

CLEC3B as a Prognostic and Immunological Biomarker in Pan-Cancer: Multi-Omics Profiling and Validation in Pancreatic Cancer and Exosomes

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Background: Despite the emergence of in vitro and in vivo experiments validating the connection between *CLEC3B* and various cancers, a comprehensive pan-cancer investigation remains elusive. In this study, we explored the potential roles of *CLEC3B* as a tumor suppressor and in immune function across multiple cancer types.

Methods: We visualized outcomes derived from Gene Expression Omnibus (GEO) and diverse online databases. The relationship between tumor-infiltrating cells, gene set enrichment analysis (GSEA) and *CLEC3B* expression and was examined using R. Additionally, we explored the potential role of *CLEC3B* in tumor malignant behavior by using siRNA-mediated knockdown.

Results: Our study identifies *CLEC3B*'s low expression in majority of cancers compared with adjacent normal tissues. Reduced *CLEC3B* expression correlated with advanced clinical stages, inferior overall survival (OS) and DNA methylation levels. We observed significant positive associations between *CLEC3B* expression and infiltration levels of various immune cell subtypes. Furthermore, markers linked with immune checkpoints, immunomodulation and RNA modification exhibited a favorable correlation with *CLEC3B* expression. Intriguingly, silencing *CLEC3B* (si-*CLEC3B*) augmented the migratory capabilities of pancreatic adenocarcinoma (PAAD) cells. Additionally, *CLEC3B* expression was notably enriched in metastatic PAAD endothelial cells and extracellular vesicles, potentially implicating its involvement in tumor vascular function by way of extracellular vesicle.

Conclusion: In conclusion, our initial pan-cancer analyses of *CLEC3B* provide insights into its associations with clinical prognosis, DNA methylation, immune cell infiltration, and tumor mutation burden, highlighting its potential as a tumor suppressor and mediator of immune infiltration in pan-cancer.

Keywords: *CLEC3B*, pan-cancer, methylation, immune infiltration, tumor microenvironment

Introduction

Cancer constitutes a significant contributor to global morbidity and mortality, presenting a substantial threat to both human health and socioeconomic stability. In 2020, there were 19.3 million reported new cancer cases and nearly 10 million cancer-related deaths worldwide.¹ Despite considerable advancements in cancer treatments, a majority of cases are diagnosed at advanced stages, leading to suboptimal treatment outcomes.² Hence, there is an urgent need to identify potential biomarkers for early cancer detection and treatment.

CLEC3B belongs to the C-type lectin domain superfamily, encoding tetranectin (TN). It regulates various physiological processes including fibrinolytic proteolysis, osteogenesis calcification, muscle development, and the occurrence of cardiovascular disease.^{3–5} Recent studies have indicated the involvement of *CLEC3B* in tumorigenesis, progression, and its close association with tumor metastasis and angiogenesis.^{6–10} However, previous research on *CLEC3B* function has

been predominantly limited to specific cancer types. Therefore, investigating the functions and molecular pathways of *CLEC3B* across pan-cancer datasets becomes imperative, offering novel insights for clinical cancer treatment.

This study comprehensively characterizes the expression profile, mutation patterns, and prognostic implications of *CLEC3B* across various cancers. Utilizing data from GEPIA, UALCAN, and TIMER databases, we explore the relationship between *CLEC3B* expression and protein expression, clinical staging, DNA methylation, and immune cell infiltration in pan-cancer. Furthermore, we evaluate the correlations between *CLEC3B* expression and tumor mutational burden (TMB), microsatellite instability (MSI), as well as various immune-related genes associated with checkpoints, immunomodulation, RNA modification, and tumor stemness. Finally, we investigate the biological functions of *CLEC3B* using GSEA. Our findings suggest that *CLEC3B* may influence immune responses by interacting with immune cell infiltration and signaling pathways such as Ca^{2+} , MAPK, and autophagy in cancer, and it positively correlates with immune checkpoints, thus influencing immune response occurrence.

Material and Methods

Data Collection

The Human Protein Atlas (HPA) database (<https://www.proteinatlas.org/>) provides comprehensive protein expression data across normal tissues, cells, and tumor tissues via its tissue atlas, cell atlas, and pathology atlas.¹¹ In this study, we examined the expression of *CLEC3B* mRNA in human tissues using data from HPA. We utilized the “ggplot2” R package to analyze *CLEC3B* expression across 33 types of normal tissues and cancers, integrating data from the Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA), which acquired from UCSC Xena (<http://xena.ucsc.edu/>). Single-cell RNA sequencing (scRNA-seq) datasets for PAAD, including GSE197177, were obtained from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>). Additionally, the UALCAN database (<http://ualcan.path.uab.edu/analysis-prot.html>) was utilized to explore protein expression and DNA methylation levels using data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and TCGA, respectively. We specifically examined the “Total protein”, “Methylation”, and “TP53 mutation status” within the “expression” module of UALCAN to compare protein expression levels, methylation status, and expression profiles of *CLEC3B* between cancers and corresponding adjacent tissues. Statistical significance was determined using a P-value threshold of < 0.05 , assessed by Students’t-test. Abbreviations and corresponding full names of various types of tumors were shown in [Table S1](#).

Genomic Alterations Analysis

The cBioPortal web platform (<https://www.cbioportal.org/>) facilitates interactive exploration of various cancer genomics datasets.¹² Genomic alteration frequency data for *CLEC3B*, including mutation, copy number amplification, deep deletion, and multiple alterations, were extracted from the TCGA database via this platform.

Survival and Prognosis Analysis

We investigated the correlation between *CLEC3B* expression and patient prognosis, focusing on OS. A forest plot was employed to visualize Hazard Ratio (HR) and associated P-values across different cancer types.

Correlation Between *CLEC3B* Expression and Immunity

TIMER online database (<https://cistrome.shinyapps.io/timer/>) was utilized to explore the correlation between *CLEC3B* expression and immune infiltration levels. Spearman correlation analysis was employed to generate heatmaps depicting the correlation between *CLEC3B* and immune genes related to checkpoints across various cancer types. The horizontal axis represents different cancer types, while the vertical axis represents different inhibitory and stimulatory markers. Correlation coefficients are depicted in different colors, with red indicating significant p-values (< 0.05). TMB is defined as the number of mutations per DNA megabases (Mb), which emerged for predicting immunotherapy response as a promising biomarker.¹³ MSI is any change in the length of a microsatellite caused by the insertion or deletion of a repeat unit in a tumor, and a new phenomenon of microsatellite alleles appears.¹⁴ We assessed TMB, MSI and their correlation with *CLEC3B* expression using Spearman correlation analysis. A lollipop map was generated to visualize the

correlation coefficients between *CLEC3B* and TMB/MSI across different tumor types, with dot size representing the magnitude of correlation and red color indicating significant p-values.

Protein–Protein Interaction (PPI) Network Construction

The GeneMANIA prediction web platform (<http://www.genemania.org>) was utilized to construct the PPI network for *CLEC3B* in this study.¹⁵ The platform integrates various types of association data, including physical interactions, co-expression, predicted interactions, co-localization, genetic interactions, pathway data, and shared protein domains.

GSEA Analysis

GSEA was employed to explore biological signaling pathways by comparing high and low expression groups based on the median level of *CLEC3B* expression. Four terms from Kyoto Encyclopedia of Genes and Genomes (KEGG) were analyzed and presented.

Correlation Between *CLEC3B* Expression and Genes of Immunomodulatory, RNA Modification and Tumor Stemness

The correlation between *CLEC3B* expression and genes associated with immunomodulation, RNA modification, and tumor stemness was investigated using the “pan-cancer analysis” module within the SangerBox online tool (<http://vip.sangerbox.com/login.html>), leveraging TCGA data.

Cell Culture and Real-Time Quantitative PCR (qPCR)

Normal human pancreatic ductal epithelial cells (hTERT-HPNE) and two pancreatic cancer cell lines (MIA PaCa-2, BXPC-3 and CFPAC-1) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured under specified conditions, and total RNA was extracted using the Fastgen 200 RNA isolation kit. Reverse transcription into cDNA was performed using the Prime Script RT reagent kit, followed by RT-qPCR using the CFX Manager 2.1 system. GAPDH served as an internal control. All primers used are listed below: *CLEC3B*, 5'-GCCTCTTCTCCCTCCTGAC-3' (forward) and 5'-ATCTTTGTGTTTCAACATCTTTCT-3' (reverse); GAPDH, 5'-TCGTCCCGTAGACAAAATGG-3' (forward) and 5'-TTGAGGTCAATGAGGGGGTC-3' (reverse).

Western Blotting and Cell Transfection

The Western blotting experimental procedure is as described in previous studies.¹⁶ The following antibodies and ladders were used: anti-*CLEC3B* (#R25891, zenbio), anti- β -actin (#AC038, ABclonal) and Epizyme Biotech (WJ102). Human *CLEC3B* siRNA sequences were synthesized by MIAOLING BIOLOGY. Transient transfection of pancreatic cancer cells was conducted using Lipo8000 and DMEM, following the manufacturer's instructions. After 48 h's transduction, cells were harvested for further experiments.

Invasion, Migration and CCK-8 Experiment

For the transwell migration assay, cells suspended in 200 μ L serum-free DMEM were seeded into the upper chambers of transwell plates (8 μ m pore polycarbonate membrane). For Matrigel-coated invasion assays, 8×10^4 cells in 200 μ L serum-free DMEM were plated in the upper chambers 4 hours post-Matrigel coating. In both assays, the lower chambers were supplemented with 700 μ L DMEM containing 20% FBS as a chemoattractant. Following incubation, cells were fixed, stained, and imaged using an upright microscope. Image analysis was performed by Image J. MIA PaCa-2 cells, infected with si-*CLEC3B* or si-NC, were inoculated into 96-well culture plates (1×10^3 cells per well). Then, 200 μ L complete medium was added to each well. Each well was added with 10 μ L CCK-8 reagent at 0 h, 24 h, 48 h and 72 h, respectively, and incubated at 37°C for 2 h. Finally, the absorbance at 450 nm wavelength was measured according to the CCK-8 kit instruction manual (WLA074, China).

Isolation and Validation of Exosomes

The specific process of isolating exosomes is shown in previous studies. In brief, dead cells and cell debris were removed by centrifugation at 300g, 2000g, and 1000g, respectively, and exosomes were separated by ultracentrifugation at 110000g. The final precipitate was resuspended in pre cooled PBS and stored at 4°C or -80°C for later use. Extracellular vesicles were suspended in PBS and dripped on carbon-coated grids. And then, the grid was air-dried and images were captured using a transmission electron microscopy (TEM) device at 80 kV for TEM analysis. In addition, NanoSight NS300 device was used to assess the size of extracellular vesicles. For identification of surface markers, lysed extracellular vesicles were subjected to immunoblotting using anti-TSG101 (#612696, BD Biosciences), anti-CD63 (#25682-1-AP, Proteintech) and anti-CLEC3B (#R25891, zenbio).

Statistical Analysis

The expression level of *CLEC3B* in cancer compared to normal tissues was assessed using Student's t-tests. The HR and p-value were determined through univariate Cox regression analysis. Kaplan-Meier analysis was employed to examine patients' survival time based on *CLEC3B* expression levels. Spearman correlation was utilized to evaluate the associations between *CLEC3B* expression and immune infiltration. GSEA was performed using R software (version 4.1.1) with packages including ggplot2, org.Hs.eg.db, clusterProfiler, DOSE, and enrichplot. Experimental data analysis and figure generation were conducted using GraphPad Prism 8.0. A significance threshold of $p < 0.05$ was established for all statistical analyses.

Results

Expression Patterns and Clinical Associations of CLEC3B

A *CLEC3B* demonstrates downregulation in pan-cancer and late-stage malignancies. In the HPA database ([Figure 1A](#)), elevated *CLEC3B* expression is observed in non-cancerous tissues compared to other cancer tissues. To elucidate the correlation between *CLEC3B* expression and cancers, we conducted differential expression analysis using data from TCGA and GTEx database via SangerBox online tool. Decreased *CLEC3B* expression is consistently found in tumor tissues across various cancer types ([Figure 1B](#)). Protein expression analysis from CPTAC database further confirms significant downregulation of *CLEC3B* in breast, colon, ovarian, clear renal cell carcinoma (RCC), lung adenocarcinoma, and uterine corpus endometrial carcinoma (UCEC) ([Figure 1C](#)). Additionally, *CLEC3B* expression exhibits significant upregulation in tumors with TP53 mutations in numerous cancer types ([Figure S1A](#)). Furthermore, *CLEC3B* downregulation correlates with advanced pathological stages in several cancers ([Figure S1B](#)).

Prognostic Significance of CLEC3B

Immunohistochemistry results showed that compared with normal tissues, *CLEC3B* expression in cancer tissues was much lower ([Figure 2A](#)). Positive rate results showed that few colorectal, breast, endometrial, lung, testicular, stomach and liver cancers showed moderate cytoplasmic positivity. Other cancers were generally negative ([Figure 2B](#)). A univariate Cox regression model was utilized to examine the correlation between *CLEC3B* expression and OS as well as DFS across various cancer types. The findings of the Cox regression analysis across multiple cancers revealed a significant correlation between *CLEC3B* expression and OS in ten cancer types, including GBMLGG, KIRC, LIHC, PAAD, LGG, CESC, HNSC, LUAD, KIPAN, and KICH ([Figure 2C](#)). Overall, these results underscore the potential of *CLEC3B* as a novel prognostic biomarker.

Epigenetic Modifications and Genetic Alterations of CLEC3B

As an epigenetic mechanism, DNA methylation significantly influences tumorigenesis and progression by regulating gene expression.¹⁷ To explore the DNA methylation status of *CLEC3B*, we utilized UALCAN and TCGA datasets. Our analysis revealed an increase in methylation levels of *CLEC3B* across various cancers including BRCA, COAD, ESCA, HNSC, LUAD, LUSC, and READ, while a decrease was observed in KIRC, LIHC, PAAD, and TGCT ([Figure 3](#)). Our findings revealed that, compared to normal cells, DNA methyltransferase DNMT1 was significantly upregulated in

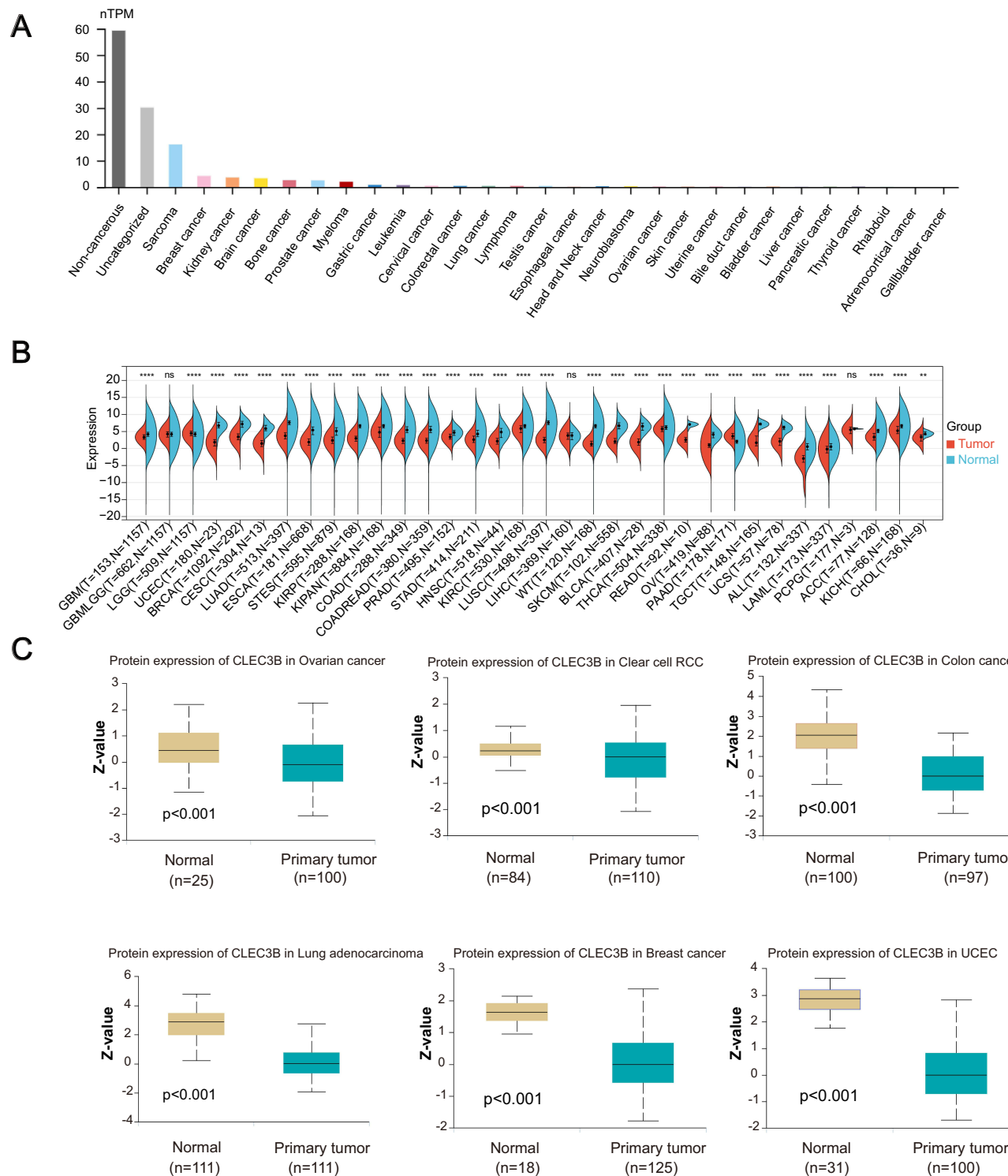


Figure 1 *CLEC3B* downregulated in pan-cancer setting and non-cancerous tissues. **(A)** Expression profile for *CLEC3B* mRNA in human different tissues displayed by HPA. **(B)** Expression profile for *CLEC3B* based on TCGA and GTEx database. **(C)** The protein expression of *CLEC3B* in UALCAN database. ** $p < 0.01$, **** $p < 0.0001$, ns $p > 0.05$.

pancreatic cancer cells ([Figure S2A](#)). In contrast, CLEC3B expression was downregulated in pancreatic cancer cells, and a negative correlation was observed between the two. Following knockdown of DNMT1, CLEC3B expression was markedly upregulated in the pancreatic cancer cell line PANC-1 ([Figure S2B](#) and [S2C](#)).

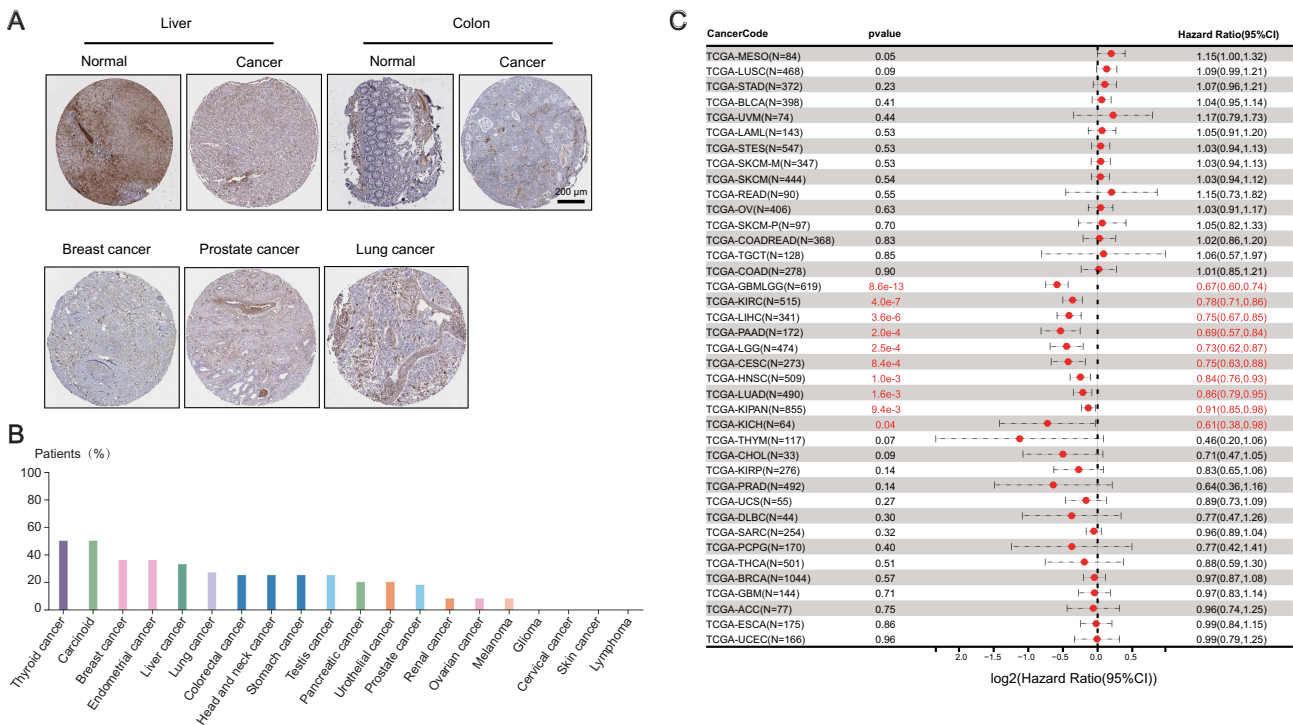


Figure 2 CLEC3B expression and prognosis in pan-cancer. **(A)** The immunohistochemical staining of CLEC3B in UALCAN database. **(B)** Immunohistochemistry positivity rate of CLEC3B in pan-cancer. **(C)** A forest plot of OS of CLEC3B in pan-cancer. The text highlighted in red in the chart refers to the data of cancer types with P<0.05.

Pan-cancer alterations of *CLEC3B* were assessed using the “TCGA PanCancer Atlas Studies” module within the cBioPortal database. The analysis revealed the highest alteration frequency of *CLEC3B*, approximately 4%, among patients with DLBC. Notably, deep deletion emerged as the most prevalent alteration type across various genetic alterations (Figure 4A). We identified 39 mutation sites within *CLEC3B* spanning amino acids 0 to 202, with AA Change: R151H/C and D166E/N/Y being the most common mutation modes (Figure 4B). Additionally, we investigated the mutation landscape of UCEC and UVM, yet found no discernible difference between high and low expression of *CLEC3B* and the expression of other highly mutated genes (Figure 4C).

PPI Network and Functional Enrichment Analysis of CLEC3B

Subsequently, the GeneMANIA online tool was employed to elucidate the potential mechanisms through which *CLEC3B* participates as a putative tumor suppressor in cancers by constructing a PPI network, as depicted in Figure 5B. This network revealed robust physical interactions between *CLEC3B* and Plasminogen (PLG), a key mediator implicated in macrophage recruitment,^{18,19} inflammation and fibrinolysis.²⁰ Among the top 100 genes exhibiting the strongest correlations with *CLEC3B* in GEPIA, notable candidates included TNXB, RP11-855A2.5, RP11-467I17.1, SCARA5, CD248, RNASE1, and GRK5, as illustrated in Figure 5A. By intersecting the 20 genes identified by GeneMANIA with the top 100 genes from GEPIA, RNASE1 and GRK5 were selected for further analysis (Figure 5C). Furthermore, GSEA was conducted to discern the functional enrichment associated with *CLEC3B* expression. KEGG enrichment analysis revealed that heightened expression of *CLEC3B* was primarily associated with cell adhesion pathway, calcium signaling pathway and focal adhesion signaling pathway (Figure 5D). Further immunofluorescence co-localization assays confirmed the interaction between CLEC3B and GRK5, suggesting that this binding may exert tumor-suppressive effects by modulating protein stability (Figure S3).

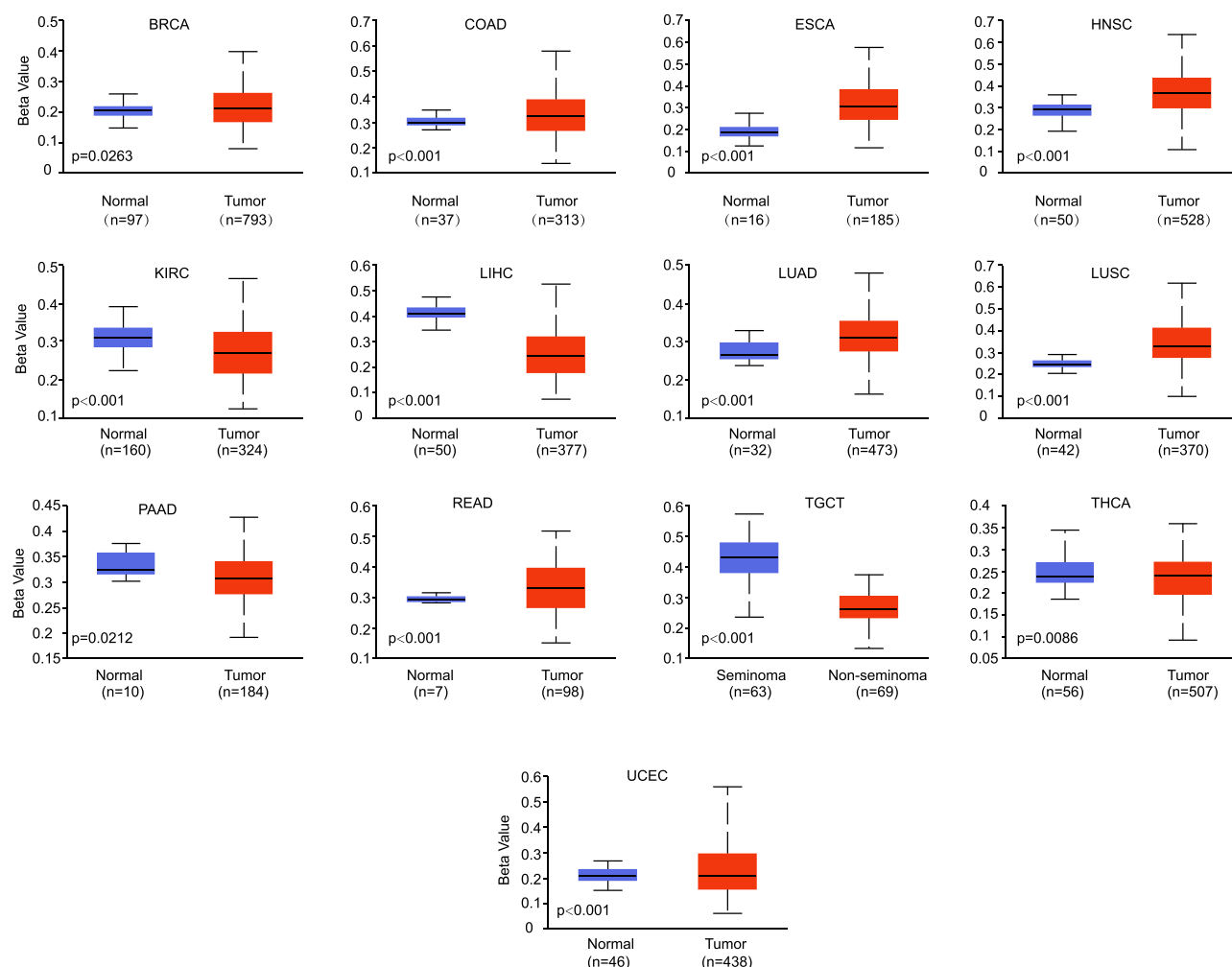


Figure 3 The level of *CLEC3B* DNA methylation in pan-cancer compared with that in normal tissues using the UALCAN database.

The Relationship Between *CLEC3B* Expression and Immune Cell Infiltration Across Pan-Cancer

TIMER2 database was employed to assess the relationship between *CLEC3B* expression, prognosis, and immune cell infiltration. Results revealed a negative correlation between *CLEC3B* expression and infiltration of B cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells in LGG. Elevated levels of these immune cells were associated with poorer prognosis (Figure 6A). Furthermore, in HNSC and LUAD, the infiltration level of B cells positively correlated with *CLEC3B* expression and was associated with a favorable prognosis (Figure 6B and C). Conversely, in STAD, high *CLEC3B* expression potentially increased macrophage infiltration, leading to reduced patient survival time (Figure 6D). Additionally, using the TISIDB online database, the immune and molecular subtypes of *CLEC3B* across pan-cancer were evaluated, indicating that the C3 subtype (inflammatory) predominated across various cancer types, aligning with previous findings,²¹ and suggesting a better prognosis in LUAD, STAD, and LIHC (Figure S4).

The Correlation Between *CLEC3B* Expression and Immune Checkpoint, TMB, and MSI Across Pan-Cancer

The surveillance function of the immune system through immune checkpoints is intricately linked to the survival and prognosis of cancer patients. In this study, we conducted a comprehensive investigation into the associations between *CLEC3B* expression and immunoinhibitor and immunostimulator gene expression using a pan-cancer dataset (Figure 7A and B). Notably, we

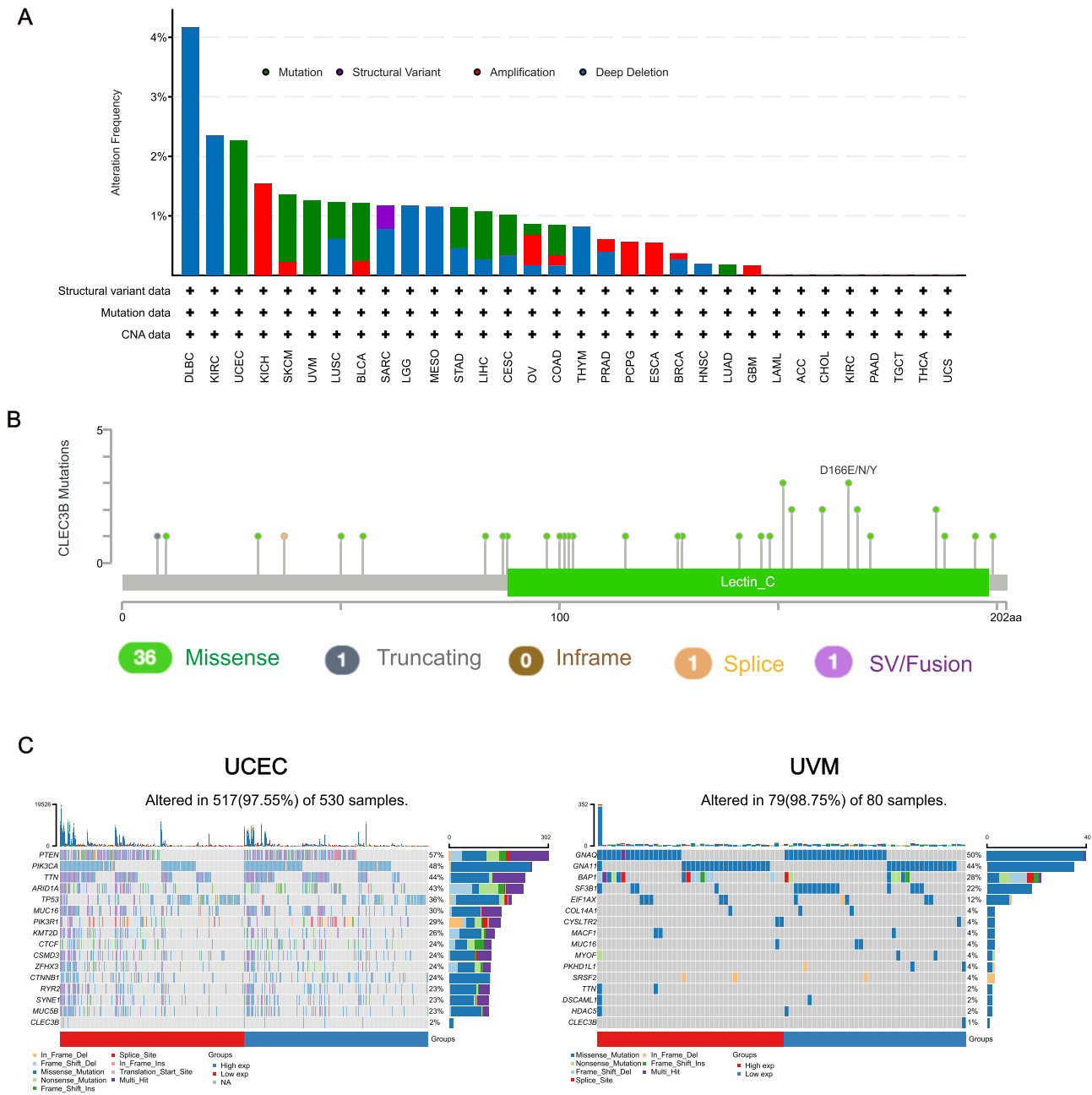


Figure 4 The mutation features of *CLEC3B* in pan-cancer. **(A)** The alteration frequency of *CLEC3B* in pan-cancer was evaluated by cBioPortal database. **(B)** Mutation diagram of *CLEC3B* in different cancer types across protein domains from cBioPortal database. **(C)** Oncoplot displaying the somatic landscape of UCEC and UVM.

observed a positive correlation between *CLEC3B* expression and most immunoinhibitor and immunostimulator genes in ACC, COAD, LUSC, OV, SARC and UCS. Conversely, in KICH, KIRC and THCA, *CLEC3B* demonstrated a negative correlation with most checkpoint genes. These findings underscore the potential role of *CLEC3B* expression in immune activation across various cancers.

Moreover, we assessed the correlation between *CLEC3B* expression and immunotherapy response using two immune-related biomarkers: TMB and MSI. In several tumors, including GBM/LGG, LGG, CESC, LUAD, COAD, COADREAD, LAML, BRCA, STES, KIPAN, STAD, PRAD, HNSC, LUSC, LIHC, THCA, READ, PAAD, SKCM, BLCA, and DLBC, *CLEC3B* expression exhibited a negative correlation with TMB (Figure 7C). Furthermore, we

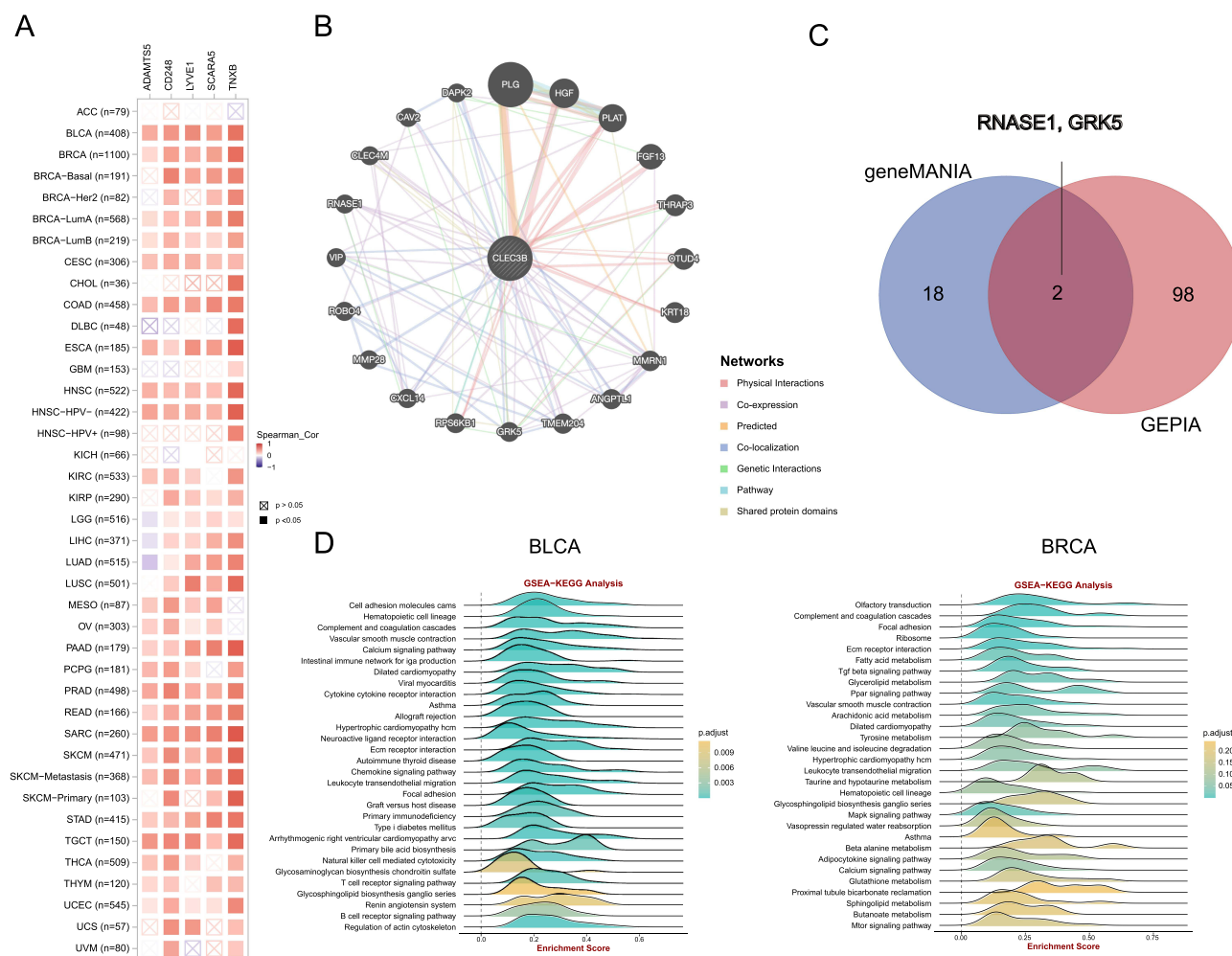


Figure 5 PPI Network of *CLEC3B* in pan-cancer. **(A)** We selected the top five of the 100 genes with the strongest correlation with *CLEC3B* in GEPIA including *TNXB*, *RPI1-855A2.5*, *RPI1-467117.1*, *SCAR5* and *CD248* and analyzed the expression correlation in pan-cancer. **(B)** The PPI network of *CLEC3B* was constructed in GeneMANIA. **(C)** An intersection analysis of *CLEC3B*-binding and correlated genes was conducted. **(D)** GSEA was performed to identify the functional enrichment of *CLEC3B* expression.

investigated the correlation between *CLEC3B* expression and MSI across different cancer types. Negative correlations were observed in STES, STAD, LUSC, LIHC, and OV, while a positive correlation was noted in GBM/LGG (Figure 7D).

The Correlation Between *CLEC3B* Expression and Genes of Immunomodulatory, RNA Modification and Tumor Stemness

The pan-cancer analysis reveals five distinct immune pathways, including chemokine and MHC gene markers. Across various tumors, the expression level of *CLEC3B* demonstrates a positive correlation with immunomodulatory genes, notably observed in ACC, KIRP, SARC and TGCT. Conversely, a negative correlation is evident in THCA and KIRC (Figure 8A). Furthermore, Figure 8B illustrates the relationship between *CLEC3B* and MHC gene markers. While ACC, KIRP, LUSC, OV, SARC, SKCM, TGCT, UCEC, UCS and UVM exhibit significant positive correlations, a different trend is observed in KICH, KIRC and THCA.

Additionally, we explored the correlation between *CLEC3B* expression and DNA stemness scores (DNAss), calculated based on pan-tumor methylation characteristics. The results indicate significant correlations with 24 tumors; among them, four tumors show significant positive correlations, including KIRP, KIPAN, KIRC, and MESO, while 20 tumors exhibit significant negative correlations. These include GBMLGG, LGG, CESC, LUAD, COAD, BRCA, ESCA, STES, SARC, STAD, HNSC, LUSC, LIHC, THCA, PAAD, TGCT, PCPG, SKCM, BLCA, and DLBC (Figure 8C).

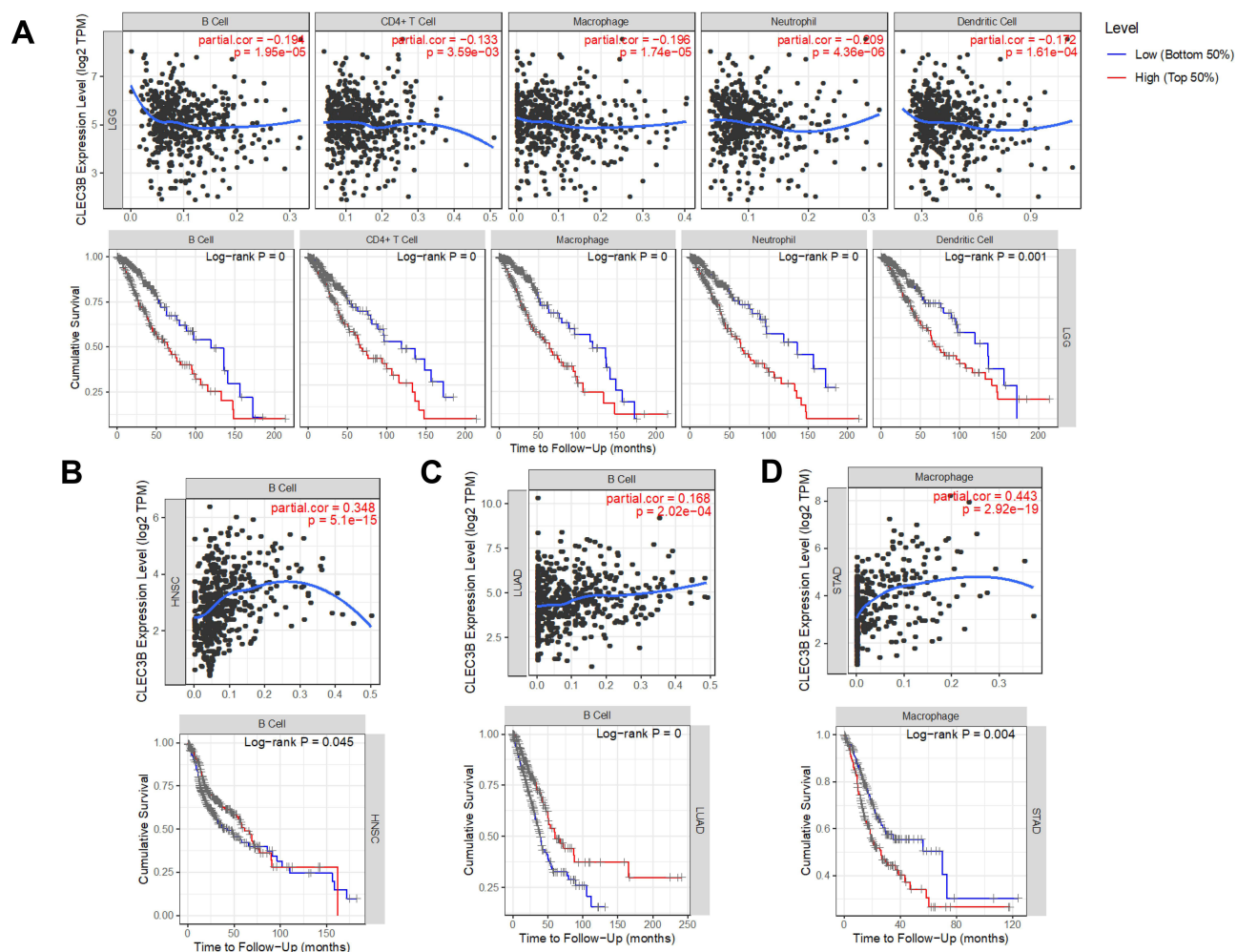


Figure 6 The relationship among *CLEC3B* expression, prognosis and immune cells infiltration of (A) LGG, (B) HNSC, (C) LUAD and (D) STAD.

Validation of the Expression and Function of *CLEC3B* in PAAD and Exosomes

Subsequently, the expression and functionality of *CLEC3B* were investigated utilizing GEO datasets. Consistent with prior findings (Figure 1B), *CLEC3B* expression was observed to be diminished in multiple cancers compared with normal or adjacent tissues (Figure 9A). Additionally, the expression of *CLEC3B* in tumor tissues was lower than that in adjacent normal tissues in GSE62452 and GSE28735 datasets of PAAD (Figure 9B). Kaplan–Meier survival analysis revealed that high *CLEC3B* expression independently influenced the prognosis of PAAD patients (Figure 9C, Figure S5). And then, qPCR was utilized to explore the expression of *CLEC3B* in PAAD cells. The results showed that *CLEC3B* expression was downregulated in HPNE than that in MIA PaCa-2, BXPC-3 and CFPAC-1 (Figure 9D). Subsequently, we validated the knockdown efficiency of *CLEC3B* in BXPC-3 cells, and qPCR and Western blotting results confirmed that the knockdown effect of sequence 1 (#1) was more significant (Figure 9E and F). CCK-8 assay results demonstrated that knockdown *CLEC3B* significantly increases the proliferation of pancreatic cancer cells (Figure 9G). Invasion and migration assay results demonstrated that the migratory capacity of BXPC-3 cells was enhanced in the *CLEC3B*-siRNA group compared to the *CLEC3B*-siNC group (Figure 10A). Finally, single-cell sequencing of normal pancreatic tissue (ZC), primary (YF), and metastatic PDAC (ZY) samples were visualized using UMAP and categorized by disease status (Figure 10B). Violin plot visualization of *CLEC3B* expression levels revealed enrichment primarily in endothelial cells (Figure 10C). The expression levels of *CLEC3B* in HUVEC cells treated with BXPC-3 CM (TEC) were significantly higher than those in wild-type HUVEC cells (Figure 10D), consistent with the findings in Figure 10C. Finally, we explored the location of *CLEC3B* in cell and found that *CLEC3B* mainly functions as a secreted protein

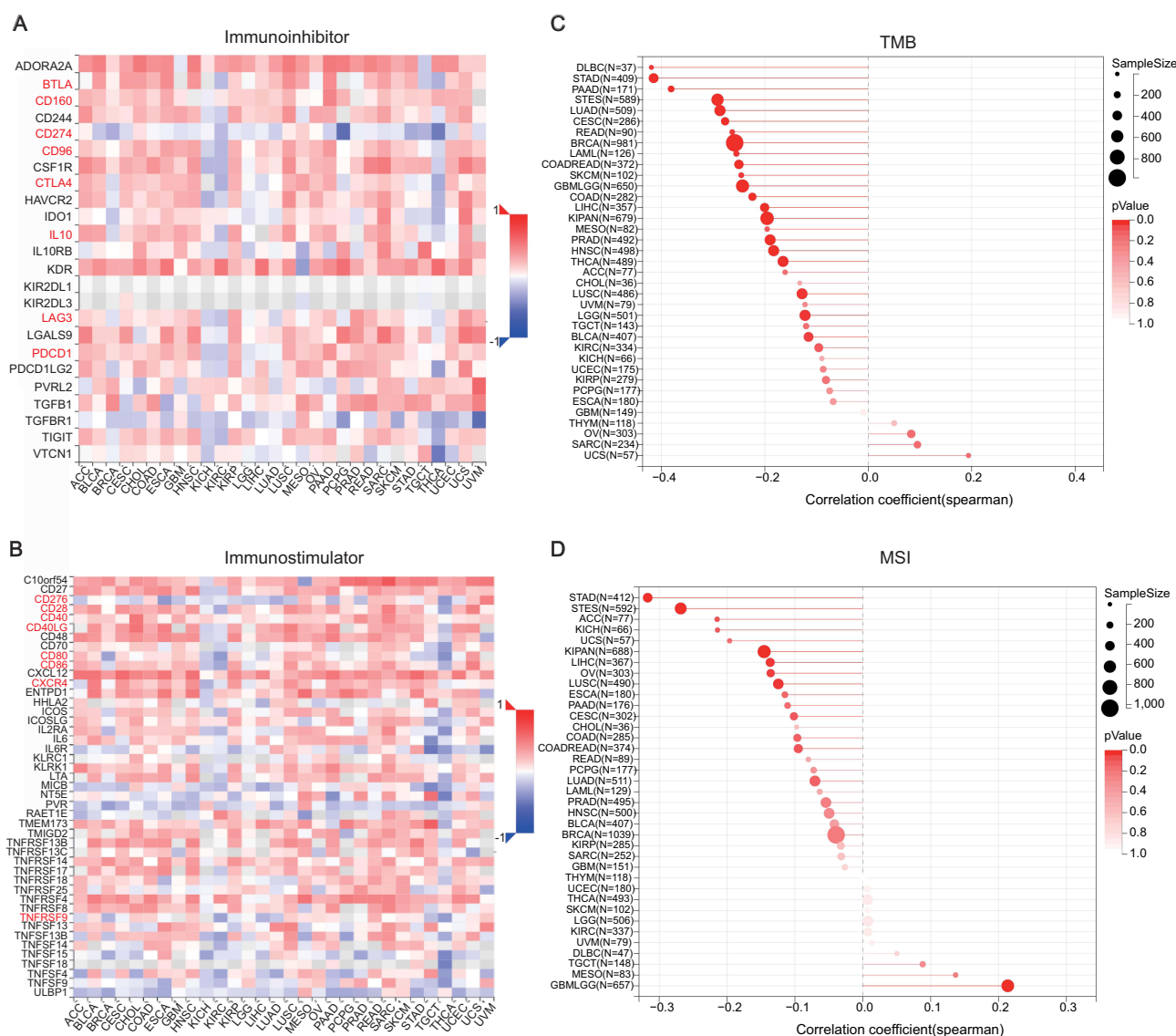


Figure 7 Correlation between the *CLEC3B* gene expression and (A) Immunoinhibitor genes, (B) Immunostimulator genes. (C) TMB and (D) MSI in pan-cancer.

(Figure 10E). Furthermore, we further isolated and characterized the exosomes of pancreatic cancer (Figure 10F and G). At the same time, it was found that *CLEC3B* was highly expressed in its extracellular vesicles (Figure 10H). The above results confirm that *CLEC3B*, as a tumor suppressor gene for various tumors, may exert its effects through secretion to the extracellular environment.

Discussion

The heightened prevalence and fatality rates of cancer on a global scale represent a substantial threat to human health. Tumors commonly exhibit poor responsiveness to treatment during advanced stages, underscoring the critical importance of early tumor detection and intervention.²² Pan-cancer analysis has emerged as a promising approach to uncovering the expression patterns and functional roles of specific genes across diverse cancer types, potentially facilitating early detection and prevention strategies.^{23,24} Numerous studies focusing on pan-cancer analysis are underway to elucidate gene expression variations among different cancers and propose effective clinical interventions.^{25–28}

CLEC3B, the gene encoding tetranectin involved in extracellular proteolysis, has garnered attention for its significant impact on multiple cancers.^{6,7,29,30} This study extensively explored the expression patterns of *CLEC3B* across various



DNA methylation, a pivotal epigenetic modification, primarily involves the addition of a methyl group to the fifth carbon of cytosine residues within CpG dinucleotides, predominantly occurring in gene promoter regions. This modification exerts a suppressive effect on gene expression through multiple mechanisms.³¹ DNA methylation was found to be elevated in the promoter region of *CLEC3B* in several tumor types compared to adjacent normal tissues, consistent with its downregulation, which modulates its interactions with immune-related factors and chemokines. Investigation of genetic alterations in *CLEC3B* across multiple cancers identified deep deletion as the most prevalent alteration, suggesting a need for further exploration of its underlying mechanisms. While previous studies have associated

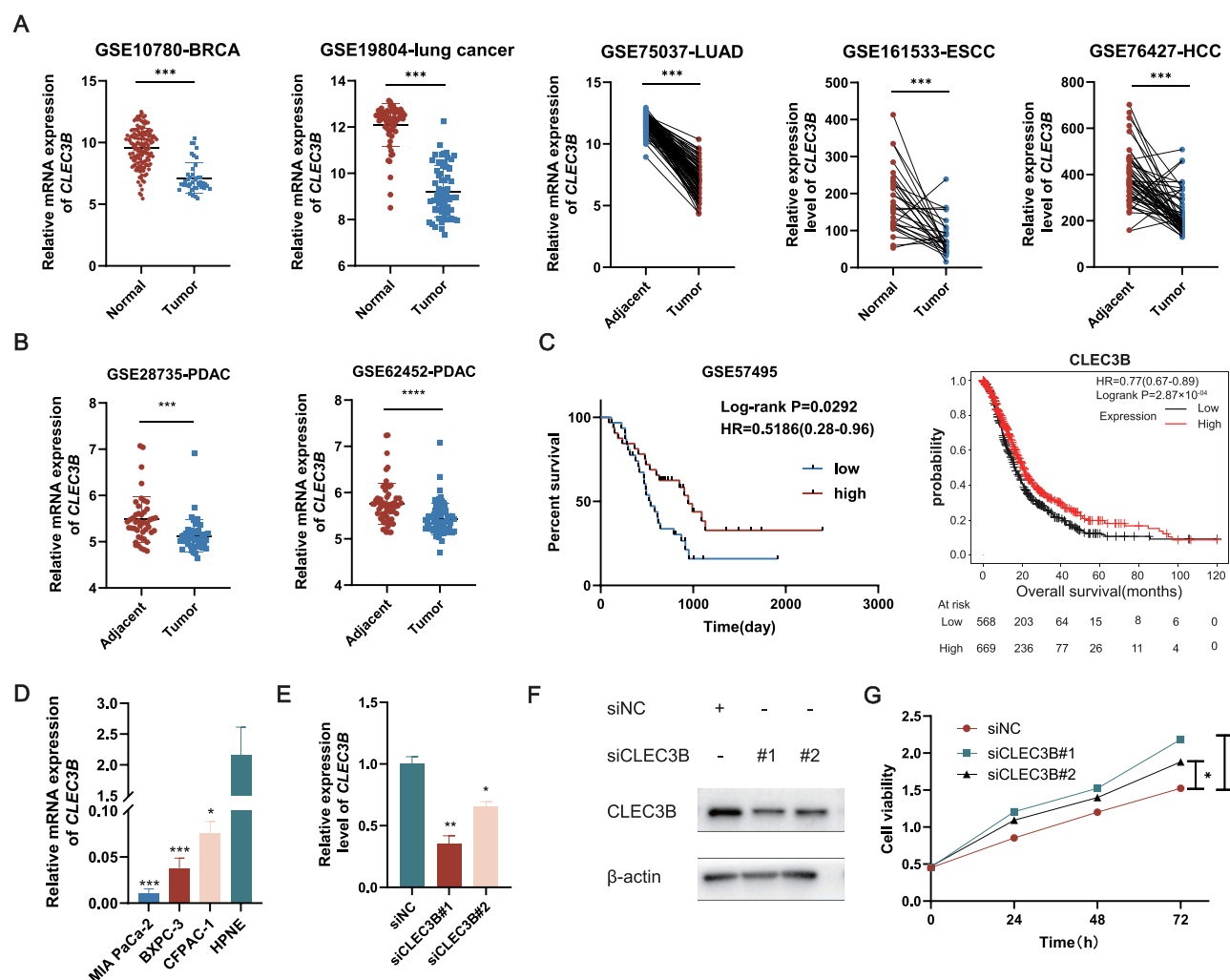


Figure 9 Validation of the Expression and Function of *CLEC3B* in multiple cancers. **(A)** The expression of *CLEC3B* was downregulated in diverse GEO datasets. **(B)** The expression of *CLEC3B* was downregulated in tumor than that in adjacent tissue in PAAD. **(C)** Kaplan–Meier survival curves revealed that high expression of *CLEC3B* was independent factors affecting the prognosis of PAAD patients. **(D)** The expression of *CLEC3B* was downregulated in PAAD cells than that in HPNE. **(E)** RT-qPCR validation of knockdown efficiency of pancreatic cancer cell line *CLEC3B*. **(F)** Western blotting validation of knockdown efficiency of pancreatic cancer cell line *CLEC3B*. **(G)** Detection of proliferation ability of pancreatic cancer cells after knockdown of *CLEC3B*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

CLEC3B missense variant p.S106G with longevity,³² its role in tumors remains unexplored. Our analysis identified R151H/C and D166E/N/Y as specific mutation sites in *CLEC3B* across various cancers, warranting further investigation of their functional implications.

Recent studies have highlighted the involvement of *CLEC3B* in tumor immune infiltration.^{9,33–36} Our results showed that *CLEC3B* was positively correlated with most part of immune cells and immune checkpoint markers. It shows that *CLEC3B* can promote immune cell infiltration and has a good ability to respond to immune checkpoint inhibitors, which provides a new strategy for clinical treatment. Experiments in-depth about the mechanism between *CLEC3B* and immune infiltration requires further exploration.

PPI analysis showed that *CLEC3B* is significantly correlated with PLG, which participate in macrophage recruitment, inflammation and fibrinolysis. In addition, the 100 genes with the strongest correlation with *CLEC3B* that we searched from GEPIA are also consistent with this result. GSEA analysis showed that high expression of *CLEC3B* was mainly associated with cell adhesion pathway, calcium signaling pathway and focal adhesion signaling pathway. Emerging studies proved that *CLEC3B* protected H9c2 cardiomyocytes from apoptosis via PI3K/Akt pathway.³⁷ *CLEC3B* located in exosome regulated metastasis and angiogenesis via AMPK and VEGF signals in hepatocellular carcinoma.⁷ At last, the markers of immunomodulatory, RNA modification and tumor stemness have a positive relationship with *CLEC3B*

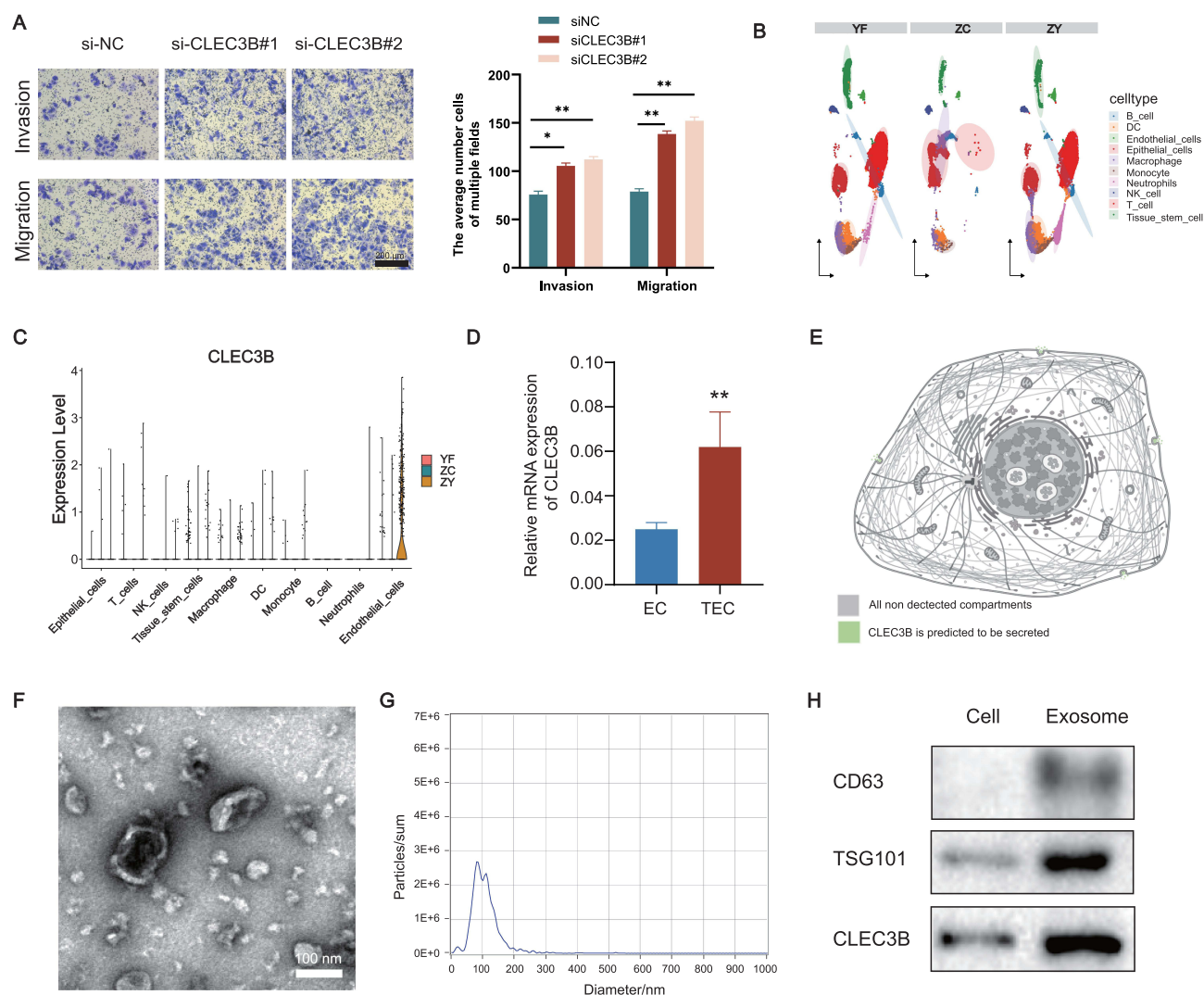


Figure 10 Validation of the Expression of *CLEC3B* in PAAD exosome. **(A)** Invasion and migration assay indicated that the migration ability of PANC-1 cells was enhanced in the *CLEC3B*-siRNA group compared with the *CLEC3B*-siNC group. **(B)** Normal pancreatic tissue (ZC), primary (YF) and metastatic PDAC (ZY) samples were visualized by UMAP grouped by disease status. **(C)** Violin plot visualization of expression levels of *CLEC3B* in normal pancreatic tissue (ZC), primary (YF) and metastatic PDAC (ZY) cancer cells. **(D)** The expression levels of *CLEC3B* in HUVEC and CM treated HUVEC (TEC). **(E)** Expression of *CLEC3B* protein inside and outside cells. **(F)** Electron microscopy image of from MIA PaCa-2 cells with a scale of 100 nm. **(G)** NTA particle size analysis of extracted. **(H)** Expression of CD63, TSG101 and *CLEC3B* in pancreatic cancer cells and extracellular vesicles. *p<0.05, **p<0.01.

expression, which further proves that this interaction existing in *CLEC3B* expression and TIME (tumor immune microenvironment).

GEO datasets and qPCR assay verified that *CLEC3B* was lower in diverse cancers than that in normal tissues and cells. In addition, knockdown of *CLEC3B* promotes the proliferation and migration of pancreatic cancer cells, which confirms that *CLEC3B*, as a tumor suppressor gene, participates in the malignant behavior of pancreatic cancer. The protein localization of *CLEC3B* revealed that it can act as a secreted protein and circulate in the body through exosomes, which is consistent with previous research results. The above results confirm that *CLEC3B* can serve as a novel tumor suppressor as an inhibitor of tumor progression. However, there are also shortcomings in this study, and further exploration is needed to determine the specific molecular mechanism of action of *CLEC3B*.

Conclusion

In summary, we integrated multi-omics analysis of *CLEC3B*. Studies have found that *CLEC3B* can be used as an effective target to predict immune infiltration and immunotherapy. In addition, *CLEC3B* inhibited the proliferation,

invasion and migration of pancreatic cancer and was expressed in the corresponding exosomes, which may be involved in the regulation of immune infiltration through secretion.

Data Sharing Statement

PAAD RNA-seq dataset and Genotype-Tissue Expression project (GTEx) data used in this study are available via the UCSC Xena (<http://xena.ucsc.edu/>) and GEO datasets (<https://www.ncbi.nlm.nih.gov/geo/>); further inquiries can be directed to the corresponding author.

Ethics Statement

This study was approved by the Ethics Committee of the Xi'an Honghui Hospital.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no competing interests in this work.

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