

LY96 Upregulation Promotes Kidney Renal Clear Cell Carcinoma (KIRC)

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Research

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Abstract

Background: LY96 has been reported to be relevant with kidney inflammatory injury but the function of this gene in kidney renal clear cell carcinoma (KIRC) remains unknown.

Methods: Various online tools were applied to analyze the roles of LY96 in KIRC using data from the Cancer Genome Atlas. Differential LY96 expression and overall survival (OS) based on different expression levels were analyzed through Oncomine and GEPIA tools. The alterations, related genes, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes pathways of LY96 were explored via cBioPortal and STRING database. LinkedOmics and Cistrome DB Toolkit were utilized to identify targets of kinase, miRNAs, and transcription factors. The relationship between LY96 and some associated genes or regulatory factors was displayed via GeneMANIA and TIMER tool. TISIDB revealed correlations between LY96 expression and immune-associated factors in the tumor microenvironment.

Results: High LY96 expression level was observed in KIRC and associated with poor prognosis and diverse clinical characteristics. LY96 often amplified in KIRC and was mostly linked to the inflammatory response. Several highly correlated genes, kinase targets, transcription factors, and DNA methyltransferase that may interact with LY96 were all identified. Our study also demonstrated that various immune-related factors were relevant to LY96 in KIRC.

Conclusions: Our study has shown the complex relationships between LY96 and KIRC from diverse angles. High LY96 expression had an adverse effect on the prognosis of KIRC. To find effective demethylation agents and transcription factors inhibitors targeting LY96 may have beneficial effects on the survival of KIRC patients.

Introduction

Renal cell carcinoma (RCC) is categorized into three subtypes: clear cell renal cell carcinoma (ccRCC), papillary renal cell carcinoma (pRCC), and chromophobe renal cell carcinoma (chRCC). Kidney renal clear cell carcinoma (KIRC) is the most common type of renal malignancy, accounting for 75% of RCC[1]. The incidence and cancer-related mortality of KIRC is increasing year by year and have become a global health problem. Early stage RCC is often treated by surgery and the overall survival (OS) can reach 60%-70%[2]. However, metastasis RCC remains incurable and is mainly treated with targeted therapies[3]. Clinically, tyrosine kinase inhibitors (TKIs) sunitinib and sorafenib are used to improve the OS of mRCC greatly[4-6]. Thus, discovery of novel therapeutic drugs or targets is an urgent need.

MD2/LY96 (Myeloid Differentiation Protein-2/Lymphocyte Antigen 96) is an accessory receptor lacking a transmembrane domain and the co-receptor for Toll-like receptor 4 (TLR4)[7]. MD2/LY96 contributes to lipopolysaccharide (LPS) recognition and the subsequent TLR4 activation[8]. It can also cooperate with TLR4 in the innate immune response to bacterial LPS, respond to cell wall components from G+ and G- bacteria with TLR-2 and enhance TLR4-dependent activation of NF- κ B[9-10]. Previous studies indicated that stimulation of TLR-4/MD2 complex by LPS increased the risk of liver metastasis from primary

colorectal cancer (CRC)[11]. Inhibition of MD2 was also confirmed to be an important target for colon cancer therapies[12]. Beyond this, TLR-4 and MD-2 were reported to be associated with gastric cell proliferation and invasion[13].

In this study, we comprehensively analyzed the relationship between LY96 expression levels and KIRC using the Oncomine, GEPIA, UALCAN, c-BioPortal, STRING, LinkedOmics, GeneMANIA, TIMER, TISIDB, CancerSEA, MEXPRESS, Cistrome DB Toolkit, ENCORI and TRRUST public database. We analyzed genomic alterations, functional network, and survival characteristics of LY96 in KIRC. And the propose of this study aims to determine whether LY96 can be a novel potential biomarker of KIRC.

Materials And Methods

Oncomine analysis

Oncomine is the largest oncogene chip database and data mining platform including 715 gene expression data sets and 86733 tumor and normal tissue samples (<http://www.oncomine.org>)[14]. We analyzed a series of KIRC studies with LY96, mainly incorporating Gumz Renal, Lenburg Renal, Beroukhim Renal, Yusenko Renal, and Jones Renal. When LY96 expression differences in KIRC tissues and normal tissues associated with $p < .01$ were considered significant.

GEPIA analysis

GEPIA (<http://gepia2.cancer-pku.cn>) is an open web that contains 9736 tumor and 8587 normal samples from TCGA and GTEx projects[15]. Correlations between LY96 expression levels and prognosis of KIRC were displayed by Kaplan-Meier (KM) survival curves. Besides, the relationships between LY96 expression and the outcomes of KICH (Kidney Chromophobe) and KIRP (kidney renal papillary cell carcinoma) were also showed via GEPIA.

UALCAN analysis

UALCAN is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data (<http://ualcan.path.uab.edu>)[16]. The ualcan database provides convenient access to public cancer transcriptome data, identification of biomarkers and patient survival information based on different gene expression, cancer stages, tumor grade and clinical characteristics.

c-BioPortal analysis

The cBio Cancer Genomics Portal (<http://cbioportal.org>) is an open-access resource for interactive exploration of multidimensional cancer genomics data sets[17]. The OncoPrint showed an overview of LY96 alterations in KIRC, mainly including mutation, CNVs, and mRNA expression.

STRING analysis

The STRING database (<https://string-db.org/>) aims to collect, score and integrate all publicly available sources of protein-protein interaction information[18]. Gene Ontology (GO) function, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, protein-to-protein interaction (PPI) networks of LY96 were performed using the STRING and related results were obtained.

LinkedOmics analysis

LinkedOmics is publicly available portal that includes multi-omics and clinical data from all [32 TCGA Cancer types](#)[19]. It also includes mass spectrometry-based proteomics data generated by the Clinical Proteomics Tumor Analysis Consortium (CPTAC) on selected TCGA tumor samples. The differentially expressed genes (DEGs) associated with LY96 were screened from the TCGA KIRC cohort via the LinkFinder module. Pearson correlation coefficient was utilized for correlation analysis. Outcomes were visualized graphically using volcano plots, heat maps or scatter plots. Data from the results of LinkFinder module were ranked and analysis of kinase target, miRNA-target, transcription factor-target were explored via LinkInterpreter module. The rank criteria was FDR<.05 and 500 stimulations were performed.

GeneMANIA analysis

GeneMANIA (<http://www.genemania.org>) is a web site for constructing protein-protein interaction (PPI) network, generating hypotheses about gene function and analyzing gene lists[20]. Given a query list, GeneMANIA can find functionally similar genes using a number of genomics and proteomics data. Another important role of GeneMANIA is gene function prediction. GeneMANIA can find genes likely to share function with LY96 according to their interactions. In addition, many genes have been proved to be related to KIRC. Thus, we used GeneMANIA to explore the relationship between LY96 and several significant genes.

TIMER analysis

TIMER is a public resource dedicated to tumor immune infiltrates across 32 cancer types incorporating 10897 samples from The Cancer Genome Atlas (TCGA) (<http://timer.comp-genomics.org/>)[21]. The relevance of LY96 expression with several related genes and regulatory factors were analyzed by TIMER.

TISIDB analysis

TISIDB (<http://cis.hku.hk/TISIDB>) is a web portal that integrates multiple heterogeneous data types for analysis of tumor and immune system interactions[22].

Spearman correlations between LY96 and diverse immune factors were analyzed from the TISIDB database including immune-inhibitory and stimulatory factors, receptors, and chemokines.

CancerSEA analysis

CancerSEA (<http://biocc.hrbmu.edu.cn/CancerSEA/>) is the first single-cell sequencing database aimed to comprehensively analyze the 14 functional states of cancer cells at single-cell level[23]. In this study, we

used it to explore the potential roles of LY96 in KIRC.

MEXPRESS analysis

MEXPRESS (<https://mexpress.be/>) is a data visualization tool for the relationships between DNA methylation status and clinical data[24]. And the methylation status of LY96 was performed by MEXPRESS web tool.

Cistrome Data Brower (DB) Toolkit analysis

The Cistrome DB Toolkit (<http://cistrome.org/db>) is a user-friendly, up to date, and well maintained resource, which was utilized to depict the genome-wide locations of transcription factor binding sites, histone post-translation modifications and regions of chromatin accessible to endonuclease activity[25]. We used this tool to infer which transcription factors (TFs) were most likely to increase the expression of LY96 in KIRC.

ENCORI analysis

ENCORI is an open-source platform for studying the miRNA-NcRNA, miRNA-mRNA, ncRNA-RNA, RNA-RNA, RBP-ncRNA, and RBP-mRNA interactions from CLIP-seq, degradome-seq and RNA-RNA interactome data[26]. This tool was performed to verify the difference of LY96 expression in KIRC and normal tissues.

TRRUST

TRRUST (<https://www.grnpedia.org/trrust/>) is an intuitive tool for human and mouse transcriptional regulatory networks[27]. The TFs of LY96 gene were predicted via TRRUST.

Results

LY96 expression in KIRC

We analyzed LY96 transcription levels in KIRC tumors by Oncomine database and observed that the mRNA expression of LY96 in KIRC tissues was significantly higher than normal tissues (**Figure 1**). Besides, the mRNA expression of LY96 was among the top 8% and the differences were all more than twofold between KIRC patients and normal tissues. To further determine the role of LY96 in KIRC, we utilized multiple clinical factors of KIRC samples in the TCGA database to compare the LY96 expression levels in each group, including histological stages, race, gender, age, tumor grade, KIRC subtype, and nodal metastasis status (**Figure 2a-h**). The results of all groups showed that KIRC patients always displayed a higher transcription level of LY96 than normal people. And this conclusion was also validated by ENCORI ($P < .01$) (**Figure 2i**). Thus, LY96 may serve as a novel biomarker in KIRC.

The relationship between LY96 expression levels and prognosis of KIRC patients

Survival curves were utilized to assess the effect of LY96 on KIRC, KICH, and KIRP prognosis. The results showed a significant association between LY96 expression levels and survival of KIRC patients. And the high expression of LY96 was prognostic for worse survival in KIRC. However, no significant relevance was observed between LY96 expression and the OS of KICH or KIRP patients (**Figure 3**).

Genomic alterations of LY96 in KIRC

We used the cBioPortal to find out the frequency of LY96 alterations in KIRC according to the data from the TCGA database. LY96 was 0.9% (16/1598) altered in KIRC patients. The alterations included amplification in 12 cases (0.75%), mutation in 3 case (0.19%), and deep deletion in 1 case (0.06%). Therefore, amplification was the most frequent type of LY96 CNV in KIRC (**Figure 4**).

Enrichment analysis of LY96 functional network in KIRC

To further explore the interactions of LY96, GO functional enrichment and KEGG pathway analysis were carried out (Table 1). The GO enrichment analysis is composed of three main parts: GO biological process (GO-BP), GO molecular function (GO-MF), and GO cellular component (GO-CC). GO-BP included 243 GO-terms, the most important of which were toll-like receptor signaling pathway, innate immune response-activating signal transduction and MyD88-dependent toll-like receptor signaling pathway. GO-MF contained 28 items and LY96 was mainly involved in signaling receptor activity, lipopolysaccharide receptor activity and lipopolysaccharide binding. Besides, LY96 was enriched for 41 GO-CC terms and endolysosome membrane, phagocytic vesicle, and lipopolysaccharide receptor complex were the top three categories. A total of 21 items were included in the KEGG pathways analysis. LY96 was predominantly related to Toll-like receptor signaling pathway, Tuberculosis, and Chagas disease (American trypanosomiasis). Based on the correlation score, the most related genes were TLR4, LY86, TLR2, TLR1, and CD180 (**Table 2**). The PPI network displayed intricate relationship between and neighboring genes in **Figure 5**. LinkedOmics was also used to analyze the mRNA sequencing data from 533 KIRC patients. The positive and negative correlations between 50 significant genes and LY96 were exhibited in the heatmap (**Figure 6a-b**). Furthermore, 1675 genes (red dots) displayed significant positive associations with LY96, while 1949 genes (green dots) showed negative correlations (FDR<0.01) (**Figure 6c**). The statistical scatter plots for most related three genes were shown in **Figure 6d-h**. These genes including LAPT M5 (cor=0.706, P=1.382e-81, FDR=1.393e-77), FCGR1B (cor=0.694, P=7.951e-78, FDR=5.343e-74), FCGR1A (cor=0.693, P=1.894e-77, FDR=9.547e-74), FCER1G (cor=0.684, P=9.555e-75, FDR=3.573e-71), and EVI2A (cor=0.684, P=1.063e-74, FDR=3.573e-71) all indicated strong correlations with LY96.

The correlation between LY96 and key genes in KIRC

Previous studies have demonstrated that many genes are associated with the prognosis of KIRC. A study stated that the most frequent mutated genes in KIRC were VHL followed by PBRM1, BAP1 and SETD2[28]. Several studies have reported some key genes or immune-related genes, such as PTTG1, RRM2, TOP2A, UHFR1, CEP55, BIRC5, UBE2C, FOXM1, CDC20, PLA2U, ISG15, IRF9, ARG2, RNASE2,

SEMA3G and UCN[29-30]. The OS of the above genes was performed using GEPIA database. The results showed that PBRM1, SETD2, PTTG1, RRM1, TOP2A, UHRF1, CEP55, BIRC5, UBE2C, FOXM1, CDC20, ISG15, RNASE2, SEMA3G, and UCN were significantly associated with survival (**Supplementary Figure 1**). The correlation between LY96 and these genes was also analyzed by GeneMANIA (**Supplementary Figure 2**).

LY96 networks of kinase, miRNA or transcription factor targets in KIRC

To further investigate the targets of LY96 in KIRC, we explored the kinase, miRNA, and transcription factor target networks of positively related gene sets generated by GSEA. The most significant kinase-target networks related primarily to the kinase LYN (v-yes-1 Yanaguchi sarcoma viral related oncogene homolog), LCK (lymphocyte-specific protein tyrosine kinase), SYK (Spleen tyrosine kinase), SGK1 (ubiquitous serum and glucocorticoid regulated kinase 1), MAPKAPK2 (mitogen-activated protein kinase-activated protein kinase 2), and HCK (hematopoietic cell kinase). The transcription factor-target network was significantly related to the ELF1_Q6, NFKB_Q6_01, ETS_Q4, PPARA_01, PEA3_Q6, IRF_Q6, PU1_Q6, ETS1_B, ETS2_B, AML_Q6, and COREBINDINGFACTOR_Q6. However, no significant miRNA-target networks met the selection criteria (FDR>0.05) (**Table 3**). In addition, we examined associations between the methylation status of LY96 and a variety of clinical characteristics. The results showed that race, stage, and T-staging all had close correlations with the methylation level of LY96 (P<.05). Higher methylation levels were observed in black and African American, stage II and T2-staging patients (**Figure 6g-i**).

LY96 expression associated with immune factors and immune gene markers

To understand the relevance between LY96 and different infiltrated immune cells including activated CD8 T cell (Act_CD8), central memory CD8 T cell (Tcm_CD8), effector memory CD8 T cell (Tem_CD8), activated CD4 T cell (Act_CD4), central memory CD4 T cell (Tcm_CD4), effector memory CD4 T cell (Tem_CD4), T follicular helper cell (Tfh), gamma delta T cell (Tgd), type 1 T helper cell (Th1), type 17 T helper cell (Th17), type 2 T helper cell (Th2), regulatory T cell (Treg), activated B cell (Act_B), immature B cell (Imm_B), memory B cell (Mem_B),

natural killer cell (NK), CD56 bright natural killer cell (CD56bright), CD56 dim natural killer cell (CD56dim), myeloid derived suppressor cell (MDSC), natural killer T cell (NKT), activated dendritic cell (Act_DC), plasmacytoid dendritic cell (pDC), immature dendritic cell (iDC), macrophage, eosinophil, mast cell, monocyte, and neutrophil, TISIDB was utilized to explain the relationships. **Figure 7A** illustrated these results. All tumor-infiltrating lymphocytes showed positive correlations with LY96 in KIRC patients beside neutrophils. Among these, macrophage (cor=0.72), MDSC (cor=0.702), Tfh (cor=0.688), Act_DC (cor=0.655), and Th1 (cor=0.652) were strongly associated with LY96 expression. Furthermore, the associations between LY96 expression and immunoinhibitors, immunostimulators, MHCs, chemokines, and receptors were all showed in **Figure 7B-F**. Correlations with P<.05 and the strength of the rho coefficient \geq 0.5 were represented by scatter plots. Figure 7G revealed the expression levels of LY96 genes

significantly varied in various immune subtypes ($P<.001$). The associations between LY96 expression and several immune gene markers were assessed via TIMER (**Supplementary Table 1**).

Relevance of LY96 across 14 functional states in RCC and the relationships among LY96 and transcription factors, DNA methyltransferase, clinical information

To further investigate the functions of LY96 in RCC, single-cell analysis was performed using CancerSEA. The results indicated that no biological process was significantly correlated with LY96 expression in RCC(**Figure 8a**). The findings suggested that LY96 was not associated with the occurrence and development of renal cell carcinoma (Table 4). LY96 was probably specific for KIRC. In addition, in order to identify members that regulate LY96 expression, transcription factors (TFs) that possibly influenced transcription of the LY96 gene were explored (**Figure 8b**). Associations between the 20 top TFs and LY96 expression levels in KIRC were investigated using the TIMER database. The results showed that CEBPB, E2F7, SNAI2, MTA2, STAT3, and RAD21 were all significantly correlated with LY96 in KIRC ($P<.001$) (**Figure 8c**). A previous study pointed out that the heterogeneity of DNA methylation was strongly associated with a high risk of RCC[31]. So, the relationships between LY96 and DNA methyltransferase (DNMT) expression levels were examined by TIMER. The result demonstrated that DNMT1 positively correlated with LY96 in KIRC ($\text{cor}=0.257$, $P<.001$) (**Figure 8c**). It seems that TFs and DNA methylation modifications regulate LY 96 expression in KIRC. Finally, we used MEXPRESS to study the relationships between LY96 expression and multiple clinical variables. The analysis indicated that history of neoadjuvant treatment (no, yes), lactate dehydrogenase result (elevated, normal), neoplasm histologic grade (g1, g2, g3, g4, gx), pathologic T (T1-T4), pathologic (m0, m1, mx), performance status scale timing (at recurrence/progression of disease, post adjuvant therapy, post secondary therapy, other), person neoplasm cancer status (tumor free, with tumor), platelet qualitative result (elevated, low, normal), white cell count result (elevated, low, normal), tumor stage (I-IV) and sample type (primary tumor, solid tissue normal) were closely related to the expression levels of LY96 ($P<.05$) (Figure 8d). Besides, the results also showed LY96 expression was negatively associated with the OS of KIRC patients ($r=-0.162$, $P<.001$).

Discussion

LY96, also known as MD2, is a co-receptor of TLR4 and is necessary for bacterial LPS binding. A previous study has reported that targeting TLR4/MD2 is an important therapeutic strategy against immunosuppressive disease[32]. Another literature proposed that MD2 was a significant contributor in the Ang II-induced kidney inflammatory injury in chronic renal diseases[33]. Our study has revealed that LY96 expression levels between tumor and normal tissues differed in various cancer types, especially in KIRC (**supplementary Figure 3**). Besides, high expression of LY96 was significantly correlated with advanced tumor stage, high histological grade and poor prognosis.

To date, molecular markers for RCC diagnosis and prognosis prediction remain lacking, so renal mass biopsy is still the most common and reliable diagnostic means. We sought to identify the relationships between developmental and progressive mechanisms of RCC and LY96 expression, however, no specific

correlation was found. Furthermore, LY96 expression was not associated with KICH and KIRP prognosis. Thus, LY96 might be an indicator of good specificity for KIRC and deserves further validation as a potential diagnostic, therapeutic, and prognostic biomarker.

Analysis of whole-genome alterations in genitourinary malignancies has reported that higher CNV was closely associated with inferior survival for KIRC[34]. Increased LY96 gene CNV was observed in KIRC and amplification was the major type of alteration. The amplification of LY96 played an important role in tumor invasion associated with poor prognosis. Abnormal expression and dysfunction of the LY96 gene may result from the alterations in chromosomal structure.

Inflammation, particularly chronic inflammation, has been proved to be closely associated with the occurrence and progression of cancers[35]. LY96 has a close relation with inflammation and inflammation-related genes, such as TLR4, LY86, TLR2, TLR1, and CD180, etc. In particular, the correlation coefficient between LY96 and TLR4 was up to 0.999. TLR4 involved in various inflammatory pathways and several studies have revealed that the activation of TLR4 was implicated in the initiation and progression of many cancer types, e.g., cancers of the liver, lung, ovary, and stomach, etc[36-39]. Other Toll-like receptors had similar functions as TLR4. Moreover, overexpression of TLR3 was observed in both primary and metastatic ccRCC[40] and MD2 was critical for generating the inflammatory response. Thus, we speculated that the high expression of LY96 might trigger inflammatory response and eventually lead to cancer.

GSEA of LinkInterpreter module in Linkedomics helped find significant networks of target kinase, miRNAs, and transcription factors. DNA damage causes genomic instability and gradually may contribute to cancer development. Kinase and their related signaling pathways frequently help balance and repair genomic DNA and have become attractive targets for developing new anticancer drugs. Our research discovered that LY96 was most relevant with kinases including LYN, LCK, and SYK. And LYN and LCK are members of the Src family of non-receptor tyrosine kinases. Src is correlated with many oncogenic cellular processes, such as migration, adhesion, invasion, and proliferation, etc. A study has investigated the role of Src family kinases (SFKs) in KIRC[41]. Nuclear accumulations of LYN were observed in solid tumors and associated with inducing cell proliferation and poor prognosis. And SFK inhibitors dasatinib induced apoptosis and inhibited cell proliferation and migration[42]. Thus, the choice of the kinase target is the most vital aspect of drug target identification. Development of inhibitors for SFKs may be an appealing novel therapeutic method for KIRC patients. The loss of cell-cycle regulation and disturbance of apoptotic mechanisms may result in the occurrence and progression of cancers. ETS transcription factor (TF) family enhanced tumorigenesis through extensive mechanisms including chromosomal translocations, DNA damage, genome instability, metabolism, and epigenetics[43]. The results showed that LY96 was closely related to ETS family, such as ELF1_Q6, ETS_Q4, ETS1_B, and ETS2_B. Many studies have confirmed that ELF1, ETS1, ETS2 were all recognized as contributors to malignancies[44-46]. In addition, other transcription factors like NFKB, PPARA, PEA3, CEBPB, E2F7, SNAI2, MTA2, STAT3, and RAD21 can also be important targets of LY96. LY96 could act through these factors to regulate cell cycle and proliferation ability. Previous studies have reported that TF CREB5 exhibited an inhibition on

LY96[47] while TF STAT1 acted as an activator[48]. The relationship between the two TFs and LY96 in KIRC deserves further investigation.

Significant DNA methylation modifications were observed in LY96 and its expression was positively related to DNMT expression. DNMT inhibitors can induce DNA demethylation and are considered as potential anticancer agents[49]. Demethylation drugs 5-Aza-2'-deoxycytidine (5-Aza) and PBA treatment in KIRC cell lines have been confirmed[50]. And 5-Aza-induced upregulation of miR-200c might inhibit invasion, migration, and EMT in KIRC cells. Therefore, we deduced that the initiation and progression of KIRC may be mediated by DNA modification alterations of LY96. To better understand how altered DNA demethylation influences the development and progression of KIRC will be the focus of future research and more effective demethylating agents are in urgent need. Meanwhile, we found that lower methylation levels of the LY96 genes were more frequently identified in Caucasian and advanced stage.

We studied tumor cell infiltration to determine whether various immune cells play a significant role in tumor growth. Immunomodulation of the tumor microenvironment is a new cancer therapy and immunotherapy based on immunomodulatory antibodies is becoming a mainstream of oncology. The composition of tumor-infiltrating lymphocytes in the tumor microenvironment can induce host immune responses to tumor cells and impact tumor growth and clinical prognosis[51-52]. Positive associations between various TILs and LY96 gene in KIRC were identified through DISIDB and the most relevant cells were macrophage (cor=0.72) and MDSC (cor=0.702) ($P<.001$). Macrophages are highly related to tumor immunity and often balance modulates cancer and inflammatory disease. Diverse macrophage subsets have been connected with either protective or pathogenic function in cancers. MDSC have potent immune suppressive functions on host immune cells to help cancers escape the immune response. High expression of the LY96 gene may promote the proliferation of macrophages and MDSCs and thereby result in a poor prognosis. Besides, we explored the correlations between lots of immunoinhibitors, Immunostimulators, MHCs, chemokines, receptors, and LY96 gene in KIRC. Among them, LGALS9, TNFSF13B, HLA-DRA, CCL18, and CCR1 displayed tight links with LY96 in KIRC.

LGALS9 encodes Galectin-9 (Gal-9), which has been demonstrated to be relevant to various cancer types. High expression level of Gal-9 was significantly associated with poor prognosis of KIRC patients[53]. TNFSF13B was reported to be involved in cell proliferation and apoptosis and correlated with a worse prognosis in cancers[54]. A study proposed that the dysregulation of HLA-DRA (major histocompatibility complex, class II, DR alpha) was relevant to systemic inflammation[55], which may lead to the occurrence of cancers. CCL18 could promote angiogenesis and induce EMT in cancer cells[56]. CCR1 was a crucial facilitator of cancer invasion and metastasis[57]. These immune checkpoints, which have a close relationship with LY96, promoted cell migration, and accelerate cancer progression. This may explain why LY96 has a negative effect on KIRC at the level of the tumor microenvironment. Different tumor-infiltrating lymphocytes varied among diverse KIRC subtypes, as well as in the immune evasion mechanisms. Therefore, we should take these factors into account when developing new strategies for immunotherapy. The results showed that LY96 expression was highest in C6 (TGF- β dominant) while lowest in C5 (immunologically quiet) immune subtypes.

In conclusion, our study showed that LY96 expression levels were strongly correlated with a worse prognosis of KIRC patients and may be a potential marker in KIRC. LY96 was crucial for generating the inflammatory response and significantly related to several kinase targets (such as LYN, LCK, SYK, etc) and transcription factors (such as ELF1, NFKB, ETS, etc). To find demethylation agents and TFs inhibitors that downregulate LY96 is meaningful. Furthermore, LY96 played an important role in the immune microenvironment and may serve as an immunotherapy target in KIRC. Our study used online tools with a large sample size and low cost, which may lead to new directions of research in KIRC. Nevertheless, further experiments are required to validate its diagnostic and therapeutic potential.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the TCGA database (<https://tcga-data.nci.nih.gov/tcga>).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Ji-li Xu: Methodology; Formal analysis and investigation; Writing-original draft preparation

Yong Guo:Conceptualization; Writing-review and editing; Funding acquisition; Supervision

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Tables

Table 1 Enriched GO and KEGG items

Category	Description	Count in network	False discovery rate
GO-BP	toll-like receptor signaling pathway	15 of 87	1.70e-25
	innate immune response-activating signal transduction	15 of 168	5.70e-22
	MyD88-dependent toll-like receptor signaling pathway	11 of 33	3.43e-21
	positive regulation of response to biotic stimulus	16 of 277	3.88e-21
	positive regulation of multi-organism process	16 of 394	6.70e-19
GO-MF	signaling receptor activity	13 of 1429	8.44e-07
	lipopolysaccharide receptor activity	3 of 5	4.18e-06
	lipopolysaccharide binding	4 of 30	4.18e-06
	transmembrane signaling receptor activity	11 of 1226	4.44e-06
	lipopeptide binding	3 of 10	1.12e-05
GO-CC	endolysosome membrane	5 of 15	6.07e-09
	phagocytic vesicle	6 of 122	6.91e-07
	lipopolysaccharide receptor complex	3 of 5	3.99e-06
	receptor complex	7 of 305	3.99e-06
	cytoplasmic vesicle	14 of 2226	3.99e-06
KEGG pathway	Toll-like receptor signaling pathway	10 of 102	3.77e-15
	Tuberculosis	5 of 172	4.97e-05
	Chagas disease (American trypanosomiasis)	4 of 101	0.00011
	Measles	4 of 133	0.00024
	Malaria	3 of 47	0.00024

BP: Biological Process; MF: Molecular Function; CC: Cellular Component

Table 2 The correlation score between FCGR1A and neighboring genes

Node1	Node2	Correlation score
LY96	TLR4	0.999
	LY86	0.994
	TLR2	0.989
	TLR1	0.982
	CD180	0.972
	HMGB1	0.963
	BGN	0.925
	TRIL	0.921
	TLR6	0.798
	TLR8	0.785
	TLR9	0.784
	TLR5	0.775
	TLR3	0.775
	TLR7	0.765
	TLR10	0.742
	ENSG00000173366	0.702

Table 3 The kinase, miRNA and transcription factor-target networks of LY96 in kidney renal clear cell carcinoma (KIRC) (LinkedOmics)

Enriched Category	Geneset	LeadingEdgeNum	FDR
Kinase Target	Kinase_LYN	22	0
	Kinase_LCK	26	0
	Kinase_SYK	17	0
	Kinase_SGK1	5	0.010
	Kinase_MAPKAPK2	11	0.019
	Kinase_HCK	9	0.020
Transcription Factor Target	V \$ ELF1_Q6	68	0.006
	GGGNNTTCC_V \$ NFKB_Q6_01	50	0.007
	V \$ ETS_Q4	74	0.012
	V \$ PPARA_01	15	0.013
	V \$ PEA3_Q6	77	0.014
	V \$ IRF_Q6	74	0.015
	V \$ PU1_Q6	58	0.023
	V \$ ETS1_B	65	0.025
	RYTTCTG_V \$ ETS2_B	261	0.033
	RACCACAR_V \$ AML_Q6	51	0.037
	V \$ COREBINDINGFACTOR_Q6	62	0.037

Table 4 Relevance of LY96 across 14 functional states in RCC

Gene	State	Correlation	P value
LY96	Angiogenesis	-0.269	0.373
	Apoptosis	0.132	0.669
	CellCycle	-0.148	0.630
	Differentiation	0.220	0.470
	DNAdamage	-0.198	0.517
	DNArepair	0.280	0.353
	EMT	0.110	0.723
	Hypoxia	-0.187	0.541
	Inflammation	0.154	0.617
	Invasion	-0.082	0.792
	Metastasis	0.132	0.669
	Proliferation	-0.099	0.751
	Quiescence	-0.016	0.964
	Stemness	-0.275	0.363

Figures

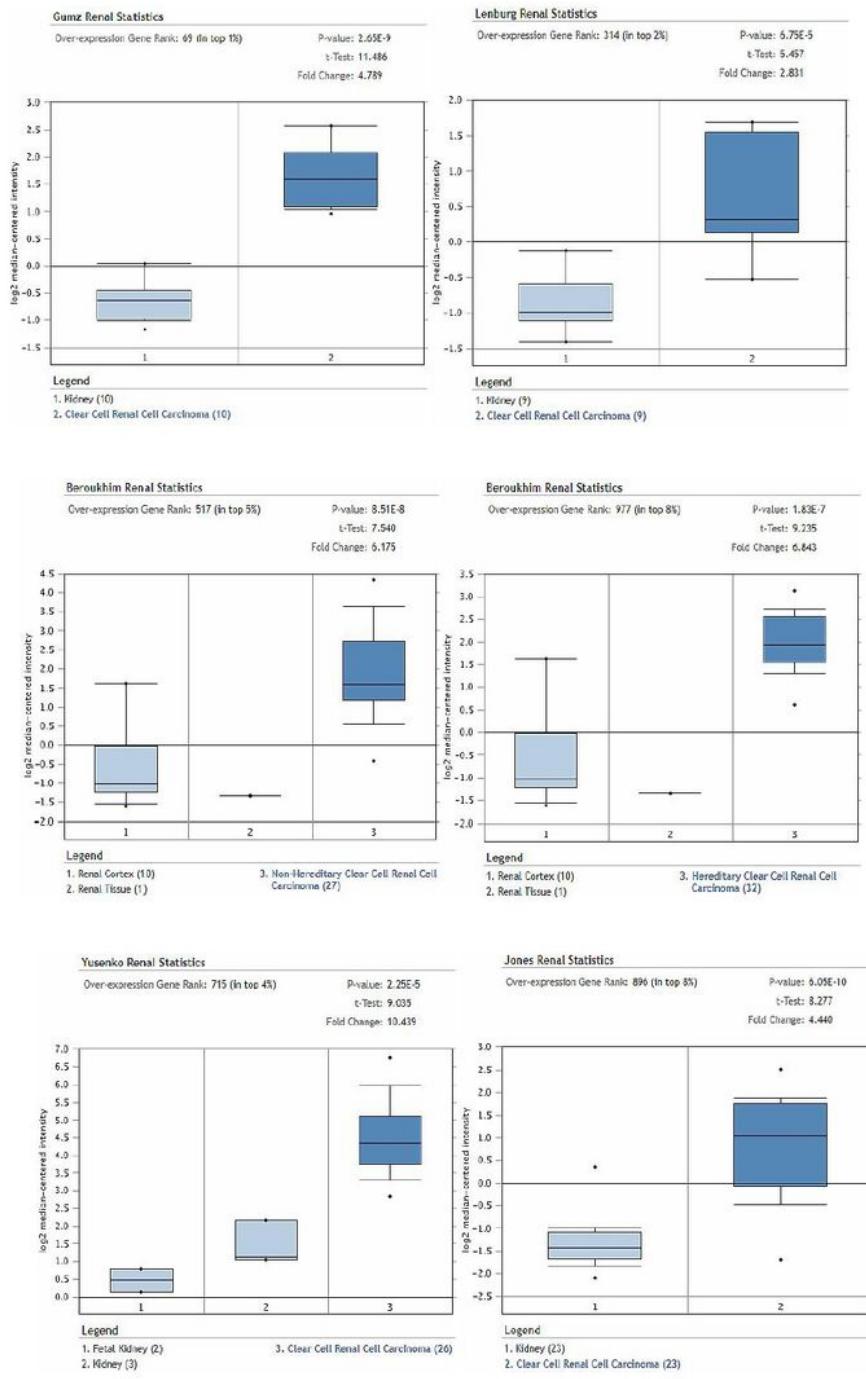
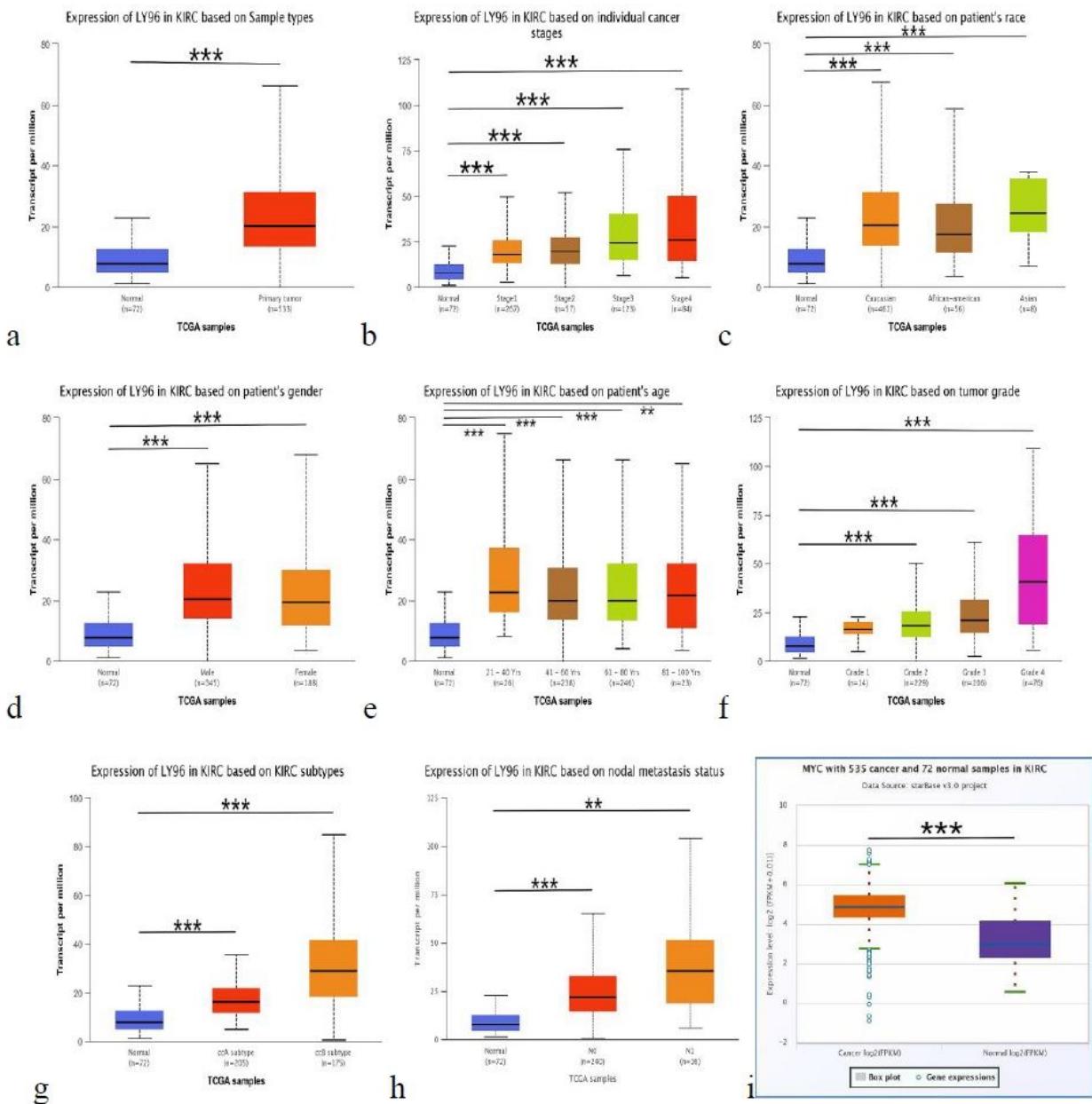


Figure 1

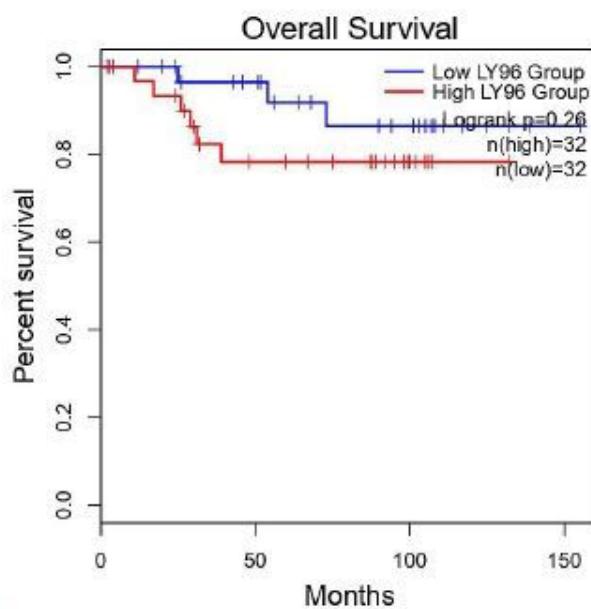
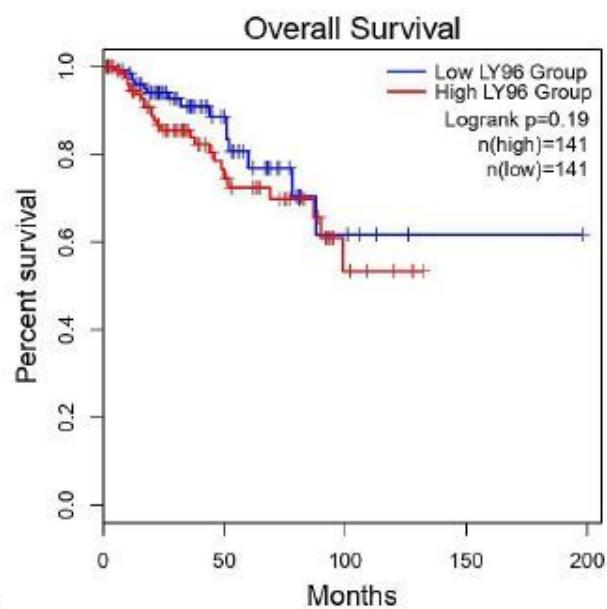
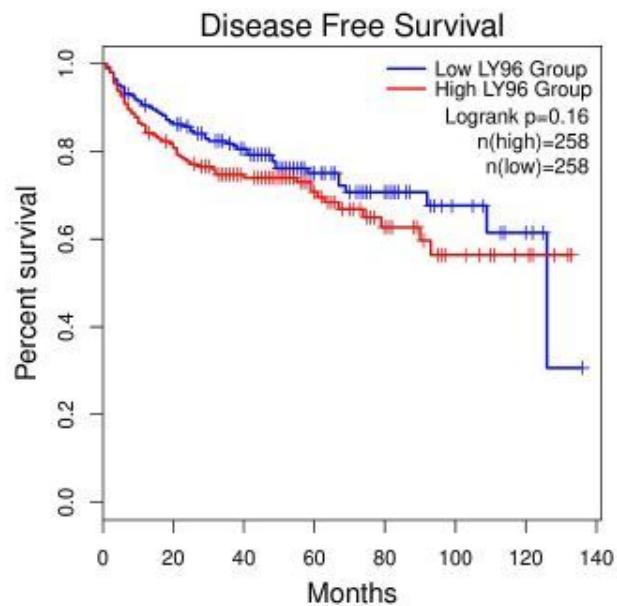
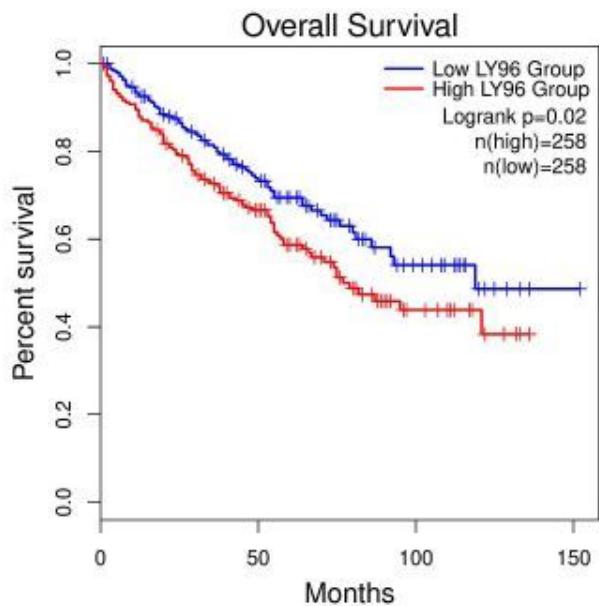
Figure 1 LY96 expression in KIRC (Oncomine)



*: P < 0.05; **: P < 0.01; ***: P < 0.001

Figure 2

LY96 expression in subgroups of KIRC patients, stratified based on stage race, gender, age, grade, subtypes, and nodal metastasis status (UALCAN)



b

a: KIRC; b: KICH; c: KIRP

c

Figure 3

Survival of LY96 in KIRC, KICH and KIRP (GEPIA)

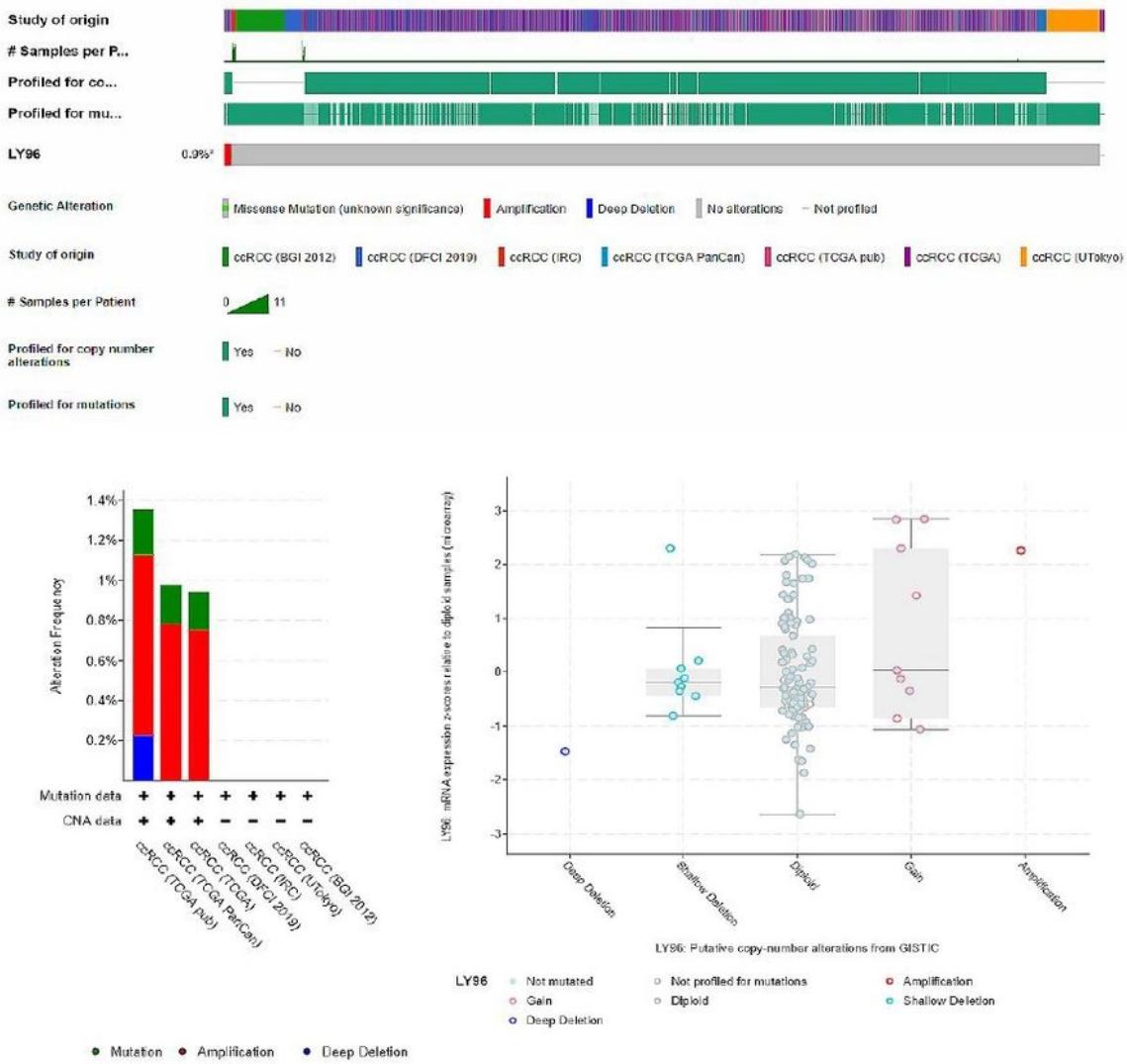
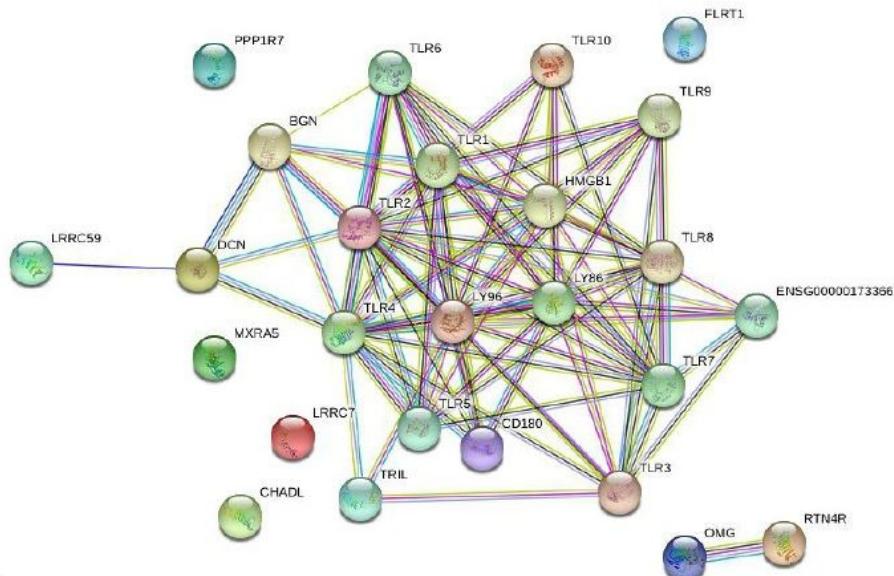
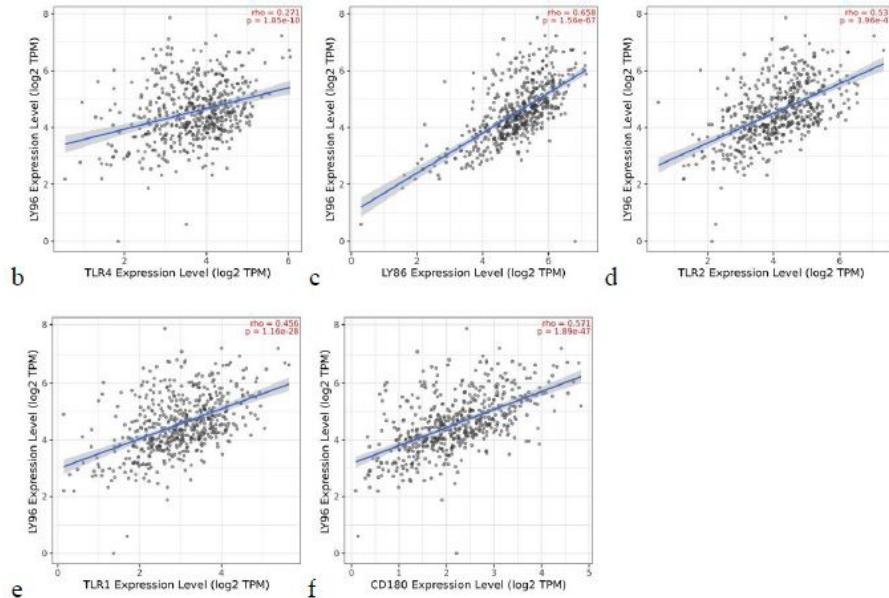


Figure 4

Visual summary of LY96 alterations and mutations in KIRC (cBioPortal)



a



a: The correlation between LY96 and related genes
 b-f: The Correlations between LY96 and top 5 related genes

Figure 5

Correlations between LY96 and highly related genes (STRING)

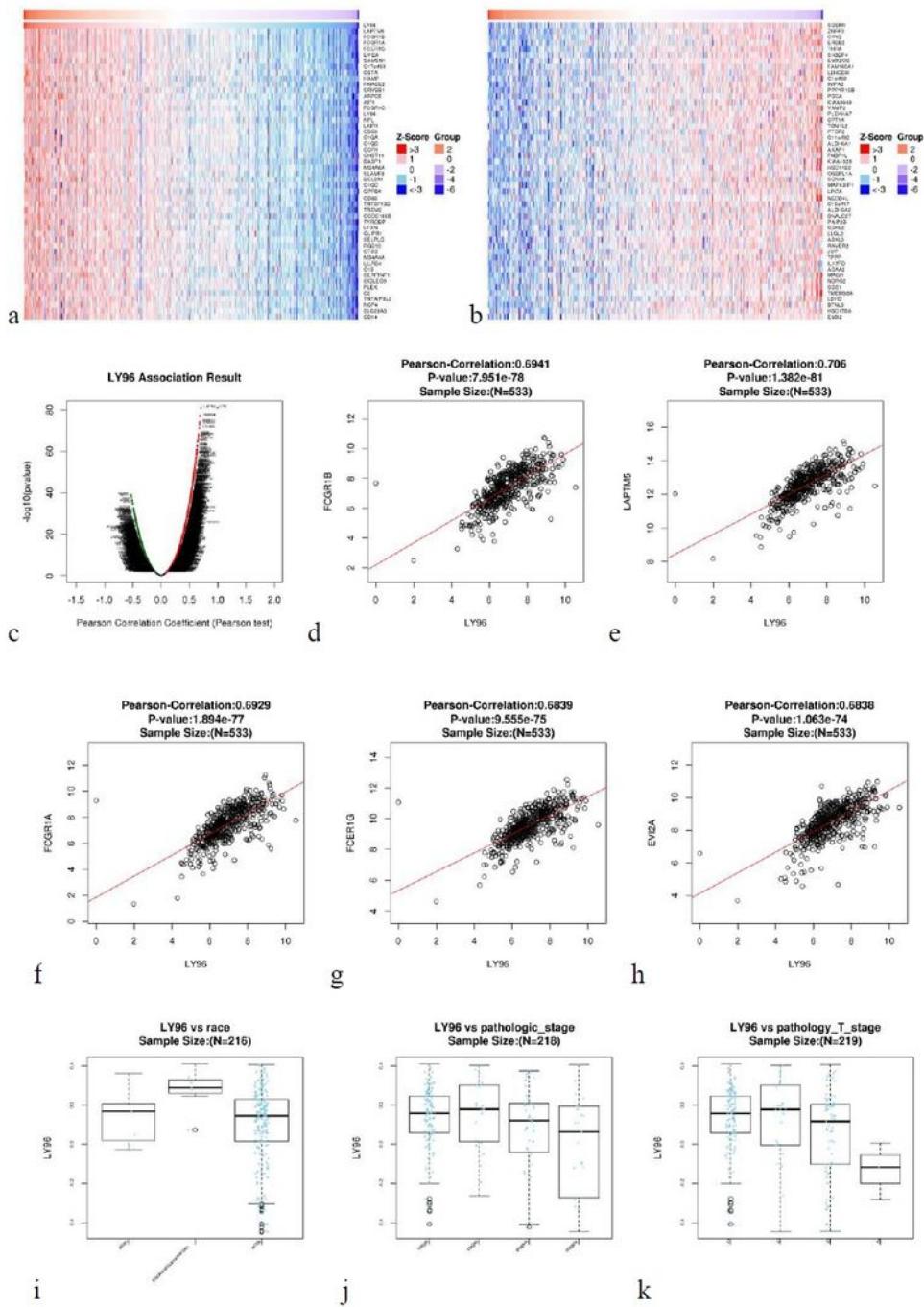


Figure 6

Genes differentially expressed in correlation with LY96 in KIRC and different LY96 methylation levels among race, stage, and T-staging (LinkerOmics)

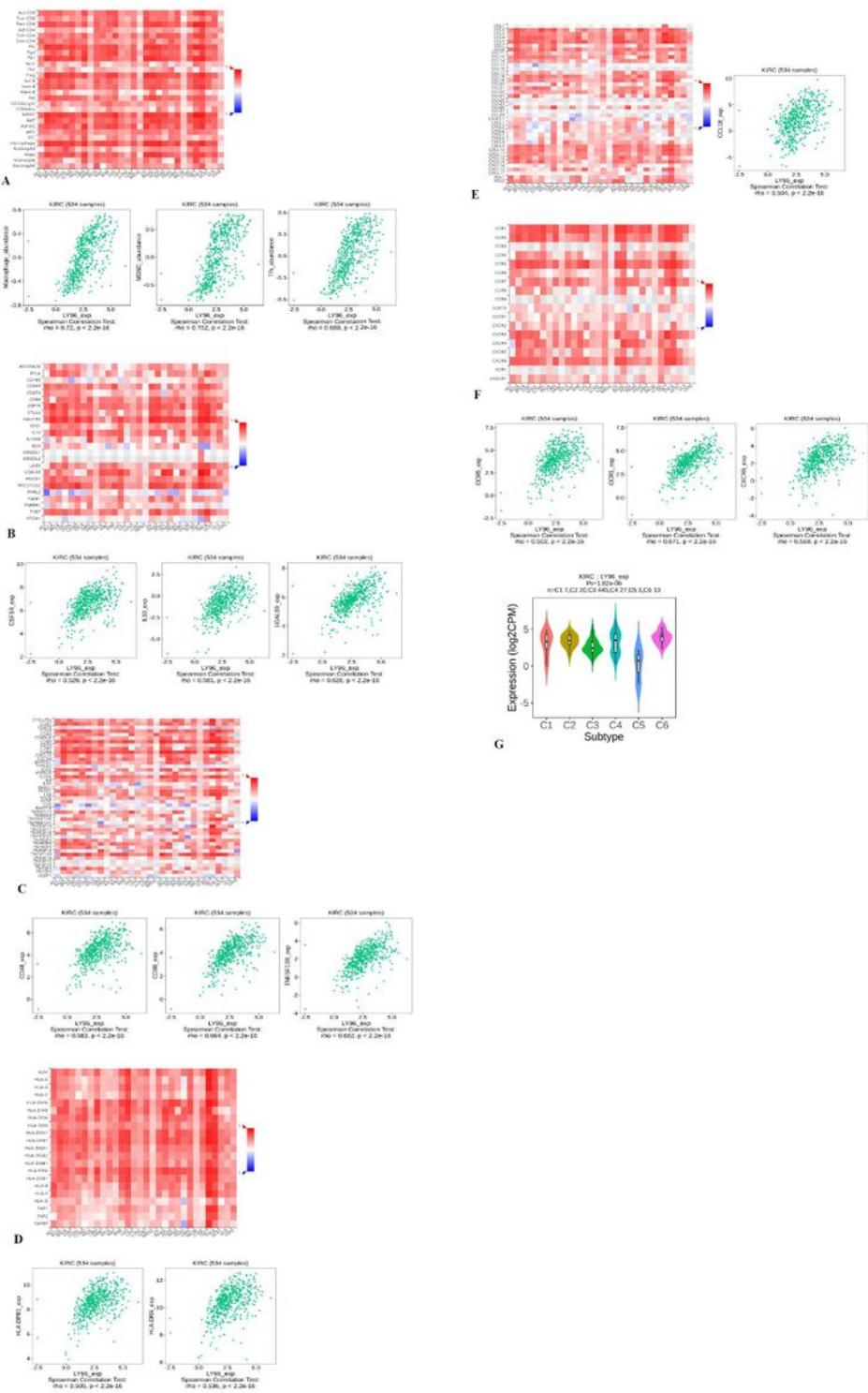


Figure 7

Correlations between LY96 and immune-related factors (TISIDB)

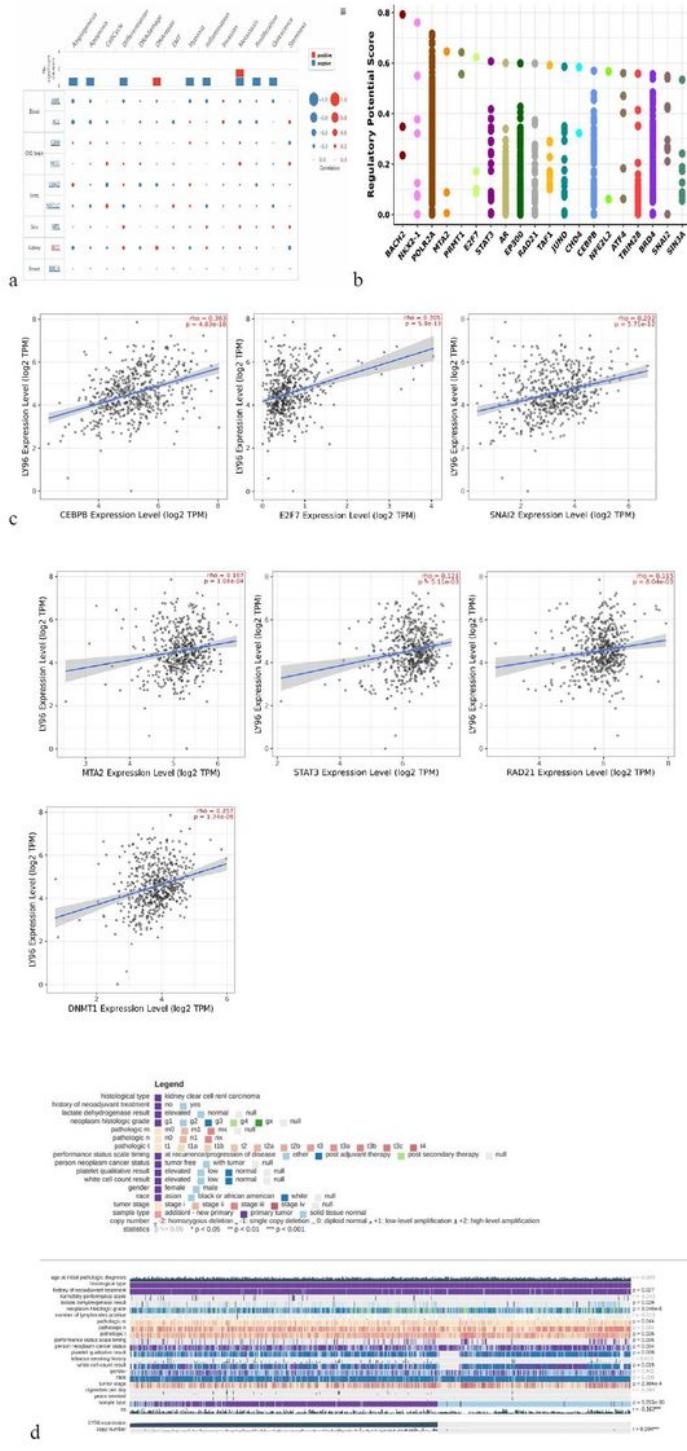


Figure 8

The function of LY96 in renal cell carcinoma (RCC) cells and LY96 is correlated with several transcription factors (TFs) and clinical features (CancerSEA, Cistrome Data Brower Toolkit and MEXPRESS)

Supplementary Files

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