



Identification of SHCBP1 as a potential biomarker involving diagnosis, prognosis, and tumor immune microenvironment across multiple cancers



Ning Wang^a, Lingye Zhu^a, Liangxing Wang^{a,*}, Zhifa Shen^{b,*}, Xiaoying Huang^{a,*}

^a Division of Pulmonary Medicine, The First Affiliated Hospital of Wenzhou Medical University, Key Laboratory of Heart and Lung, Wenzhou, Zhejiang 325000, PR China

^b Key Laboratory of Laboratory Medicine, Ministry of Education of China, Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou, Zhejiang 325035, PR China

ARTICLE INFO

Article history:

Received 27 February 2022

Received in revised form 29 May 2022

Accepted 15 June 2022

Available online 18 June 2022

Keywords:

SHCBP1

Immuno-oncology

Biomarker

Diagnosis

Prognosis

Pan-cancer

ABSTRACT

Shc SH2-domain binding protein 1 (SHCBP1), a protein specific binding to SH2 domain of Src homolog and collagen homolog (Shc), takes part in the regulation of various signal transduction pathways, which has been reported to be associated with tumorigenesis and progression. However, the pathological mechanisms are not completely investigated. Thus, this study aimed to comprehensively elucidate the potential functions of SHCBP1 in multiple cancer types. The comprehensive analyses for SHCBP1 in various tumors, including gene expression, diagnosis, prognosis, immune-related features, genetic alteration, and function enrichment, were conducted based on multiple databases and analysis tools. SHCBP1 was upregulated in most types of cancers. The results of qRT-PCR had confirmed that SHCBP1 mRNA was significantly upregulated in lung adenocarcinoma (LUAD) and liver hepatocellular carcinoma (LIHC) cell lines. Based on the receiver operating characteristic (ROC) and survival analysis, SHCBP1 was considered as a potential diagnostic and prognostic biomarker. Furthermore, SHCBP1 expression was linked with tumor immunity and immunosuppressive microenvironment according to the correlation analysis of SHCBP1 expression with immune cells infiltration, immune checkpoint genes, and immune-related genes (MHC genes, chemokines, and chemokines receptors). Moreover, SHCBP1 expression correlated with tumor mutational burden (TMB), microsatellite instability (MSI), and neoantigens. The feature of SHCBP1 mutational landscape in pan-cancer was identified. Finally, we focused on investigating the clinical significance and the potential biological role of SHCBP1 in LUAD. Our study comprehensively uncovered that SHCBP1 could be identified as an immune-related biomarker for cancer diagnosis and prognosis, and a potential therapeutic target for tumor immunotherapy.

© 2022 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations: AUC, area under the curve; CCLE, cancer cell line encyclopedia; HR, hazard ratio; ROC, receiver operating characteristic; SHCBP1, Shc SH2-domain binding protein 1; THPA, the human protein atlas; TMB, tumor mutational burden; TIME, tumor immune microenvironment; 3D, three-dimensional; MSI, microsatellite instability; TAM, tumor-associated macrophages; MDSC, myeloid-derived suppressor cells; CAF, cancer-associated fibroblasts; DEGs, differentially expressed genes; ER, endoplasmic reticulum; GO, Gene Ontology; KEGG, Kyoto encyclopedia of genes and genomes; GSEA, Gene Set Enrichment Analysis; GTEx, genotype-tissue expression; ICIs, immune checkpoint inhibitors; OS, overall survival; DSS, disease specific survival; DFS, disease free survival; PFS, progression free survival; PPI, protein-protein interaction; TCGA, the cancer genome atlas; TIMER 2.0, tumor immune estimation resource, version 2; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

* Corresponding authors.

E-mail addresses: wzyxylx@163.com (L. Wang), shenzhifa@wmu.edu.cn (Z. Shen), zjwzhxy@126.com (X. Huang).

<https://doi.org/10.1016/j.csbj.2022.06.039>

2001-0370/© 2022 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Shc SH2-domain binding protein 1 (SHCBP1), a protein specific binding to SH2 domain of Src homolog and collagen homolog (Shc), takes part in the regulation of various signal transduction pathways exerting a vital role in signal transduction [1]. Recent study evidence has confirmed that SHCBP1 nuclear translocation induced by abnormal EGFR signaling pathway activation can promote the activity of β -catenin contributing to lung cancer progression [2]. The activation of AKT-GSK3 α/β signaling mediated by the interaction between FGF13 and SHCBP1 can enhance the growth of tumor cells [3]. SHCBP1-related Wnt pathway activation contributes to the apoptosis resistance resulting in cisplatin resistance in the treatment of lung cancer [4]. The upregulation of SHCBP1 heralds a poor clinical response to trastuzumab therapy in the patients with gastric cancer owing to activation of HER2-SHCBP1-PLK1 axis [5]. SHCBP1 has been considered as an oncogenic gene correlating with the tumorigenesis and progression [6–8]. Owing to the lack of sufficient relevant studies on the pathogenic mechanisms of SHCBP1 across multiple tumors and its influence on tumor immune microenvironment, we conducted a comprehensive analysis for investigating the potential roles of SHCBP1 in TCGA cancers via RNA-sequencing data.

In the current study, we identified SHCBP1 expression characteristics, diagnostic and prognostic value across multiple cancer types, and analyzed the relevance between SHCBP1 expression and immune cells infiltration, immune checkpoint genes, and other immune-related markers, including tumor mutational burden (TMB), microsatellite instability (MSI), chemokines, chemokine receptors, MHC genes, and neoantigen. We further conducted the genetic alteration features analyses. The results indicated that SHCBP1 exerted a predictive role in diagnosis and prognosis in a variety of tumor types. Particularly, SHCBP1 expression was correlated with immunosuppressive tumor microenvironment. In summary, this study first comprehensively elucidates that SHCBP1 may function as an emerging onco-immunological biomarker involving diagnosis and prognosis and is also expected to become an encouraging therapeutic target for tumor immunotherapy.

2. Materials and methods

2.1. Gene expression analysis of SHCBP1

RNA-sequencing data and corresponding clinical data among 33 cancer and corresponding normal tissues was acquired from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>) and Genotype-Tissue Expression (GTEx) databases (<https://www.gtexportal.org/home/index.html>). The gene expression data involved tumor cell line was collected from Cancer Cell Line Encyclopedia (CCLE) database (<https://portals.broadinstitute.org/ccle>). After log₂ transformation, statistical analysis was conducted using R software, and visualized via the “ggplot2” package. Furthermore, the distribution and subcellular localization of SHCBP1 protein expression were determined by immunofluorescence staining based on the Human Protein Atlas (THPA) (<https://www.proteinatlas.org/>). SHCBP1 RNA expression in different cell cycles was also obtained from THPA database.

2.2. Cell culture and qRT-PCR

Human bronchial epithelial cells (BEAS-2B), human LUAD cell lines (H1975), human normal hepatocytes (LO2), and human LIHC cell lines (MHCC-97H) were purchased from the American Type Culture Collection (ATCC, United States). All cells were cultured in high glucose Dulbecco's Modified Eagle's media (DMEM) sup-

plemented by 10% fetal bovine serum (FBS), and 1% Penicillin/Streptomycin at 37 °C in a humidified atmosphere with 5% CO₂. Total RNA was isolated from cells using the RNA fast200 Extraction kit (Fastagen Biotech, China), and then RNA concentration was detected using NanoDrop 2000 spectrophotometer (Thermo, United States). After reverse transcription via PrimeScript RT Master Mix (Takara, Japan), qRT-PCR was conducted using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, China) via the CFX96 Real-Time System (Bio-Rad, United States). GAPDH was used for normalization. Primers were purchased from Sangon Biotech (Shanghai, China) and the sequences were shown in [Supplementary data \(Table S1\)](#).

2.3. Clinical correlation analysis of SHCBP1

The receiver operating characteristic (ROC) analysis method was utilized to estimate the diagnostic value of SHCBP1 in various cancers via the “pROC” package. Then, the value of area under the curve (AUC) (0.5 to 1.0) was calculated. The higher the AUC value, the better the diagnostic value. Generally, AUC value (0.5–0.7, 0.7–0.9, 0.9–1.0) indicates a low, middle, and high predicted effects, respectively. The relevance between SHCBP1 expression and patients' survival outcomes such as overall survival (OS), disease specific survival (DSS), disease free survival (DFS), and progression free survival (PFS) were investigated and exhibited via forest plots and Kaplan-Meier curves utilizing the “survival” package. That hazard ratio (HR) was over 1 (HR > 1) indicated that it served as a risk factor for patients' survival. On the contrary, “HR < 1” indicated that it had the protective effect on patients. The connection between SHCBP1 expression and the clinicopathologic characteristics in LUAD were exhibited via Sankey diagram accomplished by the “ggalluvial” package. The univariate and multivariate Cox regression analyses in LUAD were performed via the “forestplot” package.

2.4. Immune-related characteristics analysis of SHCBP1

We first evaluated the correlation of SHCBP1 expression with Immune Score, Stromal Score and ESTIMATE Score across multiple cancer types by Spearman's correlation analysis using the “estimate” package. Furthermore, the relevance between SHCBP1 expression and the infiltration level of immune cells, such as CD8 + T cells, M2 subtype of tumor-associated macrophages (M2-TAM), myeloid-derived suppressor cells (MDSC), cancer-associated fibroblasts (CAF), and regulatory T (Treg) cells in various cancers was conducted via tumor immune estimation resource (TIMER 2.0) web server (<http://timer.cistrome.org/>). Furthermore, the correlation between SHCBP1 expression and immune checkpoint genes such as CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT was observed by Spearman's correlation analysis.

Previously published studies demonstrate that TMB and MSI have been considered as emerging predictive biomarkers closely associated with the response to the treatment of immune checkpoint inhibitors (ICIs) [9,10]. TMB reflects the presence of somatic mutation sites in tumor genome, which contributes to producing neoantigen and immunogenicity leading to T cell responses [11]. TMB was calculated according to the somatic data acquired from the TCGA database, and MSI score of each cancer was analyzed. Then, the relevance between SHCBP1 expression and TMB or MSI was investigated via the Spearman's correlation analysis. We also investigated the association of SHCBP1 expression with the count of immune neoantigens and other immune-related genes, including MHC genes, chemokines, and their receptors in pan-cancer using Spearman's correlation analysis method.

2.5. Genetic alteration analysis of SHCBP1

SHCBP1 mutation features across multiple cancers were analyzed utilizing the cBioPortal platform (<https://www.cbioportal.org/>) containing multidimensional cancer genomics information [12,13]. “SHCBP1” was input into the “quick selection” module for the exploration of genetic alteration. The alteration frequency of SHCBP1 was observed via the “Cancer Types Summary” module. The mutation sites and three-dimensional (3D) structure could be acquired using “Mutations” module. Waterfall plot displaying genetic alterations of SHCBP1 was obtained via “OncoPrint” module. In addition, somatic mutation landscape based on SHCBP1 expression in LUAD was constructed utilizing the “maftools” package.

2.6. Construction of gene-gene and protein-protein interaction network

GeneMANIA (<https://genemania.org/>) is a web interface for establishing gene-gene interaction network [14]. STRING website (<https://string-db.org/>) is utilized to construct SHCBP1 protein-protein interaction (PPI) network containing 50 related proteins. The main parameters were as follows: active interaction sources (“experiments”), minimum required interaction score [“Low confidence (0.150)”], and max number of interactors to show (“no more than 50 interactors”). Subsequently, the visualization of PPI network was achieved utilizing Cytoscape (version 3.8.0). The degree score of each node was calculated by “CytoHubba” plug-in.

2.7. Enrichment analysis

To further investigate the functions of these tightly connected proteins and genes in PPI and gene-gene interaction network, Gene Ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) analyses were performed via the “ClusterProfiler” package. Gene Set Enrichment Analysis (GSEA) was applied to further investigate the significant pathways between the SHCBP1^{high} and SHCBP1^{low} expression samples, the most obvious signaling pathways in KEGG and HALLMARK were visualized in plots with certain criteria ($p < 0.05$) identified to be enrichment significant. In addition, the differentially expressed genes (DEGs) were explored by comparing the gene expression between SHCBP1^{high} and SHCBP1^{low} groups in LUAD samples with setting a threshold of adjusted $p < 0.05$ and fold change > 2 using the “Limma” package.

2.8. Statistical analysis

The Kruskal-Wallis test was utilized to compare gene expression across different tissues and cancer cell lines, and the Wilcoxon rank sum test was applied to assess gene expression status between tumor tissues and normal tissues. All R packages mentioned above were operated under R software version v4.0.3, and statistical significance was acknowledged in case of $p < 0.05$.

3. Results

3.1. Gene expression analysis of SHCBP1 in pan-cancer

We first analyzed the expression level of SHCBP1 in 31 types of normal tissues from the GTEx database. SHCBP1 expression varied across different types of normal tissues, and was generally at a low level according to the value of gene expression ($\log_2(\text{TPM} + 1)$) (Fig. 1A). In contrast, SHCBP1 was highly expressed in most tumor cell lines based on the value of gene expression (Fig. 1B). Furthermore, we evaluated SHCBP1 mRNA expression levels in TCGA

tumors and adjacent normal tissues, which indicated SHCBP1 expression was significantly higher in 17 cancer types, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), LIHC, LUAD, lung squamous cell carcinoma (LUSC), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), while it was significantly lower in kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), and kidney renal papillary cell carcinoma (KIRP) (Fig. 1C). Finally, based on TCGA and GTEx databases, SHCBP1 expression was significantly upregulated in 26 cancer types, involving in adrenocortical carcinoma (ACC), BLCA, BRCA, CESC, CHOL, COAD, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), ESCA, GBM, HNSC, brain lower grade glioma (LGG), LIHC, LUAD, LUSC, ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), PCPG, PRAD, READ, skin cutaneous melanoma (SKCM), STAD, testicular germ cell tumors (TGCT), THCA, thymoma (THYM), UCEC, and uterine carcinosarcoma (UCS), while it was downregulated in KICH, KIRC, KIRP, and acute myeloid leukemia (LAML) (Fig. 1D).

3.2. SHCBP1 intracellular localization, expression, and qRT-PCR validation.

To identify the intracellular localization of SHCBP1, we evaluated subcellular distribution of SHCBP1 in A-431 (human epidermoid carcinoma cells), PC-3 (human prostate cancer cells), and U-2 osteosarcoma (OS) cells through immunofluorescent staining of microtubules, endoplasmic reticulum (ER), and nucleus based on THPA database. As shown in Fig. 2A, SHCBP1 was characterized as co-localization with DAPI labelling nuclear in A-431, PC-3, and U-2 OS cells, which indicated the subcellular localization of SHCBP1 was in nuclei. Additionally, the immunofluorescent of SHCBP1 was also present in the cytoplasm in PC-3 and U-2 OS cells. Furthermore, single cell variation analysis revealed that SHCBP1 RNA expression was correlated with the cell cycle (Fig. 2B). In addition, we confirmed that SHCBP1 mRNA was remarkably upregulated in LUAD cell lines (H1975) and LIHC cell lines (MHCC-97H) with the normal cell lines as control by qRT-PCR (Fig. 2C).

3.3. Identification of the diagnostic and prognostic value of SHCBP1 in pan-cancer

AUC is characterized with sensitivity and specificity, which is generally utilized to indicate the intrinsic effectiveness of diagnostic tests [15]. The results suggested that SHCBP1 had a high accuracy ($\text{AUC} > 0.8$) in predicting the diagnosis according to AUC value in ROC curve in 20 cancer types, such as ACC ($\text{AUC} = 0.833$), BLCA ($\text{AUC} = 0.896$), BRCA ($\text{AUC} = 0.948$), CESC ($\text{AUC} = 0.995$), CHOL ($\text{AUC} = 1.000$), COAD ($\text{AUC} = 0.984$), DLBC ($\text{AUC} = 0.822$), ESCA ($\text{AUC} = 0.950$), GBM ($\text{AUC} = 0.964$), HNSC ($\text{AUC} = 0.962$), KICH ($\text{AUC} = 0.817$), LAML ($\text{AUC} = 0.883$), LIHC ($\text{AUC} = 0.894$), LUAD ($\text{AUC} = 0.850$), LUSC ($\text{AUC} = 0.960$), OV ($\text{AUC} = 0.972$), PAAD ($\text{AUC} = 0.972$), READ ($\text{AUC} = 0.974$), STAD ($\text{AUC} = 0.956$), and UCEC ($\text{AUC} = 0.981$) (Fig. 3).

We further investigated the prognostic impact of SHCBP1 on the patients with cancer encompassing OS, DSS, DFS, and PFS analyses via Cox proportional hazards model. As shown in the forest plots (Fig. 4A), high SHCBP1 expression was significantly predictive of worse OS in the patients with ACC, KIRC, KIRP, LGG, LIHC, LUAD, and MESO. The results of DSS analysis indicated that SHCBP1

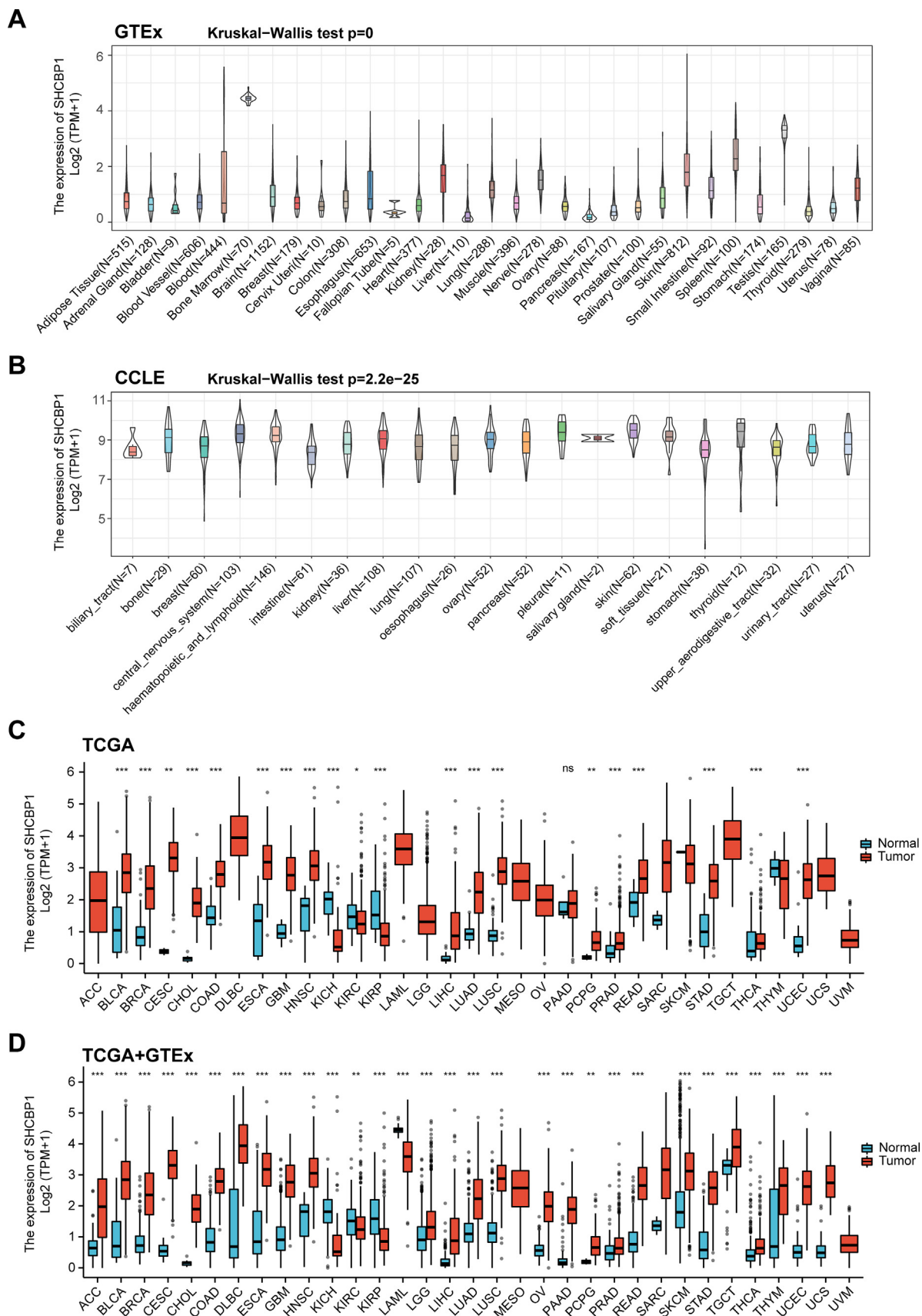


Fig. 1. The expression characteristics of SHCBP1. (A) The expression of SHCBP1 in normal tissues based on GTEx database. (B) The expression of SHCBP1 in tumor cell lines based on CCLE database. (C) Comparison of the expression of SHCBP1 in TCGA tumors and adjacent normal tissues. (D) Comparison of the expression of SHCBP1 in tumor and normal tissues based on integrated database of TCGA and GTEx. SHCBP1 expression levels are assessed using $\text{log}_2(\text{TPM} + 1)$. TPM, Transcript per million. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns, no significance).

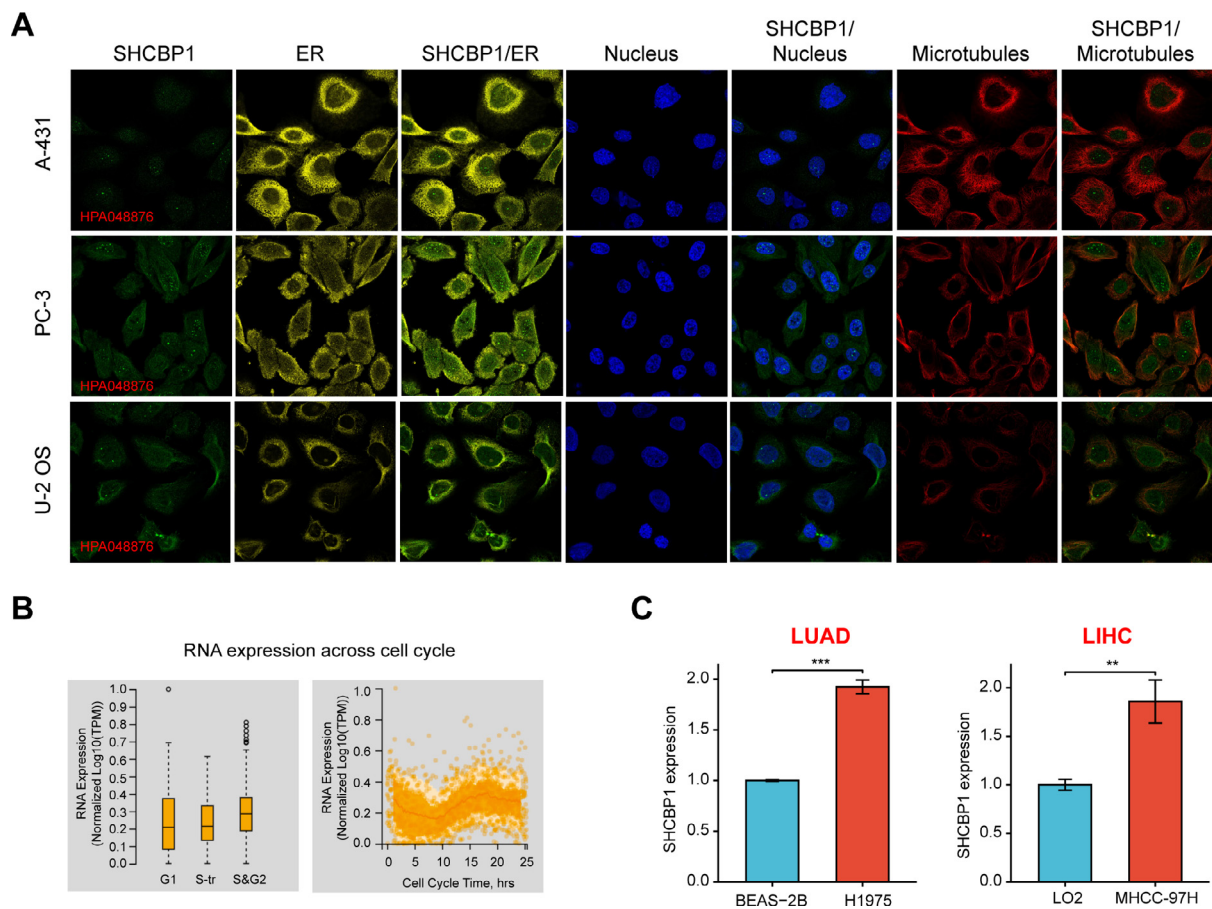


Fig. 2. SHCBP1 intracellular localization, expression, and qRT-PCR validation. (A) Immunofluorescence staining of the microtubules, endoplasmic reticulum (ER), and nucleus for the subcellular distribution of SHCBP1 expression in A-431, PC-3, and U-2 OS cells based on the Human Protein Atlas (THPA) database. (B) SHCBP1 RNA expression across cell cycle. (C) Validation of expression of SHCBP1 in tumor cells by qRT-PCR. GAPDH is used for normalization. (***p* < 0.01, and ****p* < 0.001).

exerted the hazardous effects on the patients with ACC, KIRC, KIRP, LGG, LIHC, MESO, and SARC (Fig. 4B). The results of DFS analysis confirmed the protective effects of SHCBP1 expression in the patients with ESCA, and risky effects on the patients with KIRP, LIHC, PAAD, PRAD, SARC, THCA, and UCEC (Fig. 4C). In terms of PFS analysis, SHCBP1 served as a risk factor for the patients with ACC, BLCA, KIRC, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PRAD, SARC, and UVM (Fig. 4D). Therefore, the results demonstrated that higher SHCBP1 expression closely predicted a worse prognosis in several cancers, including KIRP, LIHC, LUAD, etc. Taken together, the results of ROC and prognosis analysis suggested that SHCBP1 had high diagnostic and prognostic value across multiple cancer types.

3.4. Immune-related characteristics of SHCBP1 in pan-cancer

Tumor immune microenvironment (TIME) was largely affected by the infiltrating tumor components, which was one of the key determinants for the outcome of tumor immunotherapy [16]. We first identified the relationship between SHCBP1 expression and immune infiltration via Immune Score (Fig. 5), Stromal Score (Fig. S1) and ESTIMATE Score (Fig. S2) analyses. The top three cancers with the most significant correlation of SHCBP1 expression with immune infiltration were THCA, KIRC, and LGG. Furthermore, we analyzed the level of immune cells infiltration via several algorithms such as TIMER, EPIC, MCPOUNTER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, and XCELL using TIMER 2.0 database. As shown in Fig. 6A, a significantly positive association was observed between SHCBP1 expression and CD8 + T cells infiltration

in COAD, KIRC, TGCT, THYM, and UVM, while there appeared negative correlation or no significant correlation in many cancer types.

In addition, immunosuppressive microenvironment is considered as a critical factor contributing to uncontrollable tumor growth and even the progression and metastasis resulting in poor immunotherapy response. Thus, we assessed the correlation of SHCBP1 expression with the infiltration levels of M2-TAM, MDSC, CAF, and Treg using TIMER 2.0 database. Interestingly, SHCBP1 was significantly positively linked with the infiltration of M2-TAM, MDSC, CAF, and Treg across most cancers (Fig. 6B). The current findings revealed that high SHCBP1 expression might be associated with immunosuppressive microenvironment offering a potential new therapeutic target for tumor immunotherapy.

Next, we identified the potential relevance between SHCBP1 expression and immune checkpoint genes in pan-cancer. Significantly, SHCBP1 expression was positively related with these immune checkpoint genes, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT in multiple cancers such as LUAD, KIRC, LIHC, etc. (Fig. 6C). Meanwhile, we conducted the correlation analysis of SHCBP1 expression with TMB and MSI. As shown in Fig. 6D, the expression of SHCBP1 appeared significantly positively associated with TMB in LUAD, LUSC, STAD, BRCA, UCEC, UCS, etc. In contrast, there was a negative correlation with TMB in THYM and ESCA. For MSI, SHCBP1 expression was positively related with MSI in COAD, LIHC, LUSC, SARC, STAD, and UCEC, and negatively related with MSI in DLBC and TGCT (Fig. 6E). Gene co-expression analyses revealed positive correlations of SHCBP1 expression with most chemokines (Fig. 7A), chemokine receptors (Fig. 7B), and MHC genes (Fig. 7C) in

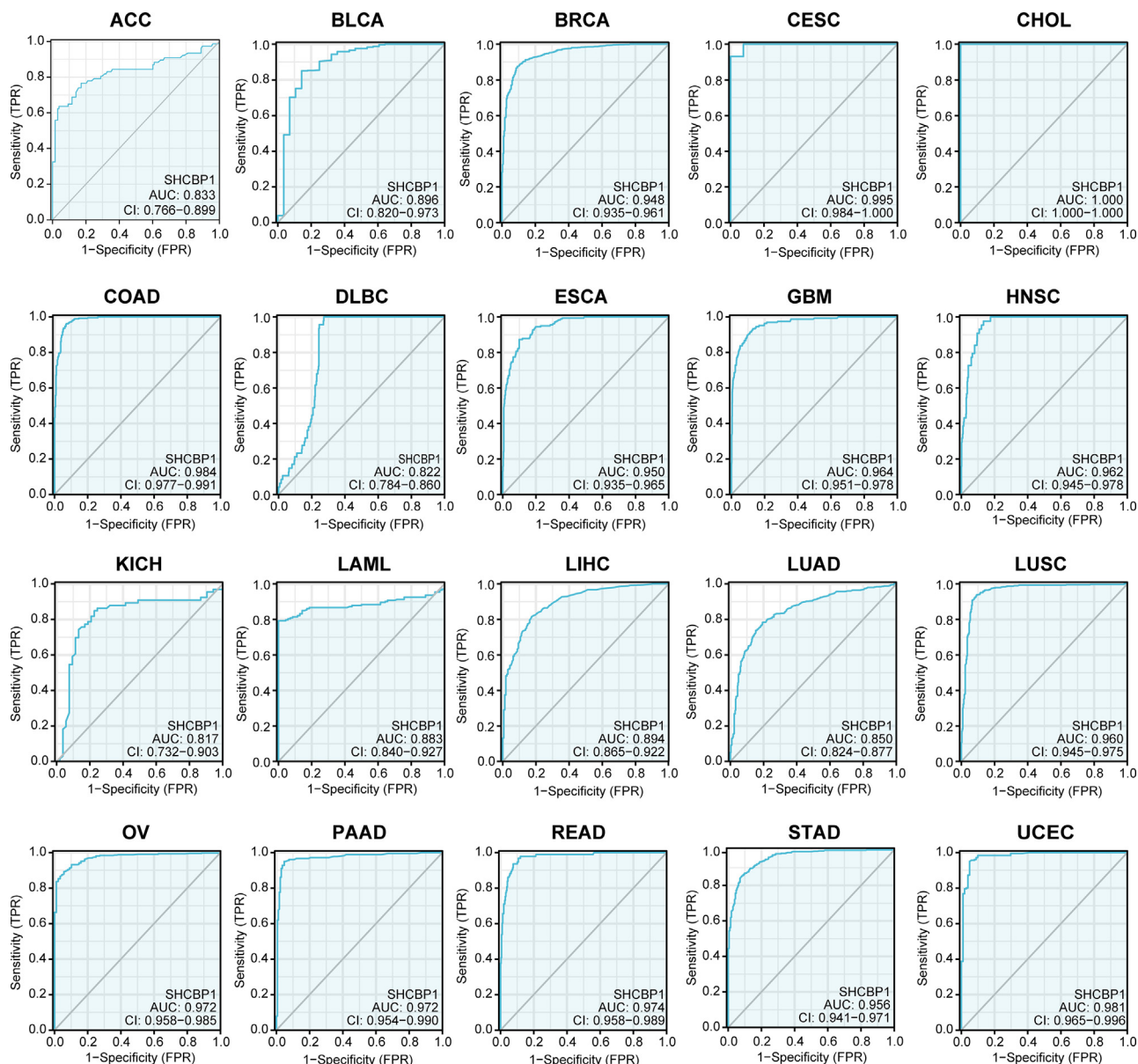


Fig. 3. Receiver operating characteristic (ROC) curve for SHCBP1 expression in pan-cancer. The abscissa indicates false positive rate (FPR) and the ordinate indicates true positive rate (TPR). The area under the receiver operating characteristic curve (AUC) value (0.5–0.7, 0.7–0.9, 0.9–1.0) indicates a low, middle, and high predicted effects, respectively. CI, confidence interval.

pan-cancer. Meanwhile, we confirmed that SHCBP1 expression was significantly positively associated with the count of immune neoantigens in LUAD, OV, BRCA, UCEC, STAD, PRAD, and LGG (Fig. S3). All these results above demonstrated that high SHCBP1 expression is significantly relevant to tumor immunity.

3.5. Genetic alteration analysis of SHCBP1

SHCBP1 mutation features in diverse types of tumor samples were explored based on the cBioPortal database. As shown in Fig. S4A, the highest alteration frequency of SHCBP1 emerged in the UCEC patients, of which “Mutation” was the major type. And the highest “amplification” type alteration occurred in the PRAD patients with an alteration frequency of ~ 3%. Moreover, the genetic alteration frequency of LUAD cases (containing “Deep Deletion” type) was higher than LUSC. The types, sites and corresponding domain of the SHCBP1 mutations were observed in Fig. S4B.

The 3D structure of SHCBP1 protein with the mutation sites (G446, T473, A487, and X489) mapped was presented in Fig. S4C. Similarly, the frequency and pattern of SHCBP1 alteration, including mutation, amplification, deep deletion, and structural variant were displayed via waterfall plot (Fig. S4D).

3.6. Construction of PPI and genes interaction network, and enrichment analysis

PPI network involved in 50 SHCBP1-interacting proteins was established utilizing STRING and visualized by Cytoscape (Fig. 8A). Each node was present as a different depth of color reflecting the extent of interaction, which revealed the interaction strength between the proteins. The gene-gene interaction network was achieved via Genemania, which exhibited top 20 most frequently altered genes linking with SHCBP1 (Fig. 8B). Subsequently, we performed the functional enrichment analysis (GO and KEGG)

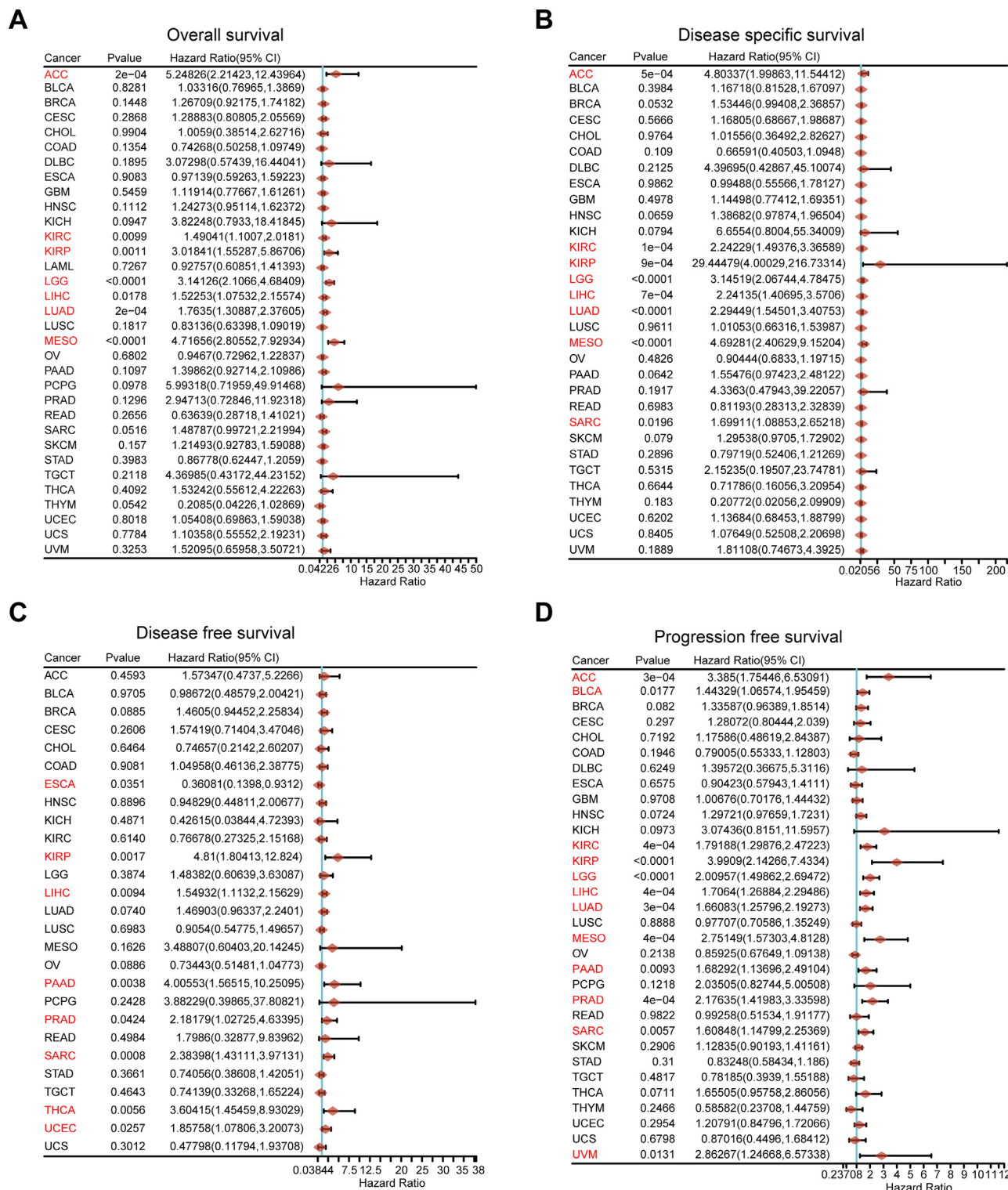


Fig. 4. The forest plots of univariate Cox regression analyses in (A) overall survival, (B) disease specific survival, (C) disease free survival, and (D) progression free survival. The red mark demonstrates that SHCBP1 expression was significantly associated with patients' prognosis. That hazard ratio (HR) is over 1 (HR > 1) indicates that it serves as a risk factor for patients' survival. HR < 1 indicates that it has the protective effect on patients. CI, confidence interval. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in PPI and genes interaction network, respectively. The results revealed that the proteins in PPI network were mainly enriched in the events such as mitosis, tubulin binding, microtubule binding, and ATPase activity (Fig. 8C). The genes in the network were mainly enriched in mitosis process, and participated in several

pathways such as microRNAs in cancer, ErbB signaling pathway, and EGFR tyrosine kinase inhibitor resistance (Fig. 8D).

GSEA analysis was conducted to investigate the potential signaling pathways in KEGG and HALLMARK from high and low SHCBP1 expression samples (Fig. 8E-H). Results of the functional

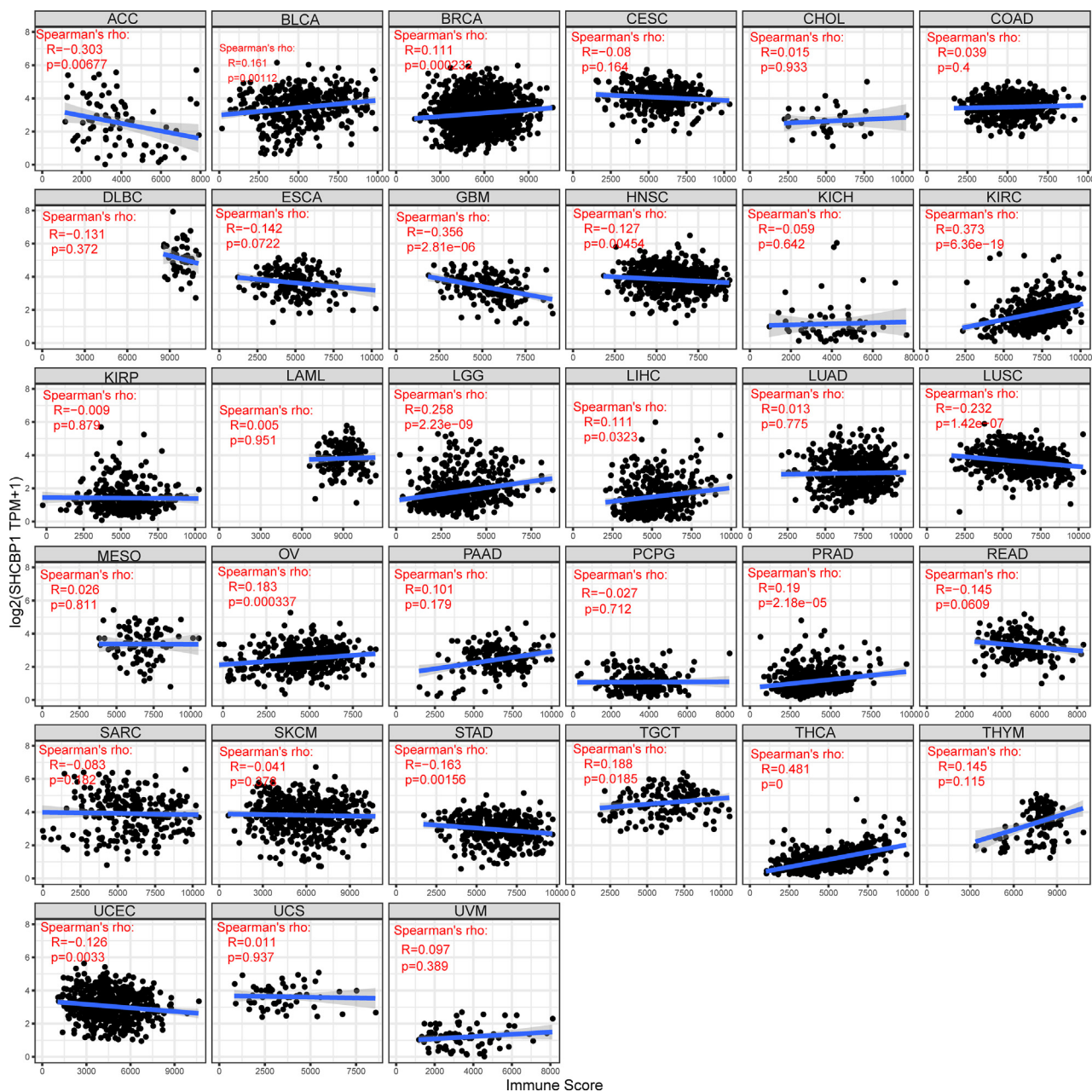


Fig. 5. Spearman's correlation analysis showing the association between SHCBP1 expression levels (log2(TPM + 1)) and Immune Score across 33 different TCGA cancer types. TPM, Transcript per million.

enrichment of KEGG terms revealed that high SHCBP1 expression was linked with P53 signaling pathway, regulation of actin cytoskeleton, and cell cycle. HALLMARK terms demonstrated that high SHCBP1 expression was mainly involving in complement, inflammatory response, and G2M checkpoint.

3.7. Clinical characteristics of SHCBP1 expression in LUAD

In addition, we focused on investigating the clinical characteristics of SHCBP1 in LUAD. We first confirmed that SHCBP1 was remarkably upregulated in tumor tissues compared to the normal tissues via TCGA and GTEx database (Fig. 9A). The SHCBP1 expression was dramatically associated with different pathological stages of LUAD patients (Fig. 9B). The distribution tendency between SHCBP1 expression and the clinicopathological characteristics

involving in ages, stages, and survival state in LUAD patients was exhibited via Sankey diagram (Fig. 9C). High SHCBP1 expression was identified to be related to poor clinical outcomes in LUAD patients according to Kaplan–Meier survival curves of OS, DSS, DFS, and PFS (Fig. 9D–G). Subsequently, SHCBP1 expression was identified as an independent prognostic factor for LUAD patients' OS through the univariate and multivariate Cox regression analyses (Fig. 9H and I).

3.8. Identification of the potential biological function of SHCBP1 expression in LUAD

To begin with, the landscape of somatic mutations in LUAD cohort revealed that the samples with high SHCBP1 expression had a high frequency of gene mutations such as TP53, TTN,

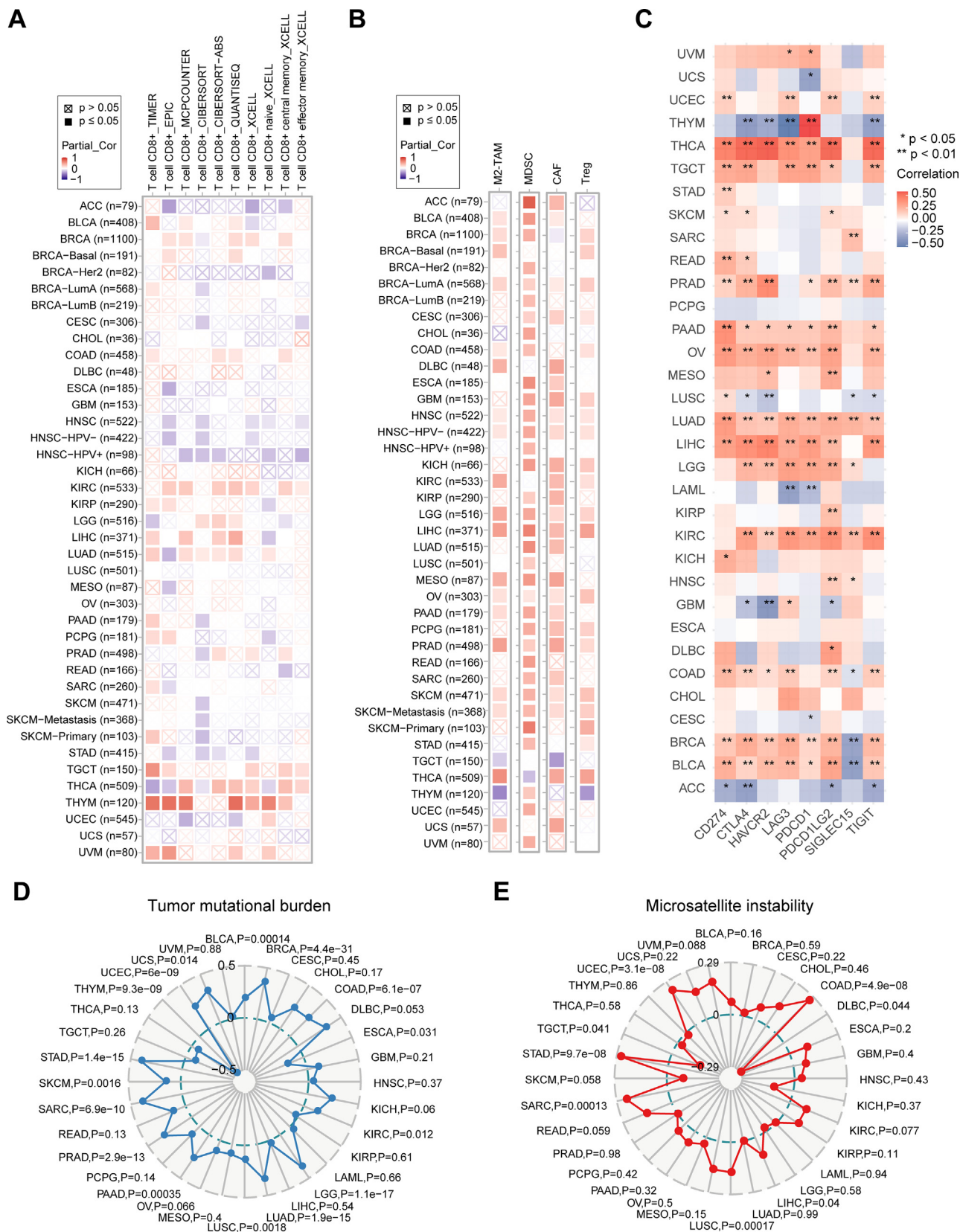


Fig. 6. Correlation of SHCBP1 expression with (A) the infiltration of CD8+ T cell, (B) M2 subtype of tumor-associated macrophages (M2-TAM), myeloid-derived suppressor cells (MDSC), cancer-associated fibroblasts (CAF), and regulatory T (Treg) cells. Red indicates a positive correlation and blue indicates a negative correlation. (C) Correlation of SHCBP1 expression with immune checkpoint genes, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT. Red indicates a positive correlation and blue indicates a negative correlation. (* $p < 0.05$ and ** $p < 0.01$) (D) Correlation of SHCBP1 expression with tumor mutational burden (TMB). (E) Correlation of SHCBP1 expression with microsatellite instability (MSI). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

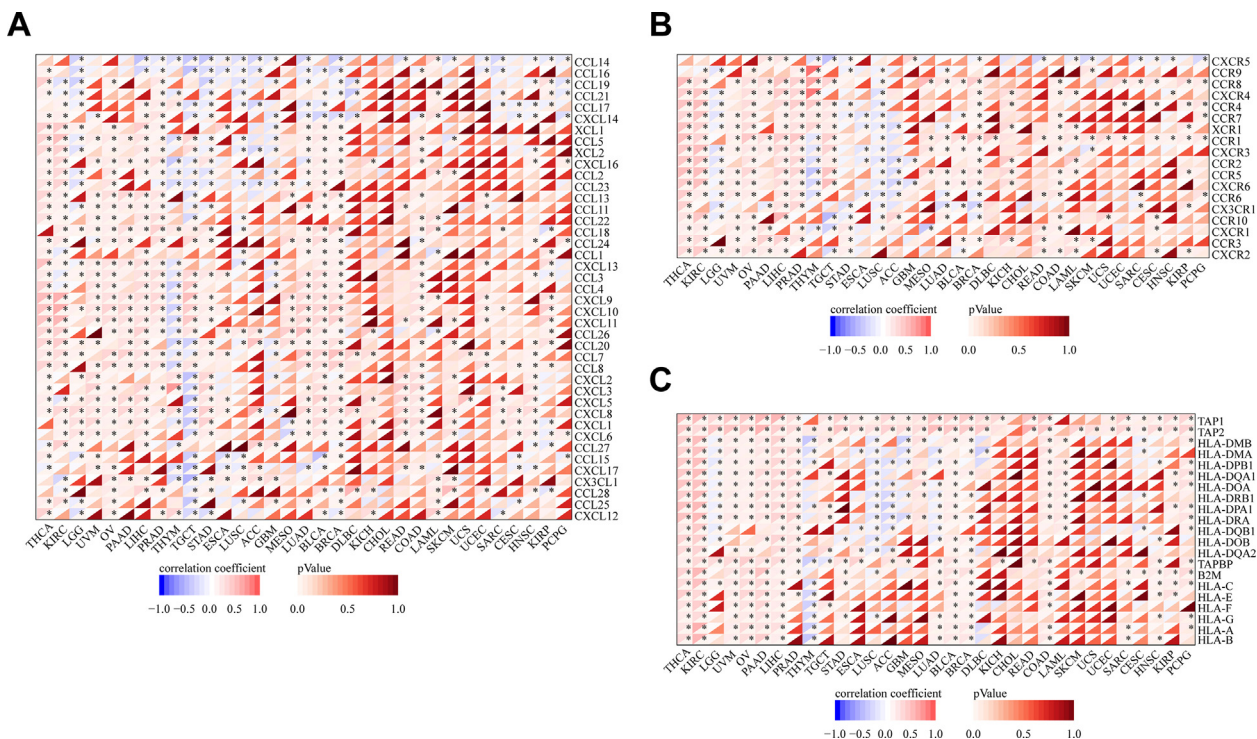


Fig. 7. Correlation of SHCBP1 expression with immune-related genes, including (A) Chemokines, (B) Chemokine receptors, and (C) MHC genes. The color scales indicate Spearman's correlation coefficient and *p* value. Upper triangle in the heatmap represents correlation coefficient. Red indicates a positive correlation and blue indicates a negative correlation. Lower triangle represents *p* value and **p* < 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

MUC16, etc. (Fig. 10A). The immune checkpoint genes were all upregulated in LUAD patients with high SHCBP1 expression (Fig. 10B). The results of DEGs analysis between high and low SHCBP1 expression in LUAD identified 199 genes upregulated and 90 genes downregulated (Fig. 10C and D). The detailed genes information can be inquired in the Supplementary data (Table S2). Then, enrichment analysis results suggested that upregulated DEGs were primarily connected with organelle fission, nuclear division, chromosome segregation, and mitotic nuclear division via GO analysis (Fig. 10E) and cell cycle via KEGG analysis (Fig. 10F).

4. Discussion

It is well established that SHC1 is considered as a key protein exerting a vital role in participating in many signal transduction pathways. SHCBP1, present in the cytoplasm, can bind specifically to SH2 domain of SHC1 participating in intracellular signal transduction and the downstream biological events. Accumulated evidences have demonstrated that SHCBP1 is closely associated with cell division, cell differentiation and proliferation. According to research findings, SHCBP1 is significantly related with the development and progression of multiple diseases including cancers. Previous studies have found that SHCBP1 plays significant roles in various cancer types [17–20]. The potential diagnostic and prognostic value of SHCBP1 and the molecular mechanisms underlying in tumor immune microenvironment remain to be explored. Thus, the comprehensive pan-cancer analysis is crucial and meaningful for determining the potential biological function of SHCBP1 among different cancers. Our present study identified the molecular expression, clinical characteristics, genetic alterations of SHCBP1 and investigated the correlation of SHCBP1 with immune cells infiltration, immune checkpoint genes expression,

TMB, MSI, immune-related genes, and neoantigen across TCGA cancer types.

Different cancer types appear distinct genetic heterogeneity owing to the genetic and epigenetic alterations during tumor evolution [21]. Gene expression profiling analysis can be applied to explore relevant biomarkers for diagnosis and prognosis in cancer [22]. In this study, we determined SHCBP1 expression features, and discovered that SHCBP1 was upregulated in almost all TCGA cancers, which indicated that SHCBP1 might serve as an oncogene for tumor development and progression. SHCBP1 had a high diagnostic value according to the AUC value in ROC curve across most cancer types, which suggested that SHCBP1 could have high sensitivity and specificity in distinguishing tumor patients from healthy individuals. Furthermore, clinical prognosis analysis suggested that SHCBP1 functioned as a risk factor predicting worse prognosis in the patients with ACC, KIRC, KIRP, LGG, LIHC, LUAD, MESO, and SARC based on DSS analysis. These results implied SHCBP1 could be considered as a significant diagnostic and prognostic biomarker in several cancer types.

Recent years have witnessed noteworthy progresses and breakthroughs in cancer immunotherapy and there has achieved greater improvement in the clinical prognosis of the patients with multiple types of cancer. TME, particularly TIME, is becoming the focus of cancer immunity research. Immune cells infiltration performs a vital role in anti-tumor immunotherapy. However, the immunosuppressive status of TME could hinder the antitumor immunity leading to poor prognosis and therapy resistance. The cells infiltration including CAFs, Tregs, TAMs, and MDSCs contribute to the formation of immunosuppressive TME. Increasing evidence has demonstrated that TAM, particularly M2-TAM, serve as key drivers participating in the establishment of an immunosuppressive TME through hampering the differentiation and function of T cells, and promoting the recruitment of MDSCs and Treg

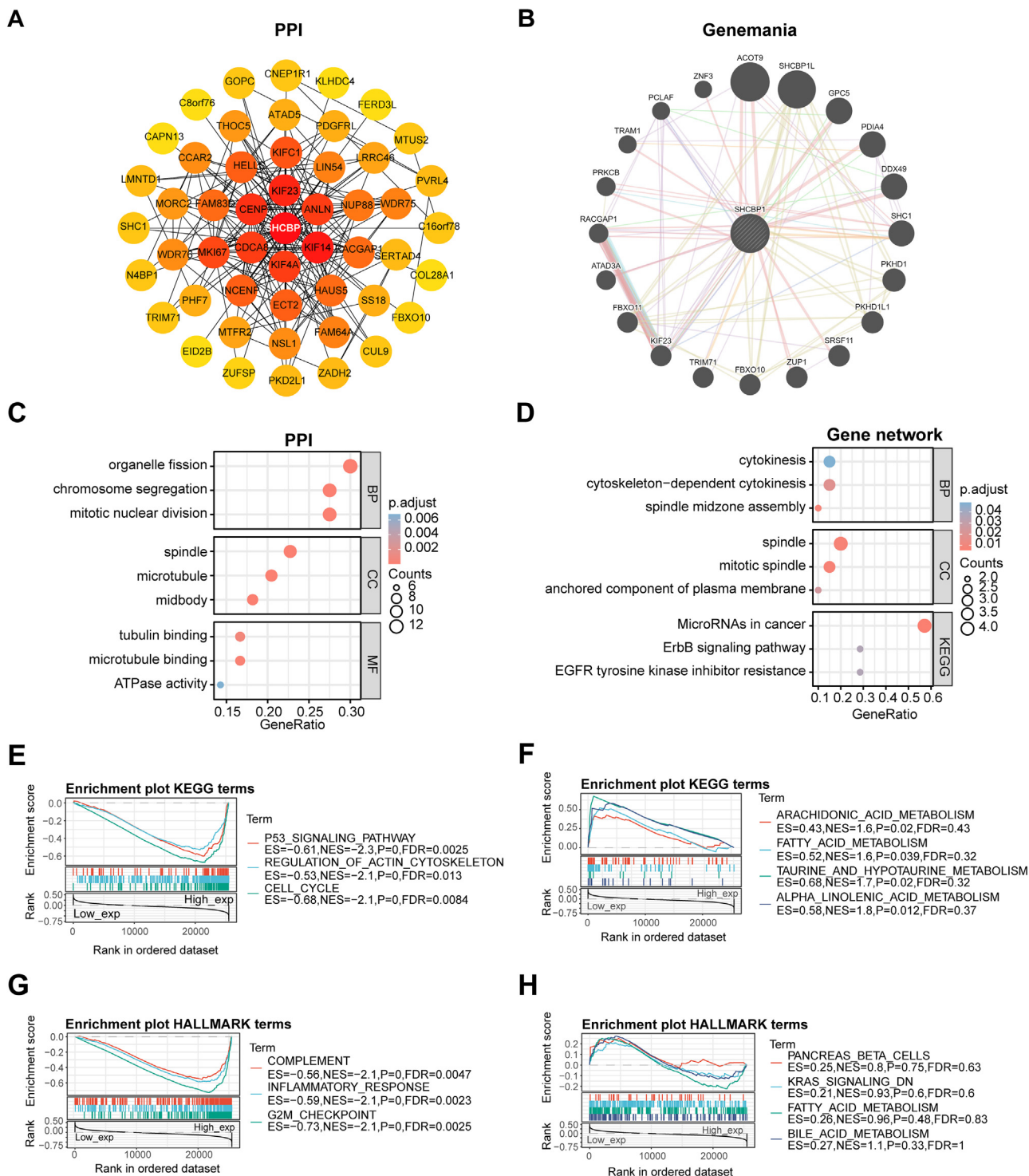


Fig. 8. Network and enrichment analyses. (A) Construction of PPI network involved in 50 SHCBP1-interacting proteins based on STRING. Each node is present as a different depth of color according to its degree score representing the extent of interaction of each node. (B) Construction of gene-gene network based on Genemania. The functional enrichment analysis in (C) PPI and (D) genes interaction network. GSEA analysis of high and low SHCBP1 expression in cancer samples. The gene sets enrichment in KEGG from the samples with (E) high SHCBP1 expression and (F) low SHCBP1 expression. The gene sets enrichment in HALLMARK from the samples with (G) high SHCBP1 expression and (H) low SHCBP1 expression. Visualization of the most obvious pathways in KEGG and HALLMARK, one gene set describing by a certain line with unique color.

cells. Furthermore, previous studies have revealed that Treg cells also exert a crucial role in immunosuppressive microenvironments in tumors [23]. Immunosuppression modulated by TAM and MDSC is considered as an underlying mechanism of the treatment resistance to ICIs [24,25]. CAF, a critical component of the tumor

microenvironment, participates in the tumor development and progression, metastasis, and immune evasion [26,27].

On the one hand, our findings confirmed that there were negative correlation or no significant correlation between SHCBP1 expression and CD8 + T cells infiltration in various cancer types.

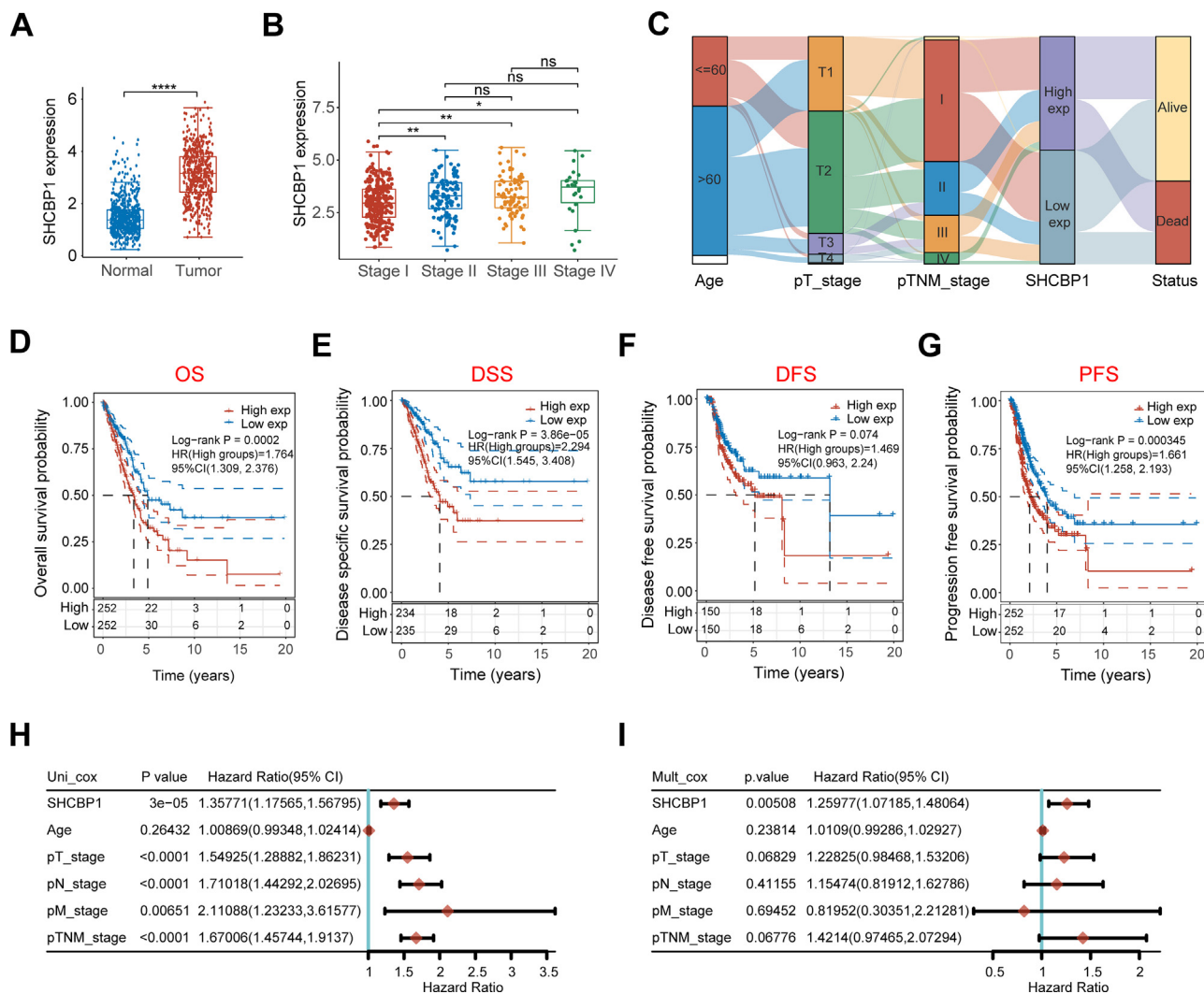


Fig. 9. Clinical landscape of SHCBP1 expression in lung adenocarcinoma (LUAD). (A) The expression level of SHCBP1 in tumor issues compared to the corresponding normal tissues. (**** $p < 0.0001$). (B) The expression level of SHCBP1 in distinct pathological stages. (* $p < 0.05$, ** $p < 0.01$, and ns, no significance). (C) Sankey diagram showing SHCBP1 Expression and clinicopathologic characteristics. Kaplan–Meier survival curves for (D) OS, (E) DSS, (F) DFS, and (G) PFS. The (H) univariate and (I) multivariate Cox regression analysis of SHCBP1 expression and clinical features. OS, overall survival; DSS, disease specific survival; DFS, disease free survival; PFS, progression free survival.

While CD8 + T cell tumor infiltration was one of key characteristics of effective cancer immunotherapy. Therefore, overexpression of SHCBP1 in several tumors might limit T cell-infiltration resulting in unsatisfied tumor-killing effects. On the other hand, SHCBP1 expression was positively correlated with the infiltration levels of M2-TAM, MDSC, CAF, and Treg, which contributed to the formation of an immunosuppressive TME. The immunosuppressive status in TME is a major barrier to successful immunotherapy. In addition, there was also a positive correlation between SHCBP1 expression and immune checkpoint genes in some cancers. Overexpression of SHCBP1 was accompanied by the upregulation of immune checkpoint molecules, which promoted these tumor cells to evade the immune surveillance. Furthermore, T cells killing effect could be blunted in tumors owing to up-regulation of immune checkpoints. In summary, these results provided supportive evidence that SHCBP1 expression was linked with immunosuppressive microenvironment in cancers.

We also found that SHCBP1 expression was highly associated with TMB, MSI, chemokines, chemokine receptors, and MHC genes in various cancer types. These results implied that SHCBP1 might exert a significant role in immune response of

tumor cells to the treatment of immunotherapy. Consequently, SHCBP1 could be further explored as a promising therapeutic target for tumor immunotherapy. Finally, we focused on investigating the clinical features and the potential biological role of SHCBP1 in LUAD. Through systematic analysis, this study reveals the important role of SHCBP1 in diagnosis, prognosis and tumor immune microenvironment across multiple cancer types. The prediction of the interacting genes may also provide new targets for cancer treatment. On the basis of our research, many studies revolving around SHCBP1 will be conducted in other cancers.

In conclusion, our study indicated that SHCBP1 had high diagnostic value, oncogenic characteristics predicting poor prognosis and a significant association with immunosuppressive TME, which suggested that it could be identified as an immune-related biomarker for cancer diagnosis and prognosis, and a potential therapeutic target for tumor immunotherapy.

Authors' contributions

Huang XY, Wang LX, and Shen ZF contributed to the conception of this work. Wang N and Zhu LY performed the experiments, data analysis and figure generation. Wang N wrote a draft and Huang

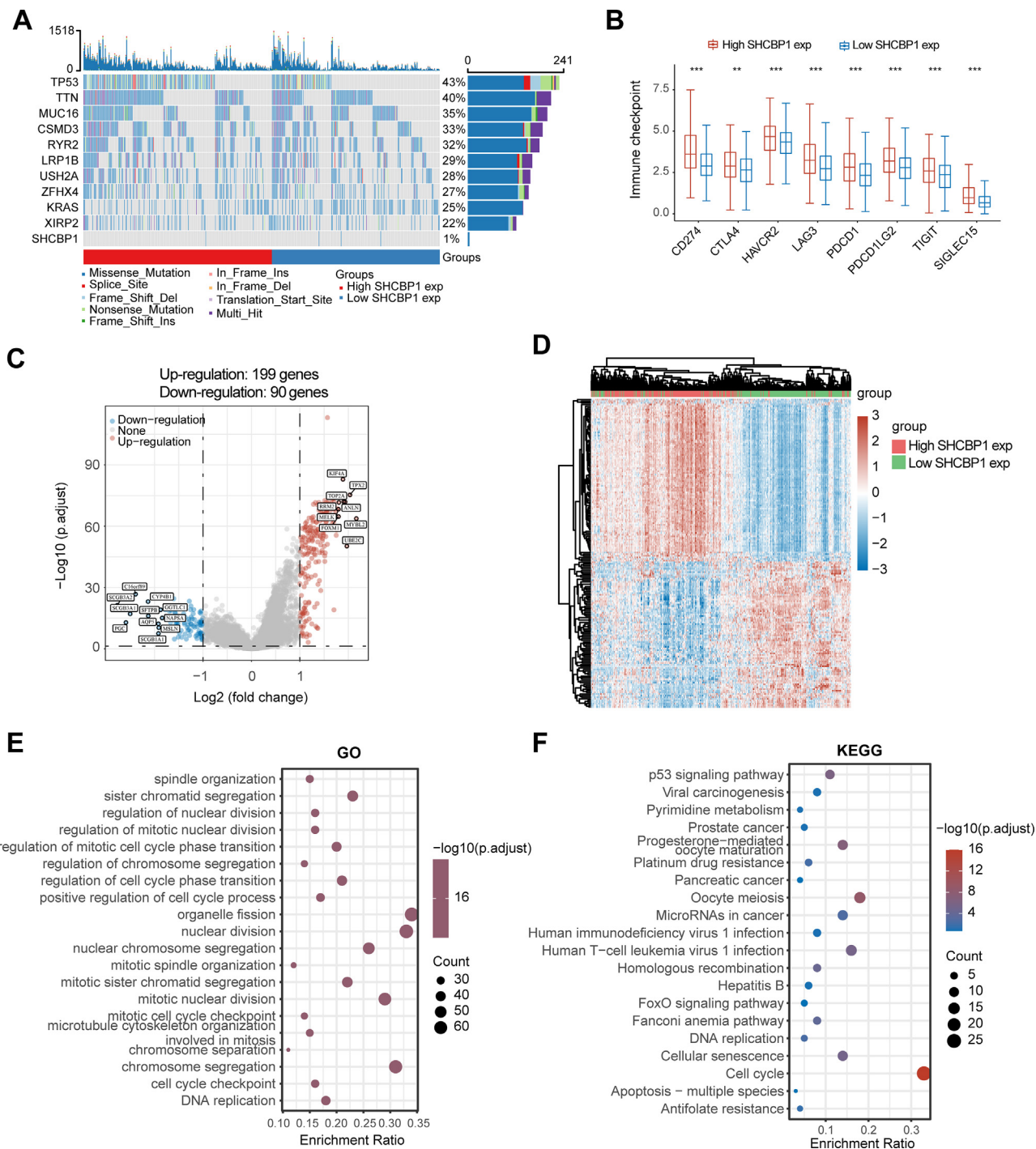


Fig. 10. Identification of the potential biological function of SHCBP1 expression in lung adenocarcinoma (LUAD). (A) The somatic mutation landscape of SHCBP1 in LUAD. Top 10 significantly different somatic mutation genes are displayed, respectively. (B) Correlation of SHCBP1 expression with immune checkpoint genes, including CD274, CTLA4, HAVCR2, LAG3, PDCD1, PDCD1LG2, SIGLEC15, and TIGIT. (** $p < 0.01$ and *** $p < 0.001$). (C) Volcano plot of DEGs (adjusted $p < 0.05$ and fold change > 2). 199 genes upregulated and 90 genes downregulated. (D) Expression heatmap of DEGs. The top 50 up-regulated genes and top 50 down-regulated genes were exhibited in this figure. (E) GO and (F) KEGG enrichment analysis of upregulated DEGs. DEGs, differentially expressed genes.

XY revised the manuscript. All authors approved the submitted version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.csbj.2022.06.039>.

References

[1] Zhang GY, Ma ZJ, Wang L, et al. The Role of Shcbp1 in Signaling and Disease. *Curr Cancer Drug Targets* 2019;19(11):854–62.

- [2] Liu L, Yang Y, Liu S, et al. EGF-induced nuclear localization of SHCBP1 activates β -catenin signaling and promotes cancer progression. *Oncogene* 2019;38(5):747–64.
- [3] Lu H, Yin M, Wang L, et al. FGF13 interaction with SHCBP1 activates AKT-GSK3 α/β signaling and promotes the proliferation of A549 cells. *Cancer Biol Ther* 2020;21(11):1014–24.
- [4] Zou A, Wu A, Luo M, Zhou C, Lu Y, Yu X. SHCBP1 promotes cisplatin induced apoptosis resistance, migration and invasion through activating Wnt pathway. *Life Sci* 2019;235:116798.
- [5] Shi W, Zhang G, Ma Z, et al. Hyperactivation of HER2-SHCBP1-PLK1 axis promotes tumor cell mitosis and impairs trastuzumab sensitivity to gastric cancer. *Nat Commun* 2021;12(1):2812.
- [6] Yang C, Hu JF, Zhan Q, et al. SHCBP1 interacting with EOGT enhances O-GlcNAcylation of NOTCH1 and promotes the development of pancreatic cancer. *Genomics* 2021;113(2):827–42.
- [7] Geng H, Guo M, Xu W, et al. SHCBP1 Promotes Papillary Thyroid Carcinoma Carcinogenesis and Progression Through Promoting Formation of Integrin and Collagen and Maintaining Cell Stemness. *Front Endocrinol (Lausanne)* 2020;11:613879.
- [8] Zhou Y, Tan Z, Chen K, et al. Overexpression of SHCBP1 promotes migration and invasion in gliomas by activating the NF- κ B signaling pathway. *Mol Carcinog* 2018;57(9):1181–90.
- [9] Ballhausen A, Przybilla MJ, Jendrusch M, et al. The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoediting during tumor evolution. *Nat Commun* 2020;11(1):4740.
- [10] Georgiadis A, Durham JN, Keefer LA, et al. Noninvasive Detection of Microsatellite Instability and High Tumor Mutation Burden in Cancer Patients Treated with PD-1 Blockade. *Clin Cancer Res* 2019;25(23):7024–34.
- [11] Reuben A, Zhang J, Chiou SH, et al. Comprehensive T cell repertoire characterization of non-small cell lung cancer. *Nat Commun* 2020;11(1):603.
- [12] Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012;2(5):401–4.
- [13] Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013;6(269):p11.
- [14] Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res.* 2010. 38(Web Server issue): W214–20.
- [15] Kumar R, Indrayan A. Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatr* 2011;48(4):277–87.
- [16] Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 2017;14(12):717–34.
- [17] Gao W, Qi CQ, Feng MG, Yang P, Liu L, Sun SH. SOX2-induced upregulation of lncRNA LINC01561 promotes non-small-cell lung carcinoma progression by sponging miR-760 to modulate SHCBP1 expression. *J Cell Physiol* 2020;235(10):6684–96.
- [18] Mo M, Tong S, Yin H, Jin Z, Zu X, Hu X. SHCBP1 regulates STAT3/c-Myc signaling activation to promote tumor progression in penile cancer. *Am J Cancer Res* 2020;10(10):3138–56.
- [19] Huang H, Cai H, Zhang L, Hua Z, Shi J, Wei Y. Oroxylin A inhibits carcinogen-induced skin tumorigenesis through inhibition of inflammation by regulating SHCBP1 in mice. *Int Immunopharmacol* 2020;80:106123.
- [20] Xu N, Wu YP, Yin HB, et al. SHCBP1 promotes tumor cell proliferation, migration, and invasion, and is associated with poor prostate cancer prognosis. *J Cancer Res Clin Oncol* 2020;146(8):1953–69.
- [21] Yang D, Denny SK, Greenside PG, et al. Intertumoral Heterogeneity in SCLC Is Influenced by the Cell Type of Origin. *Cancer Discov* 2018;8(10):1316–31.
- [22] Wang E, Lu SX, Pastore A, et al. Targeting an RNA-Binding Protein Network in Acute Myeloid Leukemia. *Cancer Cell* 2019;35(3):369–384.e7.
- [23] Hsu TS, Lin YL, Wang YA, et al. HIF-2 α is indispensable for regulatory T cell function. *Nat Commun* 2020;11(1):5005.
- [24] Takenaka MC, Gabriely G, Rothhammer V, et al. Control of tumor-associated macrophages and T cells in glioblastoma via AHR and CD39. *Nat Neurosci* 2019;22(5):729–40.
- [25] Zhang Y, Bush X, Yan B, Chen JA. Gemcitabine nanoparticles promote antitumor immunity against melanoma. *Biomaterials* 2019;189:48–59.
- [26] Duperret EK, Trautz A, Ammons D, et al. Alteration of the Tumor Stroma Using a Consensus DNA Vaccine Targeting Fibroblast Activation Protein (FAP) Synergizes with Antitumor Vaccine Therapy in Mice. *Clin Cancer Res* 2018;24(5):1190–201.
- [27] Katarkar A, Bottoni G, Clocchiatti A, et al. NOTCH1 gene amplification promotes expansion of Cancer Associated Fibroblast populations in human skin. *Nat Commun* 2020;11(1):5126.