Four and a half LIM domain protein 1 as a Novel Prognostic Biomarker and Correlation with Immune Infiltration Levels in Lung Adenocarcinoma

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Research Article

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Abstract

Background: We aimed to investigate the prognostic value of Four and a half LIM domain protein 1 (FHL1) and its correlation with FHL1 and tumor-infiltration immune cells (TIICs) in lung adenocarcinoma (LUAD).

Methods: FHL1 expression status and its influence on clinical characteristics in LUAD and Lung squamous cell carcinoma (LUSC) were collected based on GEPIA, TCGA, GEO, CPTAC, the HPA database, and the GTEx Portal. The ROC curve and Kaplan-Meier plots were used to assess the value of FHL1 expression levels in the diagnosis and prognosis of LUAD and LUSC. The interaction network revealed the related genes and proteins of FHL1 by GeneMANIA and STRING. The functional enrichment analysis based on FHL1 and FHL1-related differentially expression genes (DEGs) was conducted by the “clusterProfile” package and Metascape, respectively. The correlation analysis between FHL1 expression and tumor immunity was performed using TISIH, TIMER, and TISIDB. cBioPortal was used to investigate the mutation status between FHL1 and representative immune checkpoints.

Results: The results showed that FHL1 expression was significantly lower in tumors relative to adjacent standard samples, and downregulated FHL1 predicted a worse prognosis for LUAD than that for LUSC. Additionally, FHL1 participated in the interleukin 15 mediated signaling pathway, response to interleukin-9, and neutrophil-mediated cytotoxicity. It was also positively correlated with TIICs (B cells, $CD8^+$T, $CD4^+$T cells, macrophages, neutrophils, and DC), immune checkpoints (CD80, CD48, VTCN, and PVR), and chemokines (CCL5, CCL17, CCL20 and CXCL8).

Conclusion: FHL1 is a powerful prognostic biomarker of immune infiltration.

Background

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers[1], with an annual increase in incidence and mortality globally. LUAD has surpassed LUSC to become the most common subtype of NSCLC. Unfortunately, it has been reported that the average 5-year survival rate is only 15%[2,3] due to the high rates of invasion and metastasis. The immune system serves as a key player in identifying and removing tumor cells, and immunotherapies have demonstrated a remarkable and durable response in NSCLC[4]. However, only a small subset of patients benefits from it in clinical practice due to the lack of valid biomarkers. Therefore, it is critical to identify specific markers and immunotherapy targets to improve the survival rate of LUAD.

FHL1 is a member of the FHL protein family, characterized by four complete LIM domains and an N-terminal half LIM domain[5]. The FHL1 protein is mainly expressed in skeletal muscle and regulates skeletal growth[6]. Recent studies reported that FHL1 plays a crucial role in inhibiting cancer cell growth, migration, and invasion, and it is significantly downregulated in lung cancer, breast cancer, liver cancer, gastric cancer, kidney cancer, and prostate cancer[7-10]. However, the role of FHL1 in tumor progression is complex, and previous studies have validated that FHL1 regulates angiogenesis and the

TGF-β-like signaling pathway and induces G1 and G2/M cell cycle arrest[8,9,11]. Therefore, FHL1 is a potential prognostic biomarker for LUAD.

However, the mechanism underlying the association between FHL1 and immune infiltrates is unclear. Therefore, our study aimed to investigate the prognostic value of FHL1 and the association between FHL1 and TME based on bioinformatics techniques and multiple public databases. Furthermore, our results may present novel diagnostic and therapeutic targets and immunotherapeutic strategies for LUAD.

Method

Identification of NSCLC-related and LUAD-related differentially expressed genes

The GSE118370, GSE63459, and GSE27262 datasets were acquired from the GEO (https://www.ncbi.nlm.nih.gov/geo/) database, which includes NSCLC tissues and adjacent normal tissues. The raw data were processed and standardized with GEO2R, $|\log_2(\text{fold-change})| > 1$, and adjusted P-value < 0.05, which were considered as DEGs. The common intersection of three dataset DEGs was selected using the Venn diagram. To obtain LUAD-related DEGs, RNA sequencing data were downloaded from the TCGA database (https://portal.gdc.cancer.gov/). The data were analyzed with threshold | log (fold change) | > 1.5 and adjusted P-value < 0.05, and visualization by conducted R packages of “ggplot2.”

Validation of FHL1 expression level in LUAD and LUSC

The gene expression datasets of FHL1 in LUAD, LUSC, and normal tissues were derived from the TCGA and GEO databases. Furthermore, the FHL1 protein expression level and distribution and localization between tumor and normal tissues were shown by immunohistochemistry pictures based on CPTAC databases and the HPA (https://www.proteinatlas.org/), respectively. In addition, the corresponding clinical characteristics of FHL1 expression, such as age, sex, smoking status, TNM stage, and tumor location, were also collected from TCGA. Furthermore, receiver operating characteristic (ROC) and Kaplan–Meier (K-M) analyses were used to evaluate FHL1 expression levels in the diagnosis and prognosis of LUAD and LUSC patients.

FHL1-related Interaction Networks and Functional Enrichment Analysis

Gene-gene interaction (GGI) and protein-protein interaction (PPI) networks were used to identify potential interaction genes and proteins of FHL1 based on GeneMANIA (http://genemania.org/) and STRING (https://string-db.org/). Correlation analysis further validated the relationship between these proteins and FHL1. Functional enrichment analysis was conducted with the “ClusterProfiler” package and further visualized using the “ggplot2” package.

The Metascape database was used to investigate the functional enrichment analysis of FHL1-related DEGs in the LUAD dataset and the GO and KEGG analysis visualization by applying the “ggplot2”

package in R.

Relationship between FHL1 expression and immunity

TISCH (http://tisch.comp-genomics.org) was used to assess the abundance of FHL1 expression in different components of the TME, including immune cells, malignant cells, others, and stromal cells. In addition, correlation analysis was performed to investigate the abundance of TIICs with FHL1 expression in LUAD based on the TIMER (https://cistrome.shinyapps.io/timer/) and TISIDB (http://cis.hku.hk/TISIDB/) databases. Furthermore, TISIDB was utilized to investigate the relationship between FHL1 expression and chemokines and immune checkpoints and the prognostic value of the representative immune checkpoints in LUAD. cBioPortal (http://cbioportal.org) was used to evaluate the frequency of genetic alterations in FHL1 and immune checkpoints in LUAD samples, as well as the tendency of co-occurrence and mutual exclusivity.

Statistical Analyses

Statistical analysis was performed using R software v4.0.5 and corresponding packages. Paired *t*-tests and Mann-Whitney U tests were performed to examine the differences between tumor samples and normal samples. Kaplan–Meier (K-M) analyses and log-rank tests were used to evaluate the prognostic value of FHL1. ROC curves were constructed to investigate the FHL1 and its role in sensitivity and specificity of diagnosis. Spearman's correlation analysis was used to assess the correlation between FHL1 and immune infiltration cells, immune checkpoints, and chemokines. Differences were considered statistically significant at $P < 0.05$, except for the genetic alteration analysis. Q-value < 0.05 was considered statistically significant.

Results

Identification of NSCLC-related DEGs in LUAD

The intersection analysis showed that 76 DEGs overlapped among the GSE118370, GSE63459, and GSE27262 datasets (Figure 1A). The top ten targets were *VIPR1, ADARB1, PECAM1, CLDN18, NOTCH4, FHL1, TIMP3, TCF21, MACF1,* and *CD36*, considered key genes in NSCLC. Notably, FHL1 has been considered as a tumor suppressor gene that exerts an inhibitory effect through various mechanisms underlying cancer growth, invasion, and metastasis[9,12-14]. A recent study found that FHL1 can also play a promoting role in tumors[15]. Therefore, our study aimed to identify the function of FHL1 in NSCLC progression using bioinformatics.

Downregulated expression of FHL1 in LUAD and LUSC

Transcriptomic data were analyzed to systematically investigate the mRNA expression levels of FHL1 across diverse cancers based on TCGA and GTEx databases (Figure 1B). The results showed that the FHL1 expression level was significantly lower in 17 types of tumors than that in adjacent normal tissues, especially in LUAD and LUSC. GEPIA and GEO databases were used further to validate the findings of

TCGA and GTEx web and displayed a low expression of FHL1 in LUAD and LUSC tissues compared with that of normal samples (Figure 1C-D). In addition, UALCAN was performed to examine the protein expression level of FHL1 based on the CPTAC database (Figure 1E), and the results showed that the FHL1 protein was remarkably decreased in tumor tissues compared to that of normal samples. Immunohistochemistry images from the HPA database showed results similar to those of CPTAC (Figure 1F). These findings suggest that both mRNA and protein expression levels of FHL1 are downregulated in LUAD and LUSC compared to those of normal tissues.

Correlation Between FHL1 and Clinical Characteristics in NSCLC

To clarify the association between the transcription of FHL1 and clinicopathological parameters in LUAD and LUSC patients, the information was analyzed using Wilcoxon and logistic regression analysis (Figure 2). The results of multiple subgroup analysis showed that FHL1 expression was significantly associated with age, sex, and smoking status and was downregulated in younger (age < 65 years), male, and smoking patients ($p < 0.05$, respectively). However, FHL1 expression was not correlated with other clinicopathological parameters, such as pathologic stage, TNM stage, and anatomic neoplasm subdivision (Figure 2A). Moreover, no statistical correlation was found between FHL1 expression and clinicopathological characteristics in LUSC, including age, sex, smoking status, TNM stage, pathologic stage, and anatomic neoplasm subdivision (Figure 2B).

Diagnosis and Prognosis value of FHL1 in LUAD and LUSC

ROC curve analysis was performed to identify the role of FHL1 in distinguishing LUAD and LUSC samples from normal samples. As shown in Figure 3A and B, the area under the curve (AUC) of FHL1 was 0.993 (95% CI, 0.989–0.998) in LUAD and 0.998 (95% CI: 0.995–1.000) in LUSC, indicating that FHL1 may be a strong identification biomarker for LUAD and LUSC. The curves illustrate the association between FHL1 expression and overall survival (OS) and disease-specific survival (DSS), which helps to investigate the prognostic value of FHL1 in LUAD and LUSC based on K-M analysis. Figure 3A shows that LUAD patients with low expression of FHL1 were associated with shorter OS ($P = 0.025$) and poor DSS ($P = 0.054$). However, the levels of FHL1 were not significantly correlated with OS and DSS in LUSC patients ($P = 0.475$, $P = 0.533$, respectively) (Figure 3B). These findings suggest that low expression of FHL1 could be a promising biomarker to diagnose LUAD and LUSC, as well as the poor prognosis of LUAD patients. Because FHL1 expression is not associated with the prognosis and clinical characteristics of LUSC, the following study focused on the role of FHL1 expression in LUAD.

PPI Networks and Functional Annotations

To explore FHL1-correlated genes and FHL1-binding proteins, GGI and PPI were generated using GeneMANIA and STRING databases (Figure 4A and B). The correlation analysis suggested that the proteins (AKT1, IGFBP5, INPP5A, KCNA5, RBPJ, STAT3, STAT5A, STAT5B, and TTN) in the PPI network had a significant relationship with FHL1 expression, except for RING1 (Figure 4D). Figure 4C shows that FHL1 is associated with the biological functions of DNA-binding transcriptional activator

activity, RNA polymerase I-specific, and RNA polymerase II repressing transcription factor binding and participates in the JAK-STAT signaling pathway, interleukin 15 mediated signaling pathway, and response to interleukin-9.

Additionally, volcano plots and heatmaps were generated to identify differentially expressed genes (DEGs, | log (fold change) | > 1.5, and adjusted P-value < 0.05), based on the TCGA database (Figure 5A and B). Metascape was used to explore the role of FHL1-related DEGs in LUAD patients (Figure 5C–F). The results showed that the FHL1-related DEGs were involved in ERKl and ERK2 cascades, cell differentiation, and blood circulation. Biological process analysis further revealed that FHL1-related DEGs of LUAD participated in humoral immune response, vascular processes in the circulatory system, and neutrophil-mediated cytotoxicity. Moreover, KEGG analysis showed that these genes were closely related to the metabolism of xenobiotics by cytochrome P450. A recent study confirmed that cytochrome P450 is a crucial mediator of ferroptosis[16], considered a novel therapeutic strategy to treat NSCLC[17]. Altogether, FHL1 is strongly linked to the immune response. Therefore, the correlation between FHL1 and the anti-cancer immune response was investigated.

Correlation of FHL1 expression with infiltrating immune cells

To better understand the expression status of FHL1 in different cell types, the violin plots showed that FHL1 expression was the most frequent in immune cells, while only parts of stromal cells, malignant cells, and other cells were expressed (Figure 6A). Furthermore, establishing scatterplots to evaluate the correlation of FHL1 expression with the TIICs (Figure 6B) illustrates that FHL1 expression was positively associated with the level of B cells (r = 0.157, P = 5.21e-04), $CD8^+$T cells (r = 0.207, P = 4.05e-06), $CD4^+$T cells (r = 0.279, P = 4.41e-10), macrophages (r = 0.433, P = 1.45e-23), neutrophils (r = 0.275, P = 7.44e-10), and dendritic cells (r = 0.31, P = 2.40e-12), while being negatively related to tumor purity (r = −0.313, P = 1.03e-12). The enrichment score boxplot further validated that all six types of immune cells had a higher degree of immune infiltration in the high FHL1 expression group than that in the low FHL1 expression group (Figure 6C). Further, the correlation analysis based on the TISIDB database was used to confirm that the level of FHL1 was clearly correlated with TILs in diverse cancers (Figure 7A). As shown in Figure 7B, 26 TILs were closely related to FHL1 expression in LUAD. Specifically, FHL1 was significantly positively associated with 24 types of TILs but negatively associated with active CD4+T cells and CD56 dim cells in LUAD.

Correlation of FHL1 expression with immune molecules

TILs are important constituents of the tumor immune microenvironment and play a crucial role in antitumor efficacy and prognostic ability[18,19]. Immune checkpoints inhibit the anti-tumor immune response of TILs, contributing to tumor cell immune escape. To identify whether FHL1 impacts TIL infiltration via immune checkpoints, a correlation analysis between FHL1 expression and 47 immune checkpoint genes was performed (Figure 8A). The results showed that FHL1 was associated with most immune checkpoint genes, including CD274, CD48, CD80, VTCN, and PVR.

Then, correlation analysis was performed to explore the relationship between FHL1 expression and chemokines (Figure 8B). The results showed that FHL1 expression levels were markedly associated with CCL5 (r = 0.097, P = 0.0267), CCL17 (r = -0.138, P = 0.00166), CCL20 (r = -0.172, P = 8.31e−05), and CXCL8 (r = -0.104, P = 0.018). These results revealed that FHL1 participated widely in regulating immune molecules, thereby affecting immune cell infiltration.

Correlation of the genomic alteration between FHL1 and immune checkpoint

Further, investigation of the prognosis of immune checkpoints based on TISIDB showed that PD-L1, PD-L2, CD80, CD86, VSIR, PVR, LGALS9, and CD48 were downregulated, while VTCIN, CD112, TNFSF4, CD70, and TNFSF18 were upregulated in LUAD (Figure 9). Notably, not all the different expressions of immune checkpoints have a prognostic role in LUAD. Briefly, low expression of CD80 and CD48 and high expression of VTCN1 indicated high OS and/or DFS, but downregulated PVR and was significantly related to poor OS and DFS (P = 0.012, 0.034 respectively).

Moreover, mutation analysis revealed that FHL1 was altered in 2.3% of all study subjects, including missense mutations, splice mutations, truncating mutations, structural variants, amplifications, and deep deletions (Figure 10A). Figure 10B shows the correlation between FHL1 and immune checkpoints. In addition, alterations in PVR, NECTIN2, and HHLA2 have a co-occurrence tendency with FHL1 alterations. These results indicate that FHL1 may participate in regulating immune checkpoints in LUAD.

Discussion

Although surgical and targeted therapies have significantly improved, the mortality of LUAD continues to be high in the past decades, which seriously threatens human health. Therefore, as a novel cancer treatment, immunotherapy has attracted wide attention. Recently, an increasing number of studies have confirmed that immunotherapy is a successful option for LUAD patients[20,21]. Previous studies have validated that FHL1 is downregulated in LUAD, and it is closely related to tumor invasion and metastasis[7,9]. However, the correlation between FHL1 expression and immune infiltration has not been thoroughly investigated in LUAD. In this study, we analyzed the prognostic value of FHL1 and the relationship between FHL1 expression and immune cell infiltration based on bioinformatic analysis.

This study revealed that FHL1 expression was significantly lower in LUAD tissues than that in normal tissues, and the expression level of FHL1 was affected by age, sex, and smoking status. Currently, the role of FHL1 in LUAD has not been thoroughly investigated. Previous trials suggested that FHL1 participates in cancer cell growth, migration, and invasion, which caused a worse prognosis[9,22]. K-M and ROC analyses were conducted to confirm the diagnostic and prognostic value of FHL1 in clinical settings. The results of the K-M analysis showed that low expression of FHL1 indicated poor OS and short DSS, consistent with the results of previous studies[9,22]. ROC analysis showed that FHL1 had a substantial AUC value, which played an important role in the diagnosis of LUAD. Therefore, this study suggests that FHL1 is a powerful diagnostic and prognostic biomarker. To further investigate the functions of FHL1 in LUAD, GO and KEGG analyses were performed to reveal that FHL1 regulates cell growth via DNA-binding

transcription activator activity, RNA polymerase I-specific, and RNA polymerase II repressing transcription factor binding, as well as regulates human immunity through interleukin 15 mediated signaling pathway, and response to interleukin-9. Moreover, the FHL1-related DEGs of LUAD were co-regulated in humoral immune response and neutrophil-mediated cytotoxicity. These results show that FHL1 expression may play a crucial role in tumor growth and immune cell infiltration. However, the underlying mechanism by which FHL1 expression affects immune cells in the TME has not yet been reported.

TIICs play a critical role in tumor progression through complex intercellular interaction networks and are associated with clinical prognosis. TISCH analysis results showed that FHL1 expression was more abundant in immune cells than that in stromal cells, malignant cells, and other cells in the TME. Regarding TIMER databases, FHL1 downregulation might be closely correlated with the low degree of TIL infiltration, especially for B cells, $CD8^+$T cells, $CD4^+$T cells, macrophages, neutrophils, and DCs. Co-expression analyses based on TISIDB further validated the results of the TIMER database, revealing that FHL1 expression was positively correlated with $CD8^+$T cells, NK cells, and DCs. High $CD8^+$T cell infiltration levels were considered to be associated with excellent prognosis and more prolonged survival because $CD8^+$T cells play an important role in killing tumor cells[23]. Previous research has confirmed that NK cells release perforin and granzymes and excrete various cytokines to exert an antitumor effect[24]. DCs, as professional antigen-presenting cells, participate in T cell polarization and Th1 differentiation[25]. Altogether, FHL1 expression affects the level of TIL infiltration, which promotes the construction of an immunosuppressive environment.

Moreover, to investigate the influence of FHL1 in the TME, including immune molecules, the TISIDB database was used to analyze the correlation between FHL1 expression and representative chemokines (such as CCL5, CCL17, CCL20, and CXCL8) and immune inhibitors (such as CD80, CD48, VTCN, and PVR). Notably, CCL20 has been confirmed to enhance the migration and proliferation of A549 cells and recruit TAM cells[26]. Liu, *et al* [27] found that CXCL8 was overexpressed in LUAD and acted as a poor prognostic factor promoting tumor progression, consistent with our results. Intriguingly, beyond the classic immune checkpoints PD-L1 and CTLA-4, FHL1 expression was significantly associated with CD80, CD48, and VTCN1, closely related to patient outcomes. CD80 acts as a surface ligand on immune cells, and CTLA-4 negatively regulates T-cell activation[28]. CD48 is a member of the signaling lymphocyte activation molecule family and participates in the adhesion and activation of immune cells[29]. VTCN1 belongs to the B7 family protein, is upregulated in LUAD, and negatively regulates T-cell immunity by restraining T-cell proliferation, cytokine secretion, and the development of cytotoxicity[30]. Therefore, FHL1 expression affects the function of tumor-antagonizing immune cells, weakening the effects of immune surveillance and contributing to immune escape and tumor progression.

Interestingly, mutation analysis further revealed that PVR and NECTIN2 and HHLA2 alterations co-occurred with the FHL1 mutation. PVR and NECTIN2 inhibit the activation of T and NK cells by interacting with T cell immunoglobulin and ITIM domain (TIGIT)[31]. Furthermore, HHLA2 expression is closely correlated with CD8 T-cell infiltration status[32]. A recent study reported that HHLA2 was widely expressed in patients with PD-1-negative NSCLC, which indicated that HHLA2 might be a potential target for patients

who do not respond to PD-1 pathway blockade[33]. These findings partly demonstrate the mechanism by which FHL1 regulates the expression of immune checkpoints in LUAD. Therefore, FHL1 can function as a novel target to investigate the immunosuppressive status of LUAD.

Altogether, this study reported that FHL1 regulates anti-tumor immune responses through various mechanisms, including recruitment of different immune cells in the TME, thereby affecting the expression of chemokines and immune inhibitors, and the co-occurrence mutation of some immune checkpoints. These results suggest that FHL1 may impact patient outcomes by modulating the immunosuppressive microenvironment and could be a promising immunotherapeutic target for LUAD.

Despite comprehensive and systematic evaluation of the role of FHL1 in LUAD, based on different public databases, there are several limitations. First, the row data were collected from public repositories, and the quality standards were nonuniform, which might have influenced the study outcomes. Second, the perspective of transcriptome and genome levels could not reflect overall aspects of immune status, and there is a lack of direct evidence to demonstrate the role of FHL1 expression in regulating the immune response impacting patient prognosis. Third, more *in vivo* and *in vitro* experiments are needed to further validate the mechanism of FHL1 expression in LUAD. In the follow-up research, we will further detect the underlying function of FHL1 in LUAD.

Conclusion

In summary, this study demonstrated that FHL1 was expressed at low levels in LUAD and revealed a correlation between FHL1 expression and tumor progression. Furthermore, FHL1 expression was strongly diagnosed as a prognostic factor for LUAD. In addition, FHL1 expression was related to immune cell infiltration, immunoinhibition, and chemokines, indicating that FHL1 is a better immunotherapy target. Therefore, FHL1 is considered a potential prognostic biomarker and immunotherapy target for LUAD.

Abbreviations

FHL1, four and a half LIM domain protein 1; TCGA: The Cancer Genome Atlas; TIICs: tumor-infiltration immune cells; LUAD, lung adenocarcinoma; LUSC: lung squamous cell carcinoma; DEGs: differentially expression genes; NSCLC: non-small cell lung cancer; BP: Biological processes; MF: Molecular function; CC: Cellular component; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; OS: Overall survival; ROC: Receiver operating characteristic.

Declarations

Acknowledgements

Not applicable.

Author contributions

Jingtao Zhang wrote the original draft, Fei Xu and Minghao Guo prepared the figures and tables, Jing Zhang analyzed the data, Guangming Zhang downloaded the raw data from TCGA and GEO databases, Ning Sun reviewed the relevant literature, Wenqiang Cui proofread the manuscript, and Fei Xu edited the draft and made revisions.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

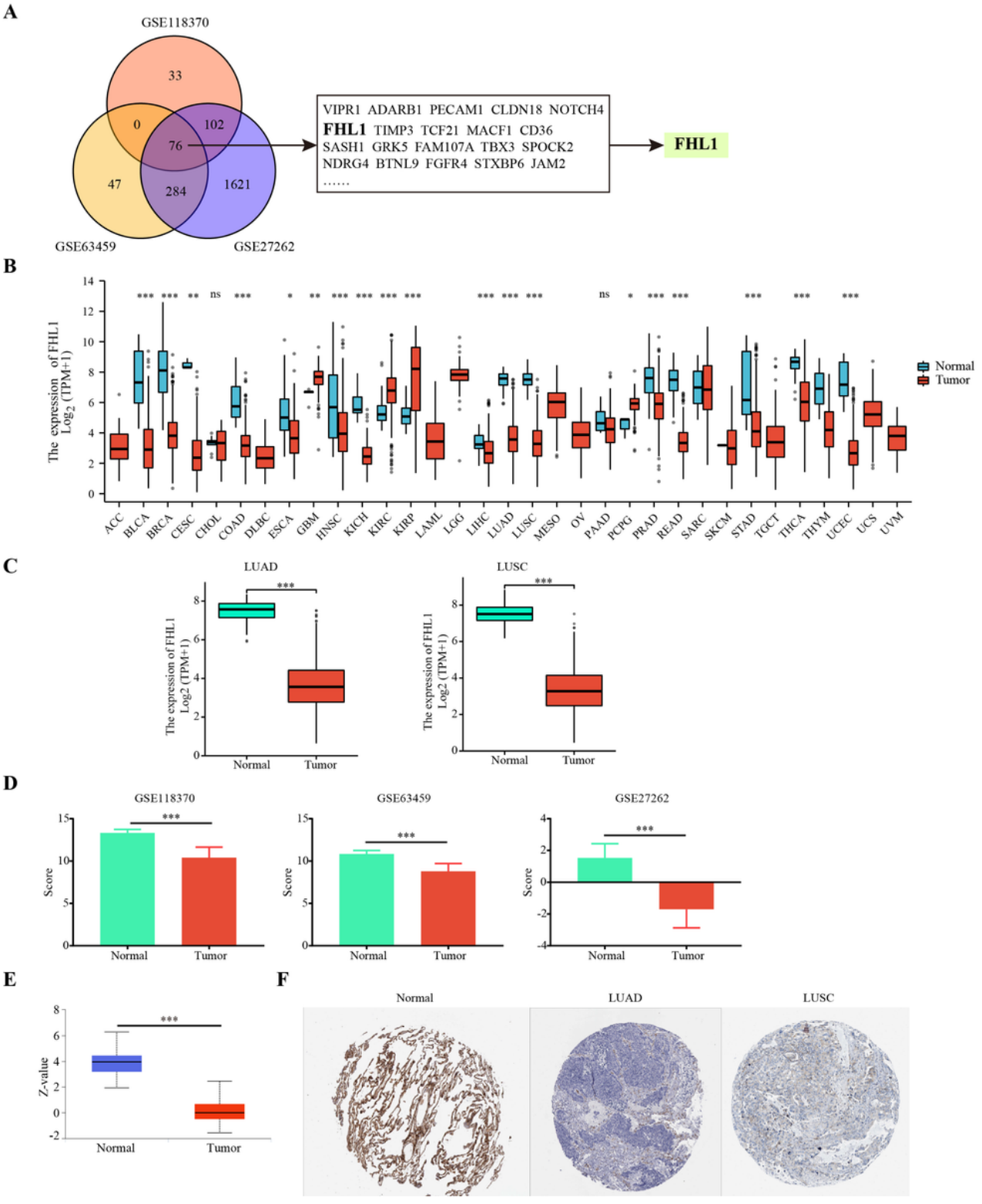


Figure 1

The mRNA and protein expression of FHL1 in LUAD and LUSC compared with normal tissues. **(A)** The Venn diagram shows the intersection of differentially expressed genes (DEGs) based on GSE63459 and GSE118370 and GSE27262 datasets. **(B)** The expression of FHL1 from a Pan-cancer perspective. **(C-D)** The mRNA expression levels of FHL1 are based on TCGA and GEO databases. **(E-F)** The protein expression status of FHL1 is based on CPTAC and HPA databases, respectively. (ns, no significance, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$)

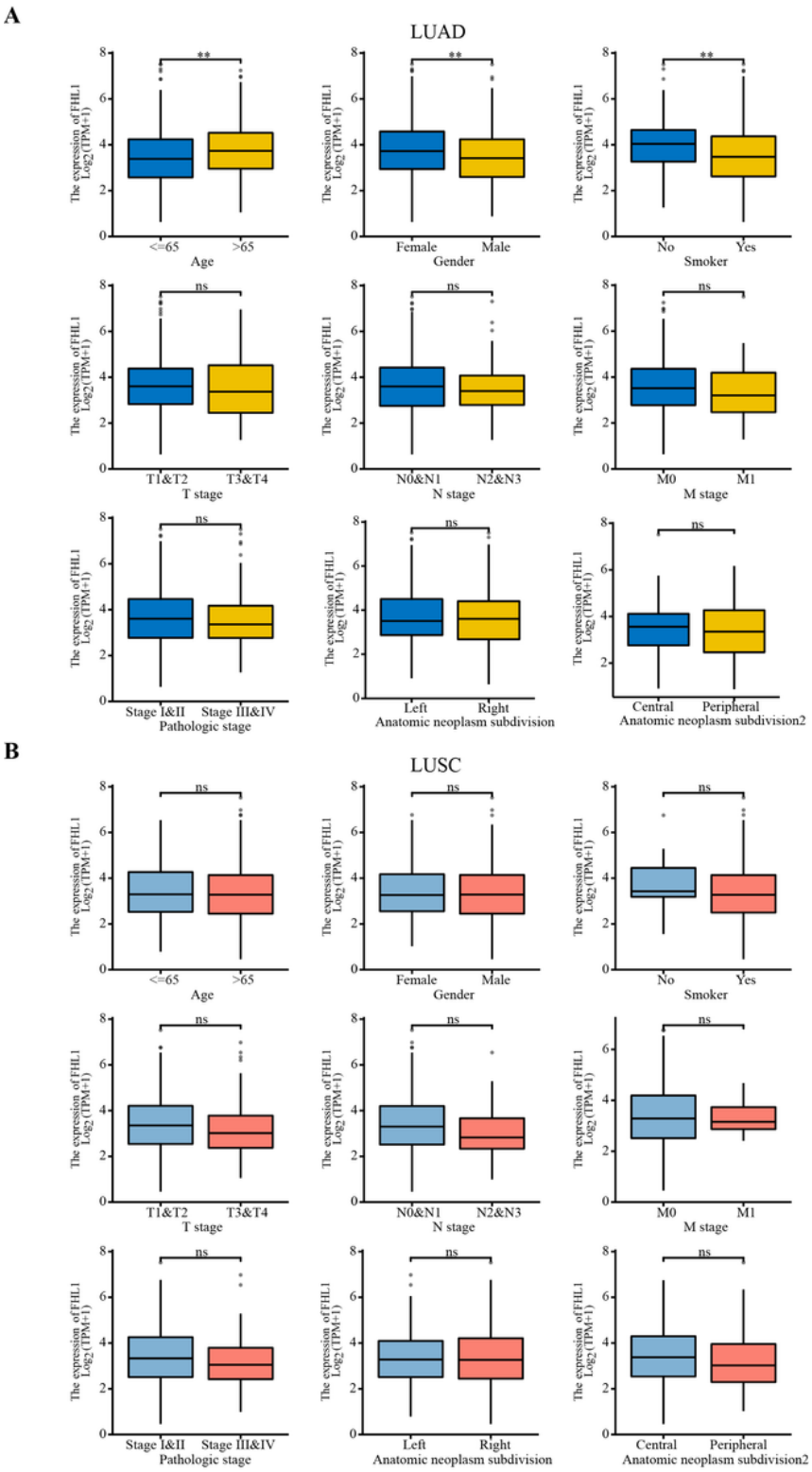


Figure 2

Correlation between FHL1 mRNA levels and clinicopathological characteristics. **(A)** FHL1 expression was significantly related to age, gender, and smoker status, while there was no significant relationship with TNM stage, pathologic stage, and anatomic neoplasm subdivision. **(B)** No statistical correlation was found between the FHL1 expression levels and the nine clinical-pathological characteristics.

Figure 3

The predictive and diagnostic value of FHL1. **(A)** FHL1 showed high sensitivity and specificity with respect to diagnostic ability in LUAD. Patients with high FHL1 expression had poor OS and DSS in LUAD. **(B)** FHL1 showed high sensitivity and specificity with respect to diagnostic ability in LUSC. Patients with high or low FHL1 expression had no significant values in OS and DSS for LUSC.

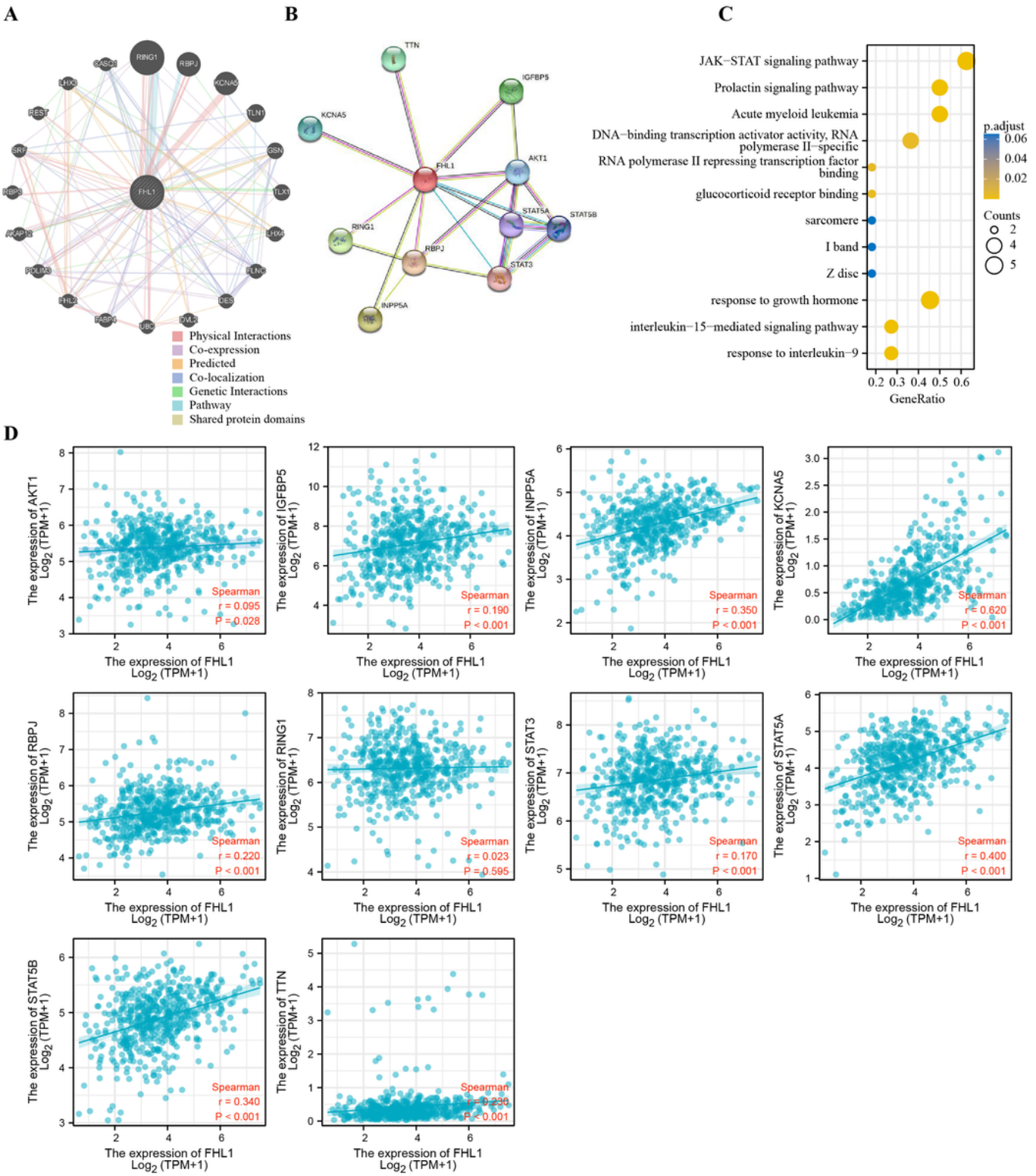


Figure 4

Interaction network, functional enrichment, and co-expression analysis of FHL1. **(A-B)** GGI and PPI of FHL1 using GeneMANIA and STRING. **(C)** The functional enrichment of FHL1. **(D)** The expression correlation between FHL1 and related protein based on STRING.

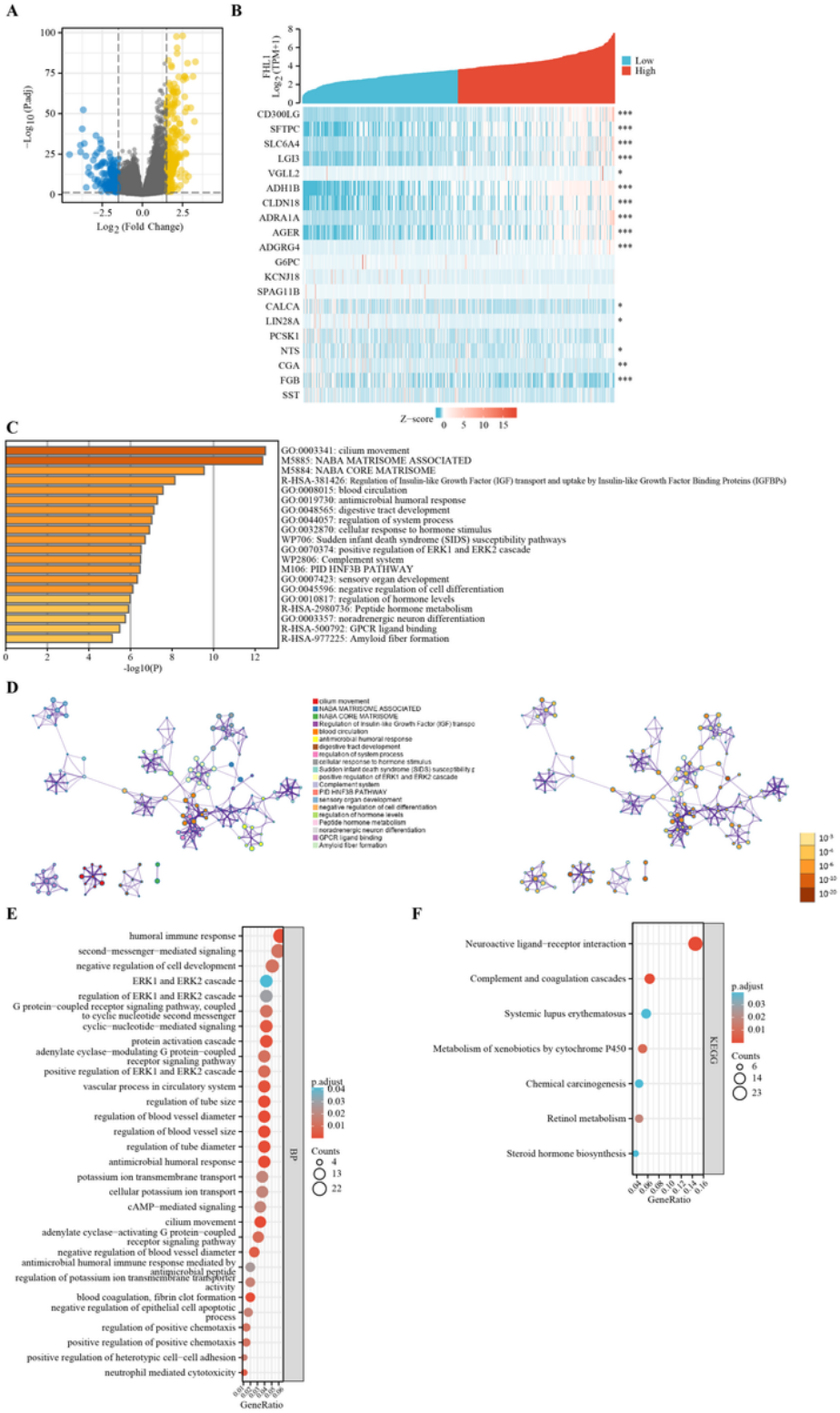


Figure 5

Identifying DEGs of FHL1 and the functional enrichment of these genes. **(A)** Volcano plot of differential gene profiles between FHL1 high expression and low expression. **(B)** Heatmap of the top 20 DEGs between FHL1 high expression and FHL1 low expression. **(C)** Metascape analysis of GO functional categories enriched in the FHL1-related genes. **(D)** The KEGG pathway analysis is displayed as a network analyzed by Metascape.

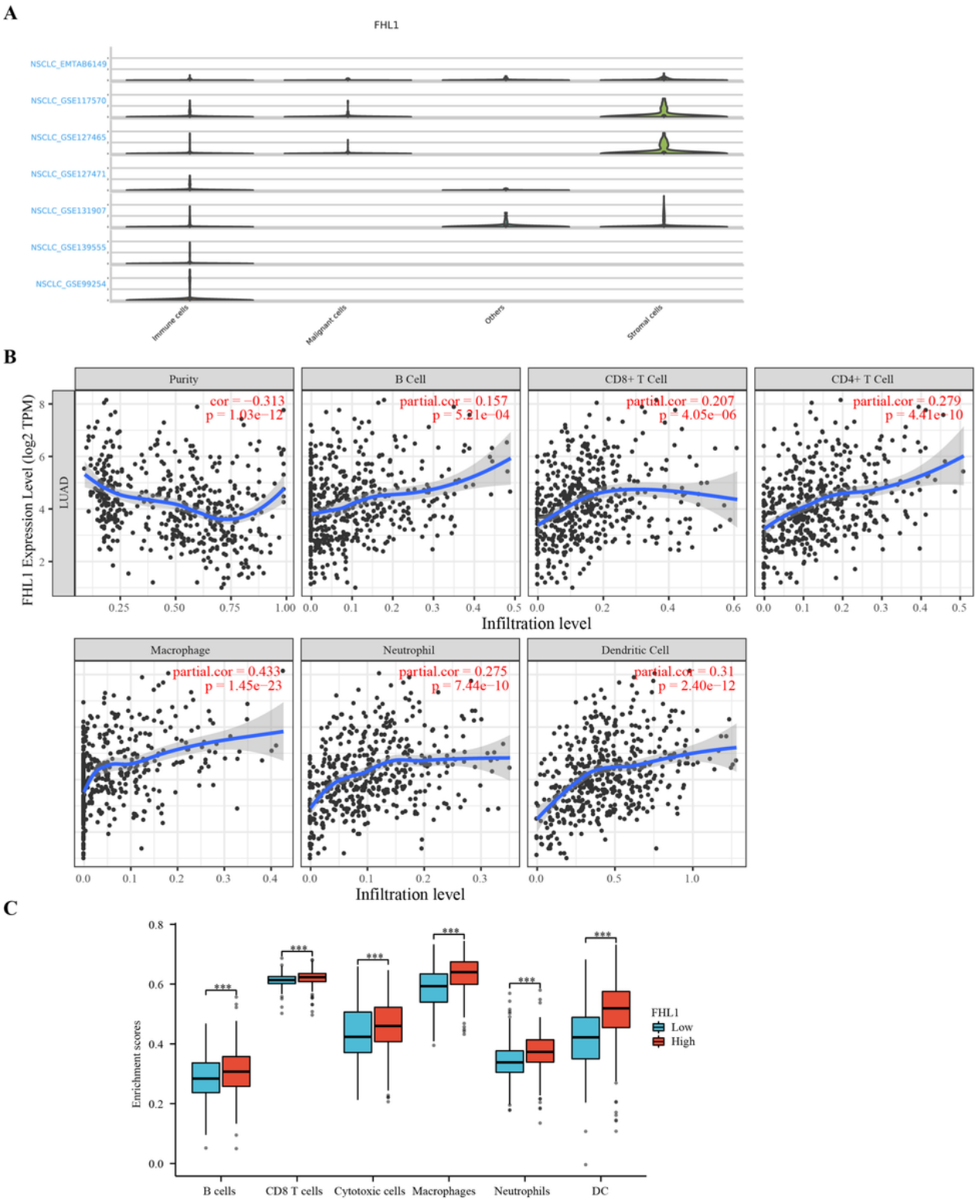


Figure 6

The correlation analysis of FHL1 and TIILs. **(A)** The violin plots show the FHL1 expression status in the immune cells, stromal cells, other cells, and malignant melanoma cells. **(B)** Association of FHL1 expression level with six types of immune infiltrations and tumor purity. **(C)** The box plot shows the differential immune infiltration between FHL1 low- and high-groups.

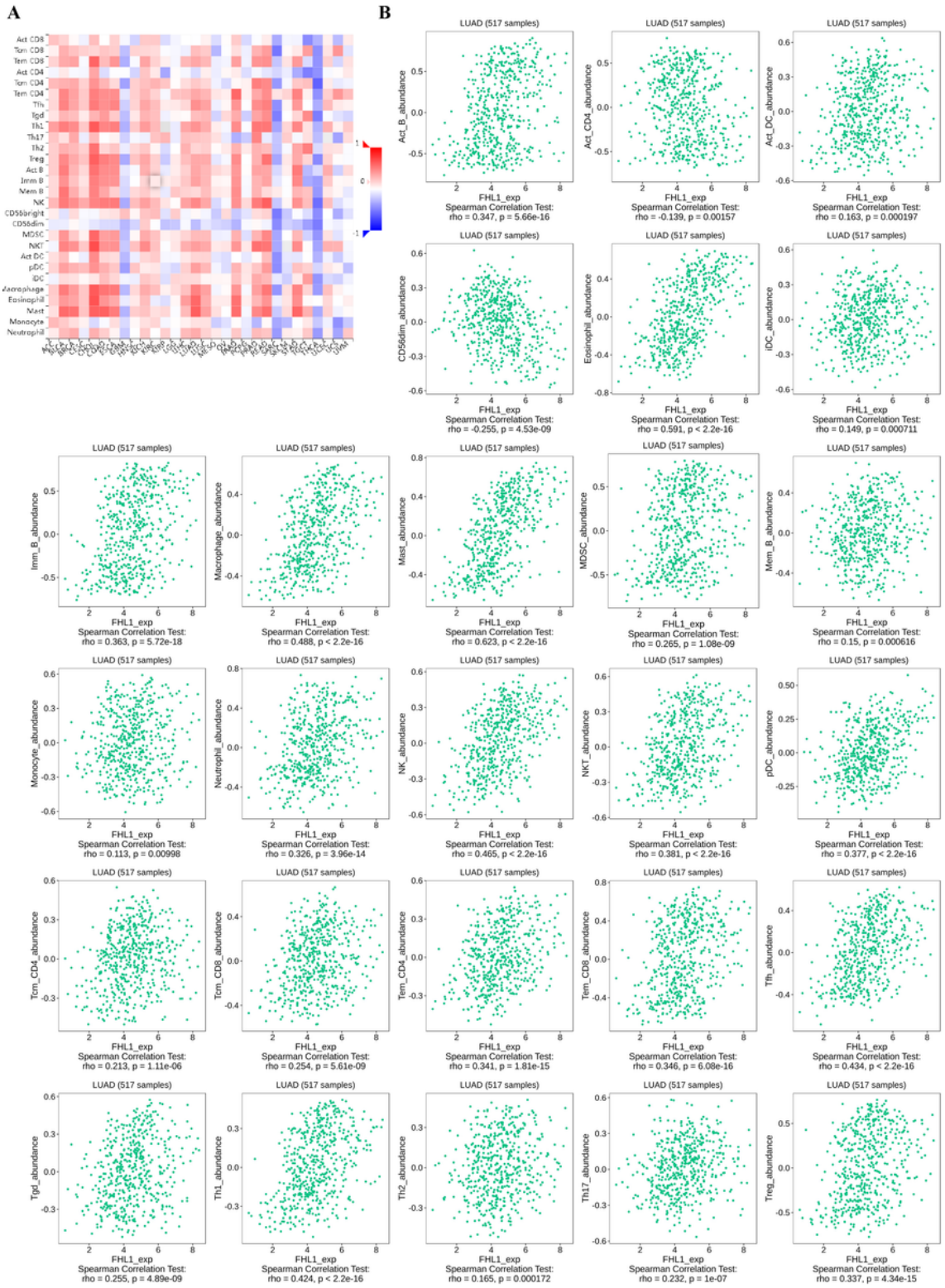


Figure 7

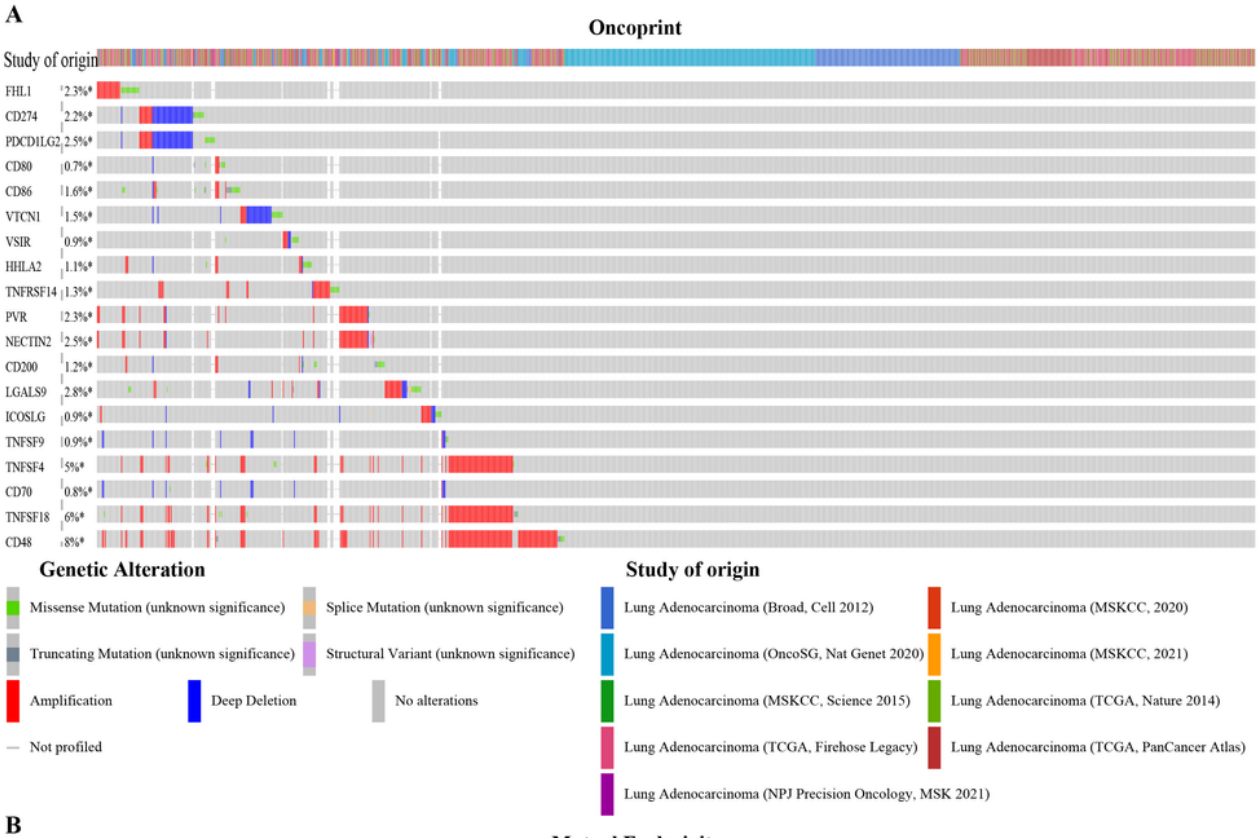
Association of FHL1 levels and lymphocytes infiltration levels from TISIDB database. **(A)** Relations between expression of FHL1 and 28 types of TILs across human heterogeneous cancers. **(B)** The infiltration degree of 26 types of TILs with FHL1 expression.

Figure 8

The association between FHL1 expression and immunomodulators and chemokines based on the TISIDB database. **(A)** Correlations between the abundance of immune checkpoints and FHL1 and FHL1 expression levels. **(B)** Correlations between chemokines and FHL1 expression levels.

Figure 9

Prognostic summary of the immune checkpoints in LUAD.



B

Mutual Exclusivity

	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency	Significant
FHL1	PVR	1427	28	30	6	>3	<0.001	0.002	Co-occurrence	**Yes**
FHL1	NECTIN2	1422	29	35	5	2.808	0.002	0.016	Co-occurrence	**Yes**
FHL1	HHLA2	1443	31	14	3	>3	0.006	0.038	Co-occurrence	**Yes**
FHL1	CD86	1434	31	23	3	2.593	0.02	0.094	Co-occurrence	No
FHL1	CD70	1447	32	10	2	>3	0.029	0.123	Co-occurrence	No
FHL1	TNFSF9	1445	32	12	2	2.912	0.039	0.141	Co-occurrence	No
FHL1	CD48	1334	28	123	6	1.217	0.067	0.197	Co-occurrence	No
FHL1	LGALS9	1417	31	40	3	1.777	0.072	0.201	Co-occurrence	No
FHL1	CD200	1439	32	18	2	2.321	0.074	0.205	Co-occurrence	No
FHL1	ICOSLG	1438	33	19	1	1.198	0.371	0.605	Co-occurrence	No
FHL1	VTCN1	1419	34	38	0	<-3	0.412	0.617	Mutual exclusivity	No
FHL1	TNFSF4	1378	33	79	1	-0.92	0.446	0.652	Mutual exclusivity	No
FHL1	TNFRSF14	1432	34	25	0	<-3	0.559	0.747	Mutual exclusivity	No
FHL1	TNFSF18	1374	32	83	2	0.049	0.588	0.769	Co-occurrence	No
FHL1	CD274	1406	33	51	1	-0.259	0.666	0.808	Mutual exclusivity	No
FHL1	PDCD1LG2	1409	33	48	1	-0.169	0.692	0.823	Mutual exclusivity	No
FHL1	VSIR	1443	34	14	0	<-3	0.723	0.833	Mutual exclusivity	No
FHL1	CD80	1448	34	9	0	<-3	0.812	0.862	Mutual exclusivity	No

Figure 10

FHL1 couples with immune checkpoints in LUAD. **(A)** The landscape of FHL1 and immune checkpoint alteration in LUAD. **(B)** Mutual-exclusivity analysis between FHL1 and multiple-immune checkpoints in LUAD. q-value < 0.05 was considered to be statistically significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RawdataFigure1D.xlsx
- RawdataFigure1A.xlsx
- RawdataLUSCclinicalraw.xlsx
- RawdataLUADclinicalraw.xlsx
- RawdataFigure8A.xlsx