World Journal of Surgical Oncology

RESEARCH

Open Access

Unveiling ficolins: diagnostic and prognostic biomarkers linked to the Tumor Microenvironment in Lung Cancer

Zeyu Zhang[1], Xueyan Geng[1], Maopeng Yin[1], Shoucai Zhang[1], Yingjie Liu[1], Dongmei Hu[3] and Guixi Zheng[1,2*]

Abstract

Background Ficolins (FCNs) are a family of proteins, comprising FCN1, FCN2 and FCN3, and integral to the immune system which have been implicated in the onset and progression of tumors. Despite their recognized roles, a comprehensive analysis of FCNs in lung cancer remains elusive.

Methods We employed a variety of bioinformatics tools, including UCSC, SangerBox, Ualcan, cBioPortal, String, Metascape, GeneMANIA, TIDE, CTD, and CAMP databases to investigate the differential expression, diagnostic and prognostic significance, genetic alterations, functional enrichment, immune infiltration, and potential immunotherapeutic implications of FCN1, FCN2, and FCN3 in lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD). Additionally, RT-qPCR and immunohistochemistry were utilized to validate the expressions of FCNs at the mRNA and protein levels in LUSC and LUAD.

Results Our comprehensive bioinformatic analysis, supported by RT-qPCR and immunohistochemistry, revealed that the expressions of FCN1, FCN2 and FCN3 were consistently downregulated in both LUSC and LUAD tumor tissues. FCNs demonstrated significant diagnostic potential for LUSC and LUAD, with the area under the receiver operating characteristic curve (AUC) for FCN1 and FCN3 exceeding 0.90. Furthermore, FCN2 and FCN3 showed a strong negative correlation with overall survival (OS) in LUSC, whereas FCN1 and FCN2 were positively correlated with OS in LUAD, suggesting their prognostic value in lung cancer. Gene enrichment analysis indicated that FCNs were predominantly associated with the complement system and complement activation pathways. Immune infiltration analysis further revealed a significant positive correlation between FCNs and the presence of neutrophils and resting mast cells. Our analysis of immunotherapy outcomes revealed a significant disparity in the immunophenoscore (IPS) among lung cancer patients treated with immune checkpoint inhibitors (ICIs), distinguishing those with high FCN expression from those with low FCN expression. Additionally, we identified small molecule compounds related to FCNs and drugs pertinent to LUSC and LUAD.

Conclusion FCNs held promise as diagnostic and prognostic biomarkers for LUSC and LUAD. This study also elucidated the relationship of FCNs with the tumor microenvironment, offering novel insights into the immunotherapeutic landscape for LUSC and LUAD.

*Correspondence:
Guixi Zheng
zhengg@sdu.edu.cn

Full list of author information is available at the end of the article

Keywords Ficolin, LUSC, LUAD, Diagnosis, Prognosis, Immune Infiltration, Immunotherapy

Introduction

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, with non -small cell lung cancer (NSCLC) being the most prevalent type, representing approximately 85% of all cases. Within NSCLC, lung squamous cell carcinoma (LUSC) and lung adenocarcinoma (LUAD) are the two primary pathological subtypes [1]. Traditional treatments, such as surgical resection and chemotherapy, have shown limited effectiveness, with the 5-year survival rate for patients with advanced or metastatic lung cancer remaining below 10% [2]. In recent years, immunotherapy and targeted therapy have emerged as prominent research areas with significant potential in the treatment of lung cancer [3].

The ficolins (FCNs) are a family of polymeric proteins characterized by an N-terminal collagen-like region and a C-terminal fibrinogen domain, comprising three members: FCN1, FCN2, and FCN3 [4]. FCN1 is secreted by neutrophils and monocytes in peripheral blood and alveoli, and is expressed in tissues such as bone marrow, spleen, and lung. It possesses the capability to bind to oligosaccharides on microbial surfaces, thereby facilitating the recognition of various pathogens [5]. FCN2 and FCN3 are proteins primarily produced by the liver and the alveoli [6]. FCN2 primarily found in the liver and adrenal glands, where it predominantly binds to N-acetylglucosamine and N-acetylgalactosamine. In contrast, FCN3 mainly expressed in the lungs and liver, where it binds to carbohydrate residues on microbial surfaces [7]. All three FCNs can activate the complement system via the lectin pathway. This activation culminates in the formation of the membrane attack complex (MAC), which disrupts the cell membranes of abnormal cells, leading to their destruction [8]. Furthermore, the complement fragments produced during activation can directly interact with immune cells, including macrophages, neutrophils, and dendritic cells. These interactions enhance the chemotaxis, phagocytic capacity, and antigen-presenting functions of these cells, thereby boosting the effectiveness of the adaptive immune response [9]. However, a comprehensive analysis of FCNs in LUSC and LUAD is still lacking.

Immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1) and its ligand (PD-L1) have demonstrated significant efficacy in patients with advanced or metastatic NSCLC, while challenges still persist in their effective application for early-stage disease [10]. Existing research indicates that combining immunotherapy with standard treatments, such as chemotherapy, may offer significant benefits for patients with resectable NSCLC, particularly those at early-stage disease or with high tumor mutation burden (TMB) [11]. TMB serves as a crucial biomarker for predicting the efficacy of immunotherapy and is linked to a more robust anti-tumor immune response [12]. Thus, investigating immunotherapy interventions in NSCLC patients and assessing their relationship with TMB can help refine treatment strategies and enhance patient outcomes.

This study conducted a comprehensive analysis of the diagnostic and prognostic significance of FCNs in LUSC and LUAD, examining their connections to the tumor microenvironment (TME) and their implications for immunotherapy, which provided valuable new insights into the diagnosis and treatment of patients with LUSC and LUAD.

Materials and methods

Differential expression analysis of FCNs in LUSC and LUAD

Three pan-cancer datasets, TCGA, TARGET, and GTEx (PANCAN, N=19,131, G=60,499), were downloaded from the UCSC database. The R package "Limma" was employed to analyze the differential expression of FCN1, FCN2 and FCN3 in LUSC and LUAD, respectively. The overall study design was illustrated in Fig. 1.

Real-time quantitative polymerase chain reaction (RT-qPCR)

A total of eight paired LUSC and adjacent non-cancerous tissues, along with 11 paired LUAD and adjacent non-cancerous tissues, were collected from the Department of Thoracic Surgery, Qilu Hospital of Shandong University. All patients provided informed consent and the study received approval from the Ethics Committee of Shandong University Qilu Hospital. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum at 37℃ in a humidified atmosphere containing 5% CO_2. The expression levels of FCNs were validated by RT-qPCR at both tissue and cellular levels.

Total RNA was extracted from tissues and cells using Trizol reagent. RNA purity and concentration were determined using a NanoDrop spectrophotometer. cDNA synthesis was subsequently performed using a reverse transcription kit (Yeasen). PCR amplification was carried out using Blaze Taq qPCR Mix, with human β-actin serving as the internal control. The primers were designed and synthesized by Platinum Shang Biotechnology, with sequences provided in Supplementary Table 1. The expressions of FCNs at the mRNA level were calculated using the $2^{-\Delta\Delta Ct}$ method. All experiments were conducted in triplicate.

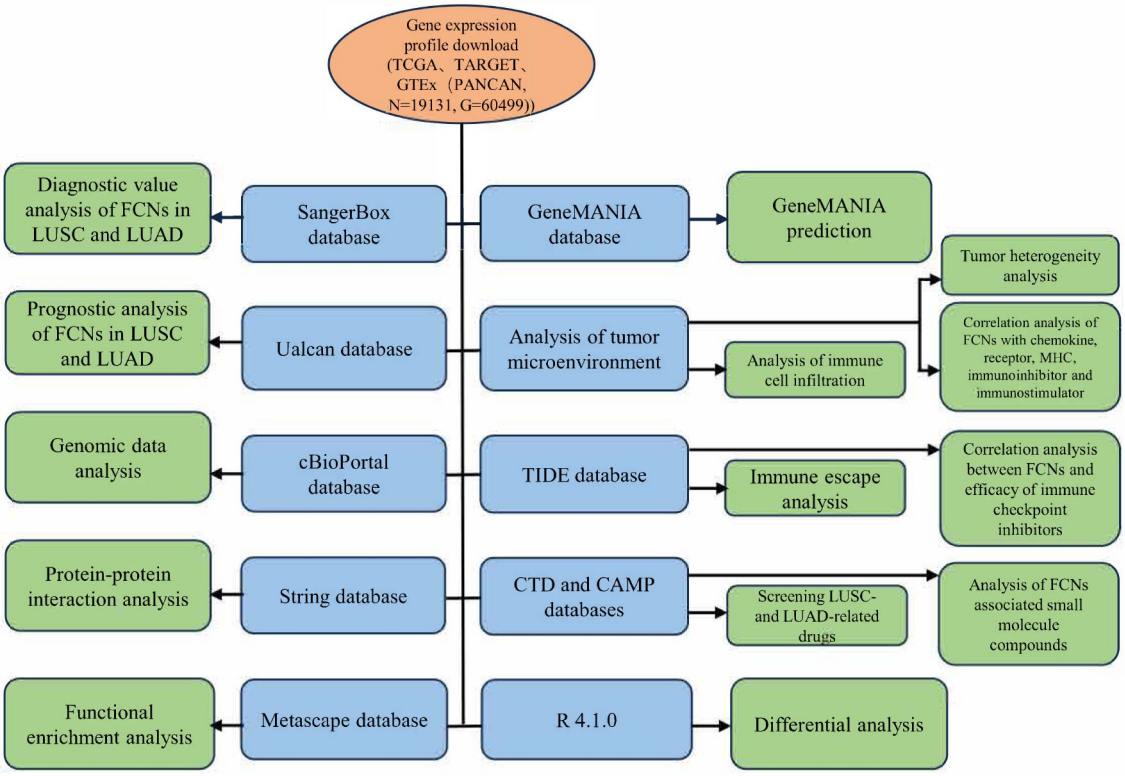

Fig. 1 Overall study flowchart

Immunohistochemistry (IHC)

Tissue samples were processed by fixing, embedding, dewaxing, and hydrating, followed by antigen retrieval using specific antigen repair methods. To prevent non-specific binding, a blocking agent was applied. Antibodies against FCN1, FCN2, and FCN3 were then added to bind to the respective target antigens, followed by the incubation with corresponding secondary antibodies. The immunoreactive bands were then visualized and photographed using a fluorescence microscope.

Diagnostic and prognostic analysis of FCNs

The diagnostic value of FCNs in LUSC and LUAD was assessed using the receiver operating characteristic (ROC) curve. Additionally, the correlation between FCN expression and patient survival was evaluated using survival curves generated from the Ualcan database. Furthermore, the R package "ComplexHeatmap" was utilized to analyze the clinical correlation between FCNs and various clinical characteristics, including stage, TNM stage, age, and gender.

Genomic data analysis

cBioPortal, a comprehensive online platform for exploring, visualizing, and analyzing large-scale cancer genomic data, was used to obtain genomic data for FCNs. These data facilitated the visual analysis of gene variation rates in LUSC and LUAD.

Analyses of protein-protein Interaction (PPI), Functional Enrichment, and GeneMANIA Prediction

The PPI network involving FCNs were visualized using data from the String database. Functional enrichment analysis of FCNs in LUSC and LUAD was conducted through the Metascape database. GeneMANIA, an online tool designed to analyze functional relationships, interactions and shared pathways among target genes and their associated genes, was employed to derive the functional enrichment of FCNs and their related genes.

Relationship of FCNs with the TME

To investigate the relationship between FCNs and the TME, three datasets, TCGA, TARGET, and GTEx, were downloaded from the UCSC database. These datasets were used to calculate immune scores for 22 types of immune cells in LUSC and LUAD samples using the R packages "e1071", "parallel", and "preprocessCore". Pearson's correlation coefficient between FCN expression and immune cell infiltration scores was determined using the R package "psych". Additionally, Spearman correlation analysis was performed to evaluate the relationship between FCNs and 150 marker genes across five immune

pathways (chemokine, receptor, MHC, immunoinhibitor, immunostimulator) in LUSC and LUAD.

The R package "estimation" was utilized to explore the correlation between FCN expression and StromalScore, ImmuneScore and ESTIMEScore. The simple nucleotide variation dataset for all TCGA samples was downloaded from the Genomic Data Commons (GDC) and used to calculate TMB for each tumor using the Maftools software. Additionally, information on microsatellite instability (MSI) scores, tumor purity (TP) values, and homologous recombination deficiency (HRD) was obtained from previous studies [13, 14]. Pearson correlation analyses were then performed to explore associations between FCN expression and TMB, MSI, TP, and HRD across LUSC and LUAD.

Correlation analysis of FCNs with Immune escape and efficacy of ICIs

The tumor immune dysfunction and exclusion (TIDE) score is a key indicator used to evaluate whether tumor cells evade immune surveillance during immunotherapy, thereby influencing the effectiveness of treatment. TIDE scores for LUSC and LUAD were obtained from the Tumor Immune Dysfunction and Exclusion database, and the relationship between FCNs and TIDE was visualized using the R package "ggpubr". Furthermore, immune data for LUSC and LUAD were downloaded from the Tumor ImmunoAtlas database to assess the impact of FCNs on the efficacy of ICI therapies.

Analysis of drug sensitivity and FCN-associated small-molecule compounds

Drugs with high sensitivity to FCN expression in LUSC and LUAD were identified using the R packages "pRRophetic" and "ggplot2". The correlation between the half maximal inhibitory concentration (IC50) of these drugs and FCN expression was then visualized. Additionally, the Comparative Toxicogenomics Database (CTD) was utilized to identify chemical substances that interacted with FCNs and their associated pathways.

Screening of LUSC- and LUAD-Related drugs

The Connectivity Map (CMAP) is a gene expression profile database developed by the Broad Institute, designed to identify functional associations of between small-molecule compounds, genes, and disease states. Differential gene expression data for LUSC and LUAD were uploaded to the CMAP database, using the "latest" version for analysis. In the results, compounds with negative scores were identified as potential therapeutic agents. Specifically, we screened compounds with Normcs > -1.6 as potential candidates for the treatment of LUSC and LUAD.

Statistical analysis

Statistical analyses in this study were conducted using R version 4.1.0 and GraphPad Prism version 8.0. Spearman or Pearson correlation analyses were performed to assess relationships between variables. Group differences were evaluated using the Unpaired Wilcoxon Rank Sum test, Signed Rank test, Mann-Whitney U test, Logrank test and Chi-square test. A P-value of <0.05 was considered statistically significant.

Results

Differential expression of FCNs in LUSC and LUAD

As shown in Fig. 2A, the expressions of FCNs at the mRNA level were significantly downregulated in LUSC tumors ($n=498$) compared to adjacent non-cancerous tissues ($n=397$). A similar trend was observed in LUAD tumors ($n=513$) compared to their corresponding non-cancerous tissues ($n=397$). This downregulation of FCN mRNA expression in LUSC and LUAD was further confirmed at both tissue and cellular levels by RT-qPCR, aligning with the results of the bioinformatics analysis (Fig. 2B-C). Additionally, the decreased protein expressions of FCNs in LUSC and LUAD were validated through IHC (Fig. 2D). These findings suggested that FCNs might function as tumor suppressors, playing a role in the development and progression of LUSC and LUAD.

Diagnostic value of FCNs in LUSC and LUAD

In LUSC, the area under the curve (AUC) for FCN1, FCN2, and FCN3 was 0.97 (95% CI: 0.96–0.98), 0.74 (95% CI: 0.71–0.77), and 0.99 (95% CI: 0.98-1.00), respectively (Fig. 3A). Similarly, in LUAD, the AUC for FCN1, FCN2 and FCN3 was 0.91 (95% CI: 0.89–0.93), 0.65 (95% CI: 0.61–0.68), and 0.99 (95%CI:0.98–0.99), respectively (Fig. 3B). These results indicated that FCN1 and FCN3 were highly effective diagnostic markers for lung cancer. Furthermore, principal component analysis (PCA) demonstrated that the fully convolutional networks established by FCNs could effectively distinguish between tumor tissues and adjacent non-cancerous tissues in both LUSC and LUAD. (Fig. 3C-D).

Potential prognostic value of FCNs in LUSC and LUAD

The prognostic potential of FCNs in LUSC and LUAD was assessed through survivorship curves, with the Ualcan database used to analyze their impact on overall survival (OS). The results revealed that FCN2 and FCN3 were significantly negatively correlated with OS in LUSC (Fig. 3E), whereas in LUAD, both FCN1 and FCN2 showed significant positive correlations with OS (Fig. 3F).

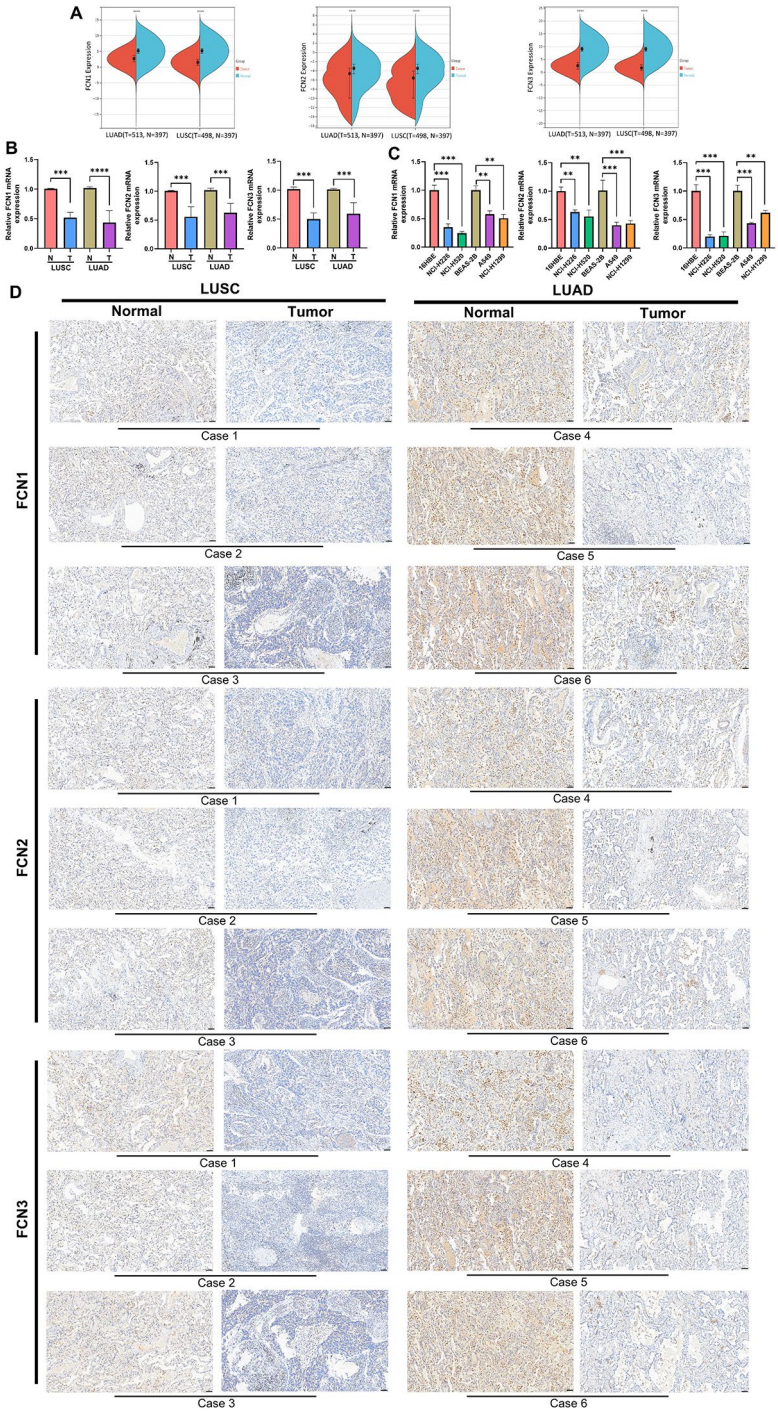

Fig. 2 The differential expression of FCNs in LUSC and LUAD. (**A**) Bioinformatics analysis of differential expression of FCNs. Unpaired Wilcoxon Rank Sum and Signed Rank Tests was used to compare the difference between two groups. (**B**) The down-regulation of FCN mRNA expression in LUSC and LUAD tissues was analyzed by Mann-Whitney U test. (**C**) The down-regulation of FCN mRNA expression at the cellular level was analyzed by Mann-Whitney U test. (**D**) The down-regulation of FCN protein levels was verified by IHC in LUSC and LUAD tumor tissues and paired non-tumor tissues using Mann-Whitney U test. **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$

Clinical correlation analysis of FCNs with LUSC and LUAD patients

The results demonstrated a significant association between FCN1 expression and gender in LUSC. FCN3 was significantly correlated with both stage and gender, while FCN2 showed no significant correlation with clinical parameters in LUSC patients (Supplementary Fig. 1A-C). In LUAD, only FCN3 was correlated with age and T

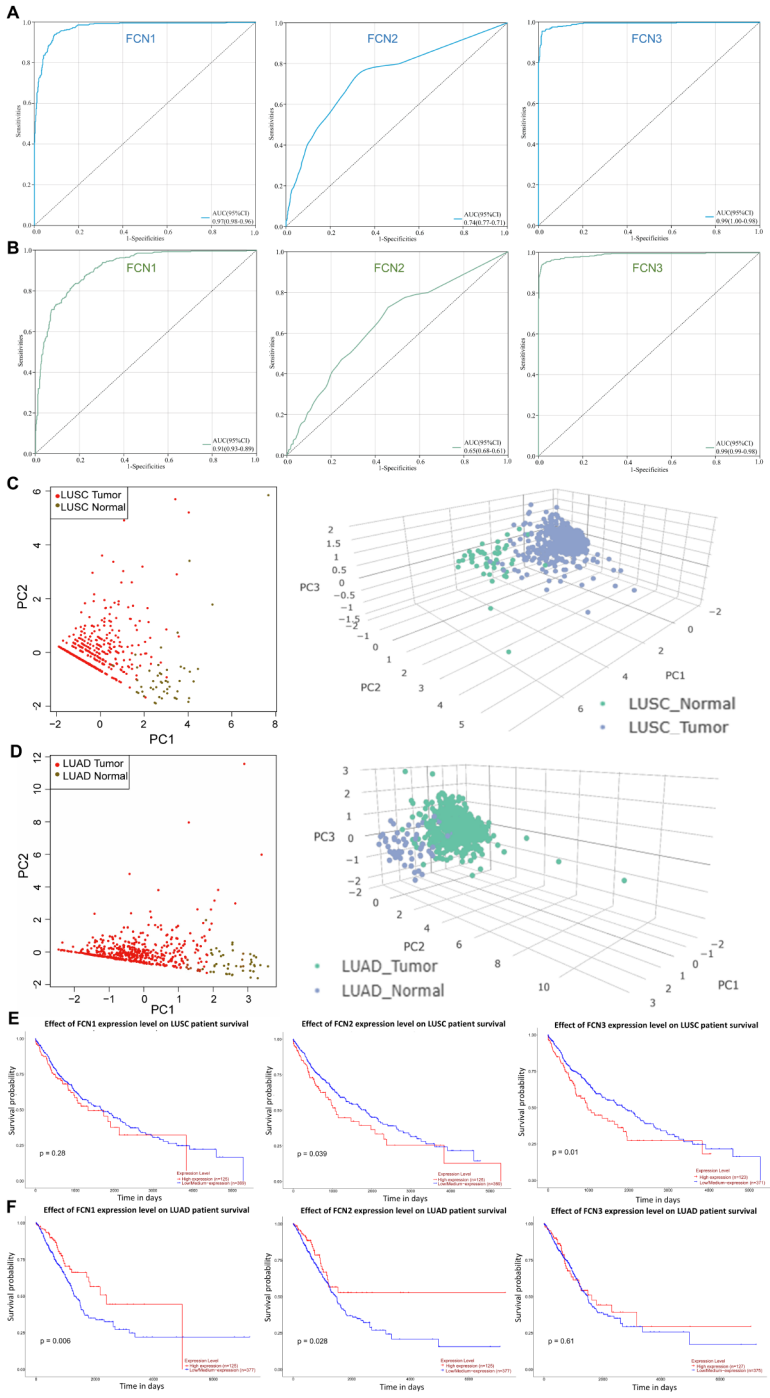

Fig. 3 Diagnostic and prognostic value of FCNs in patients with LUSC and LUAD. (**A**) ROC curves of FCNs in LUSC patients. (**B**) ROC curves of FCNs in LUAD patients. (**C**) PCA of FCNs in LUSC patients. (**D**) PCA of FCNs in LUAD patients. (**E**, **F**) The survivorship curves of FCNs in LUSC and LUAD patients from the Ualcan database were evaluated for significant prognostic differences between the two groups using the Logrank test. $P < 0.05$ was considered statistically significant

stage, whereas FCN1 and FCN2 did not show significant clinical correlations (Supplementary Fig. 1D-F).

Supplementary Fig. 1(A-F) Clinical correlation of FCNs in LUSC and LUAD patients were analyzed by Spearman test. **: $P < 0.01$; ***: $P < 0.001$.

Genetic mutations, molecular interactions, and potential functions of FCNs

In LUSC, mutations in FCNs were observed in 51 out of 1,176 patients (4.3%), with mutation rates for FCN1, FCN2, and FCN3 at 2%, 1.5% and 0.9%, respectively

(Fig. 4A). Similarly, in LUAD, 60 out of 1,382 patients (4.3%) exhibited FCN mutations, with mutation rates of 1.4% for FCN1, 1.9% for FCN2, and 1.3% for FCN3 (Fig. 4B). The mutation landscapes of the top 15 genes with the most significant differences in mutation frequencies between high- and low-FCN expression groups were separately mapped within the LUSC and LUAD cohorts (Supplementary Fig. 2).

A PPI network was constructed using data from the String database to explore potential interactions

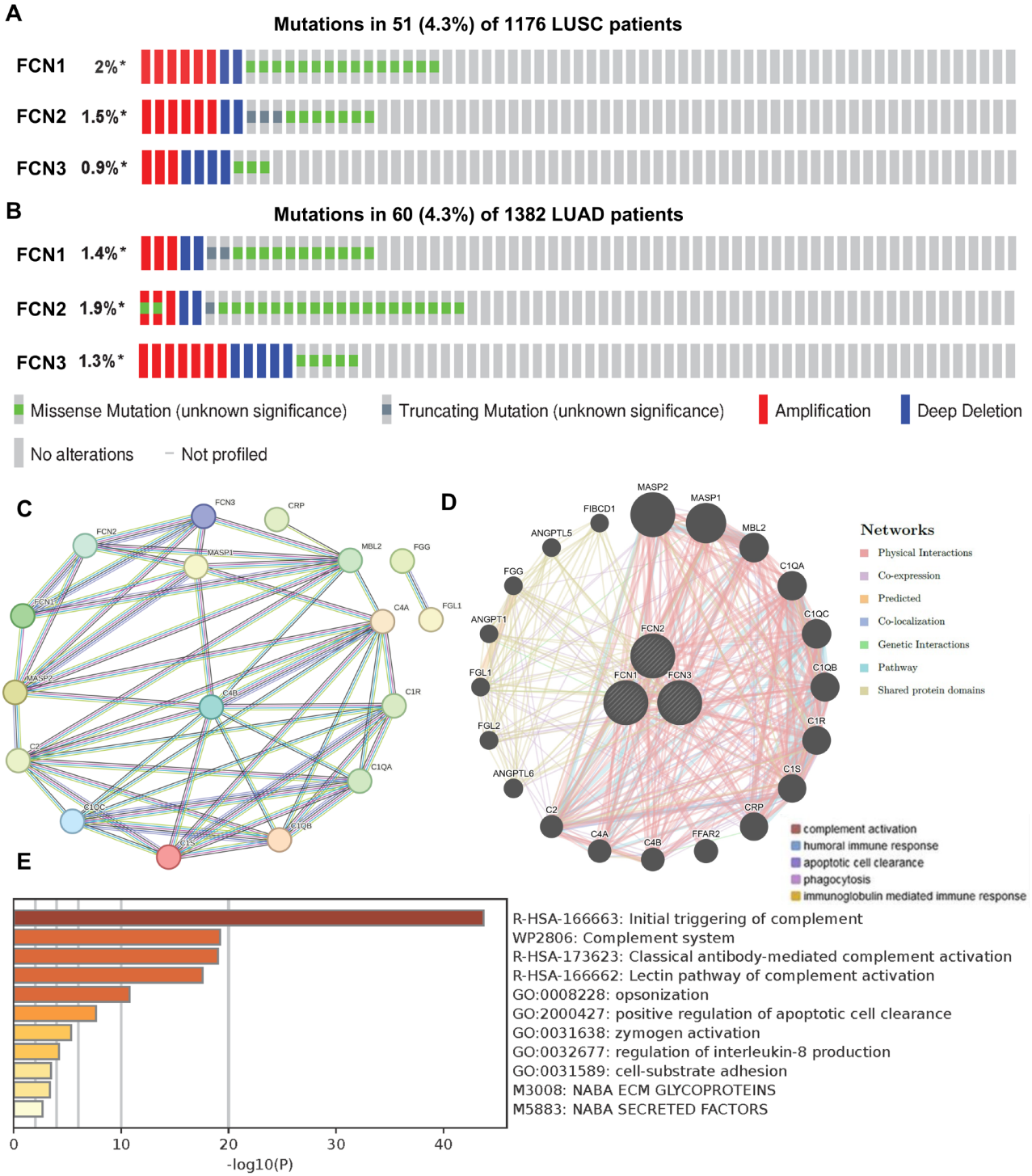

Fig. 4 Mutation, PPI network and functional enrichment analyses of FCNs. (**A**, **B**) Mutation of FCNs in LUSC patients and LUAD patients. (**C**, **D**) PPI network of FCNs. (**E**) GO and KEGG analysis of FCNs as well as their related genes

involving FCNs (Fig. 4C). Additionally, FCNs and their related genes were identified through the GeneMANIA database (Fig. 4D). Metascape analysis indicated that FCNs and their associated genes were primarily involved in the initial activation of the complement system, classical antibody-mediated complement activation, the lectin pathway of complement activation, and opsonization (Fig. 4E).

Supplementary Fig. 2 The top 15 genes with the most significant differences in mutation frequencies between the high- and low-FCN expression groups in the LUSC and LUAD cohorts were screened, and Chi-square test was used to evaluate the significance of the differences. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ****: $P<0.0001$.

Relationship of FCNs with the TME in LUSC and LUAD patients

In LUSC, the expression levels of FCN1, FCN2, and FCN3 were significantly associated with the infiltration levels of 12, seven, and eight types of immune cells, respectively (Fig. 5A-F). Similarly, in LUAD, the expressions of FCN1, FCN2, and FCN3 were significantly correlated with the infiltration of 11, four and 13 types of immune cells, respectively (Supplementary Fig. 3A-F). Notably, there was a significant positive correlation between the expression levels of FCNs and the infiltration of neutrophils and mast cells in both LUSC and LUAD. Additionally, FCNs were found to be associated with the majority of chemokines, receptors, MHC molecules, immunoinhibitors, and immunostimulators (Supplementary Fig. 4).

Furthermore, the analysis of three major TME scores, StromalScore, ImmuneScore, and ESTIMEScore, revealed that FCN expression was significantly positively correlated with all three scores in both LUSC and LUAD. These scores were higher in the high-FCN expression group compared to the low-expression group (Supplementary Fig. 5A-F).

Supplementary Fig. 4 The correlation between FCNs and marker genes of chemokine, receptor, MHC, immunoinhibitor, and immunostimulator in LUSC and LUAD was evaluated using Pearman test. *: $P<0.05$.

Supplementary Fig. 5 The correlation between FCN expression in LUSC and LUAD and StromallScore, ImmuneScore, and ESTIMEScore was analyzed by Pearman test. $P<0.05$ was considered statistically significant. (A, B) StromallScore; (C, D) ImmuneScore; (E, F) ESTIMEScore.

Association of FCNs with TMB, MSI, TP, and HRD in LUSC and LUAD

Analyzing the relationship between FCN expression and TMB, MSI, TP and HRD in LUSC and LUAD revealed a significant correlation between FCN3 expression and TMB in both LUSC and LUAD (Fig. 6A). Additionally, FCN expression was significantly associated with MSI and TP in LUSC, as well as TP and HRD in LUAD (Fig. 6B-D). These findings suggested that FCNs, particularly FCN3, held potential as targets for immunotherapy in LUSC and LUAD.

FCNs and Immune escape

To elucidate the relationship between FCNs and the efficacy of immunotherapy efficacy, the correlation between FCN expression and TIDE scores was analyzed. The results demonstrated a significant positive correlation between FCN expression and TIDE scores in LUSC, suggesting that the risk of immune escape was higher in the high-FCN expression group compared to the low-expression group (Fig. 7A). In contrast, a significant negative correlation between FCN expression and TIDE scores was observed in LUAD (Fig. 7B).

The correlation between the immunophenoscore (IPS) of two ICIs, cytotoxic T-lymphocyte-associated antigen 4 inhibitor (anti-CTLA-4) and anti-PD-1, and FCN expression was also examined. In both LUSC and LUAD, the group with high FCN3 expression exhibited a higher IPS for anti-CTLA-4, anti-PD-1 therapy, and combination therapy compared to the group with low FCN3 expression. Similarly, increased FCN1 expression was associated with enhanced IPS for both anti-PD-1 therapy and combination therapy (Fig. 8A, C, D, F). However, in LUAD, no significant correlation was found between FCN2 expression levels and IPS for anti-CTLA-4, anti-PD-1, or combination therapy. In LUSC, by contrast, FCN2 expression was significantly associated with IPS for anti-PD-1 and combination therapies (Fig. 8B, E).

Analysis of drug sensitivity, FCN-Associated Small-Molecule compounds, and LUSC- and LUAD-related drugs

The R package "pRRophetic" was used to analyze the top five drugs exhibiting high sensitivity to FCN expression. In LUSC, FCN1 showed a significant negative correlation with 5-Fluorouracil, Doxorubicin, Epothilone B, Erlotinib, and Etoposide, indicating that lower FCN1 expression was associated with increased sensitivity to these drugs. Similarly, FCN2 demonstrated a significant negative correlation with Bleomycin, Dasatinib, Doxorubicin, Tipifarnib and Etoposide. FCN3 also exhibited a significant negative correlation with Doxorubicin, Paclitaxel, Etoposide, Vinblastine and Gefitinib (Supplementary Fig. 6A-C).

In LUAD, higher expression of FCN1 was significantly positively correlated with Dasatinib, FMK, Rapamycin, Saracatinib and Sunitinib leading to increased drug sensitivity. Likewise, FCN2 expression was significantly positively correlated with Zibotentan B, Dasatinib, DMOG, FMK and Sunitnbin. In contrast, FCN3 expression showed a significant negative correlation with Axitinib,

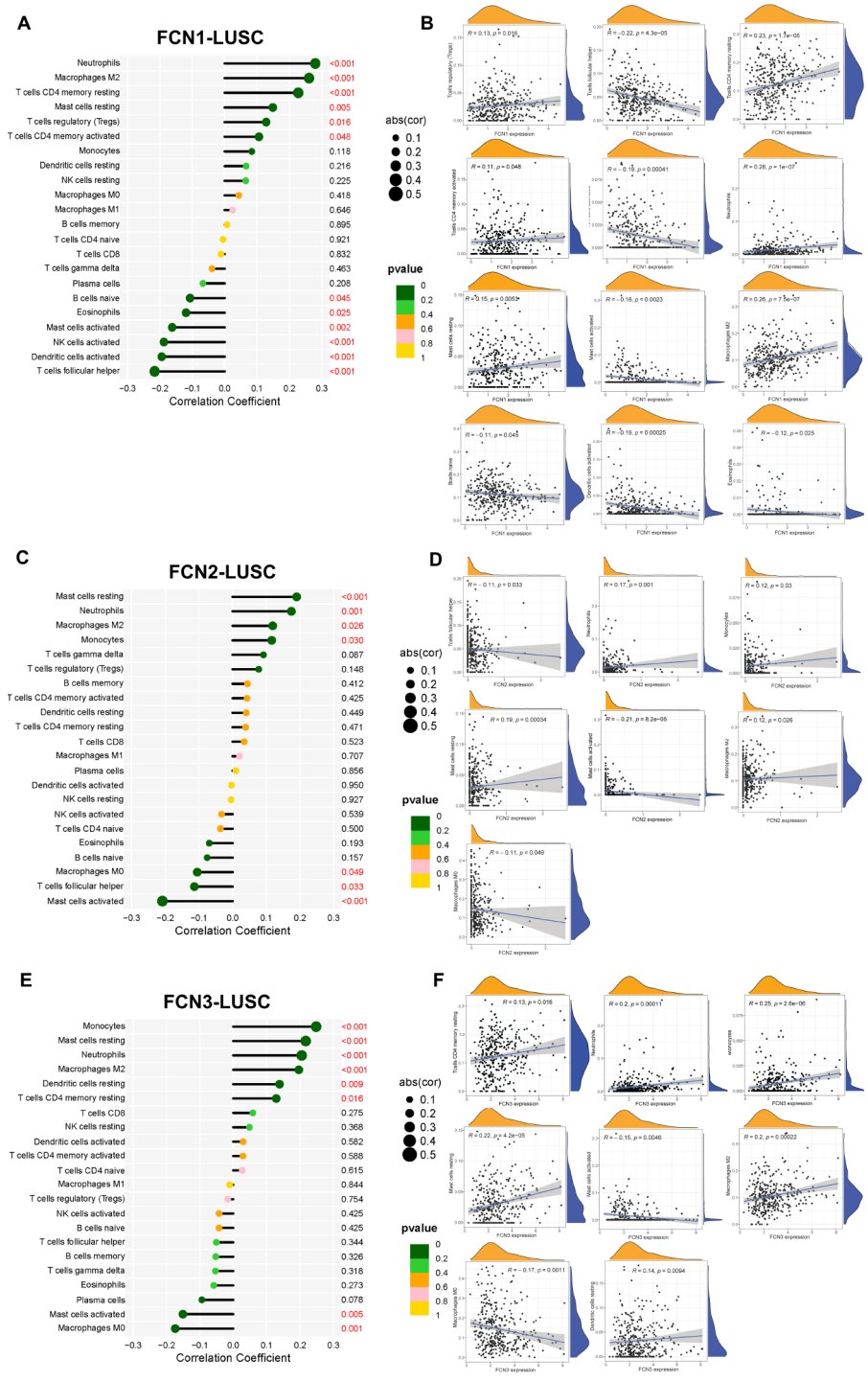

Fig. 5 Immune cell infiltration of FCNs in LUSC, and the correlation between FCNs and 22 kinds of immune cells was analyzed by Spearman test. $P < 0.05$ was considered statistically significant. (**A**, **B**) FCN1; (**C**, **D**) FCN2; (**E**, **F**) FCN3

Cisplatin, Paclitaxel, JNK Inhibitor VIII, and Ispinesib Mesylate (Supplementary Fig. 6D-F).

The CTD analysis revealed that Methotrexate, Acetaminophen and Teratogens down-regulated the expressions of FCN1, FCN2, and FCN3 at the mRNA level, respectively. Conversely, Progesterone, Benzo(a)pyrene, and Clothianidin were found to up-regulate the expressions of FCN1, FCN2, and FCN3 at the mRNA level, respectively. Additionally, Trichloroethylene, Valproic Acid and Aflatoxin B1 were identified as compounds that affect the methylation level of FCNs (Table 1).

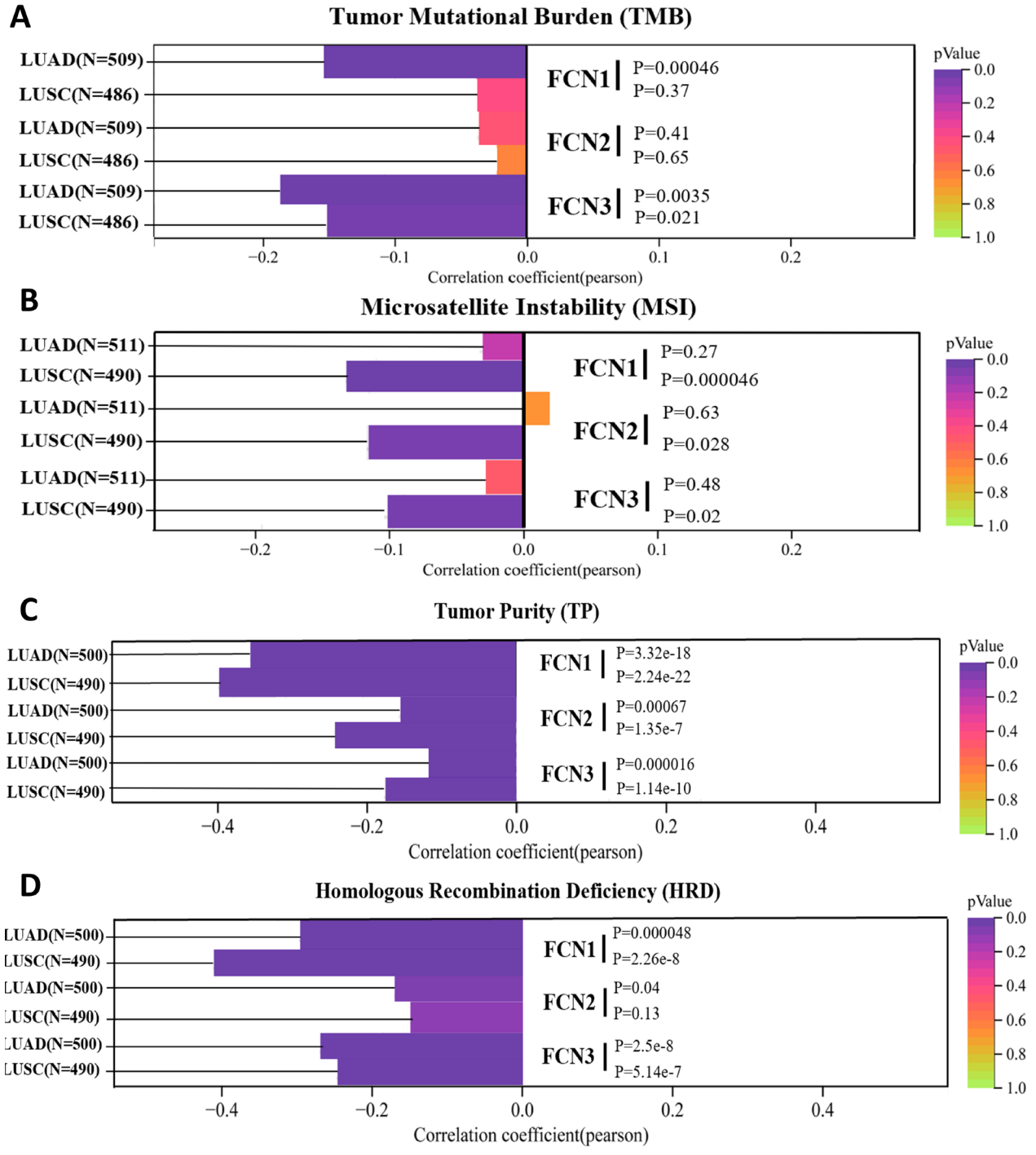

Fig. 6 Tumor heterogeneity analysis of FCNs in LUSC and LUAD, and the correlation between FCN expression and TMB, MSI, TP, and HRD was evaluated by Pearman test. $P < 0.05$ was considered statistically significant. (**A**) TMB; (**B**) MSI; (**C**) TP; (**D**) HRD

Using the CMAP database, a total of 19 small-molecule drugs were found to be highly correlated with LUSC, and 28 were highly correlated with LUAD. The top 10 drugs for each cancer type are listed in Tables 2 and 3, respectively. Notably, bisoprolol, BTS-54,505, and enalapril showed significant negative correlations with LUSC, while meglitinide, cefepime and etodolac exhibited significant negative correlations with LUAD, suggesting their potential therapeutic value.

Supplementary Fig. 6 Drug sensitivity analysis of FCNs in LUSC and LUAD. Significant differences between the two groups were assessed by the Mann-Whitney test.

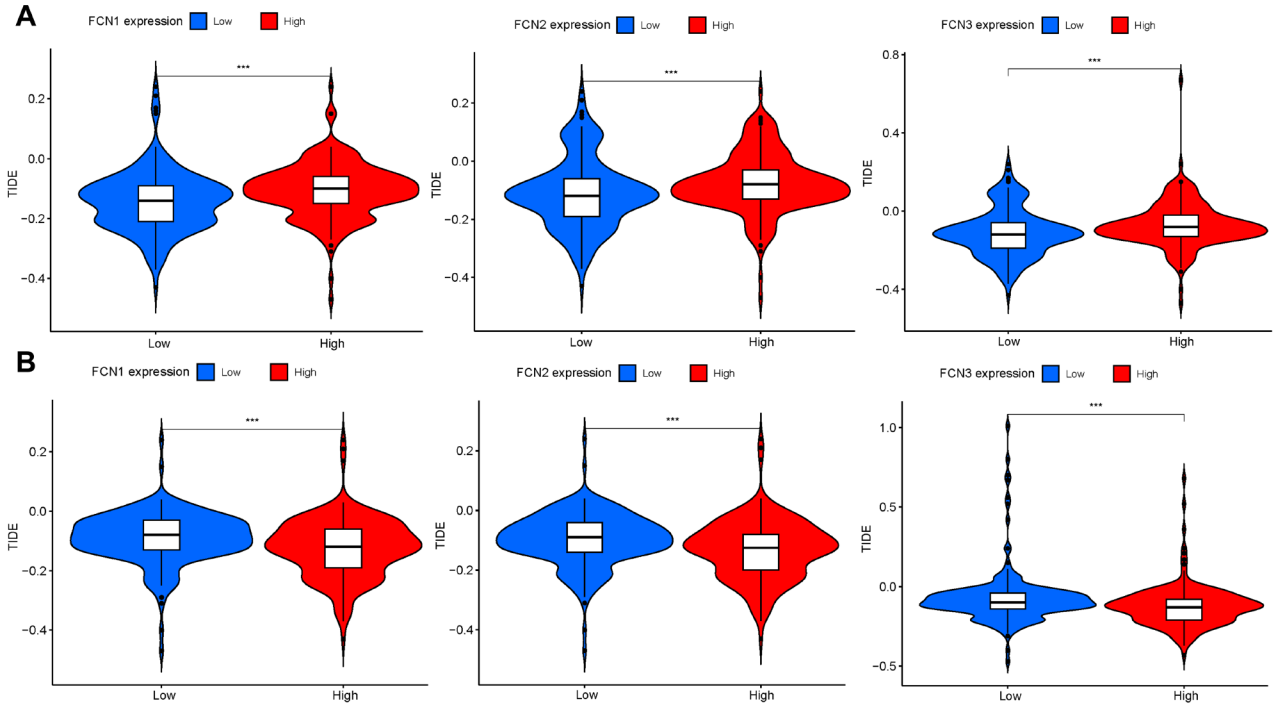

Fig. 7 Immune escape assay. Wilcoxon test was used to compare the difference between two groups. (**A**) LUSC; (**B**) LUAD. ***: $P < 0.001$

$P<0.05$ was considered statistically significant. (A-C) Drugs in LUSC that were sensitive to FCNs. (D-F) Drugs in LUAD that were sensitive to FCNs.

Discussion

In recent years, the role of FCNs in the development of various tumors has gained increasing attention. For example, FCN2 has been shown to significantly inhibit the migration, invasion, and epithelial-mesenchymal transition (EMT) of hepatocellular carcinoma (HCC) cells both in *vitro* and in *vivo* [15]. In addition, FCN3 expression is downregulated in HCC tissues, and its overexpression can induce apoptosis in HCC cells and hinder tumor progression by activating the p53 signaling pathway [16]. Elevated serum concentrations of FCN2 and FCN3 have also been observed in patients with ovarian cancer (OC) compared to normal subjects, suggesting their potential as tumor markers for OC [17]. Furthermore, research by Sokolowska et al. has highlighted the significant potential of FCNs to distinguish between non-malignant control patients and those with acute myeloid leukemia (AML), positioning FCNs as promising complementary biomarkers for AML [18]. Despite these findings, a comprehensive analysis of FCN1, FCN2, and FCN3 in lung cancer has yet to be conducted.

The study highlighted that, given the complexity of the TME and the influence of various genes and pathways, certain genes may exhibit a "dual role" in both inhibiting and promoting cancer progression [19, 20]. For instance, transforming growth factor β (TGF-β) can suppress cancer by restricting cell proliferation and inducing apoptosis in normal tissues; however, in advanced tumor stages, TGF-β may facilitate tumor invasion and metastasis [21]. Similarly, β-catenin plays a crucial role in cell adhesion and maintaining tissue integrity through the Wnt signaling pathway. Yet, when β-catenin accumulates in the nucleus, it can trigger oncogene expression, contributing to tumorigenesis [22]. In this study, the prognostic analysis revealed that low expression levels of FCN2 and FCN3 in LUSC were associated with improved patient survival, whereas higher expression of FCN1 and FCN2 in LUAD correlated with better survival outcomes. This suggested that FCN2 and FCN3 might play a "dual role" in lung cancer, underscoring the potential prognostic significance of FCNs in both LUSC and LUAD. Additionally, our findings showed that FCN1, FCN2, and FCN3 were significantly downregulated in tumor tissues of both LUSC and LUAD. FCNs demonstrated strong diagnostic capabilities, effectively distinguishing between normal lung tissue and tumor tissue, indicating their potential as diagnostic biomarkers.

Functional enrichment analysis of FCNs and their related genes further revealed that FCNs primarily exerted their effects through the activation of the complement system. Moreover, results from the GeneMANIA database indicated a strong correlation between FCNs and MASP2, aligning with previous findings. As key recognition molecules in the lectin pathway, FCNs

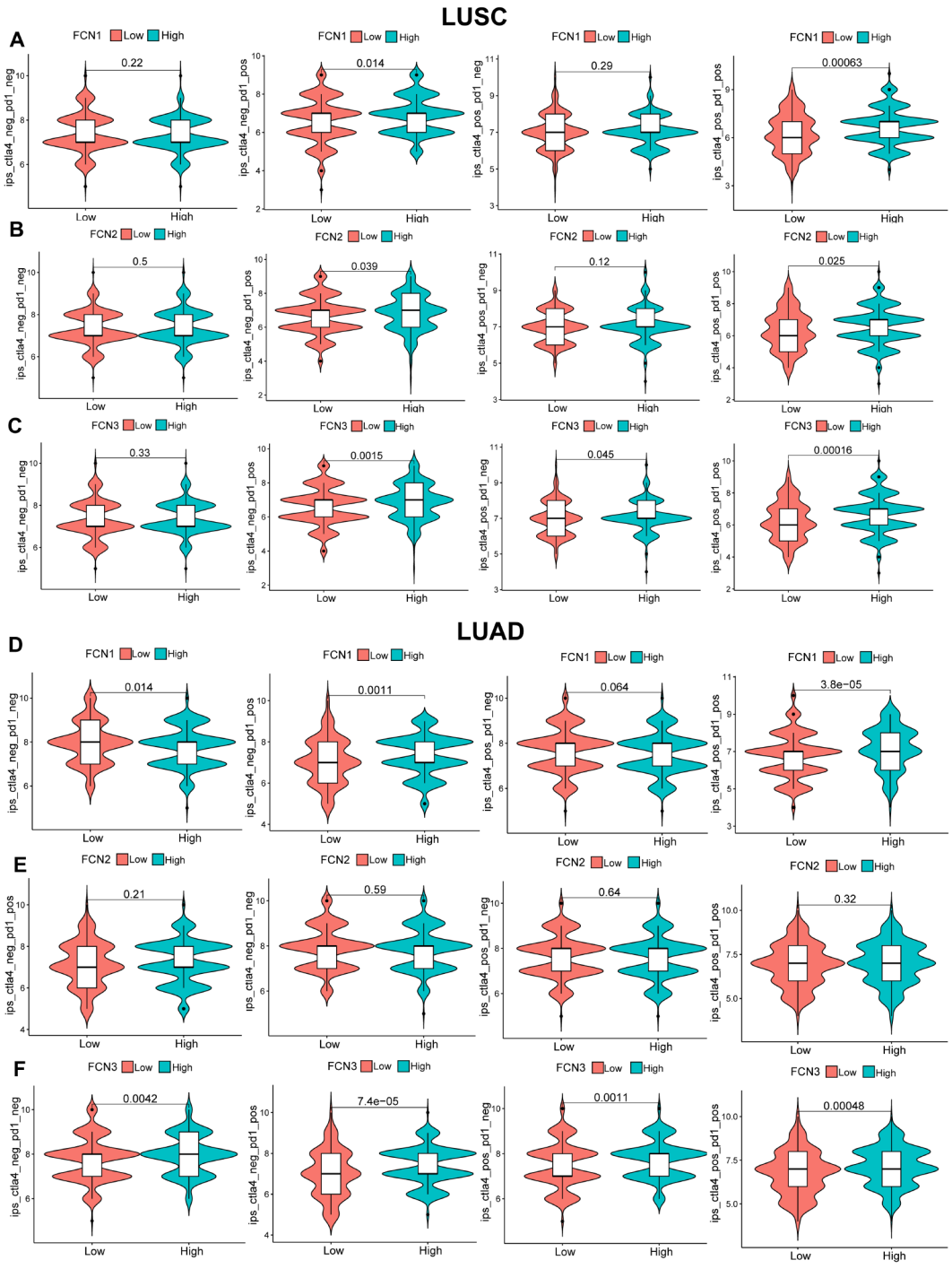

Fig. 8 Relationship between FCN expression and anti-CTLA4 as well as anti-PD-1 in LUSC and LUAD. Wilcoxon test was used to compare the differences between the two groups. $P < 0.05$ was considered statistically significant. (**A**-**C**) LUSC. (**D**-**F**) LUAD

recruit and activate mannose-binding lectin-associated serine proteases (MASPs), particularly MASP2 [23], by binding to specific carbohydrate structures on the surface of pathogens or apoptotic cells. This activation of MASPs subsequently triggers the complement cascade, including the cleavage of complement protein C3 and the formation of the C5b-9 MAC, leading to localized inflammation and the lysis of abnormal cells [24, 25].

Interestingly, our findings revealed a significant positive correlation between FCN expression and the presence of neutrophils and resting mast cells in both LUSC and LUAD. In lung cancer, Horvath et al. [26] have

Table 1 The small molecule compounds interacted with FCNs

Genes	Interacting chemical	Interaction
FCN1	Methotrexate	Decreased expression of FCN1 mRNA
	Progesterone	Increased expression of FCN1 mRNA
	Trichloroethylene	Increased methylation of FCN1 gene
FCN2	Acetaminophen	Decreased expression of FCN2 mRNA
	Benzo(a)pyrene	Increased expression of FCN2 mRNA
	Valproic Acid	Increased methylation of FCN2 promoter
FCN3	Teratogens	Decreased expression of FCN3 mRNA
	Clothianidin	Increased expression of FCN3 mRNA
	Aflatoxin B1	Decreased methylation of FCN3 gene

Table 2 Drugs with high correlation to LUSC

Pert_iname	Moa	Norm_cs
GSK-461,364	PLK inhibitor	-1.64
JNJ-26,481,585	HDAC inhibitor	-1.66
masitinib	KIT inhibitor|PDGFR inhibitor|Src inhibitor	-1.66
parthenolide	NFKB inhibitor	-1.66
proxymetacaine	Sodium channel inhibitor	-1.67
SCH-58,261	Adenosine receptor antagonist	-1.68
HLI-373	MDM inhibitor	-1.69
enalapril	ACE inhibitor	-1.7
BTS-54,505	Dopamine receptor antagonist	-1.78
bisoprolol	Adrenergic receptor antagonist	-1.81

Table 3 Drugs with high correlation to LUAD

Pert_iname	Moa	Norm_cs
SDM-25 N	Opioid receptor antagonist	-1.68
loperamide	Opioid receptor agonist	-1.68
CNX-774	BTK inhibitor	-1.68
haloperidol	Dopamine receptor antagonist	-1.72
piperine	Monoamine oxidase inhibitor	-1.73
CCMQ	Inhibitor of the binding of homoquinolinic acid	-1.76
crizotinib	ALK inhibitor	-1.78
etodolac	Cyclooxygenase inhibitor	-1.79
cefepime	Bacterial cell wall synthesis inhibitor	-1.79
meglitinide	Potassium channel antagonist	-1.87

observed that hyperoxia promotes the transformation of neutrophils into an anti-tumor phenotype, while hypoxic environment induces their shift towards a pro-tumor phenotype. Additionally, even in a resting state, mast cells can release various cytokines, including IL-1, IL-4, IL-6 and tumor necrosis factor-α (TNF-α), which can induce apoptosis in lung cancer cells by modulating the TME [27, 28]. These findings suggest that the abnormal expression of FCNs may be linked to the dysregulation of immune cell infiltration, indicating that FCNs can play an active role in the development of lung cancer.

The TIDE score serves as a comprehensive indicator that reflects the potential involvement of various immune escape mechanisms [29], while the IPS evaluates a tumor's potential response to immunotherapy, particularly ICIs [30]. CTLA-4 and PD-1 are pivotal immune checkpoint signals; PD-1, up-regulated in various tumor types, inhibits T-cell activity by binding to its ligand PD-L1 [31, 32]. Conversely, CTLA-4 suppresses T-cell activity through competitive binding to the co-stimulatory molecules CD80 and CD86 [33, 34]. Consequently, ICIs like anti-PD-1 or anti-CTLA-4 can enhance anti-tumor immunotherapy by blocking these inhibitory signals.

Nevertheless, tumors often develop resistance to such treatments through specific immune escape mechanisms. For example, IDO (Indoleamine 2,3-dioxygenase) metabolizes tryptophan into kynurenine, which suppresses T cell proliferation and fosters regulatory T cell (Treg) development, aiding tumor cells in evading immune attacks. Additionally, IDO can modulate the immune response within the TME and potentially enhance the immune system's attack on tumors when combined with other immunotherapies [35]. Similarly, Galectin-9 contributes to immune evasion by inhibiting T cell and natural killer (NK) cell functions via binding to the TIM-3 receptor. Yet, Galectin-9 can also activate certain immunoregulatory pathways or effector T cells, thereby improving the efficacy of ICIs [36]. These examples illustrate how the complexity of the TME leads to variability in the relationship between immune escape and immunotherapy outcomes.

In this study, FCNs exhibited a significant positive correlation with both TIDE and IPS in LUSC, implying that FCNs might have a dual role in both immune escape and immune activation. In contrast, for LUAD, the high FCN expression group demonstrated a lower TIDE score and a higher IPS compared to the low expression group. This suggested that FCNs might enhance the immunotherapy response in LUAD, reflecting their potential to modulate the immune landscape differently across lung cancer subtypes.

TMB is a crucial indicator closely associated with tumor development. Studies have shown that tumors with high TMB typically produce more neoantigens, which are more likely to be recognized by the immune system, thereby triggering immune responses [37]. However, in lung cancer, low TMB may be associated with a more inflammatory or immunologically active TME, characterized by the expression of a small number of highly immunogenic tumor-specific antigens, which can lead to favorable outcomes when ICIs are used alone or in combination [37, 38]. Additionally, tumors with low TMB may rely more heavily on specific immune escape mechanisms, such as evading immune surveillance through PD-L1 expression. In these cases, drugs targeting the PD-1/PD-L1 pathway may be more effective, making single or combination ICI therapy particularly beneficial [39–41]. This study's findings aligned

with these observations, as FCN expression in LUSC and LUAD was negatively correlated with TMB. Moreover, the high-FCN3 expression group exhibited higher IPS when treated with anti-CTLA-4 and anti-PD-1 therapies, either alone or in combination, compared to the low-expression group. These insights provided new perspectives and directions for lung cancer immunotherapy.

Despite the valuable insights gained from our study, there were several limitations. Notably, the LUSC and LUAD tissue samples used to verify FCN expression levels were limited in number, and we lacked experimental validation of the role of FCNs in these cancers. Moving forward, it will be essential to design comprehensive studies involving cellular models, animal experiments, and mechanistic analyses to further investigate the molecular biological functions and specific mechanisms of FCNs in LUSC and LUAD.

Conclusion

This study conducted a comprehensive analysis of FCNs in lung cancer, revealing their potential as both diagnostic and prognostic biomarkers. The findings underscored the significant role that FCNs might play in distinguishing between normal and tumor tissues, as well as in predicting patient outcomes in LUSC and LUAD. By shedding light on the expression patterns and functional implications of FCNs, this research offered valuable insights that could pave the way for more precise diagnostic tools and targeted therapeutic strategies in lung cancer. Our work not only advanced the understanding of FCNs in the context of lung cancer but also opened new avenues for exploring innovative approaches to the diagnosis, progression, and treatment of LUSC and LUAD.

Abbreviations

FCNs	Ficolins
LUSC	Lung squamous cell carcinoma
LUAD	Lung adenocarcinoma
RT-qPCR	Real-time quantitative polymerase chain reaction
AUC	Area under the receiver operating characteristic curve
OS	Overall survival
IPS	Immunophenoscore
ICIs	Immune checkpoint inhibitors
NSCLC	Non-small cell lung cancer
MAC，C5b-9	Membrane attack complex
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein ligand 1
TMB	Tumor mutation burden
TME	Tumor microenvironment
IHC	Immunohistochemistry
ROC	Receiver operating characteristic
GDC	Genomic Data Commons
MSI	Microsatellite instability
TP	Tumor purity
HRD	Homologous recombination deficiency
TIDE	Tumor immune dysfunction and exclusion
CTD	Comparative Toxicogenomics Database
CMAP	Connectivity Map
PCA	Principal component analysis
anti-CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4 inhibitor
EMT	Epithelial-mesenchymal transition
HCC	Hepatocellular carcinoma
OC	Ovarian cancer
AML	Acute myeloid leukemia
TGF-β	Transforming growth factor β
MASPs	Mannose-binding lectin-associated serine proteases
TNF-α	Tumor necrosis factor-α
IDO	Indoleamine 2,3-dioxygenase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-024-03558-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Acknowledgements

We thank members of the laboratory for comments and suggestions.

Author contributions

GXZ designed the research. ZYZ wrote the manuscript. XYG performed the experiments. MPY and SCZ plotted some figures. YJL analyzed the data. DMH acquisition of data. All authors reviewed and approved the submitted version.

Funding

This work was supported by grants from the National Natural Science Foundation of China (82172347), the Taishan scholar program of Shandong Province (tstp20221156) and the Natural Science Foundation of Shandong Province (ZR2020MH323).

Data availability

All data generated for this study are included in the article, further inquiries can be directed to the corresponding author.

Code availability

The code used in this study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This work was approved by the Ethics Committee of the Qilu Hospital of Shandong University. Human LUSC and LUAD tissues were obtained from patients undergoing surgery at the Department of Thoracic Surgery, Qilu Hospital of Shandong University. All LUSC and LUAD patients agreed to provide tissue specimens for this study. All subjects signed an informed consent form.

Consent for publication

All authors have given consent for publication.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Clinical Laboratory, Qilu Hospital of Shandong University, Jinan 250012, P.R. China
[2]Shandong Engineering Research Center of Biomarker and Artificial Intelligence Application, Jinan, China
[3]Cheeloo College of Medicine, Shandong University, Jinan, China

Received: 13 June 2024 / Accepted: 6 October 2024
Published online: 10 October 2024

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. Cancer J Clin. 2014;64(1):9–29.
2. Yang P. Epidemiology of lung cancer prognosis: quantity and quality of life. Methods Mol Biology (Clifton N J). 2009;471:469–86.
3. Vicidomini G. Current challenges and future advances in Lung Cancer: Genetics, Instrumental diagnosis and treatment. Cancers 2023, *15* (14).
4. Ichijo H, Hellman U, Wernstedt C, Gonez LJ, Claesson-Welsh L, Heldin CH, Miyazono K. Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen- and collagen-like domains. J Biol Chem. 1993;268(19):14505–13.
5. Liu Y, Endo Y, Iwaki D, Nakata M, Matsushita M, Wada I, Inoue K, Munakata M, Fujita T. Human M-Ficolin is a secretory protein that activates the lectin complement Pathway1. J Immunol. 2005;175(5):3150–6.
6. Matsushita M, Kuraya M, Hamasaki N, Tsujimura M, Shiraki H, Fujita T. Activation of the lectin complement pathway by H-ficolin (Hakata antigen). J Immunol (Baltimore Md : 1950). 2002;168(7):3502–6.
7. Liu Y, Endo Y, Iwaki D, Nakata M, Matsushita M, Wada I, Inoue K, Munakata M, Fujita T. Human M-ficolin is a secretory protein that activates the lectin complement pathway. J Immunol (Baltimore Md : 1950). 2005;175(5):3150–6.
8. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol. 2010;11(9):785–97.
9. Volanakis JE. The role of complement in innate and adaptive immunity. Curr Top Microbiol Immunol. 2002;266:41–56.
10. Pasqualotto E, Moraes FCA, Chavez MP, Souza MEC, Rodrigues A, Ferreira ROM, Lopes LM, Almeida AM, Fernandes MR, Santos N. PD-1/PD-L1 inhibitors plus Chemotherapy Versus Chemotherapy alone for Resectable Non-small Cell Lung Cancer: a systematic review and Meta-analysis of Randomized controlled trials. Cancers. 2023;15:21.
11. Yang X, Yin R, Xu L, Neoadjuvant. PD-1 blockade in Resectable Lung Cancer. N Engl J Med 2018, 379 (9), e14.
12. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, Minenza E, Linardou H, Burgers S, Salman P, Borghaei H, Ramalingam SS, Brahmer J, Reck M, O'Byrne KJ, Geese WJ, Green G, Chang H, Szustakowski J, Bhagavatheeswaran P, Healey D, Fu Y, Nathan F, Paz-Ares L. Nivolumab plus Ipilimumab in Lung Cancer with a high Tumor Mutational Burden. N Engl J Med. 2018;378(22):2093–104.
13. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen HZ, Reeser JW, Yu L, Roychowdhury S. Landscape of Microsatellite Instability Across 39 Cancer Types. *JCO precision oncology* 2017, *2017*.
14. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, Ziv E, Culhane AC, Paull EO, Sivakumar IKA, Gentles AJ, Malhotra R, Farshidfar F, Colaprico A, Parker JS, Mose LE, Vo NS, Liu J, Liu Y, Rader J, Dhankani V, Reynolds SM, Bowlby R, Califano A, Cherniack AD, Anastassiou D, Bedognetti D, Mokrab Y, Newman AM, Rao A, Chen K, Krasnitz A, Hu H, Malta TM, Noushmehr H, Pedamallu CS, Bullman S, Ojesina AI, Lamb A, Zhou W, Shen H, Choueiri TK, Weinstein JN, Guinney J, Saltz J, Holt RA, Rabkin CS, Lazar AJ, Serody JS, Demicco EG, Disis ML, Vincent, B. G.;, Shmulevich. I., The Immune Landscape of Cancer. *Immunity* 2018, *48* (4), 812–830.e14.
15. Yang G, Liang Y, Zheng T, Song R, Wang J, Shi H, Sun B, Xie C, Li Y, Han J, Pan S, Lan Y, Liu X, Zhu M, Wang Y, Liu L. FCN2 inhibits epithelial-mesenchymal transition-induced metastasis of hepatocellular carcinoma via TGF-β/Smad signaling. Cancer Lett. 2016;378(2):80–6.
16. Ma D, Liu P, Wen J, Gu Y, Yang Z, Lan J, Fan H, Liu Z, Guo D. FCN3 inhibits the progression of hepatocellular carcinoma by suppressing SBDS-mediated blockade of the p53 pathway. Int J Biol Sci. 2023;19(2):362–76.
17. Szala A, Sawicki S, Swierzko AS, Szemraj J, Sniadecki M, Michalski M, Kaluzynski A, Lukasiewicz J, Maciejewska A, Wydra D, Kilpatrick DC, Matsushita M, Cedzynski M. Ficolin-2 and ficolin-3 in women with malignant and benign ovarian tumours. Cancer Immunol Immunotherapy: CII. 2013;62(8):1411–9.
18. Sokołowska A, Świerzko AS, Gajek G, Gołos A, Michalski M, Nowicki M, Szala-Poździej A, Wolska-Washer A, Brzezińska O, Wierzbowska A, Jamroziak K, Kowalski ML, Thiel S, Matsushita M, Jensenius JC, Cedzyński M. Associations of ficolins and mannose-binding lectin with acute myeloid leukaemia in adults. Sci Rep. 2020;10(1):10561.
19. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. 2014;505(7484):495–501.
20. Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, Akhavanfard S, Cahill DP, Aldape KD, Betensky RA, Louis DN, Iafrate AJ. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. Cancer Cell. 2011;20(6):810–7.
21. Yingling JM, Blanchard KL, Sawyer JS. Development of TGF-beta signalling inhibitors for cancer therapy. Nat Rev Drug Discov. 2004;3(12):1011–22.
22. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127(3):469–80.
23. Héja D, Kocsis A, Dobó J, Szilágyi K, Szász R, Závodszky P, Pál G, Gál P. Revised mechanism of complement lectin-pathway activation revealing the role of serine protease MASP-1 as the exclusive activator of MASP-2. Proc Natl Acad Sci USA. 2012;109(26):10498–503.
24. Matsushita M, Endo Y, Hamasaki N, Fujita T. Activation of the lectin complement pathway by ficolins. Int Immunopharmacol. 2001;1(3):359–63.
25. Endo Y, Matsushita M, Fujita T. Role of ficolin in innate immunity and its molecular basis. Immunobiology. 2007;212(4–5):371–9.
26. Horvath L, Puschmann C, Scheiber A, Martowicz A, Sturm G, Trajanoski Z, Wolf D, Pircher A, Salcher S. Beyond binary: bridging neutrophil diversity to new therapeutic approaches in NSCLC. Trends cancer 2024.
27. Zhang P, Liu J, Pei S, Wu D, Xie J, Liu J, Li J. Mast cell marker gene signature: prognosis and immunotherapy response prediction in lung adenocarcinoma through integrated scRNA-seq and bulk RNA-seq. Front Immunol. 2023;14:1189520.
28. Theoharides TC, Conti P. Mast cells: the JEKYLL and HYDE of tumor growth. Trends Immunol. 2004;25(5):235–41.
29. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, Li Z, Traugh N, Bu X, Li B, Liu J, Freeman GJ, Brown MA, Wucherpfennig KW, Liu XS. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. Nat Med. 2018;24(10):1550–8.
30. Charoentong P, Finotello F, Angelova M, Mayer C, Efremova M, Rieder D, Hackl H, Trajanoski Z. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. Cell Rep. 2017;18(1):248–62.
31. Zhao Y, Ma Y, Zang A, Cheng Y, Zhang Y, Wang X, Chen Z, Qu S, He J, Chen C, Jin C, Zhu D, Li Q, Liu X, Su W, Ba Y, Hao Y, Chen J, Zhang G, Qu S, Li Y, Feng W, Yang M, Liu B, Ouyang W, Liang J, Yu Z, Kang X, Xue S, Yang G, Yan W, Yang Y, Liu Z, Peng Y, Fanslow B, Huang X, Zhang L, Zhao H. First-in-human phase I/Ib study of QL1706 (PSB205), a bifunctional PD1/CTLA4 dual blocker, in patients with advanced solid tumors. J Hematol Oncol. 2023;16(1):50.
32. Yu ZZ, Liu YY, Zhu W, Xiao D, Huang W, Lu SS, Yi H, Zeng T, Feng XP, Yuan L, Qiu JY, Wu D, Wen Q, Zhou JH, Zhuang W, Xiao ZQ. ANXA1-derived peptide for targeting PD-L1 degradation inhibits tumor immune evasion in multiple cancers. J Immunother Cancer 2023, *11* (3).
33. Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci. 2017;24(1):26.
34. Genova C, Dellepiane C, Carrega P, Sommariva S, Ferlazzo G, Pronzato P, Gangemi R, Filaci G, Coco S, Croce M. Therapeutic implications of Tumor Microenvironment in Lung Cancer: Focus on Immune Checkpoint Blockade. Front Immunol. 2021;12:799455.
35. Liu XQ, Wang X. Indoleamine 2,3-dioxygenase in tumor induced tolerance. Chin Med J. 2009;122(24):3072–7.
36. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005;6(12):1245–52.
37. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, Kaley TJ, Kendall SM, Motzer RJ, Hakimi AA, Voss MH, Russo P, Rosenberg J, Iyer G, Bochner BH, Bajorin DF, Al-Ahmadie HA, Chaft JE, Rudin CM, Riely GJ, Baxi S, Ho AL, Wong RJ, Pfister DG, Wolchok JD, Barker CA, Gutin PH, Brennan CW, Tabar V, Mellinghoff IK, DeAngelis LM, Ariyan CE, Lee N, Tap WD, Gounder MM, D'Angelo SP, Saltz L, Stadler ZK, Scher HI, Baselga J, Razavi P, Klebanoff CA, Yaeger R, Segal NH, Ku GY, DeMatteo RP, Ladanyi M, Rizvi NA, Berger MF, Riaz N, Solit DB, Chan TA, Morris. L. G. T., Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nature genetics* 2019, *51* (2), 202–206.
38. Yu J, Wu X, Ma J, Chen X, Li L. [Clinical Observation of Immunotherapy Efficacy and adverse effects in Chinese patients with lung squamous cell Carcinoma]. Zhongguo Fei ai Za Zhi = Chin J lung cancer. 2022;25(7):546–54.
39. Hellmann MD, Callahan MK, Awad MM, Calvo E, Ascierto PA, Atmaca A, Rizvi NA, Hirsch FR, Selvaggi G, Szustakowski JD, Sasson A, Golhar R, Vitazka P,

Chang H, Geese WJ, Antonia SJ. Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in combination with Ipilimumab in Small-Cell Lung Cancer. Cancer Cell. 2018;33(5):853–e8614.
40. Lin C, Shi X, Zhao J, He Q, Fan Y, Xu W, Shao Y, Yu X, Jin Y. Tumor Mutation Burden correlates with efficacy of Chemotherapy/Targeted therapy in Advanced Non-small Cell Lung Cancer. Front Oncol. 2020;10:480.
41. McGranahan N, Swanton C. Clonal heterogeneity and Tumor Evolution: past, Present, and the future. Cell. 2017;168(4):613–28.

Publisher's note