified the band as TJP1, with a molecular weight of 195 KD which is consistent with protein size of the immunoprecipitated band on the electrophoresis. A comparison of TJP1 sequence in the Uniprot database with peptide sequences from Mass spectrometry showed that the coverage rate of polypeptide fragment obtained by mass spectrometry was about 67%, supporting that TJP1 may be the target protein of CL007473 (Figure 1D).

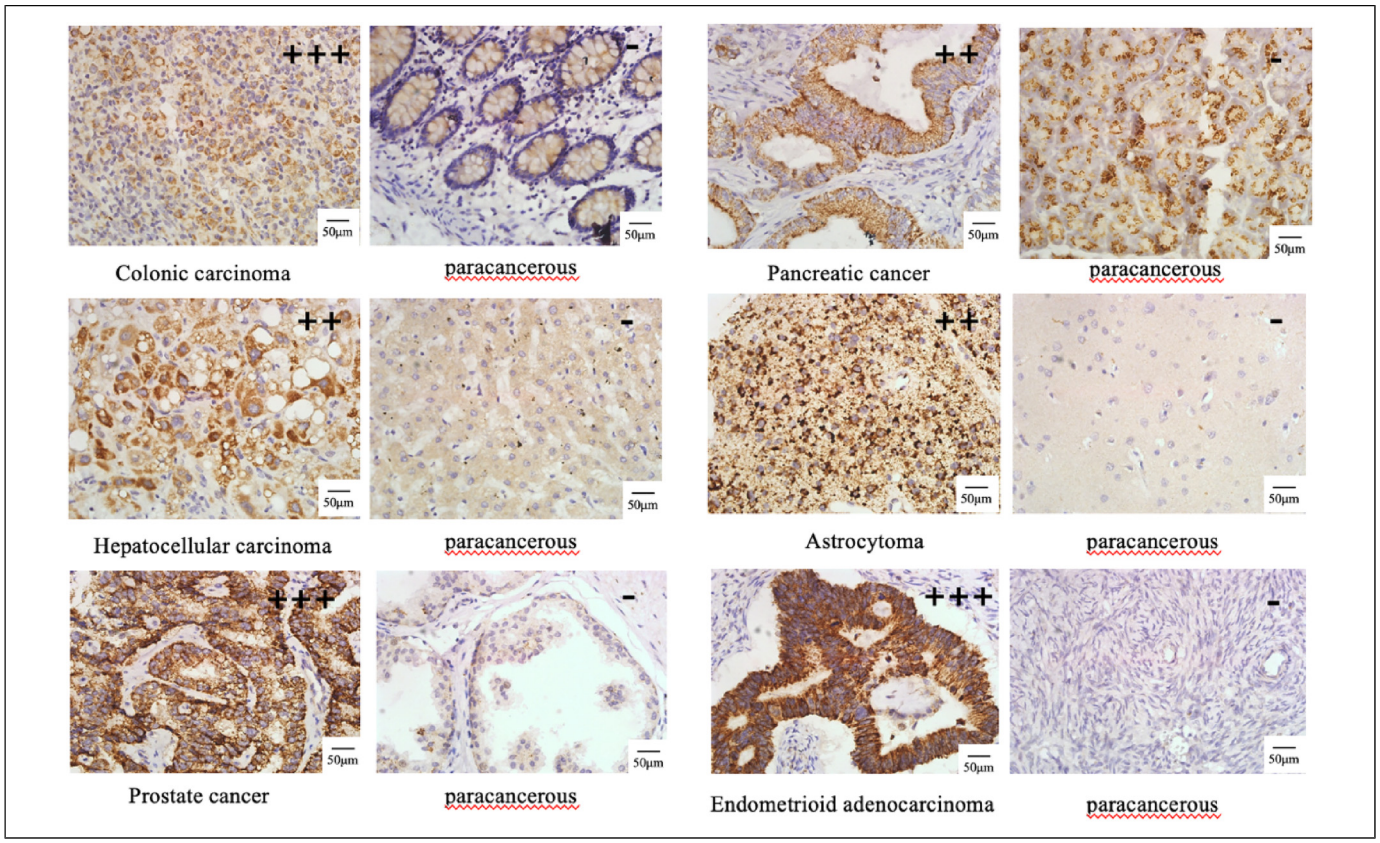

Figure 4. Immunohistochemistry was used to detect the expression of Tight Junction Protein 1 (TJP1) in multicarcinomas. Representative immunohistochemical images of CL007473 from tumor and paracancer tissues from the same patient. TJP1 is expressed in colorectal, liver, brain, prostate, pancreatic, and ovarian cancer tissues, but not in paracancer tissues. Scale: 50 μm.

Immunofluorescence Verification of Target Protein and Screening of Target Protein Expression in Lung Cancer Multi-Cell Lines

To verify that TJP1 is a membrane-expressed protein and the target protein of antibody CL007473, an immunofluorescence assay was performed. As shown in Figure 2A, TJP1 is mainly a membrane-expressed protein. The co-localization of TJP1 and CL007473 suggested that antibody CL007473 specifically binds to TJP1 with no background binding. Next, we screened the expression of TJP1 in 16 cell lines of different lung cancer subtypes by flow cytometry. TJP1 was highly expressed in lung SCC NCI-2170 and lung ADC SK-LU-1 cells (Figure 2B) with relative mean fluorescent intensity (MFI) (to isotype control IgG) of 23.8 and 14.6, respectively, while it has low expression in 1G2 and PC9 cell lines (Figure 2C) with relative MFI of 2.5 and 2, respectively. The expression of other lung cancer cell lines is shown in Table 1. To further explore the expression of TJP1 in lung ADC, we examined its expression by immunohistochemistry using antibody CL007473. CL007473/TJP1 was highly expressed in lung ADC but with much weaker expression in paracancer tissues (Figure 2D). Immunohistochemistry showed that TJP1 may be a marker for the diagnosis and treatment of lung cancer.

Knockdown of TJP1 Decreased Migration, Invasion, and Proliferation

To determine the effect of TJP1 expression on lung cancer cells, we selected lung SCC cell line NCI-2170 and lung ADC cell line SK-LU-1. As shown in Table 1, TJP1 is highly expressed in both cells. TJP1 siRNA oligonucleotides were synthesized, and siRNA was transfected into NCI-2170 and SK-LU-1 cells. qRT-PCR was used to detect the interference efficiency of 3 siRNA at the transcription level. Then, Western Blotting was used to detect the interference efficiency of protein levels. As shown in Figure 3A, SiRNA-5274 had the highest interference efficiency in NCI-2170 and SK-LU-1 cell lines, with a median interference efficiency of 25% and 16%, respectively. The interference efficiency of protein level was 36% and 44%, respectively. Therefore, siRNA-5274 was selected for subsequent cell function experiments.

We used the scratch assay to determine whether TJP1 was involved in migration capability. In SK-LU-1 cell line, the cell migration ability of siRNA-5274 treatment group was significantly lower than that of control group (NC) after 24 h, indicating that decreased TJP1 expression resulted in decreased cell migration ability (Figure 3B). In the invasion experiment, we found that the reduced expression of TJP1 significantly

attenuated the colony formation of lung cancer cells and reduced the invasion ability of the cells (Figure 3C). No obvious migration was observed in NCI-2170 cell line, suggesting that the cell line was weak in migration (Supplementary Figure 1). Knockdown of TJP1 in NCI-1975 and A549 cell lines resulted in decreased cell invasion and migration (Supplementary Figure 2). To explore the effect of TJP1 expression on cell proliferation, we tested cell activity of control cells (siNC-treated cells) and experimental cells (siRNA treated cells) at 0 h, 24 h, 48 h, and 72 h, respectively. As shown in Figure 3D, knockout of TJP1 resulted in significantly lower cell proliferation ratios of SK-LU-1 and NCI-2170 than the NC group. These results suggest that high expression of TJP1 facilitates the proliferation of lung cancer cells.

Expression of TJP1 in Tumor Tissue Microarray

To further explore the expression of TJP1 in various tumor types, immunohistochemistry was performed on tumor specimens derived from 16 different tissue types, and the statistical results are shown in Table 2. To quantify immunohistochemistry, H-score was calculated, and the scoring rules were shown in the Materials and Methods. TJP1 was found to be highly expressed in 6 types of tumor tissues but not in the control paracancer tissues, including colorectal, liver, brain, prostate, pancreatic, and ovarian cancer (Figure 4). This result suggests that TJP1 may have potential as diagnostic markers and therapeutic targets for those tumors.

Association of TJP1 Expression with Pancreatic Cancer Patient Prognosis

By mining TCGA database, we found that TJP1 was highly expressed in carcinoma tissues of diffuse large B-cell lymphoma (DBLC), low-grade glioma of brain (LGG), PAAD, and thymoma (THYM) compared with normal tissues (Figure 5A-B). In the TCGA database, 89 PAAD patients with high TJP1 expression and 89 PAAD patients with low TJP1 expression were associated with their clinical outcomes. Data showed that patients with high TJP1 expression had longer survival (Figure 5C). Immunohistochemical results also showed that TJP1 was strongly expressed in PAAD tissues, but not in paracancer tissues (Figure 4). These results suggest that TJP1 may be a diagnostic and prognostic marker

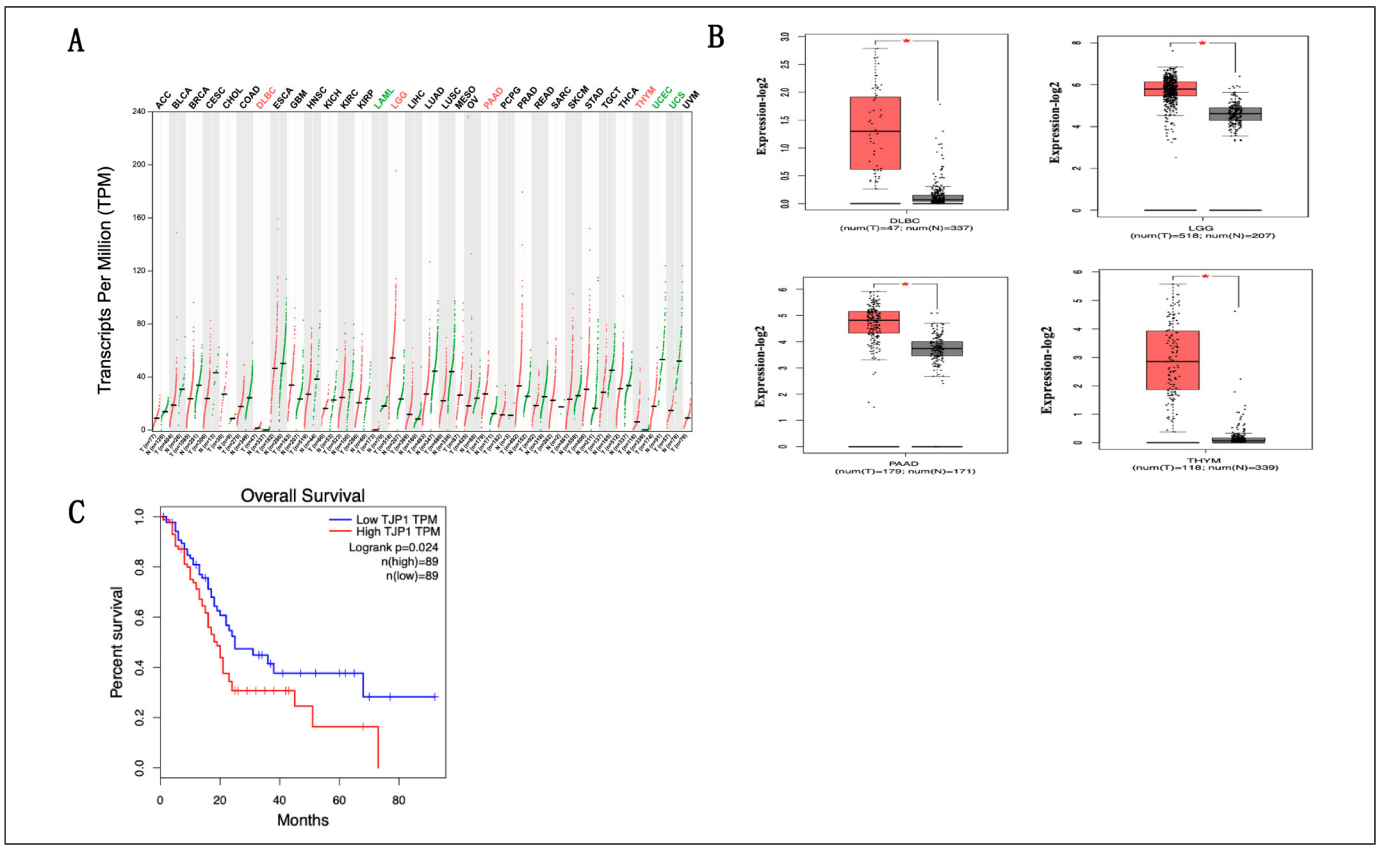

Figure 5. Expression of Tight Junction Protein 1 (TJP1) in multicarcinomas and its association with patient prognosis. (A) The expression of TJP1 in tumor tissues and normal tissues in the TCGA database. TJP1 was significantly overexpressed in tumor tissue in red carcinoma. (B) TJP1 expression was higher in diffuse large B-cell lymphoma (DBLC), low-grade glioma (LGG), pancreatic cancer (PAAD), and thymoma (THYM) than in normal tissues. Two-tailed Student's *t*-test, $P < .05$. (C) Among PAAD patients, patients with high TJP1 expression have a poor prognosis. Two-tailed Student's *t*-test, $P < .05$.

for PAAD. It also provides a strong complement to previous reports that TJP1 overexpression can enhance the motor capacity of PAAD cells.[10]

Discussion

Like most tumors, the occurrence and development of lung cancer is a multifactor, multistage, and multigene development process. The discovery of lung cancer target is needed for the development of targeted therapy, and also provides early diagnosis of lung cancer.[13] In this study, a proteome-scale antibody microarray platform was used to screen lung cancer samples to identify mAbs differentially expressed in lung cancer and normal tissues. One differentially expressed antibody, CL007473, was found to specifically target TJP1. Plasma membrane localization of TJP1 was confirmed by CL007473 using immunofluorescence. The expression of TJP1 in different lung cancer cell lines was profiled by flow cytometry using CL007473. Therefore, our approach represents a highly efficient method for the discovery and validation of the target because the same antibody was used in both steps. The availability of high-affinity monoclonal antibody greatly facilitates expression analysis of the target protein. We selected 4 lung cancer cell lines to explore the function of TJP1. We demonstrated decreased cell motility and proliferation after TJP1 deletion and expression reduction. Our data further support that TJP1 may be a potential therapeutic and prognostic target for lung cancer. Using tissue microarray, TJP1 was found to have a strong expression in colon cancer, PAAD, liver cancer, brain cancer, prostate cancer, and ovarian cancer, but not in control paracancer tissues. On the gene expression level, TCGA data analysis showed that TJP1 expression in DBLC, LGG, PAAD, and THYM was significantly higher than that in normal tissues. Gene expression and protein expression of TJP1 is consistent in PAAD with higher target expression correlating with poor prognosis. Therefore, TJP1 may be also a marker for the diagnosis and treatment of PAAD.

With the progress of pharmacogenomics/proteomics, personalized therapy based on biomarker detection is gradually leading the research and clinical treatment of cancer.[14] Here, we discovered and confirmed TJP1 at protein level as a potential diagnostic marker in multiple tumor types. Based on previous reports, TJP1 is responsible for the protein network between actin and global tight junction proteins, such as Occludin and Claudin, which maintain cell integrity,[15–17] and is directly involved in paracellular closure and membrane domain differentiation.[18,19] Metastasis is the leading cause of death in cancer patients.[20] Tight junctions prevent epithelial cell separation by means of adhesion and are the first barrier that cancer cells must overcome for metastasis.[21,22] As a key component of tight junction, TJP1 expression is associated with cell motility in breast, colorectal, and human gastrointestinal cancers.[23–25] In this study, after knockdown of TJP1 in lung cancer cell lines, it was found that the cell migration ability, cell invasion ability, and cell proliferation ability were significantly reduced, supporting its role in cancer cell motility. Together our data suggest that TJP1 may be used as a therapeutic target for lung cancer and as a prognostic marker of lung cancer.

There are some limitations in this study. First, we only explored the cell function of TJP1 in NCI-2170 and SK-LU-1 cell lines. In the future, we need to expand the No. of experiments in lung cancer cell lines to better explain the effect of TJP1 expression on lung cancer motility and proliferation. Second, in the TCGA database, there was no statistically significant relationship between TJP1 expression level and patient survival in many tumor types, so we still need to investigate the protein expression on those tumors to establish a correlation between TJP1 expression and tumor prognosis. Third, in this preliminary study, we only looked at TJP1 expression in association with lung cancer prognosis. We are starting to explore TJP1 as a therapeutic target for the development of antigen-conjugated drugs. In conclusion, a more comprehensive study of TJP1 still needs to be explored in the future.

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Declaration of Conflicting Interests

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Ethical Approval

Our study was approved by the Medical Ethics Board of Northwestern University (Approval number: No. 210120001). The date of approval: January 20, 2021.

Data Availability Statement

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Supplemental Material

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