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Comprehensive Pan-Cancer Analysis of the Prognostic Role of KLF Transcription Factor 2 (KLF2) in Human Tumors

Rong Xu[1], Yuhan Chen[2], Shicai Wei[1], Jun Chen[1]

[1]Department of Dermatology, The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing, People's Republic of China; [2]Department of Pathology, The Fourth Affiliated Hospital of Nanjing Medical University, Nanjing, People's Republic of China

Correspondence: Jun Chen; Shicai Wei, Department of Dermatology, The Fourth Affiliated Hospital of Nanjing Medical University, No. 298, Nanpu Road, Yanjiang Street, Pukou District, Nanjing, People's Republic of China, Email springchenjun@163.com; We13951651352@163.com

Background: KLF2 is a transcription factor expressed early in mammalian development that plays a role in many processes of development and disease. Recently, increasing studies revealed that KLF2 plays a key role in the occurrence and progression of cancer.

Purpose: The aim of this study was to explore the role of KLF2 in various tumor types using the Cancer Genome Atlas dataset.

Methods: Here, we set out to explore the role of KLF2 in 33 tumor types using TCGA (The Cancer Genome Atlas), GEO (Gene Expression Omnibus) dataset, Human Protein Atlas (HPA), UALCAN database, CancerSEA, GSCALite and several bioinformatic tools. Furthermore, we also performed immunohistochemistry and qPCR to further validate the role of KLF2 in multiple cancers and its correlation with prognosis.

Results: We found that KLF2 was underexpressed in most tumors and generally predicted poor OS in tumor patients. We found that amplification of KLF2 may be a risk factor for patients with OV (Ovarian serous cystadenocarcinoma). We also analyzed the abundance of checkpoints and markers of specific immune subsets including CD8+ T lymphocytes (T cells), CD4+ T cells, macrophages, and endothelial cells that significantly correlated with the expression level of KLF2 in pan-carcinoma tissues. In some cancers, KLF2 expression levels are positively correlated with gene promoter DNA methylation and drug sensitivity. In addition, we found that KLF2 is involved in single-cell level cell invasion in some cancers. In addition, KLF2 is co-expressed with several intracellular signal transduction genes involved in immune system processes. Immunohistochemistry and qPCR confirmed the low expression of KLF2 in STAD (stomach adenocarcinoma) and renal cancer.

Conclusion: Our pan-cancer analysis provides a comprehensive overview of the oncogenic roles of KLF2 in multiple human cancers and can be regarded as a potential prognostic marker and a novel target for cancer immunotherapy.

Keywords: KLF2, cancer, bioinformatics, prognosis, immune infiltration

Introduction

The incidence and mortality of malignant tumors are on the rise, which has become the second leading cause of death in the world.[1] With the in-depth understanding of the underlying mechanisms of tumorigenesis, the treatment options for malignant tumors continue to expand. In recent years, more and more attention has been paid to tumor immunotherapy, including immune checkpoint blocking therapy and immune cell therapy.[2] The specificity of immunotherapy depends heavily on the specific tumor antigen.[3] However, immunotherapy-related biomarker matching tests are still limited in most cancers.[4] Therefore, there is an urgent need to further explore effective immunotherapy-related tumor prognostic biomarkers.

KLF2 (Krüppel-like factor 2) is a member of the Kruppel-like transcription factor protein family and contains a highly conserved DNA-binding zinc finger domain.[5] The name of the family is based on the German words "cripple" and "Krüppel" describing the drosophila loss function of phenotypic variation with abnormal abdominal segmentation in the region of larvae.[6] KLF2 was named Lung KLF2 after Anderson et al (1995) found its high expression in lung.[7] Zinc finger proteins

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encoded by KLF2 are expressed early in mammalian development and are present in many different cell types.[8] This protein plays an important role in many processes of normal development and disease, including adipogenesis, embryonic erythropoiesis, intact epithelium formation, and activation of T lymphocytes (T cells), which the main mechanism is to activate the transcription process of target genes by binding to the CACCC box in the promoter of target genes.[9] There was a plethora of study data linking KLF2 to several forms of malignancies. klf2 was shown to be downregulated in gastric cancer,[10] clear cell renal cell carcinoma,[11] colorectal cancer[12] and breast cancer.[13] However, a comprehensive analysis of the function and clinical importance of KLF2 at the pan-cancer level has not been performed.

DNA methylation is critical for tumor prognosis and therapeutic targets. The results show that the polymorphic model of DNA methylation of lung cancer driver gene can effectively predict the prognosis of lung cancer patients.[14] Hydroxytyrosol inhibits tumors by altering DNA methylation levels in colorectal cancer cells.[15] KLF2 is methylated by the DNA methylation transferase DNMT3A, which affects the transcription process.[16] As a transcription factor, KLF2 can significantly affect the sensitivity of breast cancer and liver cancer to drugs.[17,18] However, studies on KLF2 DNA methylation and its role in drug sensitivity and cancer are insufficient, and more comprehensive analyses are needed to better understand its functional role in malignant tumors.

To explore the expression profile of KLF2 in various tumor types in a pan-cancer analysis, we used datasets available through the TCGA project and the GEO database. In addition to comparing KLF2 expression profiles across tumor types, we also considered survival status, genetic alterations, immune infiltration, associated cellular pathways, gene promoter DNA methylation and drug sensitivity. Moreover, immunohistochemical (IHC) analyses and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis were to further confirm the role of KLF2 in renal cancer and STAD (Stomach adenocarcinoma). This pan-cancer analysis reveals the potential molecular mechanisms of KLF2 in human cancer development and prognosis.

Materials and Methods

Dataset Information

We downloaded the unified and standardized pan-cancer dataset from the UCSC (https://xenabrowser.net/) database: TCGA TARGET GTEx (PANCAN, N=19131, G=60499), and further extracted ENSG00000127528 (KLF2) gene expression data in each sample to screened the sample sources: Solid Tissue Normal, Primary Solid Tumor, Primary Tumor, Normal Tissue, Primary Blood Derived Cancer - Bone Marrow, Primary Blood Derived Cancer - Peripheral Blood samples, and further log2(x+0.001) transformation was performed on each expression value. Finally, we also eliminated the cancer species with less than 3 samples in a single cancer species, and finally obtained the expression data of all cancer species.

Immunohistochemical (IHC) Staining

IHC images of KLF2 protein expression in normal tissues and 5 tumor tissues, including Renal cancer, PAAD (Pancreatic adenocarcinoma), PRAD (Prostate adenocarcinoma), STAD (Stomach adenocarcinoma), and UCEC (Uterine adenocarcinoma) Corpus (Endometrial Carcinoma) downloaded from HPA (Human Protein Atlas) (http://www.proteinatlas.org/)[19] and analyzed.

Prognostic Analysis

In the TCGA cohort, we assessed overall survival (OS) of patients by Kaplan-Meier analysis. Univariate Cox regression analysis was performed to assess the significance of KLF2 in predicting OS, disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) in pan-cancer.

Immune Infiltration Analysis

The immune infiltration of different types of tumors can be systematically analyzed on the Tumor Immune Estimation Resource 2.0 (TIMER2.0) web server.[20,21] We assessed the correlation between KLF2 and four types of infiltrating lymphocytes, including CD8+ T cells, CD4+ T cells, endothelial cells, macrophages.

Genetic Alteration Analysis

Using cBioPportal tool (https://www.cbioportal.org/) to collect all TCGA mutations in tumor mutation frequency and mutation type, site information and CNA (copy number changes) data.[22] Survival data were compared for all TCGA cancer types, including OS, PFI, DSS, and DFI, with or without KLF2 genetic alteration.

DNA Methylation Analysis of KLF2

DNA methylation is one of the major epigenetic modifications and plays an important role in the development of tumors.[23] We use UALCAN database to (http://ualcan.path.uab.edu/, accessed on March 21, 2024) analyze the KLF2 promoter DNA methylation level in certain types of cancer, in order to determine the difference between tumor and normal tissue. The Shiny Methylation Analysis Resource tool (SMART, http://www.bioinfo-zs.com/smartapp/, accessed on 21 March 2024) is used to discuss the distribution of methylation probes in chromosomes.[24]

Single-Cell Functional Analysis of KLF2

The Cancer single-cell state Atlas (CancerSEA, http://biocc.hrbmu.edu.cn/CancerSEA/, accessed on 22 March 2024) is an analytic tool for studying cancer cell functions at the single-cell level, containing 14 tumor-related cellular functions of 900 cancer cells from 25 cancers.[25]

Drug Sensitivity Analysis of KLF2

GSCALite (http://bioinfo.life.hust.edu.cn/web/GSCALite/, accessed on 8 April 2022) is a comprehensive platform for analyzing gene expression and drug sensitivity analysis.[26]

Collection of Clinical Tissue Samples

From the Fourth Affiliated Hospital of Nanjing Medical University, 5 patients with gastric cancer and 15 patients with kidney cancer without chemotherapy, were included, and their cancer tissues and adjacent normal tissues were preserved at −80°C. All patients signed the consent form. The Ethics Committee of the Fourth Affiliated Hospital of Nanjing Medical University approved this experiment. Collection and processing of tissue samples was performed in compliance with the Declaration of Helsinki.

Immunohistochemistry (IHC)

The tissues were incubated with the universal SP kit (Medaka Gold Bridge) and a 1:150 dilution of monoclonal antibodies against KLF2 (Thermo Fisher, MA5-26827). The sections were counterstained with hematoxylin. The sections were then passed through an alcohol gradient, dehydrated, dried and sealed, and finally, the sections were viewed under the microscope and photographed. KLF2 protein expression was scored semi-quantitatively, which was equal to expression intensity multiplied by expression area. Five different fields were randomly selected and observed under a microscope (Nikon Company, Tokyo, Japan) at 200 magnifications. The expression intensity was scored from 0 to 3, indicating negative, weakly staining (light yellow), moderately staining (light brown), and strongly staining (dark brown), respectively. Expression area was scored from 0 to 4, representing <5, 6–25, 26–50, 51–75, >75%, respectively. The degree of positive staining was defined: 1~3 as weak positive (+); 4–6 as moderately positive (++); 7–12 as strong positive (+++).[27] This study was approved by the Ethics Committee of the Fourth Affiliated Hospital of Nanjing Medical University. All the Patients signed written informed consent before collecting samples and authorized the samples to be reviewed for our study.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Analysis

RNA was extracted from tissues by Omega kit. Then the reverse transcription process was carried out according to the instructions of PrimeScript RT Reagent (TaKaRa, Japan) and all PCRs were conducted with SYBR Premix Ex Taq Kit (TaKaRa, Japan) according to the manufacturer's instructions. The 2ΔΔCT method was used to quantify the relative expression. The primers involved were as follows: KLF2-F:5′-TTCGGTCTCTTCGACGACG-3′; KLF2-R:5′-TGCGAACTCTTGGTGTAGGTC-3′; GAPDH-F:5′-GCCTCAAGATCATCAGCAATG; GAPDH-R:5′CCACGATACCAAAGTTGTCATGG-3′.

Statistical Analysis

Used Student's *t*-test and analysis of variance (ANOVA) test to compare between two groups and for comparisons of more than two groups, respectively. Used the Spearman correlation analysis to measure the degree of correlation between certain variables, and the R/rho value was used to judge the correlation. $P < 0.05$ was considered statistically significant.

Results

Pan-Cancer Expression Data of KLF2

We first assessed the differential expression of KLF2 between tumors and adjacent normal tissues using TIMER2 for tumors represented in the TCGA repository. As shown in Figure 1A, lower expression of KLF2 was observed in BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma), CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma), COAD (Colon adenocarcinoma), ESCA (Esophageal carcinoma), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIRP (Kidney renal papillary cell carcinoma), LUAD (Lung adenocarcinoma), LUSC (Lung squamous cell carcinoma), READ (Rectum adenocarcinoma), STAD (Stomach adenocarcinoma), THCA (Thyroid carcinoma) and UCEC (Uterine Corpus Endometrial Carcinoma) than in corresponding control tissues. In contrast, expression was higher only in CHOL (Cholangiocarcinoma) than in the corresponding control tissue. Notably, only a few tumor types did not show differential expression (eg GBM (Glioblastoma multiforme), KIRC (Kidney renal clear cell carcinoma), LIHC (Liver hepatocellular carcinoma), PAAD (Pancreatic adenocarcinoma), PCPG (Pheochromocytoma and Paraganglioma) and PARD (Prostate adenocarcinoma)).

Considering that the number of normal tissues in the TCGA database was too small to be statistically convincing, we integrated the GTEx and TCGA databases to reflect the KLF2 mRNA expression profile in a more convincing manner, with significant downregulation observed in 23 tumors:ACC, BLCA, BRCA, CESC, COAD, ESCA, HNSC, KICH, KIPAN, KIRC, KIRP, LAML, LUAD, LUSC, OV, PRAD, READ, SKCM, STES, TGCT, THCA, UCEC, UCS, in 4 tumors Significant upregulation was observed in: CHOL, GBM, LGG, PAAD (Figure 1B). For other tumors, such as LIHC, PCPG, STAD, we did not get significant differences (Figure 1B). Overall, we found that KLF2 expression was reduced in most human tumors.

We evaluated KLF2 protein expression in various tumors and normal tissues using the HPA database. As shown in the immunohistochemical results (Figure 2), normal kidney, pancreas, prostate, stomach, endometrium tissues showed

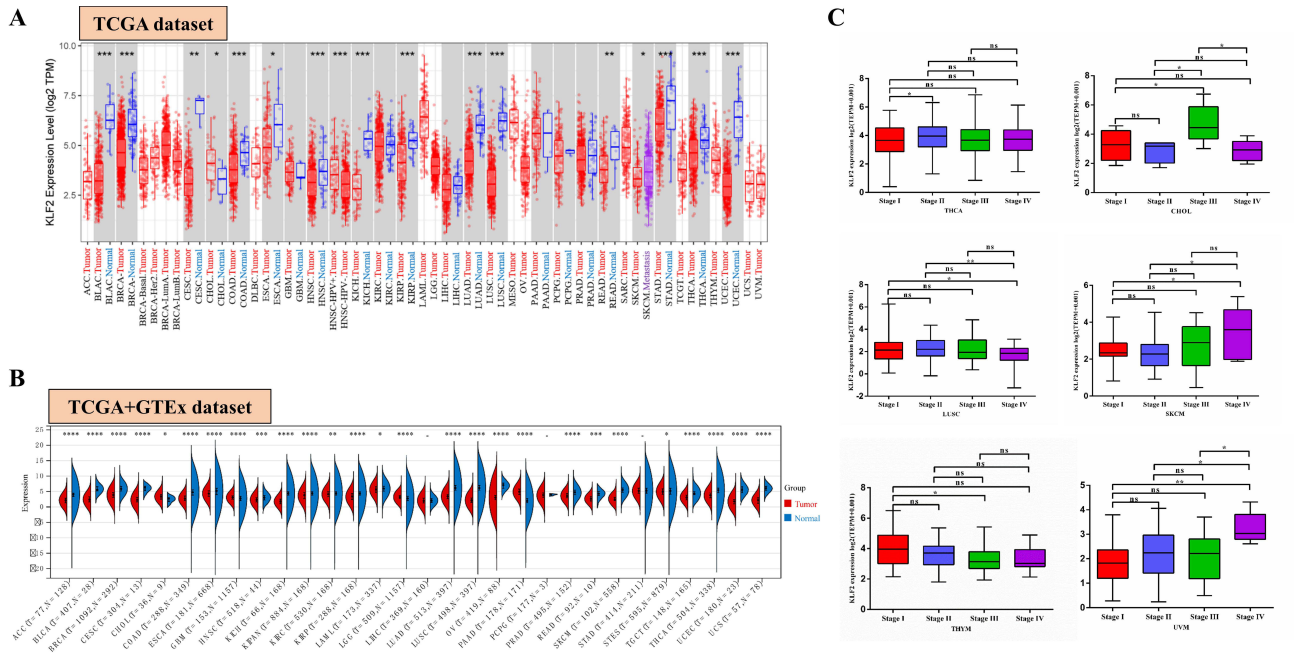

Figure 1 The Expression of KLF2 in Pan-Cancer.

Notes: (**A**) Expression level of KLF2 in TCGA tumors vs adjacent tissues (if available) as visualized by TIMER2. (**B**) Box plot representation of KLF2 expression level comparison in TCGA tumors tissues relative to the corresponding normal tissues (GTEx database). A P value less than 0.05 was considered to be statistically significant. *, **, *** and **** indicate P values less than 0.05, 0.01, 0.001 and 0.0001, respectively. A p-value of n.s means no statistical significance.

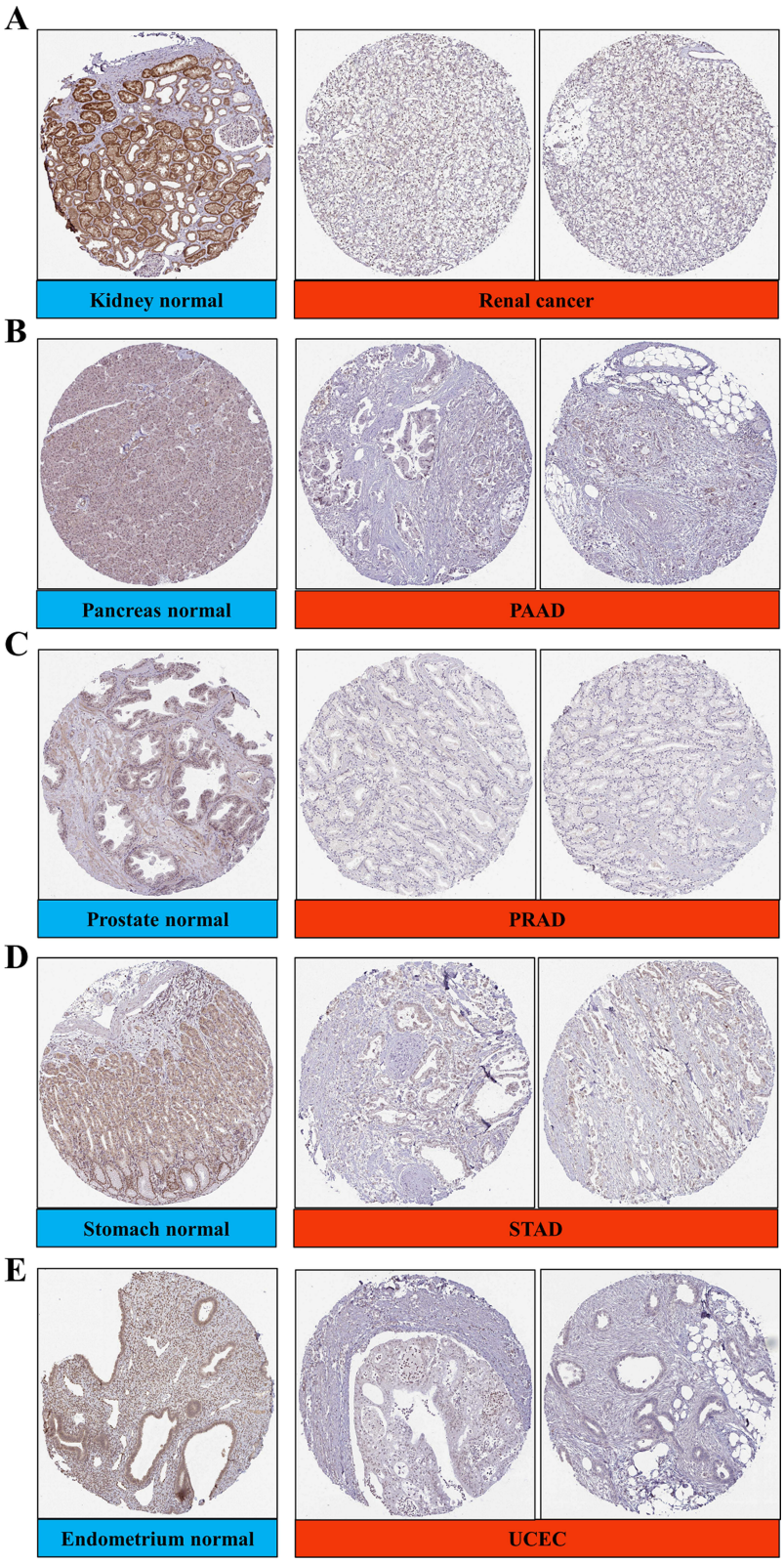

Figure 2 Comparison of immunohistochemistry images of KLF2 gene expression between normal (left) and tumor tissues.
Notes: KLF2 expression in Renal cancer, PAAD, PRAD, STAD and UCEC from the HPA database. (**A**) Kidney. (**B**) Pancreas. (**C**) Prostate. (**D**) Stomach. (**E**) Endometrium.

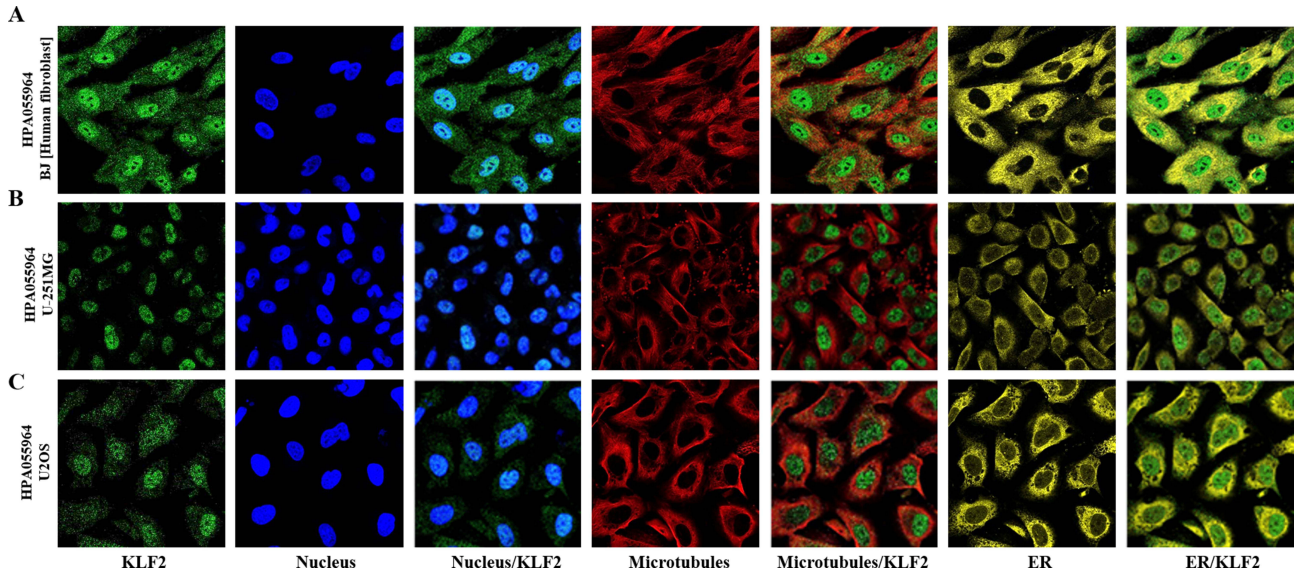

Figure 3 The subcellular localization of KLF2 in HPA database.
Note: Immunofluorescence staining of the subcellular localization of KLF2 in HPA database.

moderate or strong IHC staining for KLF2, while tumor tissues were negative or moderate. It indicated that compared with the corresponding normal tissues, KLF2 was downregulated in these cancers, which was consistent with the KLF2 gene expression results of TCGA (Figure 1A). Subcellular localization of KLF2 was obtained by immunofluorescence localization of nuclei, microtubules, and ER in BJ [Human fibroblast], U-2OS, and U-251 MG cells. KLF2 is mainly located in microtubules and nucleus. In BJ cells, KLF2 is located not only in the microtubule and nucleus, but also in the cytosol (Figure 3). Details information of the IHC results are summarized in the Table (Table 1).

The Relationship Between KLF2 Expression and Prognosis of Cancer Patients

We further evaluated the prognostic significance of KLF2 in cancer patients. Kaplan-Meier OS analysis showed that KLF2 was a protective factor in CESC, HNSC, KIPAN, KICH, LIHC, LUAD, PCPG and SARC patients (Figure 4A–I), and a risk factor in ACC, COAD and UVM patients (Figure 4J–L). For the results of univariate Cox regression analysis, OS results showed that KLF2 was a protective factor in patients with HNSC, KIRC, LIHC, PCPG and SARC, and a risk factor in patients with ACC and LUSC (Figure 5A). DSS analysis revealed that KLF2 was a protective factor in patients

Table 1 Clinical Information of KLF2 Protein in Various Tumors and Normal Tissues in HPA Database Relative Score of Immunohistochemical Results

Protein	Tissue	Histological Type	Age	Gender	Location	Quantity	Intensity
KLF2	Kidney Renal cancer	Cells in tubules Tumor cells	61 69	Male Female	C/M Nuclear	> 75% 25–75%	Strong Weak
KLF2	Pancreas Pancreatic cancer	Pancreatic endocrine cells Tumor cells	54 84	Male Male	C/M None	> 75% None	Moderate Negative
KLF2	Prostate Prostate cancer	Glandular cells Tumor cells	37 63	Male Male	Nuclear None	25–75% None	Moderate Negative
KLF2	Stomach Stomach cancer	Glandular cells Tumor cells	57 62	Female Male	C/M C/M	> 75% > 75%	Moderate Weak
KLF2	Endometrium Endometrium cancer	Glandular cells Tumor cells	34 67	Female Female	Nuclear C/M	> 75% <25%	Moderate Weak

Abbreviation: C/M, Cytoplasmic/membranous.

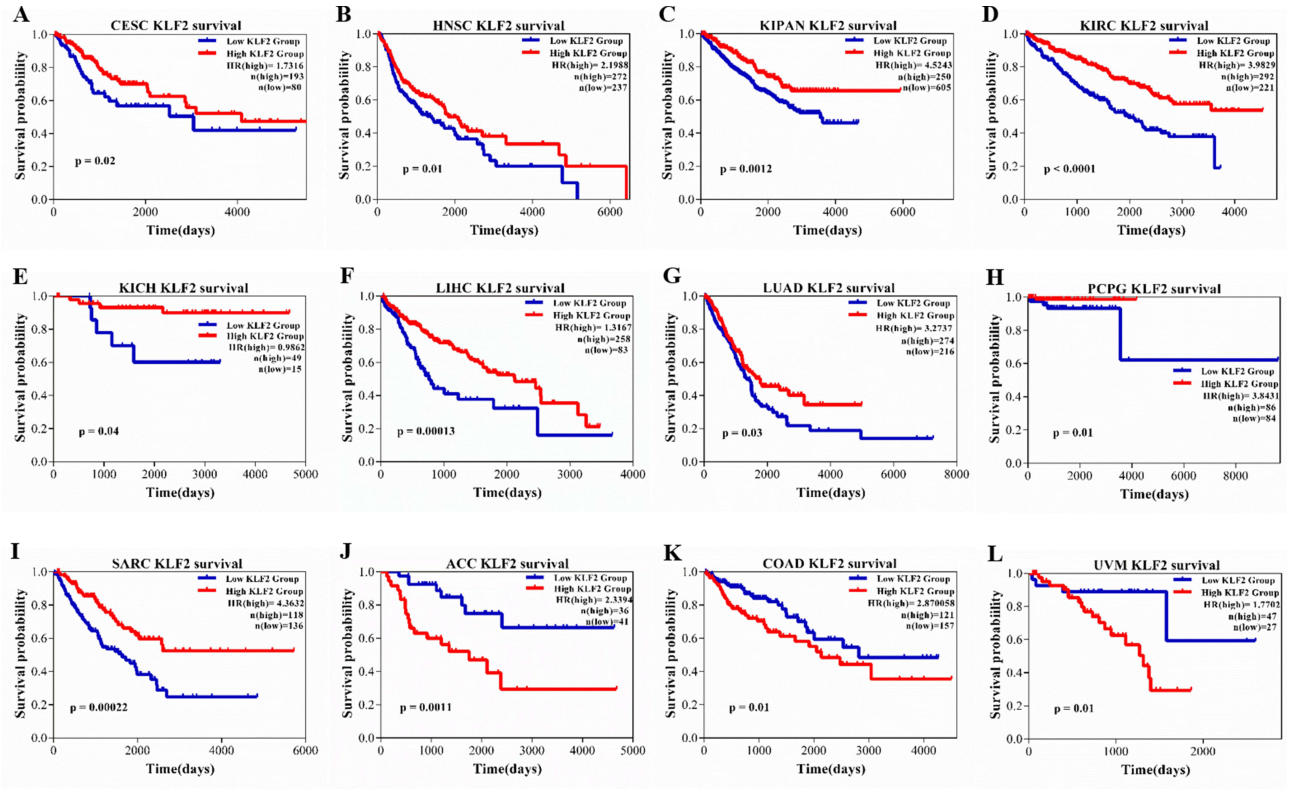

Figure 4 Kaplan-Meier overall survival of KLF2.
Notes: (**A–L**) Pan-cancer Kaplan-Meier overall survival of KLF2 in indicated tumor types from TCGA database. The median value of KLF2 in each tumor was taken as the cut-off value.

with KIPAN, KIRC, KIRP, LIHC and SARC, and a risk factor in patients with ACC and COAD (Figure 5B). DFI analysis revealed that KLF2 was a protective factor in patients with LIHC and THCA and a risk factor in patients with CESC (Figure 5C). Finally, PFI analysis showed that KLF2 was a protective factor in patients with KIPAN, KIRC, KIRP, LUAD, THCA and UCEC (Figure 5D).

The Genetic Alteration Landscape of KLF2 in Different Tumors

We used the cBioPortal to investigate Genetic alterations of KLF2 in various tumor types in the TCGA dataset. The result of analysis shows that the frequency of KLF2 alteration (>6%) is the highest in Ovarian cancer with "amplification" as the primary type. Mature B-cell lymphoma had the highest incidence of "amplification" type of CNA, with the frequency of ~5% (Figure 6A). We then explored the association between KLF2 gene alterations and clinical outcomes in cancer patients. OV patients with C1ORF112 mutation showed worse prognosis in terms of OS (p = 0.0127) and Disease Free Survival (DSF) (p = 0.0212) (Figure 6B).

Immune Cell Infiltration Analyses

To explore the relationship between KLF2 expression and immune cell infiltration, we performed correlation analysis on different immune cell infiltration data using the TIMER2 database. The results showed that KLF2 was positively correlated with the infiltration levels of CD8 + T cells, CD4 + T cells, macrophages and endothelial cells in TCGA pan-cancer, and negatively correlated with the infiltration levels of Th1 CD4 + T cells and Th2 CD4 + T cells (Figure 7A–D). We analyzed that KLF2 may play its role in most tumor types by enhancing T-cell infiltration.

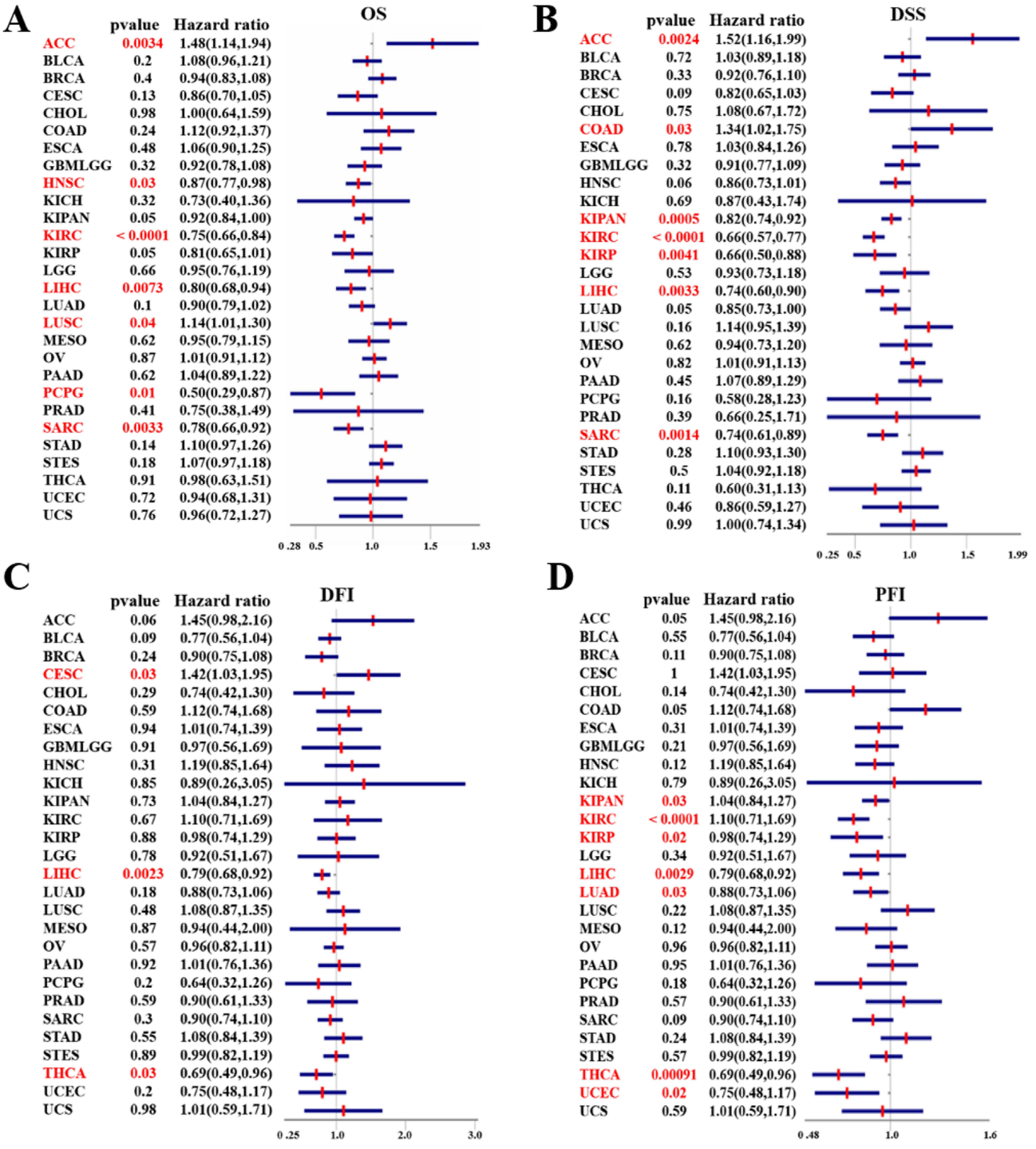

Figure 5 Univariate Cox regression analysis of KLF2.

Notes: (**A**) Forest map shows the univariate cox regression results of KLF2 for OS in TCGA pan-cancer. (**B**) Forest map shows the univariate cox regression results of KLF2 for DSS in TCGA pan-cancer. (**C**) Forest map shows the univariate cox regression results of KLF2 for DFI in TCGA pan-cancer. (**D**) Forest map shows the univariate cox regression results of KLF2 for PFI in TCGA pan-cancer. Red colors represent significant results.

Relationship Between KLF2 Expression and DNA Methylation

The DNA methylation level of KLF2 in different tumors was detected by UALCAN database. The results showed that the methylation level of KLF2 promoter in BLCA, CHOL, ESCA, HNSC, KIRC, LIHC, LUSC, READ, TGCT and UCEC was lower than that in normal tissues. This may be an explanation for the low expression of KLF2 in these

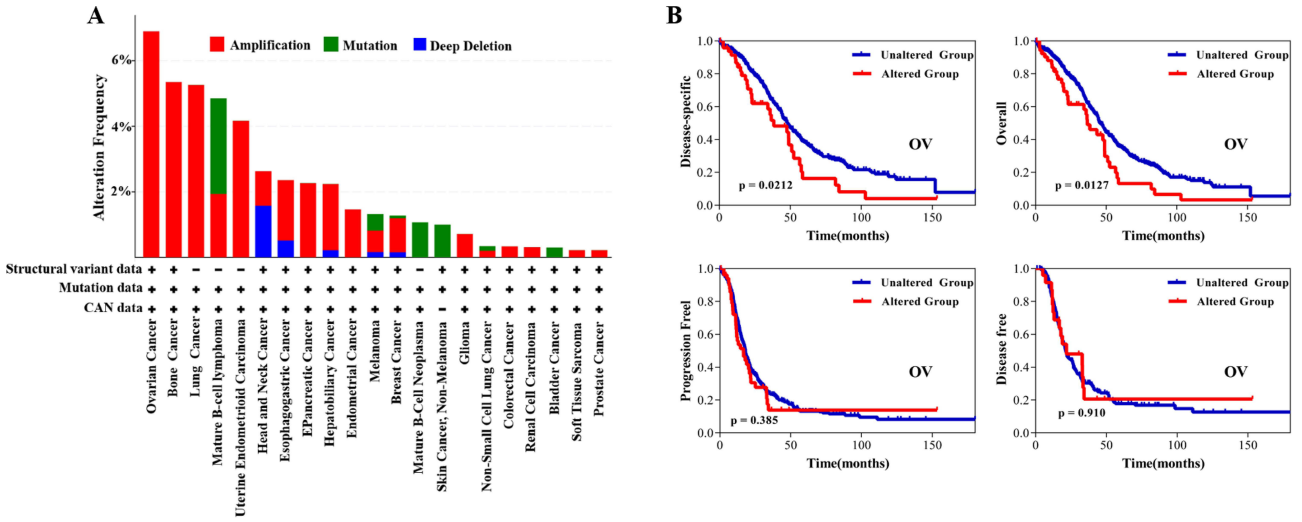

Figure 6 Mutation status of KLF2 in TCGA tumors.
Notes: (**A**) Mutation status of KLF2 in TCGA tumors was analyzed using the cBioPortal tool. (**B**) Analysis of the correlation between mutation status and OS, DSS, DFS and PFS of OV using the cBioPortal tool.

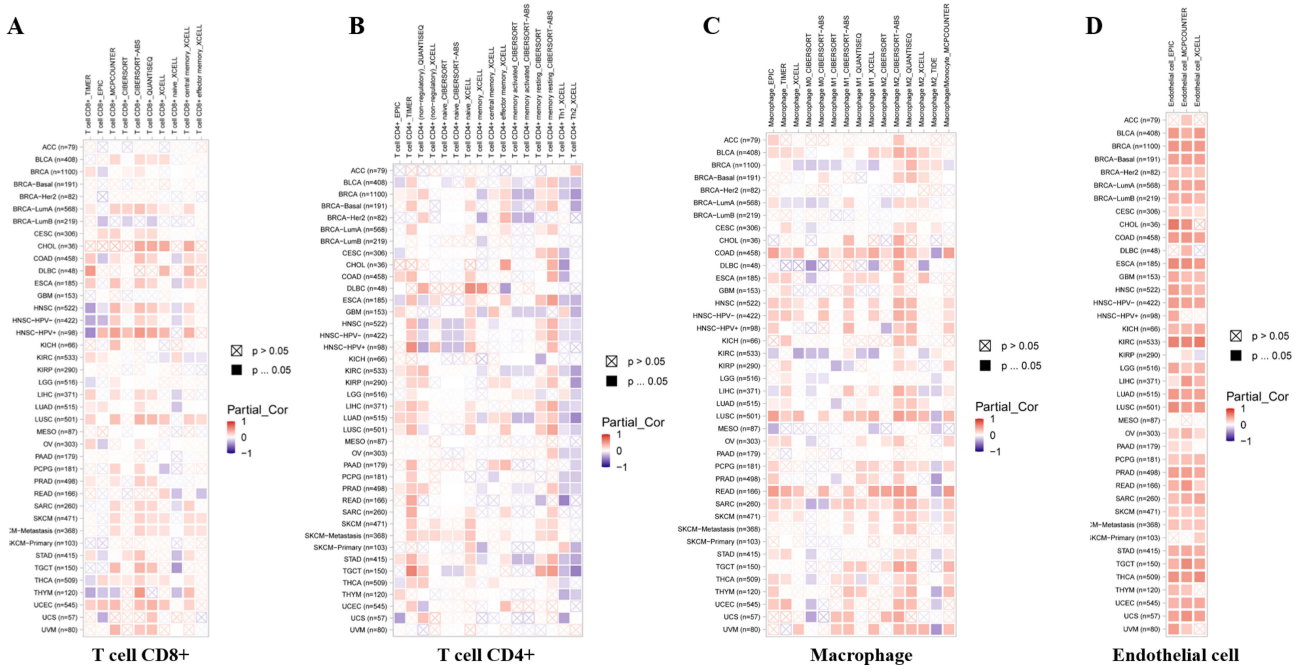

Figure 7 Correlation of KLF2 expression levels with infiltration of CD4+ T cells, CD8+ T cells, macrophages and endothelial cells.
Notes: The correlation between KLF2 and four types of infiltrating lymphocytes, including CD8+ T cells (**A**), CD4+ T cells (**B**), macrophages (**C**) and endothelial cells (**D**) was analyzed using the TIMER2.

tumors. Increased methylation levels were observed in COAD and PCPG (Figure 8A). SMART was used for DNA methylation level analysis and survival analysis of CpG sites in KLF2. As shown in Figure 8B, KLF2 has 10 methylation probes, such as cg22247553, cg15496085, cg10819847, cg03725130, cg25266327, cg26842024, cg04324758, cg05906166, CG22247553, CG15496085, CG10819847, CG03725130. cg18473733 and cg02668248. Hypermethylated cg15496085, cg10819847, cg03725130 and cg25266327 suggested a good prognosis for KIRC, but hypermethylated cg05906166 and cg18473733 in KIRC is poor. The prognosis of hypermethylation of cg15496085, cg10819847, cg03725130 and cg25266327 was poor in UCEC. cg22247553 hypermethylation had a good outcome in PCPG but a poor outcome in READ (Table 2). KLF2 may influence the prognosis of cancer patients through methylation.

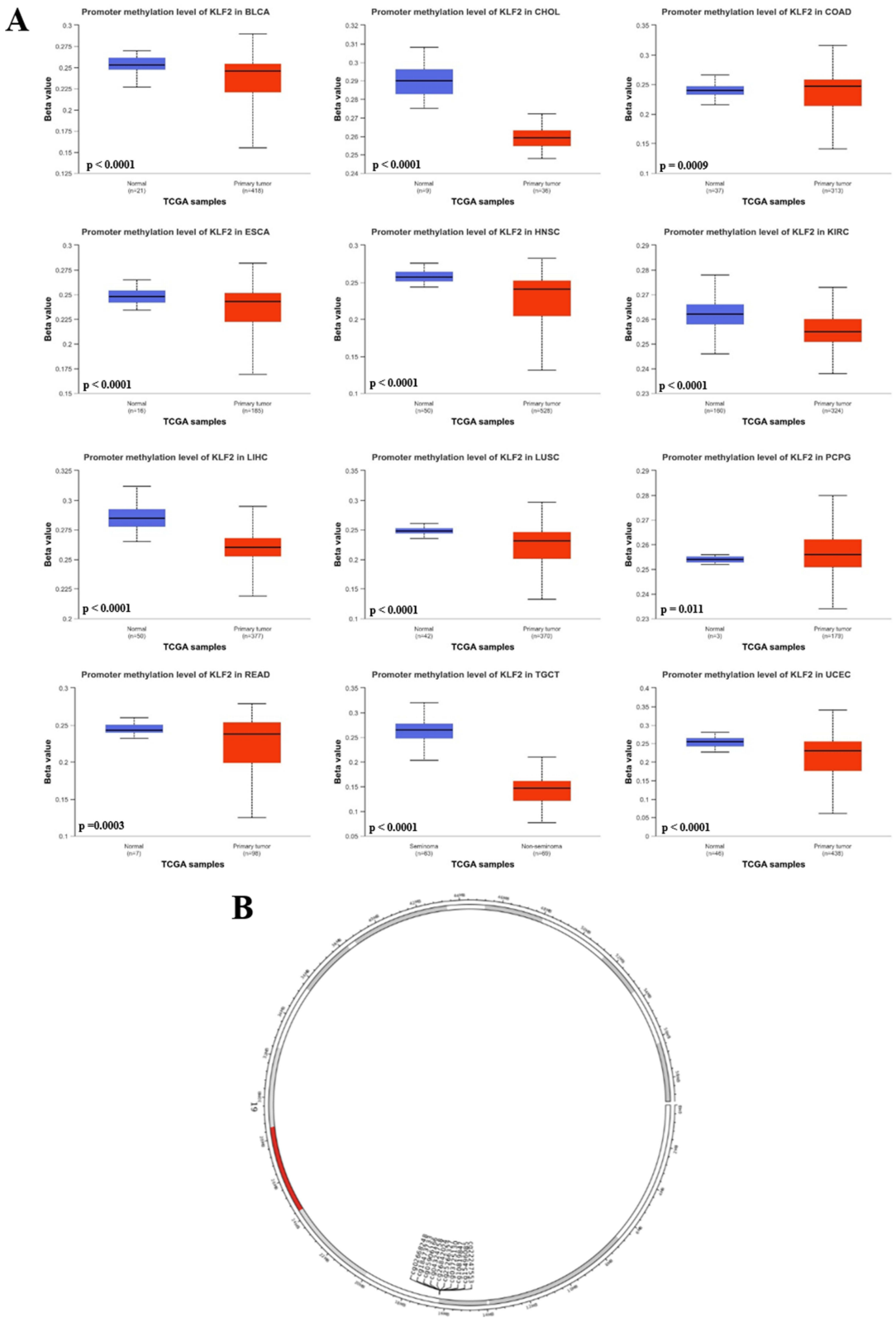

Figure 8 Relationship between KLF2 expression and DNA methylation.
Notes: (**A**) Promoter methylation level of KLF2 in BLCA, CHOL, COAD, ESCA,HNSC, KIRC, LIHC, LUSC, PCPG, READ,TGCT and UCEC. (**B**) Chromosomal distribution of the methylation probes associated with KLF2.

Table 2 Relationship Between KIF18A Methylated CpG and Survival

	CpG	HR	P value
BLCA	cg26842024	0.67	0.0067
KIRC	cg15496085	0.63	0.0166
	cg10819847	0.6	0.0088
	cg03725130	0.54	0.002
	cg25266327	0.49	0.0003
	cg05906166	2.24	<0.0001
	cg18473733	1.51	0.0398
LIHC	cg02668248	1.8	<0.0001
	cg18473733	1.47	0.0254
LUSC	cg03725130	0.71	0.0358
PCPG	cg15496085	6.36	0.047
	cg22247553	0.13	0.021
READ	cg22247553	3.14	0.0393
UCEC	cg15496085	1.67	0.0315
	cg10819847	1.68	0.0272
	cg03725130	1.86	0.0085
	cg25266327	1.76	0.0168

Single-Cell Functional Analysis of KLF2

To further study the latent role of KLF2 in tumors, we investigated the function of KLF2 at the single-cell level using CancerSEA (Figure 9A). The findings displayed that KLF2 was negatively linked with invasion and EMT in GBM. In UM (Uveal Melanoma), KLF2 had a negative relationship with DNArepair and DNAdamage. KLF2 was negatively related to inflammation in MEL (Melanoma). In OV, KLF2 had a positive relationship with EMT and had a negative relationship with invasion. However, there was a positive relationship between KLF2 with differentiation and angiogenesis. In CRC (Colorectal Cancer), KLF2 had a positive relationship with differentiation and proliferation (Figure 9B).

Drug Sensitivity Analysis

We investigated the drug sensitivity of KLF2 expression in tumors using GSCALite. The expression of KLF2 was negatively correlated with the 50% inhibitory concentration (IC50) values of 8 types of drugs, including 17−AAG, BMS −509744, CGP−60474, FH535, PD−0325901, PD−173074, RDEA119 and Trametinib. In addition, there was a positive correlation between KLF2 expression and IC50 values of Gefitinib, GSK1070916 and Navitoclax (Figure 10).

KLF2-Related Gene Enrichment Analysis

Finally, to investigate the molecular mechanism of KLF2 gene in tumorigenesis and development, we screened known KLF2-interacting proteins and genes related to KLF2 expression, and performed a series of pathway enrichment analyses. First, Using the STRING tool, we used the STRING tool to obtain a total of 10 detected KLF2-binding proteins. Figure 11A shows the interaction network of these 10 proteins. Besides, these genes are also enriched in intracellular signal transduction regulators (WWP1, EP300).

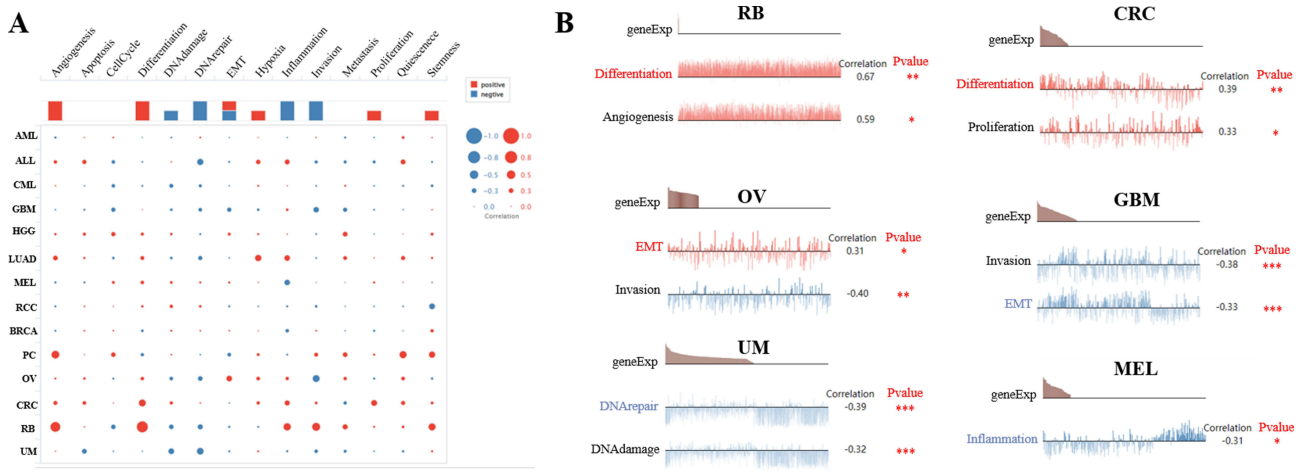

Figure 9 Single-Cell functional analysis of KLF2.
Notes: (**A**) Functional status of KLF2 in different human cancers. (**B**) Correlation analysis between functional status and KLF2 in RB, CRC, OV, GBM, UM, and MEL. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

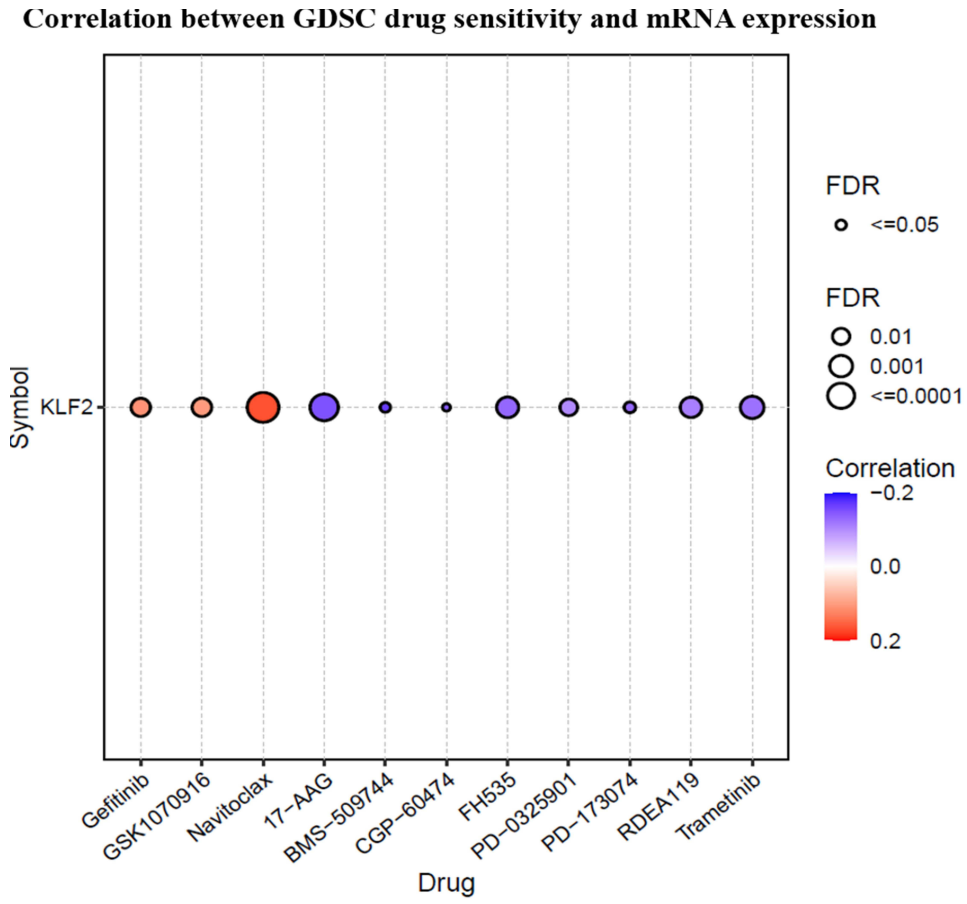

Figure 10 The associations of KLF2 expression and drug sensitivity base on GSCALite. Relationship between GDSC drug sensitivity and mRNA expression of KLF2.

These results suggest that KLF2 may play a role in these biological processes through the interaction of key proteins involved in the regulation of intracellular signal transduction. According to the BioGRID4.3 database, KLF2 physically interacts with WWP1, EP300 (Figure 11B), and they play a role in intracellular signal transduction and key

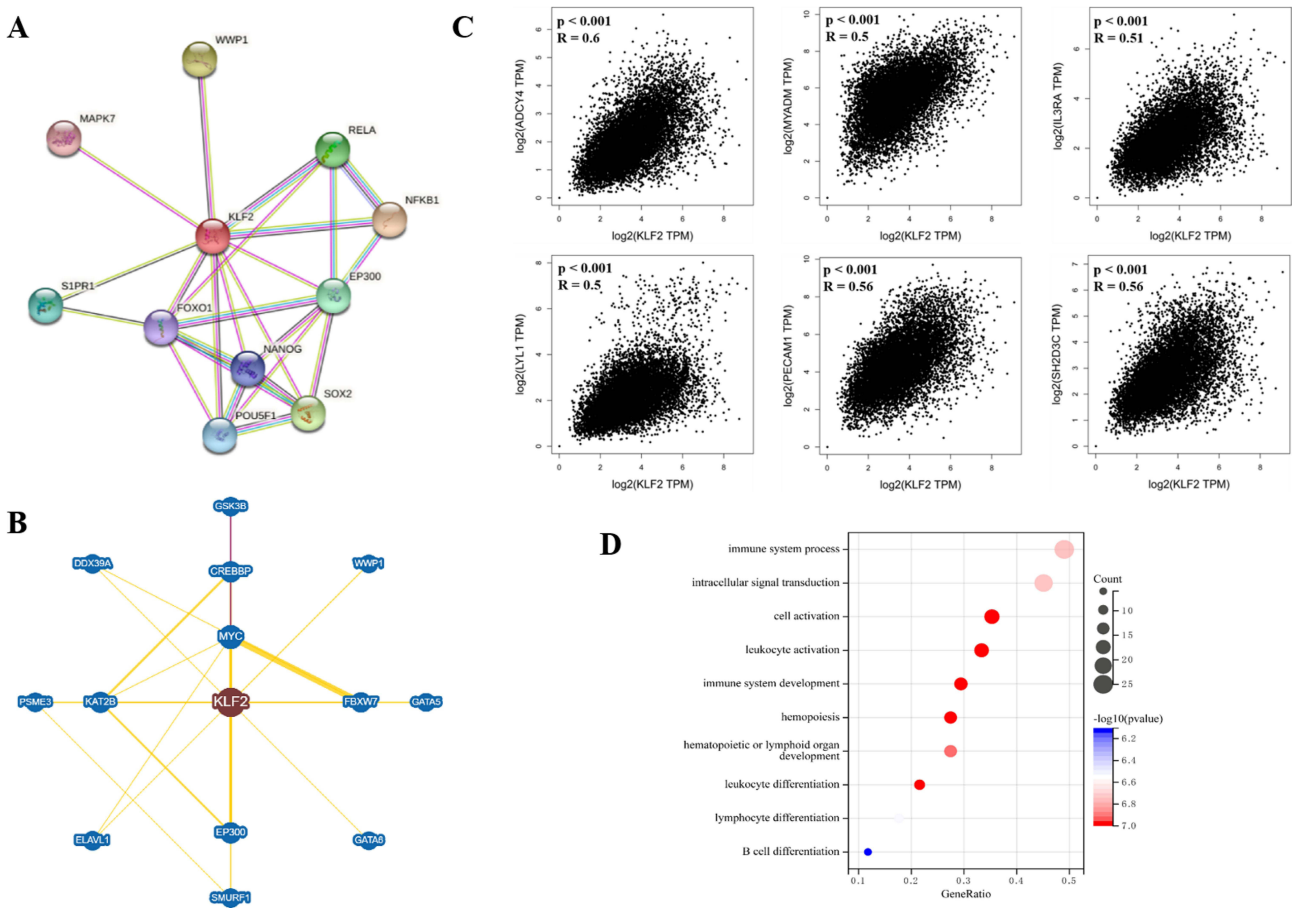

Figure 11 KLF2-related gene enrichment and pathway analysis.
Notes: (**A**) Co-expression network of 10 genes co-expressed with C1ORF112 obtained by the STRING tool. (**B**) KLF2-protein interactions obtained by BioGRID. (**C**) Correlation analysis between C1ORF112 and ADCY4, IL3RA, LYL1, PECAM1, MYADM and SH2D3C conducted by GEPIA2 across all tumor samples from TCGA. (**D**) Gene Ontology (GO) analysis of the top 100 genes co-expressed with KLF2 obtained by the GEPIA2.

molecular transport. Then, we used the GEPIA2 tool to combine all tumor expression data from TCGA to obtain the top 100 genes associated with KLF2 expression. The expression of KLF2 was positively associated with that of ADCY4 (Adenylate cyclase 4) (R=0.6), IL3RA (Interleukin 3 receptor subunit alpha) (R=0.51), LYL1 (LYL1 basic helix-loop-helix family member) (R=0.5), PECAM1 (Platelet and endothelial cell adhesion molecule 1) (R=0.56), MYADM (Myeloid associated differentiation marker) (R=0.5) and SH2D3C (SH2 domain containing 3C) (R=0.56) genes (all P<0.001) (Figure 11C).

We further performed gene ontology enrichment analysis on these top 100 genes (Figure 11D). The results show that these genes are closely related to immune system process and intracellular signal transduction, suggesting that these pathways are involved in the impact of KLF2 on tumor pathogenesis.

The Verification of the High Expression of KLF2 in Some Tumors

In order to further clarify the difference in expression of KLF2 protein between tumor tissues and normal tissues, we selected 5 cases of human gastric cancer and 15 cases of renal cancer and their corresponding normal specimens from the Department of Pathology, the Fourth Affiliated Hospital of Nanjing Medical University for immunohistochemistry and qPCR. Semi-quantitative scores of IHC showed significantly lower expression of KLF2 protein in STAD and renal cancer compared to corresponding normal tissues (Figure 12A and B). We extracted tissue RNA for qPCR studies and also showed results consistent with IHC (Figure 12C). The low expression in tumors increases the confidence of KLF2 as a new therapeutic target for these cancers.

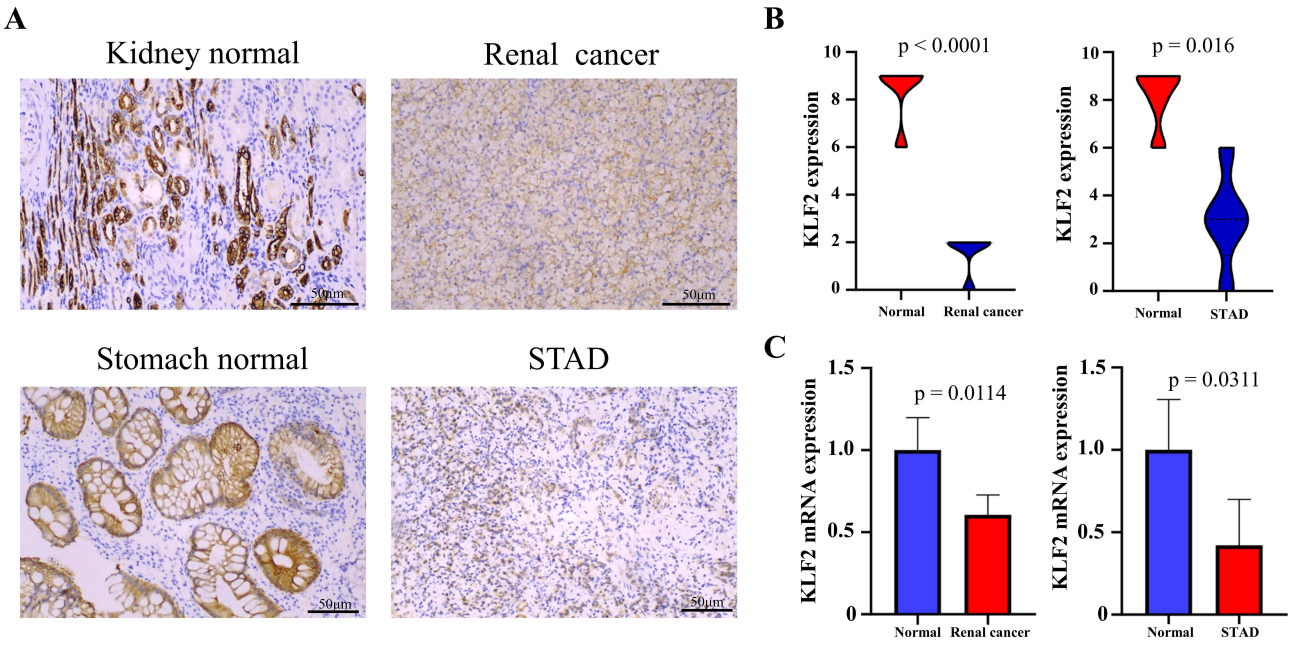

Figure 12 The Verification of the High Expression of KLF2 in Kidney and Stomach Tumors.
Notes: (**A**) Immunohistochemical expression of KLF2 protein in tumor and normal tissues (EnVision; Original magnification, x 200). (**B**) Quantitative analysis of KLF2 protein expression in tissues. (**C**) The expression of KLF2 mRNA in tissues was detected by qPCR. A P value less than 0.05 was considered to be statistically significant.

Discussion

As a member of the Kruppel-like factor regulatory protein family, KLF2 is a key factor that regulates the activation and inhibition of transcription and binds to DNA, RNA, and proteins. Recent studies have shown that KLF2 is involved in colorectal cancer, liver cancer, stomach cancer, clear cell kidney cancer, breast cancer, and prostate cancer, endometrial cancer, bladder cancer and so on the onset of a wide variety of tumor development, and also related to the survival and prognosis of these tumors.[11,13,28–32] We used a variety of databases from TCGA, GTEx, UALCAN, cBioportal, and others to reveal the molecular characteristics of KLF2 in 33 tumors from an overall perspective, including gene expression, prognosis, gene alterations, immune infiltration, DNA methylation, and drug sensitivity to clarify its role in the development and potential regulatory pathways of different tumors.

The TCGA project analyzed 33 popular tumor types through multi-omics data, facilitating molecular expression and functional analysis at the pan-cancer level.[33–36] Analysis of TCGA and GTEx data suggested that KLF2 is reduced in expression in most cancers, including BLCA, BRCA, CESC, COAD, ESCA, HNSC, KICH, KIRP, LUAD, LUSC, READ, STAD, THCA and UCEC with adjacent compared to normal controls. We further found that in patients with tumors with low KLF2 expression (eg, CESC, HNSC, KIPAN, KIRC, KICH, LIHC, LUAD, PCPG and SARC), ground expression of KLF2 generally predicted poorer OS. We evaluated the expression of KLF2 in various cancer types and found significant differences in the expression of KLF2 in tumor tissues compared with normal tissues. We validated this result by immunohistochemistry and qPCR in tumor tissue and normal adjacent normal tissues of STAD and renal cancer. Analysis of these results suggests that KLF2 is a potential molecular biomarker to predict the prognosis of tumor patients. Studies have shown that genomic mutations play an important role in tumor progression and adverse reactions after chemotherapy.[37,38] For example, TP53 mutations are critical for the development of high-grade serous ovarian cancer.[39] ARID1A mutations lead to ovarian cancer by inducing changes in the expression of multiple genes (CDKN1A, SMAD3, MLH1, and PIK3IP1) through chromatin remodeling dysfunction.[39] In this report, KLF2 mutations were the most common (>6%) in OV, followed by Bone cancer, Lung cancer and Mature B-cell lymphoma, indicating that KLF2 amplification may be a risk factor for OV patients. Together, these findings suggest that

KLF2 is a promising predictor for practical application in tumor prognosis as a tumor suppressor gene in the progression of multiple tumors.

The human KLF2 gene is highly conserved between human and mouse homolog, with 85% nucleotide sequence homology and 90% amino acid similarity, located on chromosome 19 p13.1.[40,41] Interestingly, in the two species with KLF2 homology, similar numbers of exons and introns were found in the transcriptional coding region and the 5′ and 3′ untranslated region, suggesting similar regulatory mechanisms in the two species. KLF2 is known to play an important role in lung function, cardiovascular development, and atherosclerotic protective properties.[42,43] Emerging evidence implicates KLF2 in multiple regulatory pathways, including migration, adhesion, proliferation and activation, and immune cell quiescence.[5] The expressing of KLF2 is upregulated in single-positive CD4+ and CD8+ T cells during T cell development in the thymus. Upon T cell activation, KLF2 is rapidly downregulated.[44] In addition, KLF2 is involved in regulating endothelial cell quiescence and macrophage activation.[45–47] Immune cells are extensively intertwined with cancer cells and play an important role in cancer migration and metastasis in various tumor types.[48] Another important finding of this study is that KLF2 expression in pan-cancer is highly correlated with immune cells. KLF2 expression positively correlated with the abundance of immune infiltrates, especially CD8+ T cells, CD4+ T cells, macrophages, and endothelial cells in various cancers. These results require further work to determine whether KLF2 exerts these functions. In conclusion, our report illustrates the potential role of KLF2 in tumor immunity and its prognostic value in multiple tumors.

DNA methylation is one of the most common epigenetic modifications, and changes in DNA methylation play an important role in cell cycle dysregulation, DNA repair, gene expression, silencing of tumor suppressor genes, and tumorigenesis.[49] Using the UALCAN tool, we observed a significant reduction in KLF2 promoter methylation levels in BLCA, CHOL, ESCA,HNSC, KIRC, LIHC, LUSC, READ,TGCT and UCEC compared to normal tissues and increased in COAD and PCPG. These findings indicated that KLF2 might promote tumorigenesis through DNA methylation. Interestingly, in tumors with low levels of KLF2 promoter DNA methylation compared to normal tissues, KLF2 had the lowest levels of promoter methylation in CHOL, however, was the only one with elevated KLF2 expression. In previous studies that higher DNA methylation levels in CHOL were independently associated with poorer prognosis, and that demethylation therapy improved immunotherapy outcomes.[50] Such contradictory results may suggest that changes in DNA methylation of KLF2 in CHOL are not associated with tumor progression and prognosis.

Single-cell functional analysis showed that KLF2 was negatively associated with the invasion of some tumors. Recent studies have shown that KLF2 inhibits cancer cell migration and invasion by regulating iron death in clear cell renal cell carcinoma through GPX4.[11] However, the interaction between KLF2 and cell invasion is not fully understood and needs to be further investigated in future work. In addition, the correlation between KLF2 mRNA and anti-cancer drug sensitivity was investigated. According to the GDSC database, the expression of KLF2 was negatively correlated with the IC50 value of 8 types of drugs, including 17−AAG, BMS −509744, CGP−60474, FH535, PD−0325901, PD−173074, RDEA119 and Trametinib. These drugs have the potential to prevent cancer progression. This finding will help guide clinical drug selection and patient outcomes.

Using STRING and GEPIA2, we identified a number of genes that are co-expressed with KLF2 in different tumors and other tissues. Gene enrichment analysis revealed that these genes were associated with immune system process and intracellular signal transduction, which was consistent with previous studies.[5,9] Furthermore, our results indicated that the expression of ADCY4, IL3RA, LYL1, PECAM1, MYADM and SH2D3C was closely related to the expression of C1ORF112. ADCY4, IL3RA, LYL1, PECAM1, MYADM and SH2D3C are well-characterized genes, ADCY4, IL3RA, PECAM1, MYADM and SH2D3C are proteins involved in intracellular signal transduction, and LYL1 is a protein involved in immune system. These results are consistent with our gene enrichment data and further contribute to the exploration of the molecular function of KLF2.

However, our report has some shortcomings. Firstly, all the analyses were based on bioinformatics analysis, and KLF2 expression was only confirmed by IHC and qPCR in kidney and stomach tumors. Second, analysis results for some uncommon tumor types may have batch effects or inaccurate results because of small sample sizes. Furthermore, this

study provides only preliminary findings linking KLF2 to cancer progression in various tumors, and more experimental work is required to determine the precise molecular role of KLF2 in tumorigenesis.

Conclusion

Our comprehensive investigation into KLF2 expression reveals significant correlations with clinical features, prognosis, mutational status, DNA methylation, immune cell profiles, and drug sensitivity across various cancers, enhancing our understanding of the potential role of KLF2 in pan-cancer. We ultimately validated the relationship between KLF2 expression and gastric as well as renal cancer. In summary, while further more clinical trials are necessary to substantiate these findings in the future, our study offers novel insights into the function of KLF2 in tumors and highlights its potential as a valuable target for cancer diagnosis and treatment.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors have declared that no competing interest exists.

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