Am J Transl Res 2023;15(9):5574-5593
www.ajtr.org /ISSN:1943-8141/AJTR0150716

Original Article

Identification of useful biomolecular markers in kidney renal clear cell carcinoma: an in silico and in vitro analysis-based study

Khalida Sheikh[1], Khalida Naz Memon[2], Humera Usman[3], Mostafa A Abdel-Maksoud[4], Sajid Ullah[4], Taghreed N Almanaa[4], Aqsa Chaudhary[5], Muhammad Jamil[6], Owais Bin Qadeer Gill[7], Muhammad Ahmed Yar[8], Ahmed M Hussein[9], Adel M Zakri[10]

[1]Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro 76090, Pakistan; [2]Department of Community Medicine, LUMHS, Jamshoro 76090, Pakistan; [3]Fazaia Medical College, Air University, Islamabad 44000, Pakistan; [4]Badr Al Samaa Hospital, Ruwi, Oman; [5]Department of Biochemistry, University of Central Punjab, Lahore, Pakistan; [6]PARC Arid Zone Research Center, Dera Ismail Khan 29050, Pakistan; [7]Department of Radiology, PMO, PINUM, Faisalabad 38000, Pakistan; [8]Mufti Mehmood Memorial Teaching Hospital, Dera Ismail Khan 29050, KPK, Pakistan; [9]Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University of Vienna, Vienna 1090, Austria; [10]Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia

Received April 14, 2023; Accepted August 28, 2023; Epub September 15, 2023; Published September 30, 2023

Abstract: Background: Kidney renal clear cell carcinoma (KIRC) is the most prevalent type of renal cell carcinoma (RCC), with a high incidence and mortality rate. There is a lack of sensitive biomarkers. Therefore, the discovery of accurate biomarkers for KIRC patients is critical to improve prognosis. Methods: We determined hub genes and their associated pathways involved in the pathogenesis of KIRC from the GSE66272 dataset consisting of KIRC (n = 26) and corresponding control (n = 26) samples and later validated the expression and methylation level of the identified hub genes on The Cancer Genomic Atlas (TCGA) datasets and Human RCC 786-O and normal HK-2 cell lines through RNA sequencing (RNA-seq), Reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and targeted bisulfite sequencing (bisulfite-seq) analyses. Results: The identified up-regulated four hub genes include TYROBP (Transmembrane Immune Signaling Adaptor TYROBP), PTPRC (Protein tyrosine phosphatase, receptor type, C), LCP2 (Lymphocyte cytosolic protein 2), and ITGB2 (Integrin Subunit Beta 2). Moreover, the higher expression of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC patients insignificantly correlates with a poor prognosis in KIRC patients. In addition, hub genes were involved in the "Fc epsilon RI signaling pathway, asthma, natural cell killer mediated cytotoxicity, T cell receptor signaling pathway, primary immunodeficiency, Fc gamma R-mediated phagocytosis, malaria, leukocyte transendothelial migration, and legionellosis" pathways and associated with the infiltration level of CD8+ T, CD4+ T, and macrophage cells. Conclusion: Our integrated in silico and in vitro analysis identified important hub genes (TYROBP, PTPRC, LCP2, and ITGB2) involved in the pathogenesis of KIRC as possible diagnostic biomarkers.

Keywords: KIRC, RCC, hub genes, biomarkers

Introduction

According to recent cancer statistics, kidney cancer is ranked as the 9th most prevalent cancer type in men and is ranked 14th in women [1]. Approximately 400,000 (2.2% of the total cancer cases) new kidney cancer cases and nearly 175,000 deaths due to it occurred around the world in 2021 [2]. Recent studies have also highlighted that the rate of new kidney cancer cases is increasing rapidly worldwide [3]. Among all kidney cancers, kidney renal clear cell carcinoma (KIRC) is the most common [4]. Nearly 30% of KIRC patients experience metastasis during disease progression [5]. Moreover, the 5-year survival rate is less than 10%, with a median survival of only 13 months [6]. The prognosis of KIRC patients is poor mainly due to late detection and resistance to existing chemo or radiotherapies [7, 8].

KIRC development and progression involve the dysregulation of numerous genes, including VHL (Von Hippel-Lindau), BAP1 (BRCA1 associated protein-1), PBRM1 (Protein polybromo-1), and many more [9]. So far, a range of targeted therapies has been utilized to treat KIRC based on different molecular markers, such as the use of chemotherapeutic drugs against PDGF (platelet derived growth factor), VEGF (vascular endothelial growth factor), and immunologic checkpoints [9]. However, due to the heterogeneity of KIRC behavior, the efficacy of already targeted therapies varies substantially between patients. The choice of chemotherapeutic drugs for treating KIRC is a challenging task in clinical practice; thus, the exploration of novel molecular biomarkers for KIRC diagnosis, prognosis, and treatment is high on the agenda.

Integrative analysis of KIRC Gene Expression Omnibus (GEO) expression datasets in order to determine diagnostic and prognostic biomarkers for KIRC is urgently required. The discovery of novel signature genes will not only aid in KIRC diagnosis but also provide new chemotherapeutic drug targets for KIRC treatment. In this work, we determined differentially expressed genes (DEGs) from the GSE66272 [10] dataset. We further employed Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), Cytoscape, MCODE, and CytoHubba to explore hub genes. Based on the degree method, the top four hub genes include TYROBP (Transmembrane Immune Signaling Adaptor TYROBP), PTPRC (Protein tyrosine phosphatase, receptor type, C), LCP2 (Lymphocyte cytosolic protein 2), and ITGB2 (Integrin Subunit Beta 2). Lastly, we analyzed hub genes expression and promoter methylation levels using KIRC cell lines (786-O and HK-2) through in vitro experiments and different The Cancer Genome Atlas (TCGA) databases.

Methods

Data acquisition

A messenger RNA (mRNA) expression-based GSE66272 [10] dataset consisting of KIRC (n = 26) and corresponding control (n = 26) samples was acquired from the GEO database [11].

Data preprocessing and DEG analysis

The acquired GSE66272 dataset was based on the GPL3921, Affymetrix HT Human Genome U133A Array platform. After downloading, preprocessing of the raw data in GSE66272 was carried out by adjusting the background, performing normalization, and performing gene annotation and re-annotation. Moreover, the identification of differentially expressed genes (DEGs) among KIRC samples and corresponding controls was done with the help of the R package “limma” [12]. The filtering criteria for DEG identification was selected as absolute log2 fold-change > 1 and $P < 0.05$.

Construction of protein-protein interaction (PPI) network, module identification, and the selection of hub genes

The Search Tool for the Retrieval of Interacting Genes (STRING) database is known to explore mutual effects among proteins of interest across a biological system [13]. Using STRING, we first constructed the PPIs of the identified DEGs with default settings, and then we applied a threshold of > 0.9 interaction score to further refine the PPIs by removing unconnected nodes. Cytoscape is a recently developed easy-to-use software for visualizing and analyzing PPIs [14]. In the current study, this tool was initially applied to visualize the constructed PPI networks of the DEGs. Then, a plug-in of the Cytoscape software, the MCODE, which is an application for exploring densely connected regions in PPI networks [15] was applied to identify the significant module with default settings. Finally, another plug-in of the Cytoscape software, the CytoHubba application [16], was used to screen out the top four hub genes in the module based on the degree method.

UALCAN database

To further enhance the reliability of our data, we reanalyzed the transcriptional expression of the hub genes on the KIRC TCGA dataset. For this purpose, we utilized the UALCAN database. This database helps users to document expression variations in query gene(s) among the specified cancer subtypes [17].

GEPIA, OncoDB, and GENT2 databases

Then, to further validate hub genes’ expression across KIRC tissues and cell lines, we employed the GEPIA [18], OncoDb [19], and GENT2 [20] databases. All these online databases are cancer microarray-based expression analysis platforms that provide expression analysis results

in the form of box plots. Moreover, we also used GEPIA for survival analysis for the hub genes.

Receiver operating curve (ROC) generation

To calculate the diagnostic values of hub genes in KIRC, ROC curves were generated using SPSS 26 (IBM SPSS Statistics, Chicago, IL, USA).

The Human Protein Atlas (HPA)

The Human Protein Atlas (HPA) is a valuable tool for analyzing protein expression patterns across various cancer research projects [21]. This database was utilized in the present study to validate the hub genes' expression in KIRC tissue samples relative to control tissues. Additionally, this database was also used to find the subcellular localization of proteins encoded by the hub genes in KIRC cells.

UALCAN and OncoDB

The UALCAN [22, 23] and OncoDB [19] were conducted in our study employed to check the DNA promoter methylation level of identified hub genes in KIRC.

cBioPortal

The cBioPortal web-resource provides multi-omic information on nearly 5,000 cancer samples from 20 cancer-related studies in the medical literature [23]. A TCGA KIRC dataset, "TCGA PanCancer Atlas (512 cases)", was utilized in this work to analyze genetic mutations, co-expressed genes, and the influence of mutations on KIRC patient survival.

Functional enrichment analysis

The Gene Ontology (GO) analysis provides functional annotation of the gene(s) of interest. While KEGG (Kyoto Encyclopedia of Genes and Genomes) provides an interpretation of the user-defined genes in biological pathways [24]. The GO and KEGG analysis of hub and hub genes enriched genes was performed using the Gene Set Enrichment Analysis (GSEA) program [25].

TIMER database

The TIMER database utilizes RNA sequencing data for detecting immune cell infiltration in cancer tissues and also helps to analyze associations between hub gene expression and immune cell infiltration [26]. In this research, levels of immune cell infiltration in KIRC were plotted against hub gene expression.

miRNA network analysis

ENCORI platform is developed for analyzing the miRNA-mRNA, RNA-RNA, and lncRNA-miRNA networks across various types of cancer [27]. In this investigation, the ENCORI database was used to create the miRNA network of the identified hub genes [27].

Hub genes' drug prediction analysis

We used the DrugBank database to uncover a variety of drugs associated with the identified hub genes because we believe that the identified hub genes could be promising therapeutic targets. This database compiles information on chemotherapeutic drugs that target hub genes from a variety of reliable sources [28].

In vitro validation of the hub genes expression and methylation levels

Cell lines: Human RCC cell lines (786-O), and normal renal tubular epithelial cell line (HK-2) were purchased from the American Type Culture Collection (ATCC, USA) and cultivated in accordance with the manufacturer's instructions.

Total RNA and RNA extraction: Total RNA extraction from both KIRC and normal cell lines was done by isopycnic centrifugation as described previously [29]. The extracted RNA was then processed for DNA digestion step of incubation with RNase-free DNase I (Roche, Germany) at 37°C for 15 minutes. The DNA extraction was done by the organic method [30]. The quality of the extracted RNA and DNA was checked by a 2100 Bioanalyzer (Agilent Technologies, Germany).

RNA sequencing (RNA-seq) and targeted bisulfite sequencing (bisulfite-seq) analysis: RNA and DNA samples were sent to Beijing Genomics Institute (BGI) Company for RNA-seq bisulfite-seq analysis. Following RNA-seq and targeted bisulfite-seq analyses, the gene expression values of the hub genes were normalized using reads per kilobase million reads (RPKM) and

fragments per kilobase million reads (FPKM). Methylation values were normalized as beta values. The obtained FPKM and beta values against hub genes in RCC and the normal control cell line were compared to identify differences in the expression and methylation levels.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR), validation analysis: The specific protocols are as follows: First, the PrimeScript™ RT reagent kit (Takara, Japan) was used for reverse transcription of the extracted RNA from HK-2, and 786-O, cell lines into complementary DNA. Then, the RT-qPCR was carried out on a ABI ViiA 7 Real Time PCR System (Thermo Fisher, USA) with a SuperReal SYBR Green Premix Plus (Tiangen Biotech, China) as a fluorescent dye. GAPDH was chosen as the internal reference in the present study. All the experiments were in triplicate independently. The $2^{-\Delta\Delta Ct}$ method was employed to evaluate the relative expression of each hub gene [31]. Following primers were selected for RT-qPCR analysis: GAPDHF 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDHR 5'-CTGTTGCTGTAGCCAAATTCG-3'; TYROBPF 5'-TGGTGCTGACAGTGCTCATTGC-3', TYROBPR 5'-CTGATAAGGCGACTCGGTCTCA-3'; PTPRCF 5'-CTTCAGTGGTCCCATTGTGGTG-3', PTPRCR 5'-CCACTTTGTTCTCGGCTTCCAG-3'; LCP2F 5'-GGAAGAAGCCACCTGTGCCAAA-3', LCP2R 5'-GCTCATAGGAAGTAGTGCTGGC-3'; ITGB2F 5'-AGTCACCTACGACTCCTTCTGC-3', ITGB2R 5'-CAAACGACTGCTCCTGGATGCA-3'.

Statistical procesing

DEGs were identified using a t-test [32]. For GO and KEGG enrichment analysis, we used Fisher's Exact test for computing a statistical difference [33]. Correlational analyses were carried out using Pearson method. For comparisons, a Student t-test was adopted in the current study. All the analyses were carried out in R version 3.6.3 software.

Results

DEGs and hub gene identification

In the current work, the GSE66272 [10] dataset, comprising 26 KIRC and 26 corresponding control samples, was downloaded from the GEO database. Genes with a *P* value less than 0.05 and |log2 fold change| > 1 was considered as DEGs. Using this filtering criterion, we extracted a total of 26676 DEGs by applying the R package "limma" (**Figure 1A**). After, STRING and Cytoscape analyses of the DEGs, there were 140 nodes and 543 edges in the PPI network of the DEGs (**Figure 1B**). Based on the degree method, CytoHubba analysis of the constructed PPI network revealed four hub genes, including TYROBP, PTPRC, LCP2, and ITGB2 (**Figure 1B**).

Expression analysis of hub genes using the TCGA dataset

UALCAN tool was employed to analyze hub gene (TYROBP, PTPRC, LCP2, ITGB2) expression using the TCGA data set. Results showed that those hub genes were upregulated in KIRC samples compared to the controls (**Figure 1C**, **1D**). The difference in hub gene expression between KIRC and control tissues was significant ($P < 0.05$).

Higher hub gene expressions were correlated with the adverse clinical data

Next, we further explored the potential roles of TYROBP, PTPRC, LCP2, and ITGB2 up-regulation in KIRC patients with different clinicopathologic data. For this purpose, clinicopathologic data were obtained and analyzed by the UALCAN, including cancer stage, race, gender, and age. Then relationships between hub genes mRNA expressions and clinicopathological data in the KIRC cohort were documented. Analysis results revealed that TYROBP, LCP2, and ITGB2 expressions were up-regulated in KIRC patients belonging to cancer stages 3 and 4 relative to stages 1 and 2. However, PTPRC showed higher expression in cancer stages 2 and 3 KIRC patients as compared to stages 1 and 4. Moreover, concerning hub gene expressions in KIRC patients of different races, genders, and age groups, TYROBP, PTPRC, LCP2, and ITGB2 hub genes exhibited higher expressions in KIRC patients belonging to the Asian population, male gender, 41-61 yr, and 61-80 yr age group (**Figure 2**).

Expression verification, survival analysis, and diagnostic values of TYROBP, PTPRC, LCP2, and ITGB2

The use of additional databases for expression verification analysis strengthened our results. Therefore, we utilized 3 more databases,

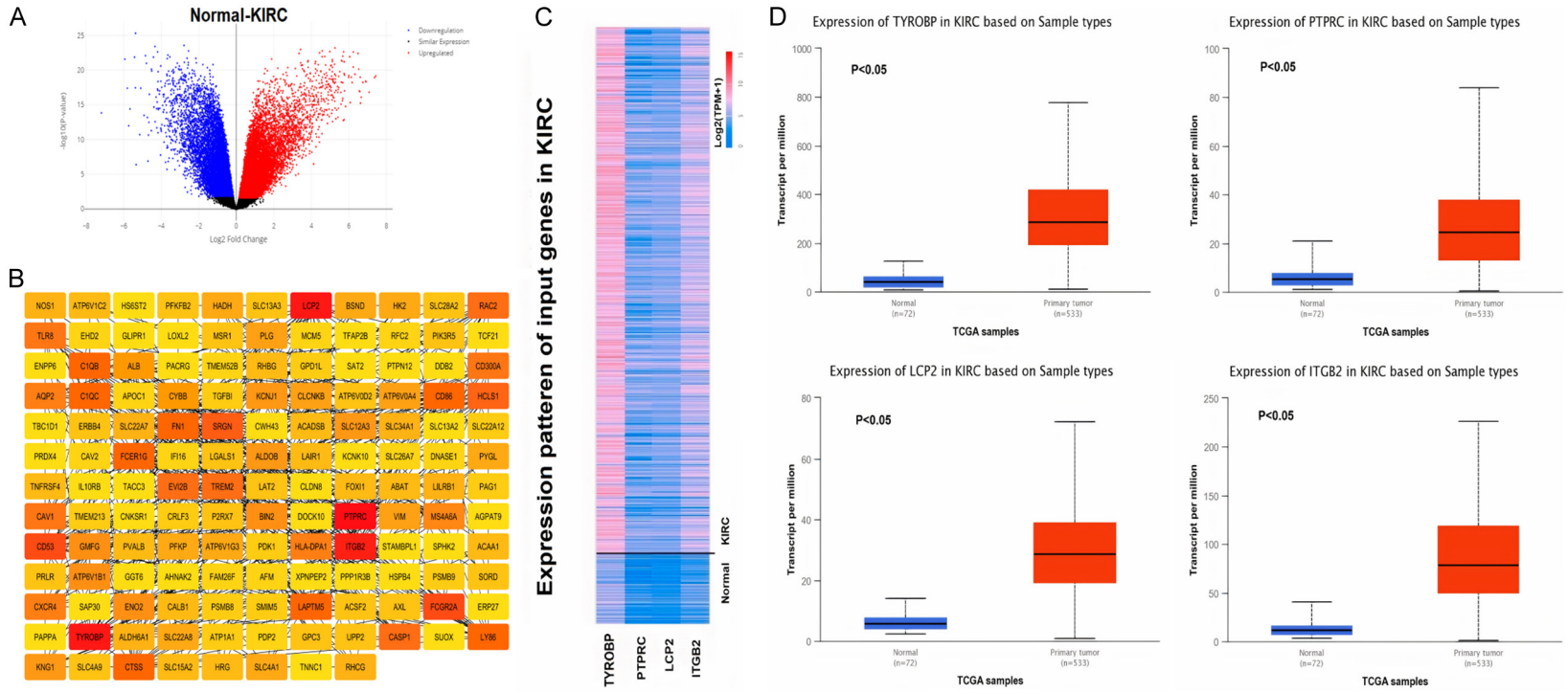


Figure 1. A volcano graph of overall DEGs, a PPI network of the top 250 DEGs identified in the GSE66272 microarray dataset, and mRNA expression profiling of TYROBP, PTPRC, LCP2, and ITGB2 hub genes by UALCAN. (A) A volcano graph of the overall DEGs, (B) A PPI network of the top 250 DEGs, (C) A heatmap of TYROBP, PTPRC, LCP2, and ITGB2 hub genes in KIRC sample group and normal control group, and (D) Box plot presentation of TYROBP, PTPRC, LCP2, and ITGB2 hub genes mRNA expression in KIRC sample group and normal control group. DEGs = differentially expressed genes, PPI = Protein-protein interaction, KIRC = Kidney renal clear cell carcinoma, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2.

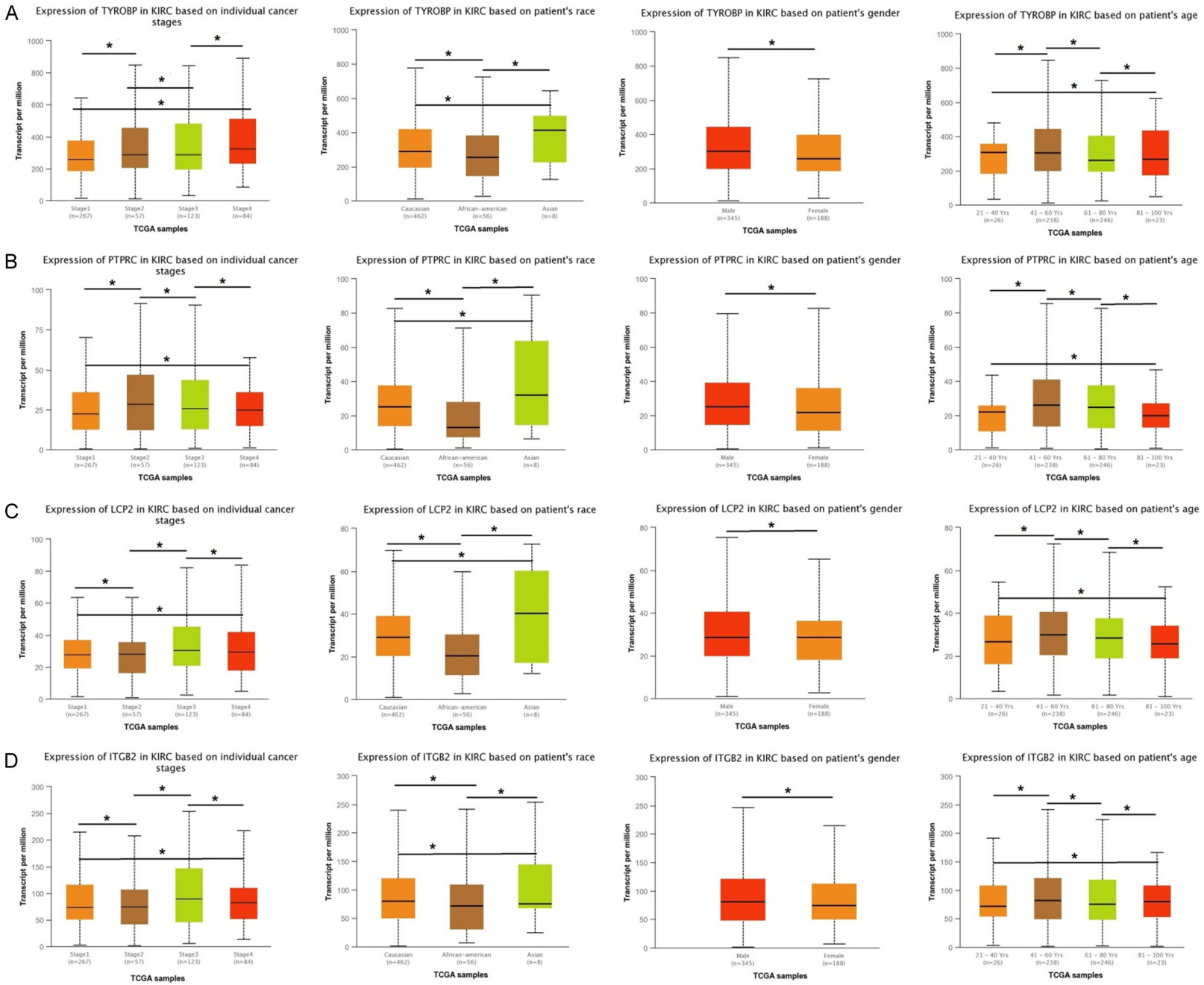

A
Expression of TYROBP in KIRC based on individual cancer stages
Expression of TYROBP in KIRC based on patient's race
Expression of TYROBP in KIRC based on patient's gender
Expression of TYROBP in KIRC based on patient's age
B
Expression of PTPRC in KIRC based on individual cancer stages
Expression of PTPRC in KIRC based on patient's race
Expression of PTPRC in KIRC based on patient's gender
Expression of PTPRC in KIRC based on patient's age
C
Expression of LCP2 in KIRC based on individual cancer stages
Expression of LCP2 in KIRC based on patient's race
Expression of LCP2 in KIRC based on patient's gender
Expression of LCP2 in KIRC based on patient's age
D
Expression of ITGB2 in KIRC based on individual cancer stages
Expression of ITGB2 in KIRC based on patient's race
Expression of ITGB2 in KIRC based on patient's gender
Expression of ITGB2 in KIRC based on patient's age
Transcript per million
TCGA samples
Stage1 (n=267)
Stage2 (n=57)
Stage3 (n=123)
Stage4 (n=84)
Caucasian (n=462)
African-american (n=56)
Asian (n=8)
Male (n=345)
Female (n=188)
21 - 40 Yrs (n=26)
41 - 60 Yrs (n=238)
61 - 80 Yrs (n=246)
81 - 100 Yrs (n=23)

Figure 2. Expression profiling of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC samples of different clinical variables relative to controls by UALCAN. (A) Expression profiling of TYROBP in KIRC samples of different clinical variables, (B) Expression profiling of PTPRC in KIRC samples of different clinical variables, (C) Expression profiling of LCP2 in KIRC samples of different clinical variables, and (D) Expression profiling of ITGB2 in KIRC samples of different clinical variables. KIRC = Kidney renal clear cell carcinoma, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2.

including GEPIA, OncoDB, and GENT2 to verify the mRNA expression and for the survival analysis of the TYROBP, PTPRC, LCP2, and ITGB2 in KIRC and control tissues. The results of mRNA expression verification analysis showed that the levels of these four hub gene mRNAs were significantly ($P < 0.05$) higher in KIRC samples relative to the non-cancer samples (**Figure 3A**). Moreover, this study also utilized the GEPIA database for the survival analysis of the hub genes. Outcomes of survival analysis showed that higher expressions of TYROBP, PTPRC, LCP2, and ITGB2 were correlated with the worst overall survival (OS) of KIRC patients. However, the results were not significant ($P > 0.05$) (**Figure 3B**). Additionally, ROC analysis was performed to assess the diagnostic values of TYROBP, PTPRC, LCP2, and ITGB2 expression in KIRC patients. The observed AUCs (TYROBP = 0.57, PTPRC = 0.575, LCP2 = 0.54, and ITGB2 = 0.60) suggest that TYROBP, PTPRC, LCP2, and ITGB2 expression have poor diagnostic value for KIRC (**Figure 3C**). Therefore, further research work should be carried out to explore accurate diagnostic values of TYROBP, PTPRC, LCP2, and ITGB2 expression on a large cohort of KIRC patients.

Subcellular localization and protein expression validation analysis

With the help of HPA, the subcellular location of the TYROBP, PTPRC, LCP2, and ITGB2 was visualized in KIRC cells. TYROBP protein was mainly enriched in plasma membrane (**Figure 4A**), PTPRC localization was found nucleoplasm and vesicles (**Figure 4A**), LPC2 localization was enriched in vesicles (**Figure 4A**), and ITGB2 localization was seen in plasma membrane and rods and ring (**Figure 4A**). Based on the HPA data, obtained from the immunohistochemistry (IHC) analysis, the protein expressions of the TYROBP, PTPRC, LCP2, and ITGB2 were validated in KIRC samples relative to controls. Results showed that the expressions of all four genes were higher (staining = medium) in KIRC samples (**Figure 4B**) relative to control samples (staining = low) (**Figure 4B**).

Possible mechanisms behind hub gene expression dysregulation

Effect of DNA mutation and promoter methylation on TYROBP, PTPRC, LCP2, and ITGB2 upregulation and KIRC patient survival: Since TYROBP, PTPRC, LCP2, and ITGB2 hub gene expressions were up-regulated and correlated with the worst OS and different clinical measures of KIRC, the regulatory mechanisms behind hub gene overexpression were explored. For this purpose, we first detected genetic mutations in hub genes across the TCGA KIRC cohort by the cBioPortal database. The extremely low genetic alteration frequencies of TYROBP (0.2%), PTPRC (1.2%), and ITGB2 (1.2%) themselves explain their lower involvement in the expression regulation of the hub gene (**Figure 5A**). On the other hand, a much higher genetic alteration frequency in the LCP2 (15%) hub gene was the only predicted alteration that was associated with its overexpression in KIRC patients. Moreover, the observed genetic alterations in the hub genes were also found to be linked with the better OS and DFS of KIRC patients (**Figure 5B**). Concerning the effect of the genetic mutations on the overall expression profile of the KIRC patients, the overall mRNA and protein profiles of the hub genes in mutated KIRC samples were higher than in the non-mutated KIRC samples (**Figure 5C**). Genes co-expressed with TYROBP, PTPRC, LCP2, and ITGB2 were explored through cBioPortal. Results revealed that TYROBP-FCER1G, PTPRC-IK2F1, LCP2-IKZF1, and ITGB2-VAV1 were the top co-expressed genes (**Figure 5D**). Finally, using UALCAN and OncoDB, promoter methylation levels of the TYROBP, PTPRC, LCP2, and ITGB2 genes were documented. As shown in **Figure 6**, the promoter regions of the hub genes were hypomethylated in KIRC samples relative to non-cancer samples (**Figure 6**).

GO and biological pathways analysis: Next, we analyzed hub genes and their co-expressed genes to find their GO and KEGG pathways in KIRC. In the CC, "Fc-gamma receptor signaling pathway, Fc receptor mediated stimulatory

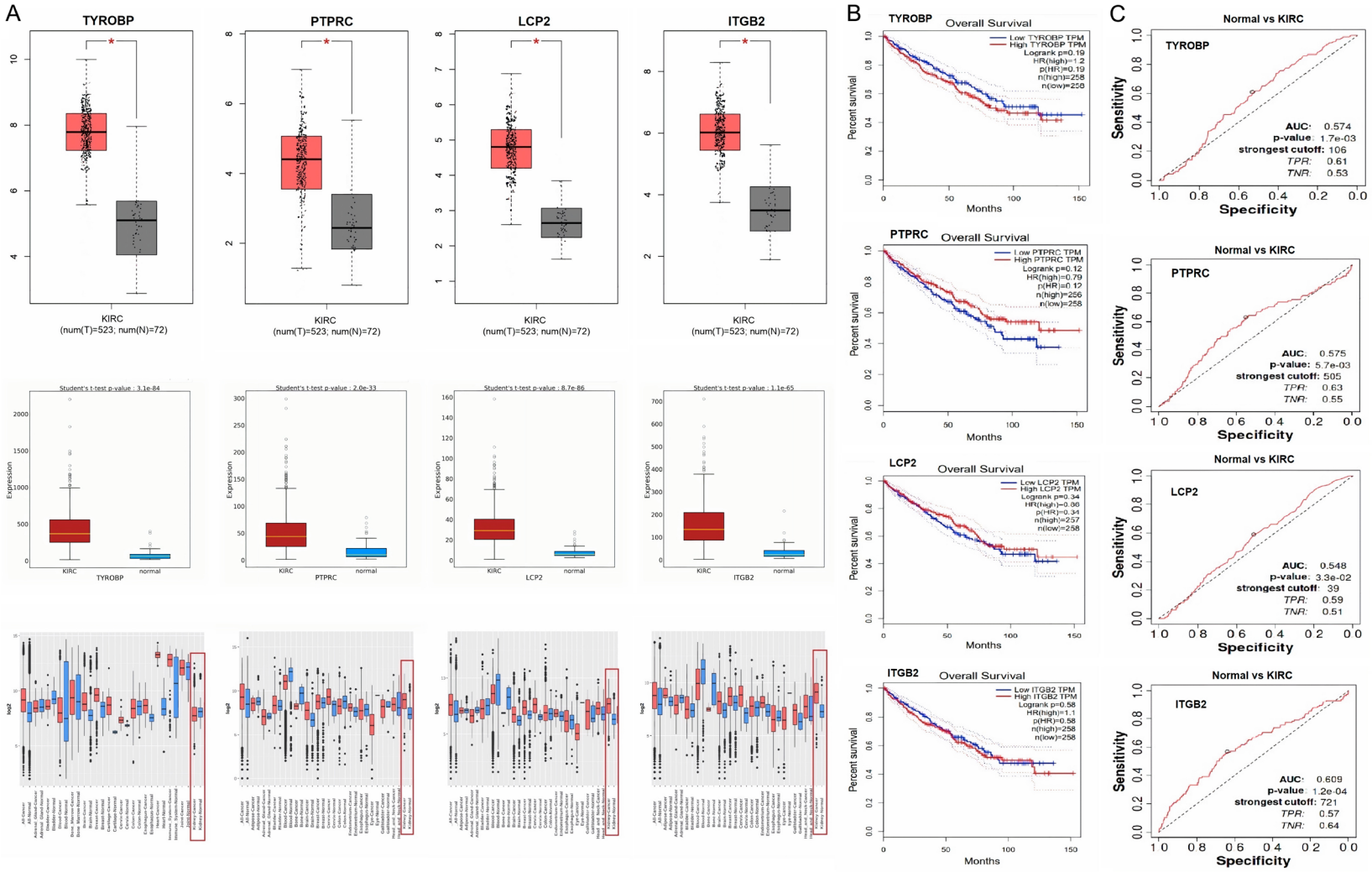


Figure 3. Expression validation, survival analysis, and diagnostic values of TYROBP, PTPRC, LCP2, and ITGB2. (A) Expression validation of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC and normal samples using GEPIA, OncoDB, and GENT2 databases, (B) Survival analysis of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC and normal samples via GEPIA database, and (C) Diagnostic values of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC through ROC analysis. AUC = area under ROC curve, KIRC = Kidney renal clear cell carcinoma, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2.

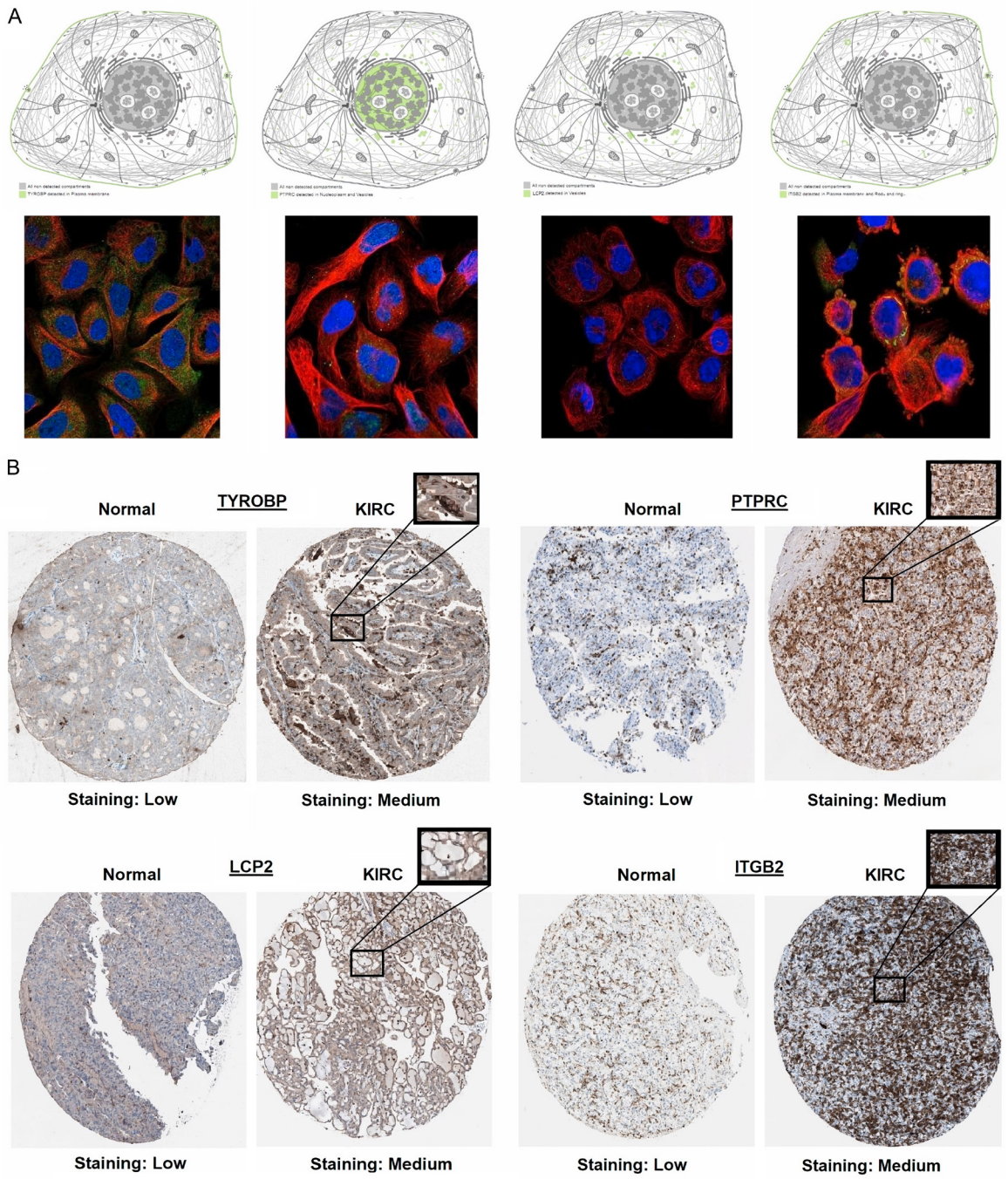


Figure 4. Subcellular localization and protein expression validation of TYROBP, PTPRC, LCP2, and ITGB2 by the HPA database. (A) Subcellular localization prediction of TYROBP, PTPRC, LCP2, and ITGB2, and (B) Protein expression analysis of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC and normal samples. TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2, HPA = Human Protein Atlas.

signaling pathway, neutrophil chemotaxis, Fc-epsilon receptor signaling pathway, and neutrophil migration" were significantly associated with the analyzed genes (**Figure 7A**). Concerning MF, the "Fc receptor complex, Fc-epsilon receptor I complex, Bleb, Ficolin-1-rich granule membrane, tertiary granule membrane, plasma membrane raft, and secretory granule membrane" were closely associated with the hub genes (**Figure 7B**). In BP, some vital functions

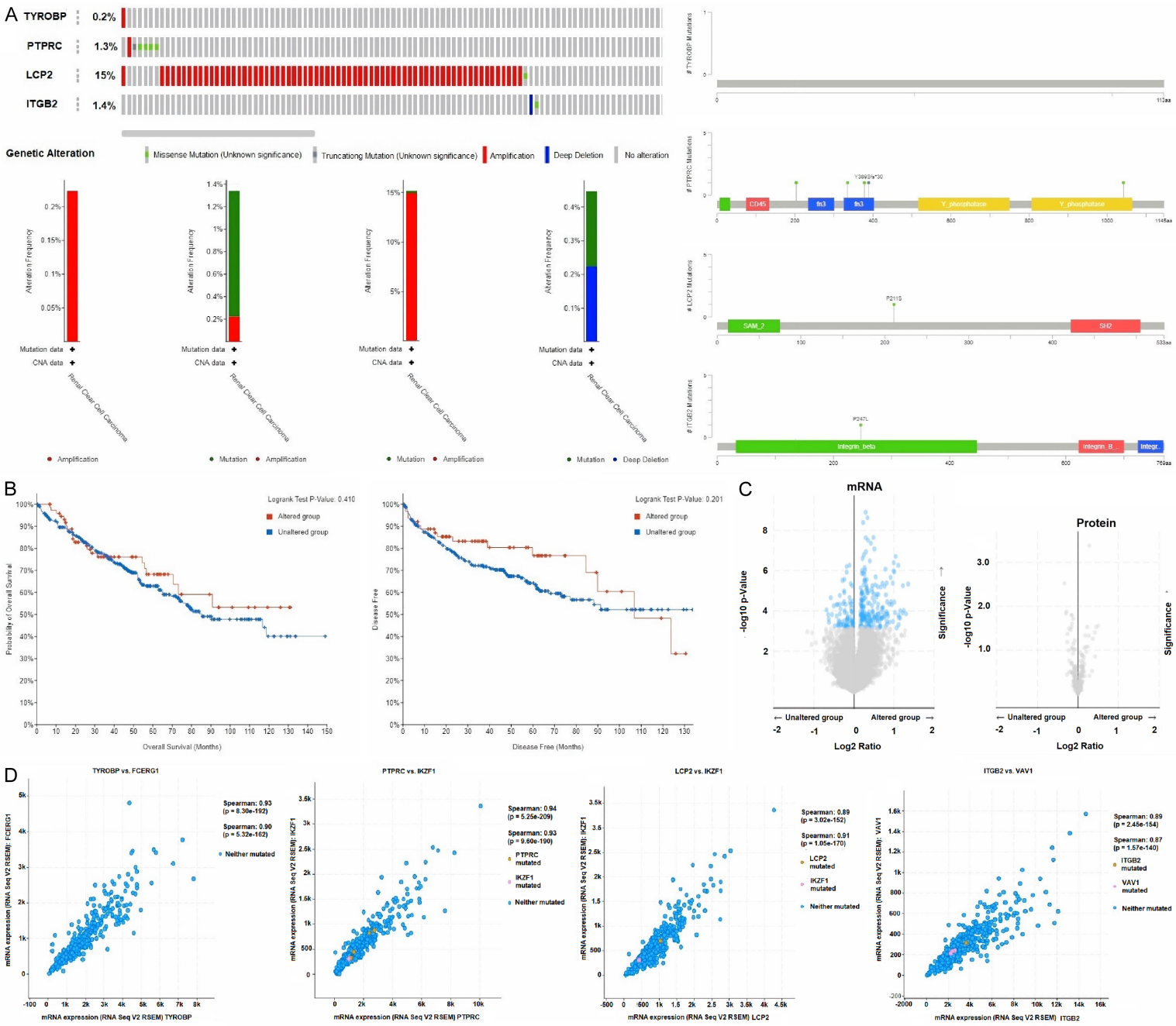
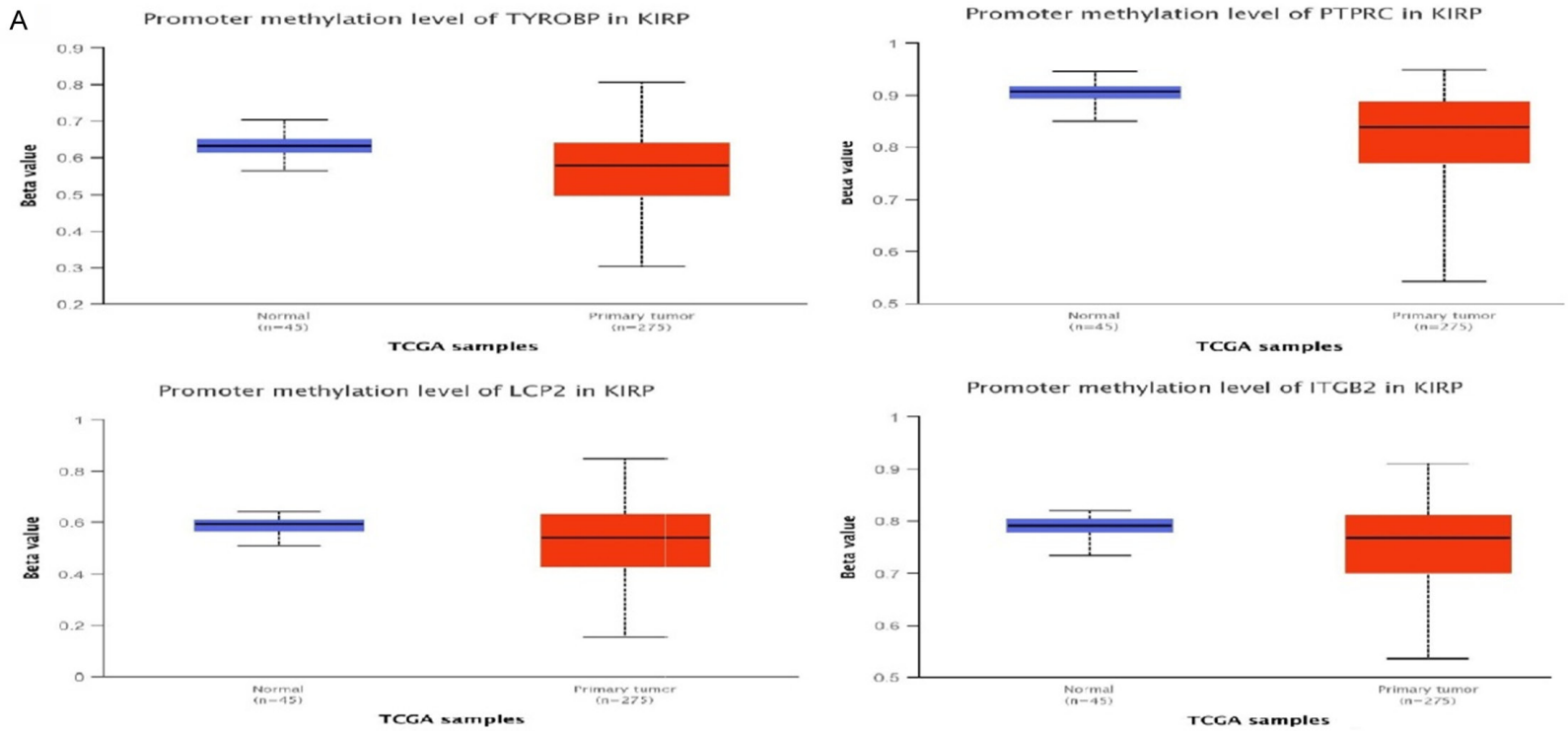

A
TYROBP 0.2%
PTPRC 1.3%
LCP2 15%
ITGB2 1.4%
Genetic Alteration
Missense Mutation (Unknown significance)
Truncating Mutation (Unknown significance)
Amplification
Deep Deletion
No alteration
Alteration Frequency
Mutation data
CNA data
Renal Clear Cell Carcinoma
Amplification
Mutation
Deep Deletion
TYROBP Mutations
PTPRC Mutations
CD45
fn3
Y_phosphatase
LCP2 Mutations
SAM_2
SH2
ITGB2 Mutations
integrin_beta
B
Logrank Test P-Value: 0.410
Logrank Test P-Value: 0.201
Altered group
Unaltered group
Probability of Overall Survival
Overall Survival (Months)
Disease Free
Disease Free (Months)
C
mRNA
Protein
-log10 p-Value
Significance
Unaltered group
Altered group
Log2 Ratio
D
TYROBP vs. FCERG1
PTPRC vs. IKZF1
LCP2 vs. IKZF1
ITGB2 vs. VAV1
mRNA expression (RNA Seq V2 RSEM) TYROBP
mRNA expression (RNA Seq V2 RSEM) PTPRC
mRNA expression (RNA Seq V2 RSEM) LCP2
mRNA expression (RNA Seq V2 RSEM) ITGB2
Neither mutated
PTPRC mutated
IKZF1 mutated
LCP2 mutated
ITGB2 mutated
VAV1 mutated

Figure 5. Exploration of genetic alteration frequencies, mutational hotspots, OS, DFS analyses, co-expressed genes, and effect of mutations on the overall expression profiling of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC samples by cBioPortal. (A) Types, frequencies, and location of the genetic alterations in TYROBP, PTPRC, LCP2, and ITGB2, (B) OS and DFS analysis of TYROBP, PTPRC, LCP2, and ITGB2 in genetically altered and unaltered HNSC groups, (C) Effect of mutations on the overall mRNA and protein expression profiling of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC samples, and (D) Identification of co-expressed genes with TYROBP, PTPRC, LCP2, and ITGB2 in KIRC samples. KIRC = Kidney renal clear cell carcinoma, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2, OS = Overall survival, DFS = Disease-free survival.



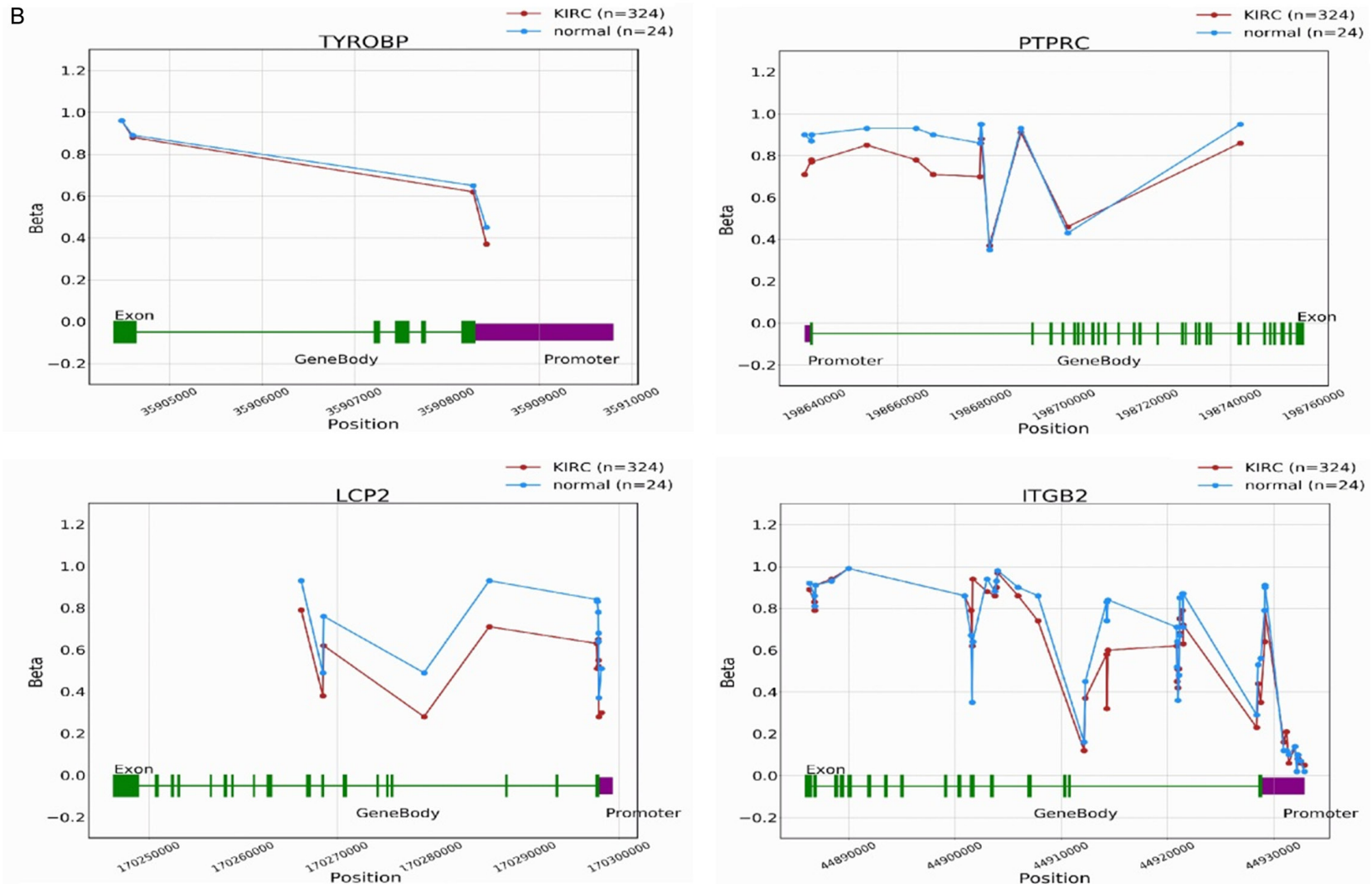


Figure 6. Methylation status exploration of TYROBP, PTPRC, LCP2, and ITGB2 by UALCAN and OncoDB in KIRC and normal samples. (A) Methylation status exploration of TYROBP, PTPRC, LCP2, and ITGB2 by UALCAN, and (B) Methylation status exploration of TYROBP, PTPRC, LCP2, and ITGB2 by OncoDB. KIRC = Kidney renal clear cell carcinoma, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2.

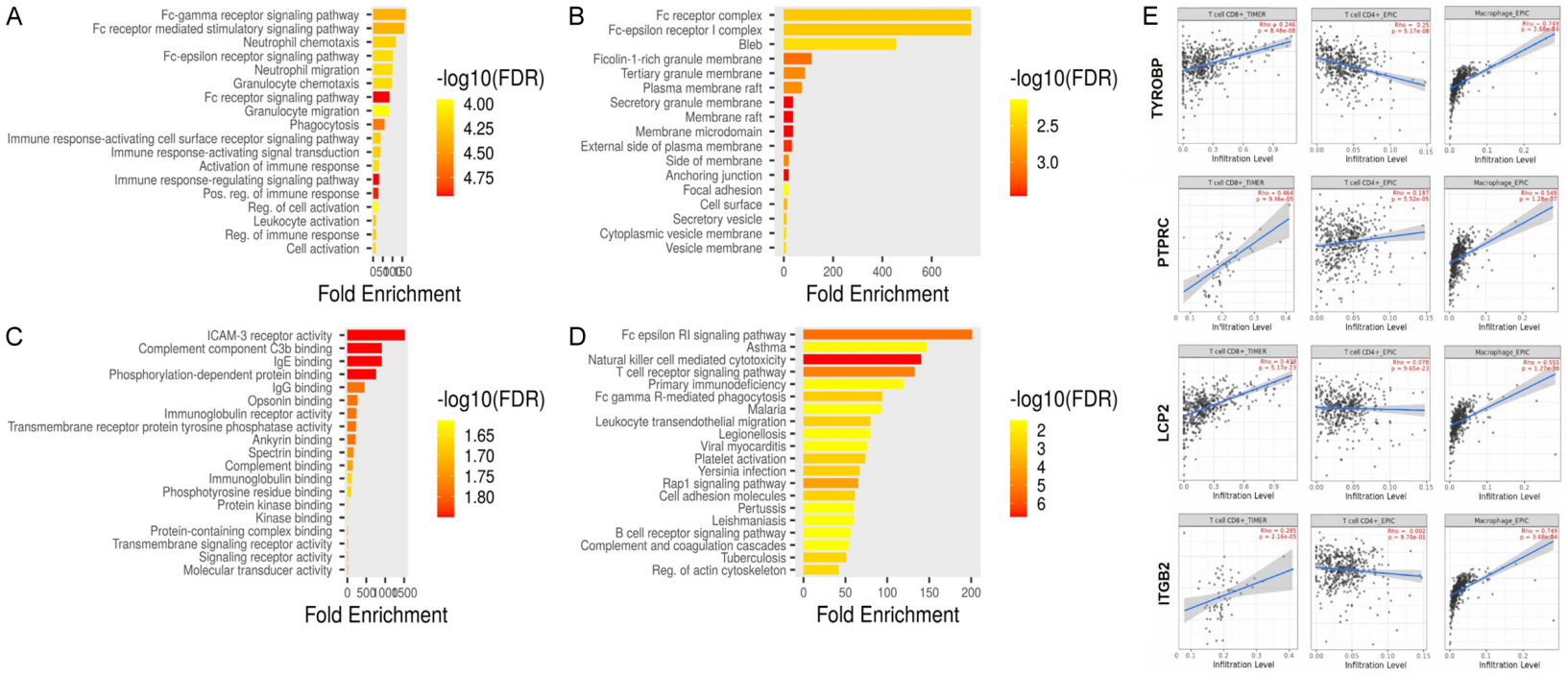


Figure 7. Gene enrichment and immune cell infiltration analyses of TYROBP, PTPRC, LCP2, and ITGB2. (A) TYROBP, PTPRC, LCP2, and ITGB2 associated CC terms, (B) TYROBP, PTPRC, LCP2, and ITGB2 associated BP terms, (C) TYROBP, PTPRC, LCP2, and ITGB2 associated MF terms, (D) TYROBP, PTPRC, LCP2, and ITGB2 associated KEGG terms, and (E) Immune cell infiltration analyses of the TYROBP, PTPRC, LCP2, and ITGB2 in KIRC. TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2. CC = Cellular Component, MF = Molecular Function, BP = Biological Process, KEGG = Kyoto Encyclopedia of Genes and Genomes.

including "ICAM-3 receptor activity, complement component C3b binding, IgE binding, phosphorylation-dependent activation of proteins, IgG binding, and opsonin binding" were significantly associated with hub genes (**Figure 7C**). Hub genes and their co-expressed genes associated KEGG pathways include "Fc epsilon RI signaling pathway, asthma, natural cell killer mediated cytotoxicity, T cell receptor signaling pathway, primary immunodeficiency, Fc gamma R-mediated phagocytosis, malaria, leukocyte transendothelial migration, and legionellosis" (**Figure 7D**).

Immune cells analysis of the hub genes: Using TIMER, we then identified relationships among different immune cell infiltration (CD8+ T, CD4+ T, and macrophages) and hub genes (TYROBP, PTPRC, LCP2, and ITGB2) expression. The TYROBP, PTPRC, LCP2, and ITGB2 expressions were positively correlated ($P < 0.05$) with the infiltration level of CD8+ T, CD4+ T cells, and macrophage cells (**Figure 7E**).

miRNA network of the hub genes: Using ENCORI and Cytoscape, we constructed the lncRNA-miRNA-mRNA co-regulatory networks of TYROBP, PTPRC, LCP2, and ITGB2. In the constructed networks, the total count of lncRNAs, miRNAs, and mRNAs were 68, 10, and 4, respectively (**Figure 8A**, **8B**). Based on the constructed networks, we identified one miRNA (has-mir-27a-3p) that targets all hub genes simultaneously. Therefore, we speculate that the identified lncRNAs, has-mir-27a-3p, and hub genes (TYROBP, PTPRC, LCP2, and ITGB2) (**Figure 8C**) as an axis, may also be inducers of KIRC.

Drug prediction analysis of the hub genes: For patients suffering from KIRC, medical treatment is the first option for treatment. Therefore, a selection of appropriate candidate drugs is required. In the current study, by DrugBank database, we explored drugs that might reverse the gene expressions of identified hub genes for the treatment of KIRC. We noted that Methotrexate and Calcitriol along with many other drugs were negative expression regulators of TYROBP, PTPRC, LCP2, and ITGB2 mRNA expression (**Table 1**).

In vitro validation of the hub genes expression and methylation levels: By performing RNA-seq, RT-qPCR, and targeted bisulfite-seq analyses of one RCC cell line (786-0) and one normal renal tubular epithelial cell line (HK-2), the expression and methylation levels of the TYROBP, PTPRC, LCP2, and ITGB2 were validated. As shown in **Figure 8D**, **8E**, TYROBP, PTPRC, LCP2, and ITGB2 genes were expressed in both normal and RCC cell lines, and expressions of TYROBP, PTPRC, LCP2, and ITGB2 were notably higher, in the RCC cell line (786-0) as compared to a normal cell line (HK-2) (**Figure 8D**, **8E**). Moreover, it was also noted that the beta values of TYROBP, PTPRC, LCP2, and ITGB2 were lower in the RCC cell line as compared to the normal cell line (**Figure 8F**).

Discussion

Kidney carcinomas are a heterogeneous group of diseases characterized by a variety of genetic alterations that are usually resistant to chemo or radiotherapy [34]. There are clear signs and symptoms of this disease, and nearly 30% of kidney cancer patients had metastasis at the time of diagnosis [35]. To discover novel markers for diagnosis, prognosis, and treatment, we analyzed GSE66272 gene expression datasets to uncover molecular mechanisms underpinning KIRC.

Through integrative analysis, we pinpoint four hub genes including TYROBP, PTPRC, LCP2, and ITGB2. We surprisingly noted that the mRNA expressions of those genes were significantly increased across KIRC samples relative to normal controls in the GSE66272 dataset. Further expression validation on TCGA datasets through bioinformatic analysis and on KIRC cell lines by in vitro analysis further confirmed the up-regulation of TYROBP, PTPRC, LCP2, and ITGB2 in KIRC cell lines and TCGA KIRC samples of different clinical variables. Of note, the TYROBP, PTPRC, and ITGB2 genes were the least mutated, while the LCP2 gene was relatively highly mutated in KIRC samples. Moreover, the TYROBP, PTPRC, LCP2, and ITGB2 genes were hypomethylated and insignificantly correlated with worseOS of the KIRC patients.

The protein encoded by the TYROBP gene is involved in activating various receptors found on the surfaces of different immune cells for mediating a variety of biologically important signaling pathways [36, 37]. Previous studies also highlighted that TYROBP may act as an activator or inhibitor of different immune cells

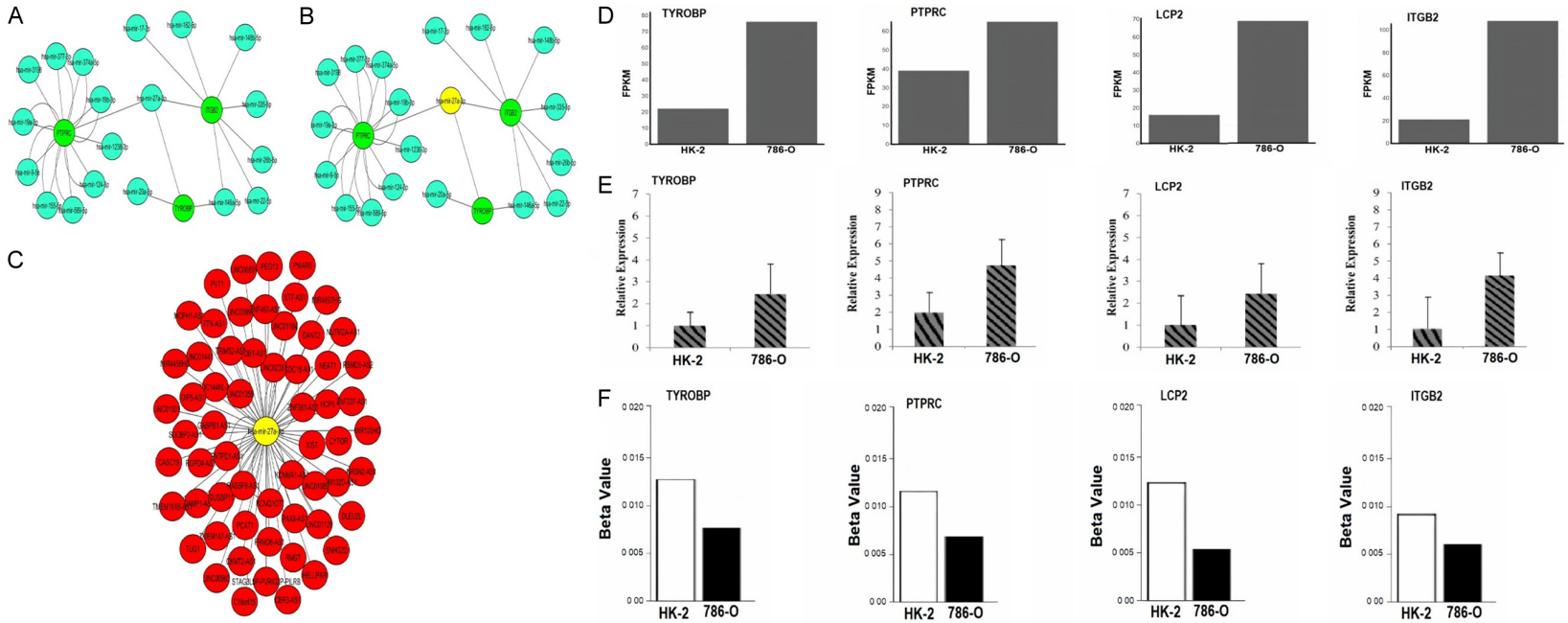


Figure 8. lncRNA-miRNA-mRNA co-regulatory network and validating TYROBP, PTPRC, LCP2, and ITGB2 expressions and promoter methylation levels in RCC cell line (786-0), and the normal renal tubular epithelial cell line (HK-2) by RNA-seq, RT-qPCR, and targeted bisulfite-seq analysis. (A) A PPI of miRNAs targeting hub genes, (B) PPI highlighting most important miRNA (hsa-mir-27a-3p) targeting all hub genes, and (C) A PPI of lncRNAs targeting hsa-mir-27a-3p, (D) FPKM values based expression validation of TYROBP, PTPRC, LCP2, and ITGB2, (E) RT-qPCR based expression validation of TYROBP, PTPRC, LCP2, and ITGB2 and (F) Beta values-based promoter methylation based validation of TYROBP, PTPRC, LCP2, and ITGB2. Blue nodes: miRNAs, green nodes: mRNAs, Yellow node: hsa-mir-27a-3p, and Red nodes: lncRNAs. lncRNA = long ncRNAs, miRNAs = Micro RNA, mRNA = Messenger RNA, TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2, RNA-seq = RNA sequencing, targeted bisulfite sequencing = targeted bisulfite-seq, RT-qPCR = Reverse transcription-quantitative polymerase chain reaction.

Table 1. DrugBank-based hub gene-associated drugs

Sr. No	Hub gene	Drug name	Effect	Reference	Group
1	TYROBP	Calcitriol	Decrease expression of TYROBP mRNA	A22299	Approved
		Methotrexate		A23201	
2	PTPRC	Calcitriol	Decrease expression of PTPRC mRNA	A22299	Approved
		Methotrexate		A23202	
		Tamibarotene		A20516	
		Silicon dioxide		A24195	
3	LCP2	Estradiol	Decrease expression of LCP2 mRNA	A21098	Approved
		Acetaminophen		A20420	
		Methotrexate		A23199	
4	ITGB2	Cyclosporine	Decrease expression of ITGB2 mRNA	A20661	Approved
		Azacitidine		A20985	
		Simvastatin		A24273	
		Hydroquinone		A23008	
		Methotrexate		A23202	

TYROBP = Transmembrane Immune Signaling Adaptor TYROBP, PTPRC = Protein tyrosine phosphatase, receptor type, C, LCP2 = Lymphocyte cytosolic protein 2, ITGB2 = Integrin Subunit Beta 2.

such as natural killer cells, granulocytes, monocytes, myeloid cells, and many others. When TYROBP acts as an activator, it is involved in activating natural killer cells by mediating signaling between PI3K-Akt, MAPK, PLCγ, and their other downstream pathways. However, when TYROBP acts as an inhibitor, it is involved in the inhibition of Toll-like receptor-mediated activation, which is very important for stimulating DNA damage response pathways [38-40]. Recently, the overexpression of the TYROBP gene has been established as an oncogenic factor in gastric cancer [41]. Besides this, other studies in the medical literature showed that overexpression of TYROBP in breast cancer (BC) is correlated with worse prognosis and bone metastasis [42]. However, little is reported about the function of TYROBP in KIRC.

The PTPRC gene encodes for a highly conserved protein tyrosine phosphatase CD45, which may be a regulator of B and T cell receptor signaling pathways [43-45]. The role of CD45 protein in cancer initiation, progression, and metastasis is still contradictory. Chen et al. in their study showed that CD45 and CD71+ erythroid cells accusation in liver cancer cells may play a vital role in tumor growth [46]. More studies demonstrated that the higher expression of CD45 protein was significantly associated with the worst prognosis of non-small cell lung cancer (NSCLC) patients [47]. However, on the other hand, CD45 overexpression was also correlated with a better prognosis of different other cancer patients [48]. For example, a study by Wei et al. revealed the significant down-regulation of PTPRC in LUAD and correlated it with a better prognosis of LUAD patients [49].

LCP2 is an actin-binding protein that is involved in numerous cell signaling pathways [50]. LCP2 is an activator of T cells, whose activation is linked with the secretion of IL-2 and IFN-γ [51, 52]. The lymphocyte cytoplasmic protein family is largely expressed by hematopoietic cells under normal conditions. Recently, the higher expression of LCP2 has been documented across different types of malignant tumors [53, 54]. It was observed by previous studies that LCP2 was correlated with worse prognosis in gastric cancer BC patients [55]. However, nothing was reported about the function of LCP2 in KIRC.

ITGB2 gene encodes for the β2 integrins that are important for immune cell trafficking [56]. According to earlier reports, ITGB2 dysregulation was associated with the development of various diseases, such as carotid atherosclerosis and different types of cancer [57]. ITGB2 was overexpressed across atheroma plaque samples compared to intact tissue samples and was a risk factor for carotid atherosclerosis development [58]. Moreover, the down-regulation of this gene results in the hyperresponsive-

ness of the Toll-like receptor and causes the pathogenesis of necrotizing enterocolitis [59]. In cancer-based studies, the expression of ITGB2 was overexpressed in malignant gliomas and was correlated with worse prognosis of cancer patients [60]. Overexpressed ITGB2 was proven to be linked to the enhanced invasion and metastatic abilities of breast cancer cells [61]. In addition to this, ITGB2 higher expression was associated with worse prognosis in patients with acute myeloid leukemia [62]. In sum, the outcomes of all these studies indicate that ITGB2 dysregulation is a key alteration in cancer development and progression.

Immune cell infiltration, particularly of CD8+ T cells, CD4+ T cells, and macrophages, is critical in tumor growth and successful immunotherapy [63]. In the present study, the TYROBP, PTPRC, LCP2, and ITGB2 expressions were positively correlated with the infiltration levels of CD8+ T, CD4+ T, and macrophage cells. Earlier, the increased infiltration levels of CD8+ T cells, CD4+ T, and macrophages cells were correlated with the worse prognosis of cancer patients [64]. Therefore, we speculate that the increased infiltration levels of CD8+ T, CD4+ T, and macrophage cell with respect to TYROBP, PTPRC, LCP2, and ITGB2 gene expression may also participate in the development and worse prognosis of KIRC. We further noticed that TYROBP, PTPRC, LCP2, and ITGB2 hub genes' expression were regulated simultaneously by hsa-mir-27a-3p miRNA in KIRC patients. Previously, the dysregulation of hsa-mir-27a-3p in multiple human cancers has been reported in published studies, for example in breast cancer, bladder cancer, glioblastoma, and esophageal cancer [65, 66]. However, no tumor suppressor or tumor-causing role of hsa-mir-27a-3p in KIRC has been reported. Therefore, to the best of our knowledge, this study is the first to report a probable cancer-driving role of the hsa-mir-27a-3p miRNA with respect to the TYROBP, PTPRC, LCP2, and ITGB2 hub genes in KIRC.

To further enhance the understanding of molecular pathways underpinning KIRC initiation and progression, we conducted the KEGG analysis of hub genes and their co-expressed genes. Through this analysis, it was revealed that hub genes and their co-expressed genes were enriched in "Fc epsilon RI signaling pathway, asthma, natural cell killer mediated cytotoxicity, T cell receptor signaling pathway, primary immunodeficiency, Fc gamma R-mediated phagocytosis, malaria, leukocyte transendothelial migration, and legionellosis". Previously, a number of studies had shown the involvement of these pathways in tumor development and metastasis [67-71].

Conclusion

Use of GEO expression dataset and high throughput RNA-seq and bisulfite-seq analyses, enabled us to explore TYROBP, PTPRC, LCP2, and ITGB2 hub genes as diagnostic, and adverse prognostic biomarkers of KIRC. This study also discovered some important KIRC-associated pathways, such as "Fc epsilon RI signaling pathway, asthma, natural cell killer mediated cytotoxicity, T cell receptor signaling pathway, primary immunodeficiency, Fc gamma R-mediated phagocytosis, malaria, leukocyte transendothelial migration, and legionellosis". In addition, TYROBP, PTPRC, LCP2, and ITGB2 hub genes may also become novel therapeutic targets for KIRC therapy.

Acknowledgements

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSPD2023R1089), King Saud University, Riyadh, Saudi Arabia.

Disclosure of conflict of interest

None.

Address correspondence to: Muhammad Jamil, PARC Arid Zone Research Center, Dera Ismail Khan 29050, Pakistan. E-mail: jamilmatrah@parc.gov.pk

References

[1] Sial N, Saeed S, Ahmad M, Hameed Y, Rehman A, Abbas M, Asif R, Ahmed H, Hussain MS, Rehman JU, Atif M and Khan MR. Multi-omics analysis identified TMED2 as a shared potential biomarker in six subtypes of human cancer. Int J Gen Med 2021; 14: 7025-7042.

[2] Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, Rawla P and Barsouk A. Epidemiology of renal cell carcinoma. World J Oncol 2020; 11: 79-87.

[3] Chow WH, Dong LM and Devesa SS. Epidemiology and risk factors for kidney cancer. Nat Rev Urol 2010; 7: 245-257.

[4] Sanchez DJ and Simon MC. Genetic and metabolic hallmarks of clear cell renal cell carcino-

ma. Biochim Biophys Acta Rev Cancer 2018; 1870: 23-31.
[5] Mao J, Huang X, Okla MK, Abdel-Maksoud MA, Mubarak A, Hameed Z, Noreen R, Chaudhary A, Ghazanfar S, Liao Y, Hameed Y and Li C. Risk factors for TERT promoter mutations with papillary thyroid carcinoma patients: a meta-analysis and systematic review. Comput Math Methods Med 2022; 2022: 1721526.
[6] Liao G, Wang P and Wang Y. Identification of the prognosis value and potential mechanism of immune checkpoints in renal clear cell carcinoma microenvironment. Front Oncol 2021; 11: 720125.
[7] Hameed Y, Usman M and Ahmad M. Does mouse mammary tumor-like virus cause human breast cancer? Applying bradford hill criteria postulates. Bull Natl Res Cent 2020; 44: 1-13.
[8] Cui H, Shan H, Miao MZ, Jiang Z, Meng Y, Chen R, Zhang L and Liu Y. Identification of the key genes and pathways involved in the tumorigenesis and prognosis of kidney renal clear cell carcinoma. Sci Rep 2020; 10: 4271.
[9] Barata PC and Rini BI. Treatment of renal cell carcinoma: current status and future directions. CA Cancer J Clin 2017; 67: 507-524.
[10] Wotschofsky Z, Gummlich L, Liep J, Stephan C, Kilic E, Jung K, Billaud JN and Meyer HA. Integrated microRNA and mRNA signature associated with the transition from the locally confined to the metastasized clear cell renal cell carcinoma exemplified by miR-146-5p. PLoS One 2016; 11: e0148746.
[11] Clough E and Barrett T. The gene expression omnibus database. Methods Mol Biol 2016; 1418: 93-110.
[12] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.
[13] von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res 2003; 31: 258-261.
[14] Demchak B, Hull T, Reich M, Liefeld T, Smoot M, Ideker T and Mesirov JP. Cytoscape: the network visualization tool for GenomeSpace workflows. F1000Res 2014; 3: 151.
[15] Bandettini WP, Kellman P, Mancini C, Booker OJ, Vasu S, Leung SW, Wilson JR, Shanbhag SM, Chen MY and Arai AE. MultiContrast delayed enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: a clinical validation study. J Cardiovasc Magn Reson 2012; 14: 83.
[16] Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 2014; 8 Suppl 4: S11.
[17] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.
[18] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.
[19] Tang G, Cho M and Wang X. OncoDB: an interactive online database for analysis of gene expression and viral infection in cancer. Nucleic Acids Res 2022; 50: D1334-D1339.
[20] Park SJ, Yoon BH, Kim SK and Kim SY. GENT2: an updated gene expression database for normal and tumor tissues. BMC Med Genomics 2019; 12 Suppl 5: 101.
[21] Thul PJ and Lindskog C. The human protein atlas: a spatial map of the human proteome. Protein Sci 2018; 27: 233-244.
[22] Koch A, De Meyer T, Jeschke J and Van Criekinge W. MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. BMC Genomics 2015; 16: 636.
[23] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.
[24] Zhang J, Xing Z, Ma M, Wang N, Cai YD, Chen L and Xu X. Gene ontology and KEGG enrichment analyses of genes related to age-related macular degeneration. Biomed Res Int 2014; 2014: 450386.
[25] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
[26] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B and Liu XS. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017; 77: e108-e110.
[27] Huang DP, Zeng YH, Yuan WQ, Huang XF, Chen SQ, Wang MY, Qiu YJ and Tong GD. Bioinformatics analyses of potential miRNA-mRNA regulatory axis in HBV-related hepatocellular carcinoma. Int J Med Sci 2021; 18: 335-346.
[28] Freshour SL, Kiwala S, Cotto KC, Coffman AC, McMichael JF, Song JJ, Griffith M, Griffith OL and Wagner AH. Integration of the drug-gene

interaction database (DGIdb 4.0) with open crowdsource efforts. Nucleic Acids Res 2021; 49: D1144-D1151.
[29] Feng Z, Li Y, McKnight KL, Hensley L, Lanford RE, Walker CM and Lemon SM. Human pDCs preferentially sense enveloped hepatitis A virions. J Clin Invest 2015; 125: 169-176.
[30] Ghatak S, Muthukumaran RB and Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013; 24: 224-231.
[31] Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25: 402-408.
[32] Kim TK. T test as a parametric statistic. Korean J Anesthesiol 2015; 68: 540-546.
[33] Kim HY. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. Restor Dent Endod 2017; 42: 152-155.
[34] Testa U, Pelosi E and Castelli G. Genetic alterations in renal cancers: identification of the mechanisms underlying cancer initiation and progression and of therapeutic targets. Medicines (Basel) 2020; 7: 44.
[35] Usman M, Okla MK, Asif HM, AbdElgayed G, Muccee F, Ghazanfar S, Ahmad M, Iqbal MJ, Sahar AM, Khaliq G, Shoaib R, Zaheer H and Hameed Y. A pan-cancer analysis of GINS complex subunit 4 to identify its potential role as a biomarker in multiple human cancers. Am J Cancer Res 2022; 12: 986-1008.
[36] Lanier LL, Corliss B, Wu J and Phillips JH. Association of DAP12 with activating CD94/NKG2C NK cell receptors. Immunity 1998; 8: 693-701.
[37] Lanier LL, Corliss BC, Wu J, Leong C and Phillips JH. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. Nature 1998; 391: 703-707.
[38] Takaki R, Watson SR and Lanier LL. DAP12: an adapter protein with dual functionality. Immunol Rev 2006; 214: 118-129.
[39] Bouchon A, Dietrich J and Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. J Immunol 2000; 164: 4991-4995.
[40] Turnbull IR and Colonna M. Activating and inhibitory functions of DAP12. Nat Rev Immunol 2007; 7: 155-161.
[41] Jiang J, Ding Y, Wu M, Lyu X, Wang H, Chen Y, Wang H and Teng L. Identification of TYROBP and C1QB as two novel key genes with prognostic value in gastric cancer by network analysis. Front Oncol 2020; 10: 1765.
[42] Moretta L and Moretta A. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J 2004; 23: 255-259.
[43] Porcu M, Kleppe M, Gianfelici V, Geerdens E, De Keersmaecker K, Tartaglia M, Foa R, Soulier J, Cauwelier B, Uyttebroeck A, Macintyre E, Vandenberghe P, Asnafi V and Cools J. Mutation of the receptor tyrosine phosphatase PTPRC (CD45) in T-cell acute lymphoblastic leukemia. Blood 2012; 119: 4476-4479.
[44] Courtney AH, Shvets AA, Lu W, Griffante G, Mollenauer M, Horkova V, Lo WL, Yu S, Stepanek O, Chakraborty AK and Weiss A. CD45 functions as a signaling gatekeeper in T cells. Sci Signal 2019; 12: eaaw8151.
[45] Rheinländer A, Schraven B and Bommhardt U. CD45 in human physiology and clinical medicine. Immunol Lett 2018; 196: 22-32.
[46] Chen J, Qiao YD, Li X, Xu JL, Ye QJ, Jiang N, Zhang H and Wu XY. Intratumoral CD45(+) CD71(+) erythroid cells induce immune tolerance and predict tumor recurrence in hepatocellular carcinoma. Cancer Lett 2021; 499: 85-98.
[47] Gao P and Ren G. Identification of potential target genes of non-small cell lung cancer in response to resveratrol treatment by bioinformatics analysis. Aging (Albany NY) 2021; 13: 23245-23261.
[48] Zhao X, Kallakury B, Chahine JJ, Hartmann D, Zhang Y, Chen Y, Zhang H, Zhang B, Wang C and Giaccone G. Surgical resection of SCLC: prognostic factors and the tumor microenvironment. J Thorac Oncol 2019; 14: 914-923.
[49] Wei J, Fang D and Zhou W. CCR2 and PTPRC are regulators of tumor microenvironment and potential prognostic biomarkers of lung adenocarcinoma. Ann Transl Med 2021; 9: 1419.
[50] Huo Y, Zhang K and Han S. Lymphocyte cytosolic protein 2 is a novel prognostic marker in lung adenocarcinoma. J Int Med Res 2021; 49: 3000605211059681.
[51] Sommers CL, Menon RK, Grinberg A, Zhang W, Samelson LE and Love PE. Knock-in mutation of the distal four tyrosines of linker for activation of T cells blocks murine T cell development. J Exp Med 2001; 194: 135-142.
[52] Maltzman JS, Kovoor L, Clements JL and Koretzky GA. Conditional deletion reveals a cell-autonomous requirement of SLP-76 for thymocyte selection. J Exp Med 2005; 202: 893-900.
[53] Moncayo G, Grzmil M, Smirnova T, Zmarz P, Huber RM, Hynx D, Kohler H, Wang Y, Hotz HR, Hynes NE, Keller G, Frank S, Merlo A and Hemmings BA. SYK inhibition blocks proliferation and migration of glioma cells and modifies the tumor microenvironment. Neuro Oncol 2018; 20: 621-631.

[54] Dezorella N, Katz BZ, Shapiro M, Polliack A, Perry C and Herishanu Y. SLP76 integrates into the B-cell receptor signaling cascade in chronic lymphocytic leukemia cells and is associated with an aggressive disease course. Haematologica 2016; 101: 1553-1562.
[55] Usman M, Hameed Y, Ahmad M, Iqbal MJ, Maryam A, Mazhar A, Naz S, Tanveer R, Saeed H, Bint-E-Fatima, Ashraf A, Hadi A, Hameed Z, Tariq E and Aslam AS. SHMT2 is associated with tumor purity, CD8+ T immune cells infiltration, and a novel therapeutic target in four different human cancers. Curr Mol Med 2023; 23: 161-176.
[56] Fagerholm SC, Guenther C, Llort Asens M, Savinko T and Uotila LM. Beta2-integrins and interacting proteins in leukocyte trafficking, immune suppression, and immunodeficiency disease. Front Immunol 2019; 10: 254.
[57] Zu L, He J, Zhou N, Zeng J, Zhu Y, Tang Q, Jin X, Zhang L and Xu S. The profile and clinical significance of ITGB2 expression in non-small-cell lung cancer. J Clin Med 2022; 11: 6421.
[58] Meng Y, Zhang C, Liang L, Wei L, Wang H, Zhou F, Li R, Zou D, Huang X and Liu J. Identification of potential key genes involved in the carotid atherosclerosis. Clin Interv Aging 2021; 16: 1071-1084.
[59] George L, Menden H, Xia S, Yu W, Holmes A, Johnston J, Reid KJ, Josephson CD, Patel RM, Ahmed A, Mulrooney N, Miller NA, Farrow E and Sampath V. ITGB2 (Integrin β2) immunomodulatory gene variants in premature infants with necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 2021; 72: e37-e41.
[60] Sai Krishna AVS, Ramu A, Hariharan S, Sinha S and Donakonda S. Characterization of tumor microenvironment in glioblastoma multiforme identifies ITGB2 as a key immune and stromal related regulator in glial cell types. Comput Biol Med 2023; 165: 107433.
[61] Liu M, Gou L, Xia J, Wan Q, Jiang Y, Sun S, Tang M, He T and Zhang Y. LncRNA ITGB2-AS1 could promote the migration and invasion of breast cancer cells through up-regulating ITGB2. Int J Mol Sci 2018; 19: 1866.
[62] Wei J, Huang XJ, Huang Y, Xiong MY, Yao XY, Huang ZN, Li SN, Zhou WJ, Fang DL, Deng DH and Cheng P. Key immune-related gene ITGB2 as a prognostic signature for acute myeloid leukemia. Ann Transl Med 2021; 9: 1386.
[63] Li Y, Tao L and Cai W. Profiles of immune infiltration and prognostic immunoscore in lung adenocarcinoma. Biomed Res Int 2020; 2020: 5858092.
[64] Tanaka A and Sakaguchi S. Regulatory T cells in cancer immunotherapy. Cell Res 2017; 27: 109-118.
[65] Zhu B, Chen W, Fu Y, Cui X, Jin L, Chao J, Yun X, Gao P, Shan S, Li J, Yin X, Zhu C and Qin X. MicroRNA-27a-3p reverses adriamycin resistance by targeting BTG2 and activating PI3K/Akt pathway in breast cancer cells. Onco Targets Ther 2020; 13: 6873-6884.
[66] Salmani T, Ghaderian SMH, Hajiesmaeili M, Rezaeimirghaed O, Hoseini MS, Rakhshan A, Nasiri MJ, Ghaedi H and Akbarzadeh R. Hsa-miR-27a-3p and epidermal growth factor receptor expression analysis in glioblastoma FFPE samples. Asia Pac J Clin Oncol 2021; 17: e185-e190.
[67] Portales-Cervantes L, Dawod B and Marshall JS. Mast cells and natural killer cells-a potentially critical interaction. Viruses 2019; 11: 514.
[68] Karimi K and Forsythe P. Natural killer cells in asthma. Front Immunol 2013; 4: 159.
[69] Amiah MA, Ouattara A, Okou DT, N'Guetta SA and Yavo W. Polymorphisms in Fc gamma receptors and susceptibility to malaria in an endemic population. Front Immunol 2020; 11: 561142.
[70] Fall AKDJ, Dechavanne C, Sabbagh A, Guitard E, Milet J, Garcia A, Dugoujon JM, Courtin D and Migot-Nabias F. Susceptibility to plasmodium falciparum malaria: influence of combined polymorphisms of IgG3 Gm allotypes and Fc gamma receptors IIA, IIIA, and IIIB. Front Immunol 2020; 11: 608016.
[71] Nunnink JC, Gallagher JG and Yates JW. Legionnaires' disease in patients with cancer. Med Pediatr Oncol 1986; 14: 81-85.