

ORIGINAL RESEARCH ARTICLE

Reduced FOXO1 expression is associated with tumor progression through metabolic and immune dysregulation in kidney renal clear cell carcinoma

Wenyi Hou¹ , Mengwei Wang^{1,2} , Xiaozheng Zhang^{3,4,5*} , and Yongqiang Fu^{6*} 

¹Department of Pharmacy, Academy of Medical Sciences, Shanxi Medical University, Taiyuan, Shanxi, China

²Department of Medical Statistics, School of Public Health, Shanxi Medical University, Taiyuan, Shanxi, China

³Key Laboratory of Coal Environmental Pathogenicity and Prevention (Shanxi Medical University), Ministry of Education, Taiyuan, Shanxi, China

⁴Shanxi Key Laboratory of Birth Defect and Cell Regeneration, Shanxi Medical University, Taiyuan, Shanxi, China

⁵Department of Basic Medicine, Shanxi Medical University, Taiyuan, Shanxi, China

⁶Department of Urology, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Third Hospital of Shanxi Medical University, Tongji Shanxi Hospital, Taiyuan, Shanxi, China

***Corresponding authors:**

Xiaozheng Zhang
(zhangxiaozheng@sxmu.edu.cn)
Yongqiang Fu
(fuyongqiang@sxqbqeh.com.cn)

Citation: Hou W, Wang M, Zhang X, Fu Y. Reduced FOXO1 expression is associated with tumor progression through metabolic and immune dysregulation in kidney renal clear cell carcinoma. *Cancer Plus*.
doi: 10.36922/CP026190029

Received: May 5, 2026

Revised: July 3, 2026

Accepted: July 7, 2026

Published online: July 21, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Kidney renal clear cell carcinoma (KIRC), the most aggressive subtype of renal cancer, is characterized by metabolic reprogramming and an immunosuppressive microenvironment. While forkhead box O1 (FOXO1) is recognized as a pleiotropic regulator of cellular metabolism and immune responses, its potential tumor-suppressive roles in KIRC remain undefined, particularly regarding metabolic-immune crosstalk. We integrated The Cancer Genome Atlas bulk RNA sequencing and clinical data, immunohistochemical validation (The Human Protein Atlas database), and bioinformatics algorithms (single-sample Gene Set Enrichment Analysis [GSEA] and GSEA). Survival modeling (Cox regression and Kaplan–Meier), differential gene expression profiling, and protein–protein interaction network construction were systematically performed to delineate FOXO1's prognostic relevance and candidate associated mechanisms. Lower FOXO1 expression was associated with unfavorable overall clinical outcomes and more advanced pathology stages and histologic grades. Reduced FOXO1 expression was accompanied by an immunosuppressive tumor microenvironment, marked by elevated regulatory T cell infiltration and diminished effector and memory T cell signatures. Gene Ontology together with GSEA analyses showed a strong association between FOXO1 and lipid metabolism-related pathways. In addition, FOXO1 expression correlated with altered expression of epigenetic regulators, including higher lysine acetyltransferase 2A (KAT2A) and lower E1A-binding protein P300 (EP300), which may be associated with T cell functional states. This study, based on bulk RNA sequencing data and in silico analyses, suggests that FOXO1 could represent a potential regulator of metabolic-immune interactions in KIRC and is associated with tumor progression and remodeling of the immune landscape. Our results support that FOXO1 emerges as a potential indicator of patient

prognosis. However, additional experimental studies are required to further substantiate these observations.

Keywords: Forkhead box O1; Kidney renal clear cell carcinoma; Metabolic reprogramming; Immune infiltration; Epigenetic modification

1. Introduction

Renal cell carcinoma, a predominant malignancy of the urinary system, exhibits a rising global incidence. It comprises three major histological entities: kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), and kidney chromophobe renal cell carcinoma (KICH). Among these, KIRC represents the predominant and most aggressive pathological subtype¹, with an increasing incidence over time.² While surgery achieves favorable outcomes in localized KIRC, advanced and metastatic stages of the disease remain clinically challenging, with limited therapeutic efficacy of conventional radiotherapy and chemotherapy contributing to dismal survival rates.^{3,4} Although the introduction of immune checkpoint blockade and anti-angiogenic agents has substantially extended survival and improved disease control in patients with advanced KIRC^{5,6}, a considerable proportion of patients fail to achieve durable responses because of intrinsic or acquired therapeutic resistance⁷, highlighting the importance of elucidating the biological processes that drive KIRC progression and treatment response. Therefore, elucidating the molecular mechanisms driving tumor progression and metastasis represents a critical imperative for developing innovative therapeutic strategies with enhanced oncological efficacy.

The hallmark metabolic remodeling observed in KIRC exemplifies how transcriptional dysregulation drives oncogenic metabolic adaptation, with altered cellular energetics propagating tumorigenic expansion.⁸ Histopathological analyses corroborate this metabolic paradigm, as cytoplasmic clearing—a diagnostic hallmark of KIRC—manifests as pathognomonic accumulations of neutral lipids and cytoplasmic glycogen stores, indicative of profound lipolysis and glucometabolic reprogramming.⁹ The von Hippel–Lindau (*VHL*) tumor suppressor gene is genetically inactivated in approximately 90% of KIRC cases, a molecular defect that constitutively activates hypoxia-inducible factor (HIF) signaling through impaired proteasomal degradation.¹⁰ KIRC exhibits distinct metabolic reprogramming, marked by glucose metabolism dysregulation, impaired oxidative phosphorylation, and tumor-specific dependencies, such

as glutamine anaplerosis.¹¹

The immune landscape within the tumor microenvironment serves not only as a critical determinant of patient prognosis but also as a promising therapeutic frontier in oncology.^{7,12} While KIRC exhibits substantial infiltration of T lymphocytes and myeloid populations¹³, these immune effectors demonstrate compromised cytotoxic functionality.^{14–16} Metabolic reprogramming serves as a pivotal mechanism regulating functional adaptation in lymphocytes, with particular relevance to T cell differentiation processes.^{17,18} While effector T cells predominantly utilize glucose and amino acid catabolism to sustain clonal expansion and cytolytic potential^{19–22}, exhausted T cells develop distinct bioenergetic profiles marked by mitochondrial dysfunction^{23–25}, preferential utilization of glycolysis²⁶, and accumulation of lipid oxidation byproducts that collectively drive functional impairment.²⁷

As a constituent of the evolutionarily conserved forkhead box (FOX) protein superfamily, FOXO1 functions as a master regulatory switch governing metabolic homeostasis.^{28,29} This pleiotropic transcription factor mediates canonical insulin/insulin-like growth factor 1 (IGF-1) signal transduction networks while maintaining pan-tissue expression patterns in vertebrates.³⁰ As a biological switch governing T lymphocyte responsiveness and immunological memory commitment, FOXO1's functional coordination with its inherent metabolic regulatory circuits remains mechanistically undefined.^{31,32}

FOXO1 demonstrates paradoxical oncogenic regulation across cancer subtypes. The expression signature of FOXO1 demonstrates significant depletion across advanced renal cell carcinoma histotypes, particularly in KIRC.³³ Genetic restoration of *FOXO* transcriptional activity induces conserved tumor-suppressive responses through apoptotic activation and Myc-dependent transcriptional network remodeling.³⁴ Paradoxically, FOXO-mediated potentiation of AKT catalytic domain phosphorylation establishes compensatory signaling circuits that sustain neoplastic progression under phosphoinositide 3-kinase (PI3K)-targeted therapeutic interventions.³⁵

Although FOXO1 expression was initially surveyed

across multiple renal cancer histotypes, this study focuses exclusively on KIRC for all subsequent mechanistic and clinical analyses. Based on transcriptomic and clinical data obtained from The Cancer Genome Atlas (TCGA), we analyzed the clinical relevance of FOXO1 expression in KIRC. The relationship between the immune microenvironment in KIRC patients and FOXO1 mRNA levels was investigated using bioinformatics tools, including single-sample Gene Set Enrichment Analysis (ssGSEA) and GSEA. This study aims to evaluate the prognostic value of FOXO1 and to explore its potential role in immune regulation in KIRC.

2. Materials and methods

2.1. Data sources and pre-processing

We obtained gene expression data, clinical data, and other relevant information corresponding to different types of renal cancer from the TCGA database (<https://portal.gdc.cancer.gov>). RNA sequencing (RNA-seq) data were generated using the Spliced Transcripts Alignment to a Reference (STAR) workflow and processed into transcripts per million (TPM) format according to the platform's standardized pipeline. The processed TPM expression matrix was subsequently used for all downstream analyses. For visualization and additional comparisons, expression data were log₂-transformed. Comparisons between two groups were performed using paired or unpaired two-tailed *t*-tests, as appropriate. Specifically, for samples with matched tumor and adjacent normal tissues from the same patient, paired *t*-tests were applied to account for inter-patient variability. A *p*-value < 0.05 was considered statistically significant. For analyses involving multiple comparisons, *p*-values were adjusted using the Benjamini–Hochberg method (see Sections 2.5 and 2.6).

2.2. Immunohistochemistry

The Human Protein Atlas (HPA, <https://www.proteinatlas.org/>) is an open-access database that provides comprehensive information on protein expression and localization across a wide range of human tissues and cell types. Immunohistochemical images of normal and renal cancer tissues were downloaded from HPA to compare FOXO1 expression levels between normal and tumor samples.

2.3. Survival analysis

Overall survival (OS), disease-specific survival (DSS), and disease progression-free interval (PFI) were used as clinical endpoints. These survival data were obtained from the TCGA Pan-Cancer Clinical Data Resource (TCGA-CDR) developed by Liu *et al.*³⁶, which provides standardized

survival endpoints for TCGA cohorts. FOXO1 expression was analyzed as a continuous variable in Cox proportional hazards regression models. The associations between FOXO1 expression and OS, DSS, or PFI in patients with different kidney cancer types were evaluated to assess the prognostic value of FOXO1. The Kaplan–Meier method was used to plot survival curves.

2.4. Clinical risk factor analysis

Based on the clinical information from the TCGA database, the association between FOXO1 expression levels and clinicopathological characteristics, including gender, age, pathologic T stage, and pathologic M stage, was analyzed. Patients were stratified into FOXO1-high and FOXO1-low groups according to the median FOXO1 expression level. OS and DSS were used as the study endpoints. OS was defined as the time from diagnosis to death from any cause or the last follow-up, whereas DSS was defined as the time from diagnosis to death attributable to KIRC or the last follow-up. Patients alive at the last follow-up were treated as censored observations. Univariate and multivariate Cox proportional hazards regression analyses were performed using the “survival” package in R. Age, gender, pathological T stage, pathological M stage, and FOXO1 expression group were included to identify independent prognostic factors. Samples with missing values for variables included in the survival analyses were excluded from the corresponding analyses.

2.5. Tumor infiltration level analysis of immune cells

Immune cell infiltration was quantified using ssGSEA with the GSVA R package. Gene expression profiles were used as input, and enrichment scores representing the relative abundance of immune cell populations were calculated for each sample. The ssGSEA method was applied to estimate the relative infiltration levels of 24 immune cell subsets in each sample. Spearman correlation analysis was performed to evaluate the association between FOXO1 expression and the infiltration levels of the 24 immune cell types (marker genes were selected from a previous study³⁷), and *p*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) method. A *p*-value < 0.05 was considered statistically significant.

To further validate the ssGSEA findings, immune cell composition was independently estimated using the Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) algorithm based on the LM22 leukocyte signature matrix. Associations between FOXO1 expression and the inferred immune cell proportions were subsequently evaluated using Spearman correlation analysis.

2.6. Analysis of differentially expressed genes

Differentially expressed genes (DEGs) were identified between FOXO1-high and FOXO1-low KIRC samples using the DESeq2 package with the design formula $\sim$ FOXO1_group. The screening threshold was set at $|\log_2FC$ (fold-change) > 1 , and the Benjamini–Hochberg method was used for multiple-comparison adjustment, with adjusted p -values < 0.05 considered statistically significant. A positive $\log_2FC$ indicates higher expression in the FOXO1-low group, whereas a negative $\log_2FC$ corresponds to higher expression in the FOXO1-high group. As the analysis was intended to explore FOXO1-associated transcriptional programs, no adjustment for tumor stage or histological grade was performed. Volcano plots were drawn using the “ggplot2” package in the R software, in which red markers represented upregulated genes and blue markers represented downregulated genes.

2.7. Gene Ontology analyses

Gene Ontology (GO) enrichment analyses are commonly used functional enrichment methods in bioinformatics to parse gene expression data and reveal gene function and pathway information in organisms. The screened DEGs were imported into the R software, and the “enrichGO” function in the “clusterProfiler” toolkit was used to perform GO annotation. Significance thresholds were set at p -value < 0.05 and FDR < 0.05 to identify significant biological processes in which the DEGs were involved. The default background gene universe defined by the clusterProfiler package was used for enrichment analyses.

2.8. Gene Set Enrichment Analysis

Gene Set Enrichment Analysis was performed using the “clusterProfiler” R package. Genes were ranked by $\log_2FC$ between the FOXO1-high and FOXO1-low groups, with the FOXO1-high group serving as the reference. Therefore, a positive normalized enrichment score represents pathway enrichment in the FOXO1-low group, whereas a negative normalized enrichment score represents pathway enrichment in the FOXO1-high group. The predefined gene set “c2.cp.all.v2022.1.Hs.symbols.gmt” from the Molecular Signatures Database (MSigDB), which contains canonical pathways from multiple pathway databases (including Kyoto Encyclopedia of Genes and Genomes [KEGG], Reactome, WikiPathways, BioCarta, and Pathway Interaction Database [PID]), was used as the reference gene set. The number of permutations was set to 1,000, and gene sets with sizes between 10 and 500 genes were included. The enriched pathways were considered statistically significant when the FDR was < 0.05 . The most significantly enriched KEGG pathways are visualized, whereas representative metabolism-related pathways from

the Reactome and WikiPathways databases are presented in this article.

2.9. Protein–protein interaction analysis of differentially expressed genes

Protein–protein interaction (PPI) network analysis was performed using the STRING database (<https://string-db.org/>) to investigate the interactions among DEGs identified between the FOXO1-high and FOXO1-low expression groups. The resulting PPI network was visualized and further analyzed in Cytoscape. Hub genes were identified based on network topology, and the top 14 genes ranked by connectivity (degree) were selected for visualization.

3. Results

3.1. FOXO1 expression is decreased in kidney renal clear cell carcinoma

We first characterized *FOXO1* mRNA expression across the three major renal cell carcinoma subtypes (KICH, KIRC, and KIRP) using TCGA data. In the unpaired sample analysis, *FOXO1* expression was significantly reduced in tumor tissues from both KIRC and KIRP cohorts compared with their corresponding normal tissues (Figure 1A; sample sizes, exact p -values, and effect sizes $[\log_2FC]$ are shown in Table S1). Paired analysis of matched samples confirmed this downregulation (Figure 1B; sample sizes, exact p -values, and effect sizes $[\log_2FC]$ are shown in Table S2). To confirm the robustness of the TCGA findings, an independent Gene Expression Omnibus dataset (GSE40435) containing 101 paired KIRC and adjacent non-tumorous renal tissues was analyzed. Consistent with the TCGA results, *FOXO1* expression in KIRC tissues was also markedly decreased relative to their normal counterparts (Figure S1). Immunohistochemical data obtained from the HPA database showed higher FOXO1 protein expression in normal renal samples, whereas more than half of the tumor samples exhibited low or undetectable FOXO1 expression (Figure 1C). Because KIRC is the most common and aggressive subtype, all subsequent analyses in this study focused exclusively on KIRC. The initial observation of FOXO1 downregulation in KIRC suggested a potential association with tumor biology, prompting further investigation.

3.2. Low FOXO1 expression is associated with significantly worse survival in kidney renal clear cell carcinoma

To explore the association between FOXO1 expression and clinical outcome status in KIRC, FOXO1 expression levels were compared among patients with different OS, DSS, and PFI outcomes. FOXO1 expression differed significantly

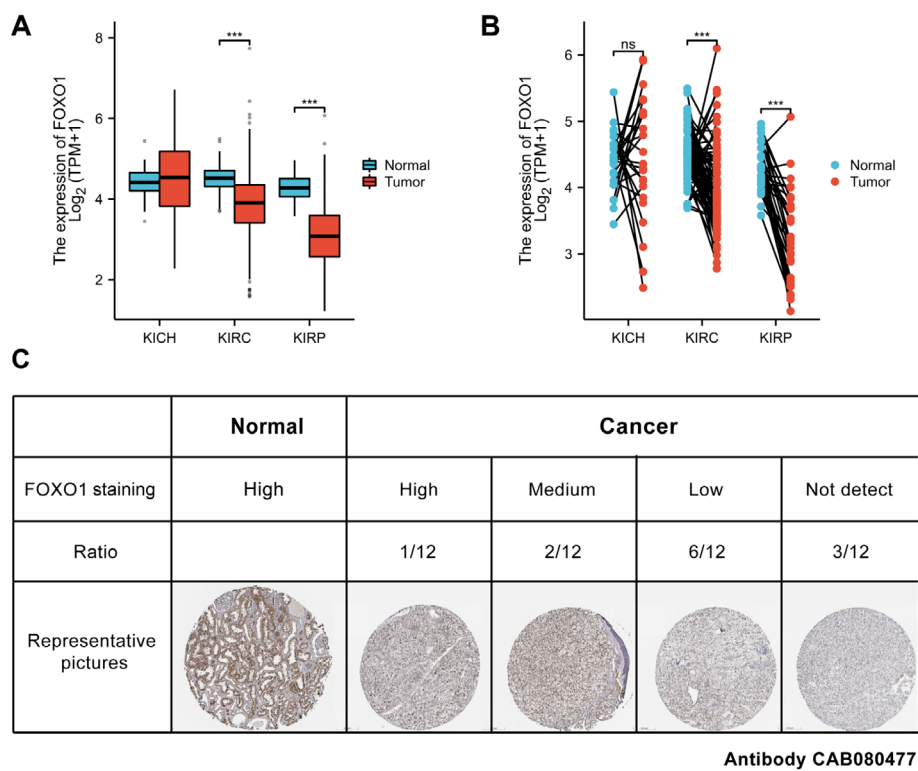


Figure 1. Comparison of FOXO1 expression in normal and renal cancer tissues. (A) Comparison of FOXO1 expression between tumor and normal tissues in KIRC, KICH, and KIRP. (B) Comparison of FOXO1 expression between tumor and paired normal tissues in KIRC, KICH, and KIRP. (C) Immunohistochemical expression characteristics of FOXO1 in normal renal samples and KIRC samples. Notes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: FOXO1: Forkhead box O1; KICH: Kidney chromophobe renal cell carcinoma; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; TPM: Transcripts per million.

between outcome groups for all three endpoints (Figure 2A–C). Specifically, patients who experienced death (OS or DSS events) or disease progression (PFI events) exhibited significantly lower FOXO1 expression than patients without these events.

A univariate Cox proportional hazards regression analysis was conducted using FOXO1 expression to examine its relationship with survival outcomes. To visualize survival differences, Kaplan–Meier survival curves were plotted by dividing patients into FOXO1-high and FOXO1-low groups according to the median FOXO1 expression (3.91, log₂[TPM + 1]). Patients with lower FOXO1 expression experienced significantly poorer OS (Figure 2D), DSS (Figure 2E), and PFI (Figure 2F) than those with higher FOXO1 expression (number of events and median follow-up time are presented in Table S3). As a preliminary observation, FOXO1 expression showed no significant correlation with clinical outcomes in the KIRP and KICH subtypes (Figure S2); however, given that the

present study focuses on KIRC, these findings are not discussed further.

3.3. Distribution of FOXO1 expression in clinical characteristics sub-groups

Based on the transcriptomic and clinicopathological data obtained from TCGA-KIRC, FOXO1 expression levels varied significantly across tumor stages. FOXO1 expression decreased with increasing pathologic stage (Figure 3A) and histologic grade (Figure 3B). As shown in Figure 3C, FOXO1 expression exhibited notable differences across pathologic T stages, with lower levels in T3 and T4. Similarly, FOXO1 expression was reduced in patients with tumor metastasis (Figure 3D) and decreased with increasing N classification (Figure 3E). Furthermore, FOXO1 expression was not associated with gender (Figure 3F).

To determine whether FOXO1 expression serves as an independent determinant of survival in KIRC, multivariate

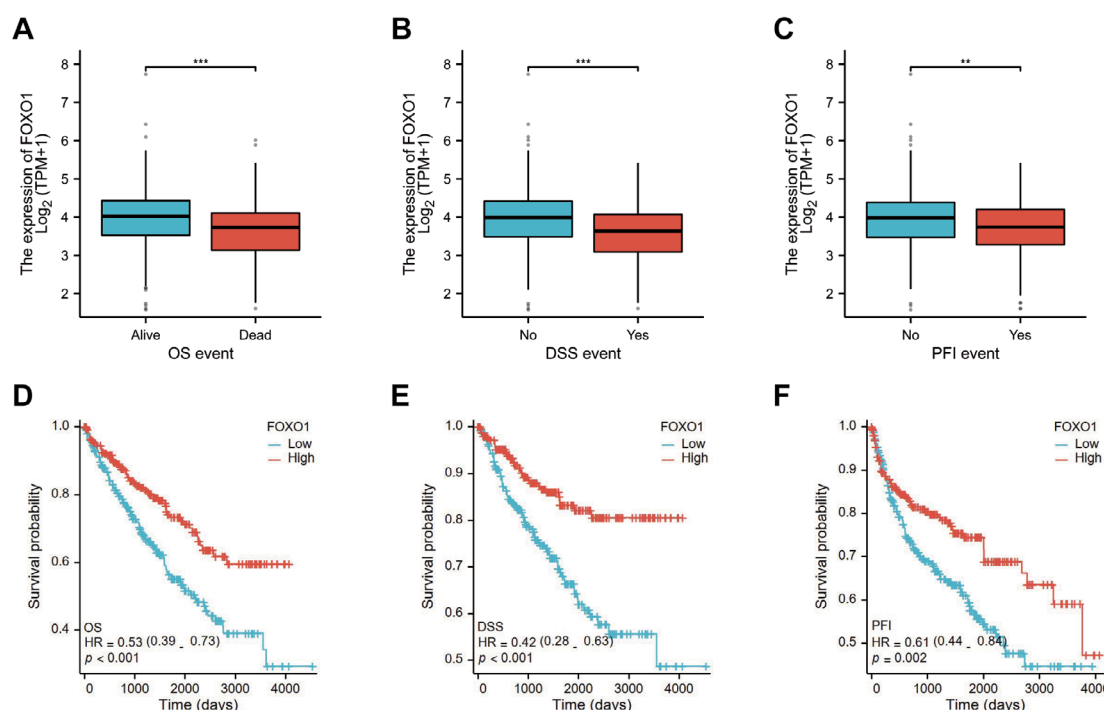


Figure 2. Prognostic significance of FOXO1 expression in KIRC. FOXO1 expression was significantly associated with clinical outcomes of (A) overall survival (OS), (B) disease-specific survival (DSS), and (C) progression-free interval (PFI). Kaplan-Meier survival analysis demonstrated poorer (D) OS, (E) DSS, and (F) PFI in patients with low FOXO1 expression compared with those exhibiting high FOXO1 expression.

Notes: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Abbreviations: FOXO1: Forkhead box O1; HR: Hazard ratio; KIRC: Kidney renal clear cell carcinoma; TPM: Transcripts per million.

Cox regression analyses were conducted. The results demonstrated that pathologic T stages, pathologic M stages, and FOXO1 expression were significant predictors of OS and DSS in KIRC patients (Table 1). These data support the notion that FOXO1 expression has independent prognostic value, with higher expression levels correlating with longer OS and DSS.

These findings strongly suggest that investigating the molecular mechanism of FOXO1 in KIRC provides valuable insights and may represent a potential therapeutic target for KIRC patients.

3.4. Correlation between FOXO1 expression and tumor-infiltration levels of immune cells in kidney renal clear cell carcinoma

To explore the association between FOXO1 and the immune landscape of KIRC, we systematically assessed FOXO1 levels and immune cell enrichment using the ssGSEA algorithm. FOXO1 expression exhibited significant correlations with multiple immune cell infiltration signatures (Figure 4A,B; specific p and FDR values are shown in Table S4). Specifically, higher FOXO1 expression was associated with increased enrichment scores for mast

cells ($r = 0.417$, $\text{FDR} = 2.91 \times 10^{-20}$), central memory T cells (Tcm; $r = 0.343$, $\text{FDR} = 2.14 \times 10^{-15}$), effector memory T cells (Tem; $r = 0.290$, $\text{FDR} = 1.19 \times 10^{-10}$), $\gamma\delta$ T cells (Tgd; $r = 0.290$, $\text{FDR} = 4.07 \times 10^{-9}$), and natural killer (NK) cells ($r = 0.263$, $\text{FDR} = 5.17 \times 10^{-8}$). Conversely, FOXO1 expression was negatively correlated with the enrichment scores of NK CD56⁺ cells ($r = -0.410$, $\text{FDR} = 4.37 \times 10^{-22}$) and regulatory T cells (Treg; $r = -0.267$, $\text{FDR} = 4.60 \times 10^{-10}$). Representative scatter plots for these immune cell populations are shown in Figure 4C.

To further validate the robustness of the immune infiltration analysis, we also estimated immune cell composition using CIBERSORT. Overall, the CIBERSORT analysis revealed a similar pattern of immune cell infiltration associated with FOXO1 expression, supporting the robustness of the ssGSEA-based findings (Figure S3).

In summary, our findings imply that FOXO1 may be associated with the immune landscape of KIRC, involving processes such as antigen presentation, the inflammatory response, T cell activation, and immune memory. These mechanisms potentially enhance immune system activation and effector functions, ultimately influencing

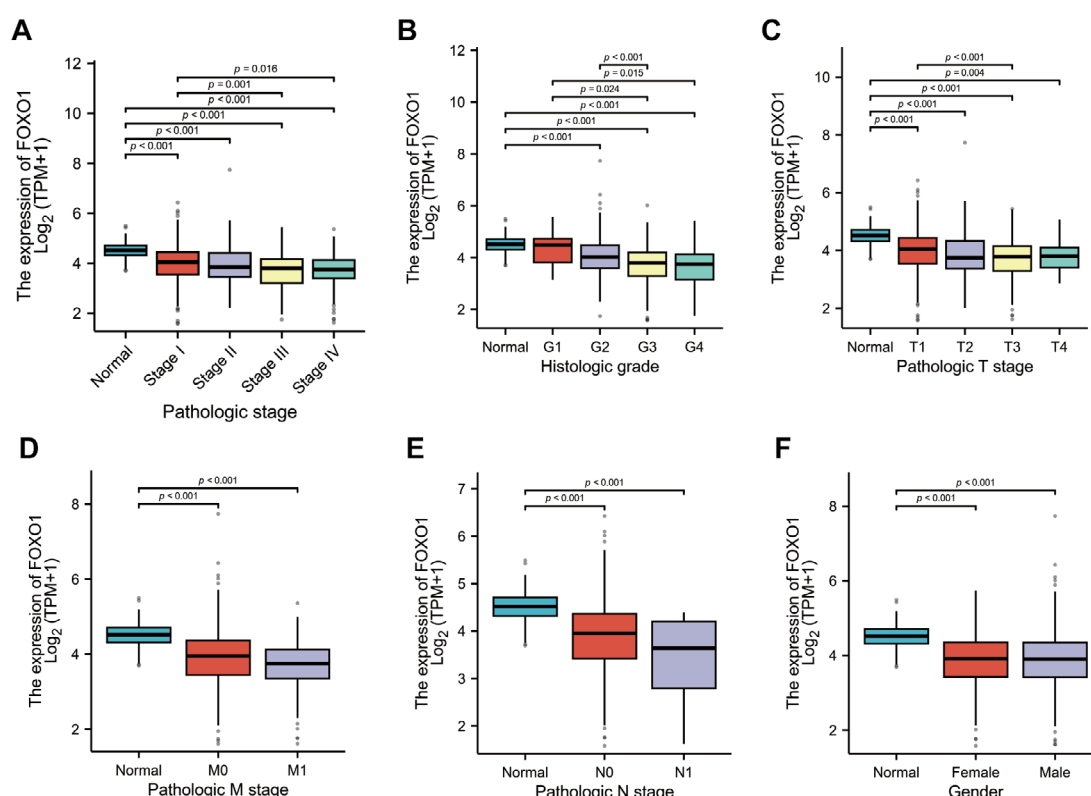


Figure 3. Associations between *FOXO1* expression and clinicopathological characteristics in KIRC. *FOXO1* expression across (A) different pathologic stages, (B) histologic grade, (C) pathologic T stages, (D) pathologic M stages, (E) pathologic N stages, and (F) gender. Statistical comparisons among multiple groups were performed using the Kruskal–Wallis test with Dunn's post hoc test, whereas comparisons between two groups (gender) were conducted using the Wilcoxon rank-sum test.

Abbreviations: FOXO1: Forkhead box O1; KIRC: Kidney renal clear cell carcinoma; TPM: Transcripts per million.

tumor prognosis. As these results are derived from bulk RNA-seq data, which reflect mixed signals from tumor, immune, stromal, and other cells, they are correlative and do not indicate the cellular source of these associations.

3.5. Gene sets enriched in *FOXO1*-low phenotype

Comparative analysis between *FOXO1*-high and *FOXO1*-low groups identified 3,396 DEGs ($|\log_2 FC| > 1$, adjusted p -value < 0.05), with 2,796 genes upregulated and 600 genes downregulated in the *FOXO1*-low cohort (Figure 5A).

To explore biological pathways associated with *FOXO1* expression in KIRC, we performed GSEA by comparing samples with high and low *FOXO1* expression. A number of canonical KEGG pathways were significantly enriched according to *FOXO1* expression status. Among these, the top 10 KEGG pathways with the highest normalized enrichment scores are shown in Figure 5B. These pathways included several cancer-associated signaling pathways (e.g., renal cell carcinoma, prostate cancer, small cell lung cancer,

endometrial cancer), cell adhesion-related pathways (e.g., adherens junction, focal adhesion, extracellular matrix–receptor interaction), and immune-modulatory pathways (e.g., transforming growth factor beta [TGF- β] signaling, *Helicobacter pylori* infection-mediated epithelial cell signaling, and *Vibrio cholerae* infection).

Gene Ontology enrichment analysis further elucidated the biological functions associated with *FOXO1*. Downregulation of *FOXO1* is not only associated with immune-related processes (e.g., acute inflammatory response, acute-phase response) but also significantly correlates with lipoprotein metabolism. Key enriched biological processes included cellular lipid catabolic process, glycerolipid catabolic process, protein-containing complex remodeling, plasma lipoprotein particle remodeling, protein–lipid complex remodeling, high-density lipoprotein (HDL) particle remodeling, very-low-density lipoprotein particle remodeling, and triglyceride-rich lipoprotein particle remodeling (Figure 5C). The complementary GSEA further confirmed *FOXO1*-

Table 1. Univariate and multivariate Cox regression analysis of clinicopathological features (including FOXO1 expression) with clinical outcomes in KIRC patients

Characteristics	Total (<i>n</i>)	Univariate analysis		Multivariate analysis	
		HR (95% CI)	<i>p</i> -value	HR (95% CI)	<i>p</i> -value
Overall survival					
Gender	530				
Female	181	Reference			
Male	349	1.183 (0.786–1.781)	0.420		
Age	530				
≤60	265	Reference			
>60	265	1.351 (0.926–1.971)	0.118		
Pathologic T stage	530				
T1 & T2	347	Reference		Reference	
T3 & T4	183	5.606 (3.697–8.502)	<0.001	2.632 (1.619–4.281)	<0.001
Pathologic M stages	497				
M0	422	Reference		Reference	
M1	75	9.219 (6.294–13.504)	<0.001	5.148 (3.314–7.996)	<0.001
FOXO1	530				
Low	262	Reference		Reference	
High	268	0.423 (0.282–0.634)	<0.001	0.569 (0.377–0.858)	0.007
Disease-specific survival					
Gender	541				
Female	187	Reference			
Male	354	0.924 (0.679–1.257)	0.613		
Age	541				
≤60	269	Reference		Reference	
>60	272	1.791 (1.319–2.432)	<0.001	1.735 (1.272–2.367)	<0.001
Pathologic T stage	541				
T1 & T2	350	Reference		Reference	
T3 & T4	191	3.210 (2.373–4.342)	<0.001	1.965 (1.386–2.787)	<0.001
Pathologic M stages	508				
M0	429	Reference		Reference	
M1	79	4.401 (3.226–6.002)	<0.001	2.919 (2.044–4.167)	<0.001
FOXO1	541				
Low	270	Reference		Reference	
High	271	0.533 (0.391–0.726)	<0.001	0.639 (0.467–0.874)	0.005

Abbreviations: HR: Hazard ratio; KIRC: Kidney renal clear cell carcinoma.

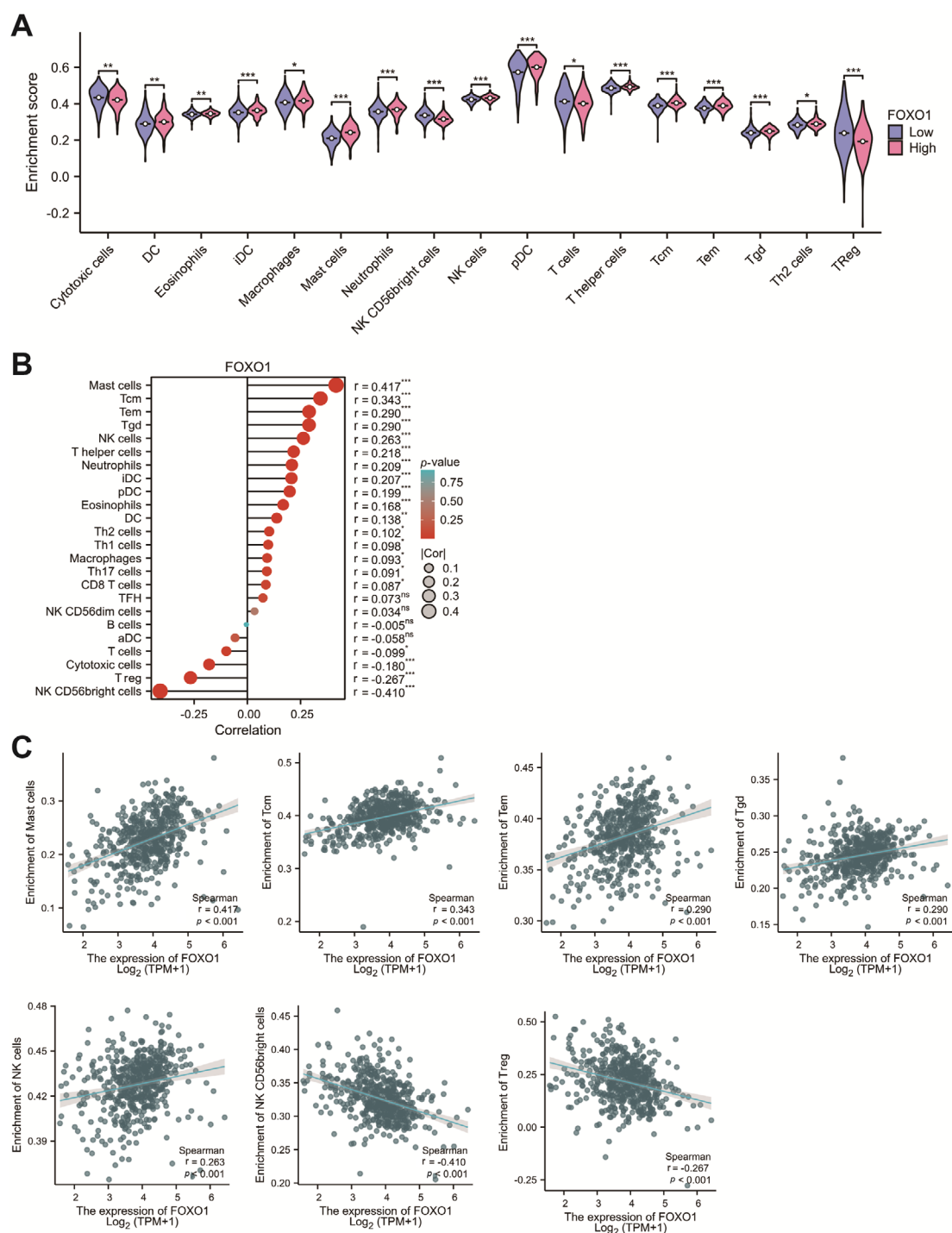


Figure 4. Association of *FOXO1* expression with the immune infiltration in KIRC. (A) ssGSEA-based evaluation of the relationship between *FOXO1* expression and immune cell infiltration. (B) Correlation coefficients between *FOXO1* expression and 24 immune cell subsets in KIRC. (C) Scatter plots illustrating the correlations between *FOXO1* expression and the immune cell subsets that showed statistically significant associations in panel B.

Notes: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Abbreviations: aDC: Activated dendritic cells; DC: Dendritic cells; iDC: Immature dendritic cells; KIRC: Kidney renal clear cell carcinoma; NK: Natural killer; pDC: Plasmacytoid dendritic cells; ssGSEA: Single-sample Gene Set Enrichment Analysis; Tcm: Central memory T cell; Tem: Effector memory T cells; TFH: T follicular helper cells; Tgd: $\gamma\delta$ T cell; Th: T helper; TPM: Transcripts per million; Treg: Regulatory T cell.

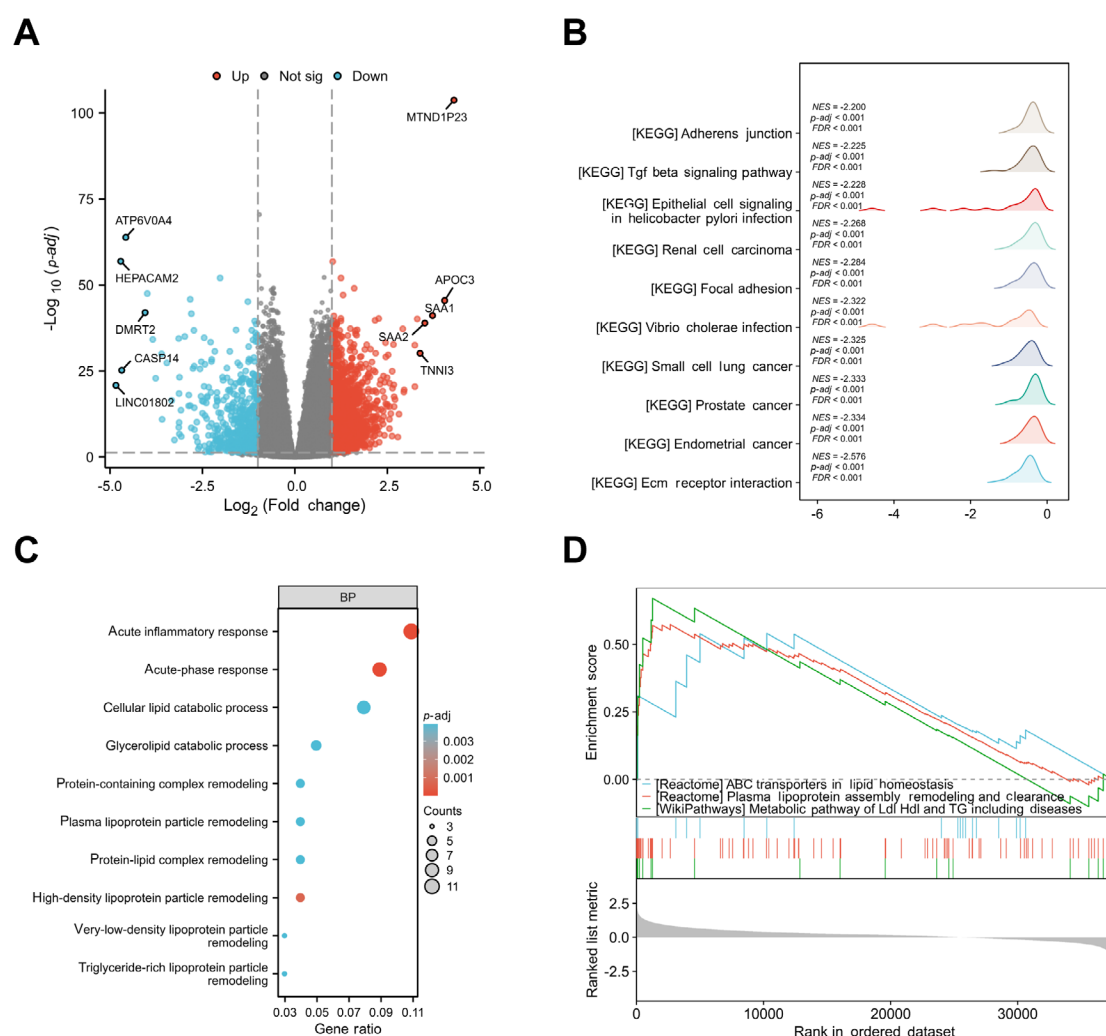


Figure 5. Differential expression analysis and enrichment analysis of genes between the FOXO1-high and FOXO1-low groups. (A) Volcano map of differentially expressed genes (DEGs). Red points indicate upregulated genes, blue points indicate downregulated genes, and gray points indicate genes without significant differential expression. (B) Top 10 significantly enriched KEGG pathways identified by GSEA. (C) Top 10 GO enrichment terms in biological process. (D) Differentially enriched metabolism-related pathways identified from the Reactome and WikiPathways databases by GSEA. Abbreviations: ABC: ATP-binding cassette; FDR: False discovery rate; FOXO1: Forkhead box O1; GO: Gene Ontology; GSEA: Gene Set Enrichment Analysis; HDL: High-density lipoprotein; KEGG: Kyoto Encyclopedia of Genes and Genomes; LDL: Low-density lipoprotein; TG: Triglyceride.

low-associated lipid metabolic dysregulation, highlighting key pathways such as ATP-binding cassette (ABC) transporters in lipid homeostasis, plasma lipoprotein assembly and remodeling, and clearance, as well as metabolic pathways of low-density lipoprotein (LDL), HDL, and triglycerides, and related diseases (Figure 5D).

3.6. Analyses of FOXO1-related hub genes in kidney renal clear cell carcinoma

The PPI network of DEGs included 1,090 nodes (protein-coding genes) and 968 edges. From this network, we identified 14 hub genes with the highest node degrees,

including *APOA1*, *APOA2*, *APOA4*, *APOA5*, *APOC2*, *APOC3*, *SAA1*, *SAA2*, *SAA4*, *AHSG*, *FGA*, *SERPINC1*, *F2*, and *HPR* (Figure 6A). Gene co-expression analysis demonstrated strong negative correlations between most hub genes and FOXO1 expression (Figure 6B). Clinically, elevated expression of *APOA1*, *APOC3*, *SAA1*, *SAA2*, and *F2* correlated with poorer OS in KIRC patients (Figure 6C–G), whereas the remaining nine hub genes exhibited no statistically significant association with OS outcomes (Figure S2). The five FOXO1-associated hub genes exhibit robust functional interconnectivity and collectively form a multi-gene prognostic biomarker panel for KIRC.

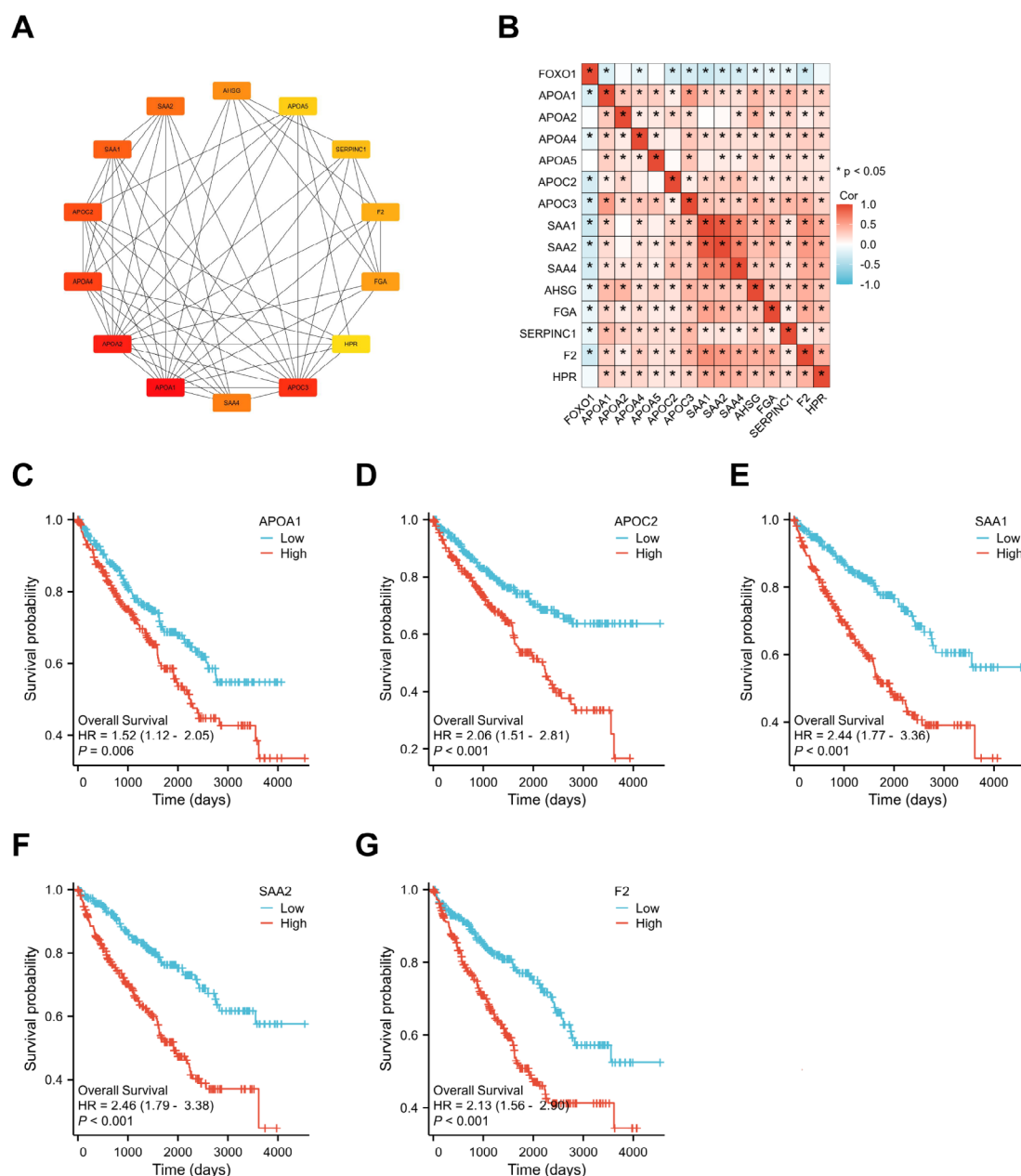


Figure 6. Analyses of *FOXO1*-related hub genes in KIRC. (A) The top 14 genes in the protein–protein interaction network. (B) Heatmap for differential co-expression analysis between hub genes and *FOXO1* expression. (C–G) Associations between *APOA1*, *APOC3*, *SAA1*, *SAA2*, or *F2* expressions and overall survival (OS) in kidney renal clear cell carcinoma (KIRC) patients. Abbreviation: HR: Hazard ratio.

3.7. FOXO1 is associated with metabolic–epigenetic crosstalk

FOXO1 has been reported to participate in T-cell activation and memory differentiation. Emerging evidence suggests that nutrient metabolism-mediated epigenetic modifications play a key role in determining T-cell fate. To investigate whether *FOXO1* expression is associated with

metabolic programs in KIRC, GSEA was conducted by comparing tumors with high and low *FOXO1* expression. The analysis identified significant enrichment in several nutrient metabolism-related gene sets, including energy metabolism integration, triglyceride metabolism, inositol phosphate metabolism, ascorbate and aldarate metabolism, arachidonic acid metabolism, and linoleic acid metabolism (Figure 7A–I).

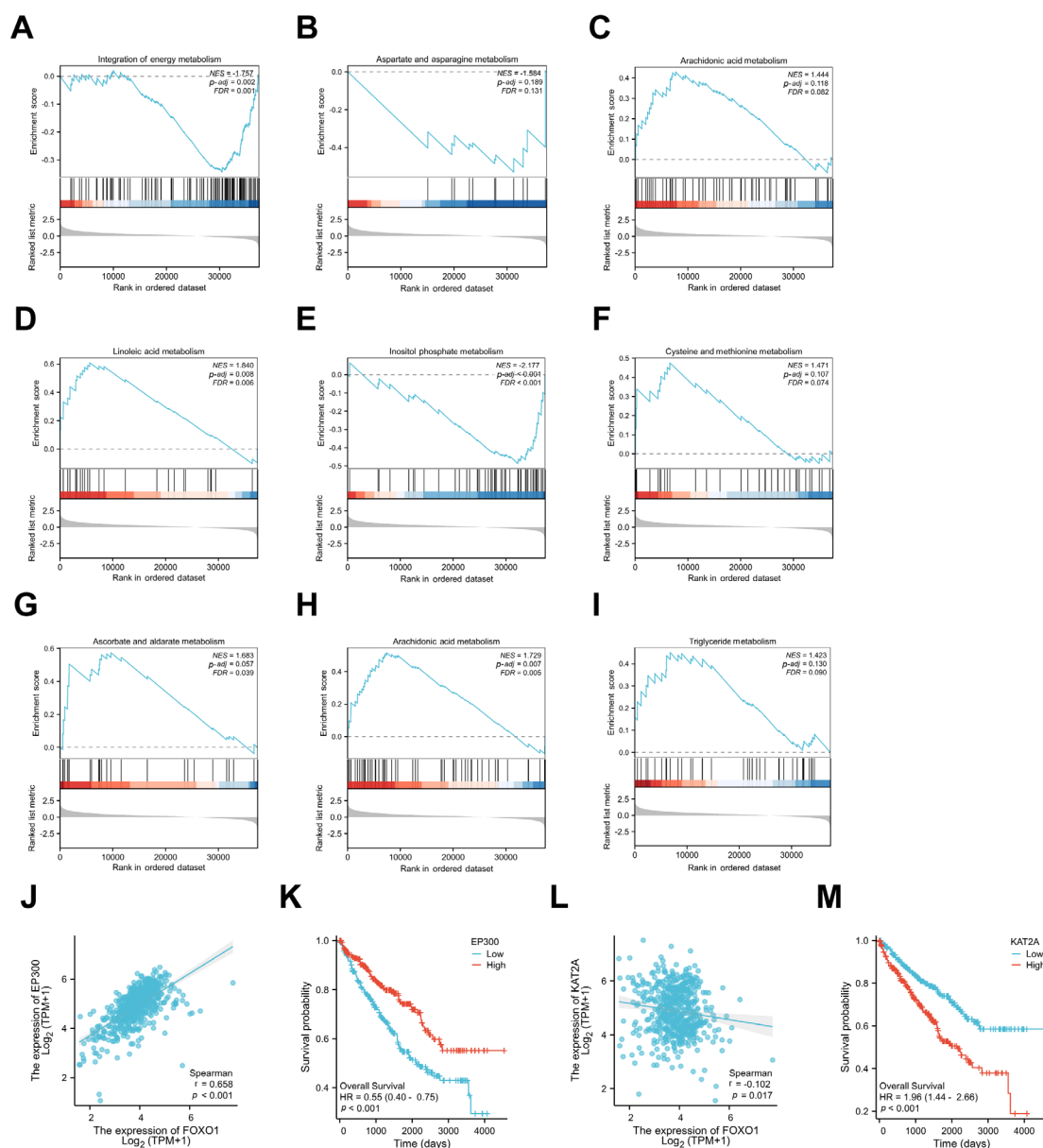


Figure 7. FOXO1 is linked to nutrient metabolism in KIRC. (A–I) FOXO1 is associated with multiple nutrient metabolic pathways: (A) energy metabolism integration, (B) aspartate and asparagine metabolism, (C) arachidonic acid metabolism, (D) linoleic acid metabolism, (E) inositol phosphate metabolism, (F) cysteine and methionine metabolism, (G) ascorbate and aldarate metabolism, (H) arachidonic acid metabolism, (I) and triglyceride metabolism. (J and K) EP300 correlates with (J) FOXO1 expression and (K) patient prognosis. (L and M) KAT2A correlates with (L) FOXO1 expression and (M) patient prognosis.

Abbreviations: FDR: False discovery rate; HR: Hazard ratio; KIRC: Kidney renal clear cell carcinoma; TPM: Transcripts per million.

Acetyl-CoA synthetase 2 (ACSS2) and ATP citrate lyase (ACLY) are key enzymes that generate acetyl-CoA from acetate and citrate, respectively. In prior T cell studies, the ACSS2–E1A binding protein P300 (EP300) axis has been linked to histone acetylation at effector/memory T cell gene loci, whereas the ACLY–lysine acetyltransferase 2A (KAT2A) axis has been associated with epigenetic

modifications at exhausted T cell gene loci.^{38,39} Although these findings were derived from T-cell models and have not been validated in KIRC, they may provide a conceptual framework for generating hypotheses regarding the potential interplay between FOXO1, metabolism, and epigenetic regulation in KIRC. In our bulk KIRC transcriptomic dataset, EP300 expression showed a

significant positive correlation with *FOXO1* levels ($p < 0.001$), and lower *EP300* levels were correlated with worse patient prognosis. Conversely, *KAT2A* exhibited an inverse correlation with *FOXO1* expression ($p < 0.001$), and elevated *KAT2A* levels were associated with poorer prognosis (Figure 7J–M). Because these correlations were derived from bulk KIRC transcriptomic data and because the functional ACSS2–EP300 and ACLY–KAT2A links originate from prior T-cell studies that have not been validated in KIRC, this metabolic–epigenetic relationship should be regarded as a hypothesis-generating observation rather than an established mechanism in KIRC.

4. Discussion

Accumulating evidence indicates that FOXO1 serves as a central regulator of immune cell development, metabolic programming, and stress response—functions that are indispensable for maintaining effective antitumor immunity.⁴⁰ FOXO1 activity enhances macrophage-mediated innate immune responses⁴¹ and promotes dendritic cell function^{40,42}, thereby shaping early immune surveillance. In adaptive immunity, FOXO1 orchestrates B-cell differentiation⁴³ and distribution⁴⁴ while exerting nuanced control over T-cell fate decisions. In CD4⁺ T cells, it supports the transition of naïve cells into effector and memory populations.⁴⁵ In CD8⁺ T cells, although FOXO1 limits terminal effector differentiation⁴⁶, it is indispensable for the generation and long-term maintenance of the memory T-cell compartment.^{47,48}

Among solid malignancies, KIRC is recognized by an exceptionally immune-infiltrated microenvironment.^{49,50} Patterns of this immune-rich contexture—particularly T-cell activation and differentiation states^{51,52}—profoundly influence tumor biology and dictate responses to systemic therapies.⁵³ Accordingly, comprehensive characterization of the KIRC immune landscape and the identification of prognostic or predictive immune signatures are considered essential for advancing biomarker discovery and therapeutic development.^{53,54}

Although FOXO1 dysregulation has been implicated across multiple cancer types, including hepatocellular cancer⁴¹, pancreatic cancer⁵⁵, and colorectal cancer^{56,57}, its immune-related functions in the context of KIRC remain largely unexplored. Our study reveals that *FOXO1* mRNA levels are closely correlated with the abundance of immune cell populations in KIRC, especially T-cell subsets. Differential expression analyses further demonstrate that FOXO1-high and FOXO1-low tumors exhibit enrichment of immune-modulatory and cell-adhesion-related pathways, suggesting that FOXO1 may be associated with the structural and functional organization of the immune

microenvironment. Because these observations derive from bulk transcriptomic data, they indicate associations rather than direct effects, and the cellular source of FOXO1 expression cannot be resolved.

Beyond its highly immune-infiltrated nature, KIRC is distinguished by widespread metabolic remodeling, particularly with respect to cellular energetic metabolism.^{58,59} Loss of *VHL* function—a hallmark event in KIRC—drives constitutive HIF activation, promoting a metabolic shift toward glycolysis, impaired mitochondrial oxidative phosphorylation, and widespread alterations in lipid and amino acid metabolism.⁵⁹ In parallel, aberrant lipid handling, including dysregulated fatty acid utilization and lipid storage remodeling, further contributes to tumor progression and aggressiveness. These metabolic deviations are accompanied by phenotypic changes such as decreased lipid droplet content, enhanced nutrient uptake, increased proliferative capacity, and ultimately poorer clinical outcomes.⁶⁰

Consistent with established metabolic vulnerabilities in KIRC, our GO enrichment analysis further revealed that *FOXO1* downregulation is associated with alterations in lipid metabolic processes. Specifically, FOXO1-low tumors showed significant impairment in lipoprotein metabolism, with enrichment of biological processes related to cellular and glycerolipid catabolism, protein–lipid complex remodeling, and the structural remodeling of HDL, LDL, VLDL, and triglyceride-rich lipoprotein particles. These findings align with the known reliance of KIRC cells on aberrant lipid processing and nutrient scavenging to sustain proliferation. Complementary GSEA reinforced this metabolic phenotype, demonstrating that *FOXO1* deficiency is associated with dysregulation of ABC transporter-mediated lipid homeostasis and pathways governing plasma lipoprotein assembly, remodeling, and clearance.

Our study suggests that *FOXO1* downregulation was significantly associated with ACLY–KAT2A axis activation and ACSS2–EP300 axis suppression. These associations are consistent with emerging concepts of metabolic–epigenetic coupling described in T cell biology.^{38,39} However, it should be emphasized that this conceptual framework is derived primarily from T-cell-based studies and has not been validated in the context of KIRC. The positive FOXO1–EP300 correlation suggests that *FOXO1* expression may be linked to pathways related to acetate metabolism and acetyl-CoA homeostasis. However, whether FOXO1 directly regulates ACSS2 or related metabolic processes requires further experimental validation. In addition, the observed expression pattern, characterized by relatively increased *ACLY/KAT2A* and decreased *ACSS2/EP300*,

resembles metabolic features reported in exhausted T cells, particularly the shift from acetate utilization toward citrate-derived acetyl-CoA production. These observations raise the possibility that FOXO1 expression is linked to metabolic states relevant to T cell function. Therefore, the FOXO1-associated metabolic–epigenetic axis should be interpreted as a hypothesis-generating observation rather than an established mechanistic pathway in KIRC, and should be further investigated in future mechanistic and cell-type–resolved studies.

The observed associations also suggest that restoring FOXO1 activity may represent a therapeutic strategy for reprogramming the metabolic and immune landscapes of KIRC. Nevertheless, translating this concept into clinical practice presents important challenges. FOXO1 is a pleiotropic transcription factor that regulates glucose metabolism, immune homeostasis, cellular differentiation, and stress responses across multiple normal tissues.^{61,62} Consequently, systemic activation of FOXO1 could produce undesirable metabolic and immunological effects, thereby limiting its therapeutic window. Future therapeutic development should therefore focus on tumor-selective approaches, such as nanoparticle-mediated drug delivery^{63,64}; tissue-specific gene modulation⁶⁵; or targeting upstream signaling pathways that preferentially restore FOXO1 activity in tumor cells.⁶¹ These strategies may enable the antitumor benefits of FOXO1 activation while minimizing systemic toxicity and improving its translational potential.

This study had two main limitations. First, this study is primarily based on secondary analyses of TCGA-derived transcriptomic and clinical data, which may be influenced by sample heterogeneity, data processing, and incomplete clinical annotation. Although the integration of multiple bioinformatics algorithms enhances the robustness of our findings, the analyses are limited by the size and scope of the TCGA-KIRC cohort. Second, no independent validation cohort or experiments using cellular/animal models were performed to confirm the proposed mechanisms linking *FOXO1* deficiency to metabolic and immune dysregulation.

5. Conclusion

FOXO1 may serve as a metabolic checkpoint in KIRC, with its inactivation associated with alterations in cellular energy metabolism and immune-related signaling pathways. These changes may contribute to the immunosuppressive tumor microenvironment through lipid metabolic dysregulation and epigenetic-associated transcriptional alterations involving acetyl-CoA-related processes. It should be emphasized that these findings are correlative and are based on bulk transcriptomic

data, which cannot identify the cellular source of FOXO1 expression or demonstrate direct biological regulation. Therapeutic strategies targeting FOXO1 or its upstream regulatory networks may represent a potential avenue for future investigation in overcoming tumor progression and improving immunotherapy responses. However, these hypotheses require extensive experimental validation in functional and preclinical models.

Acknowledgments

None.

Funding

This work was supported by the Fundamental Research Program of Shanxi Province (202203021221244).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Xiaozheng Zhang, Yongqiang Fu

Data curation: Mengwei Wang

Formal analysis: Wenyi Hou

Funding acquisition: Yongqiang Fu

Investigation: Wenyi Hou

Methodology: Wenyi Hou

Software: Mengwei Wang

Supervision: Xiaozheng Zhang, Yongqiang Fu

Validation: Wenyi Hou, Mengwei Wang

Visualization: Wenyi Hou, Mengwei Wang

Writing–original draft: Wenyi Hou

Writing–review & editing: Wenyi Hou

All authors have read and agreed to the published version of the manuscript.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The datasets analyzed in this study are publicly available from The Cancer Genome Atlas (TCGA) database (<https://portal.gdc.cancer.gov/>). RNA-seq expression profiles and corresponding clinical information for kidney renal clear cell carcinoma (KIRC) were obtained from the TCGA-KIRC project. All data used in this study are publicly accessible and can be downloaded via the Genomic Data Commons Data Portal using project ID TCGA-KIRC.

References

1. Lopez-Beltran A, Scarpelli M, Montironi R, Kirkali Z. 2004 WHO classification of the renal tumors of the adults. *Eur Urol.* 2006;49(5):798-805.
doi: 10.1016/j.eururo.2005.11.035
2. Bukavina L, Bensalah K, Bray F, *et al.* Epidemiology of Renal Cell Carcinoma: 2022 Update. *Eur Urol.* 2022;82(5):529-542.
doi: 10.1016/j.eururo.2022.08.019
3. Young M, Jackson-Spence F, Beltran L, *et al.* Renal cell carcinoma. *Lancet.* 2024;404(10451):476-491.
doi: 10.1016/s0140-6736(24)00917-6
4. Rose TL, Kim WY. Renal Cell Carcinoma: A Review. *JAMA.* 2024;332(12):1001-1010.
doi: 10.1001/jama.2024.12848
5. Choueiri TK, Powles T, Burotto M, *et al.* Nivolumab plus Cabozantinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* 2021;384(9):829-841.
doi: 10.1056/NEJMoa2026982
6. Rini BI, Plimack ER, Stus V, *et al.* Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med.* 2019;380(12):1116-1127.
doi: 10.1056/NEJMoa1816714
7. Braun DA, Hou Y, Bakouny Z, *et al.* Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat Med.* 2020;26(6):909-918.
doi: 10.1038/s41591-020-0839-y
8. Jonasch E, Walker CL, Rathmell WK. Clear cell renal cell carcinoma ontogeny and mechanisms of lethality. *Nat Rev Nephrol.* 2021;17(4):245-261.
doi: 10.1038/s41581-020-00359-2
9. Du W, Zhang L, Brett-Morris A, *et al.* HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. *Nat Commun.* 2017;8(1):1769.
doi: 10.1038/s41467-017-01965-8
10. Kim WY, Kaelin WG. Role of VHL gene mutation in human cancer. *J Clin Oncol.* 2004;22(24):4991-5004.
doi: 10.1200/jco.2004.05.061
11. Hakimi AA, Reznik E, Lee CH, *et al.* An Integrated Metabolic Atlas of Clear Cell Renal Cell Carcinoma. *Cancer Cell.* 2016;29(1):104-116.
doi: 10.1016/j.ccell.2015.12.004
12. Díaz-Montero CM, Rini BI, Finke JH. The immunology of renal cell carcinoma. *Nat Rev Nephrol.* 2020;16(12):721-735.
doi: 10.1038/s41581-020-0316-3
13. Chevrier S, Levine JH, Zanutelli VRT, *et al.* An Immune Atlas of Clear Cell Renal Cell Carcinoma. *Cell.* 2017;169(4):736-749.e18.
doi: 10.1016/j.cell.2017.04.016
14. Hah YS, Koo KC. Immunology and Immunotherapeutic Approaches for Advanced Renal Cell Carcinoma: A Comprehensive Review. *Int J Mol Sci.* 2021;22(9):4452.
doi: 10.3390/ijms22094452
15. Giraldo NA, Becht E, Pagès F, *et al.* Orchestration and Prognostic Significance of Immune Checkpoints in the Microenvironment of Primary and Metastatic Renal Cell Cancer. *Clin Cancer Res.* 2015;21(13):3031-3040.
doi: 10.1158/1078-0432.Ccr-14-2926
16. Porta C, Bonomi L, Lillaz B, *et al.* Renal cell carcinoma-induced immunosuppression: an immunophenotypic study of lymphocyte subpopulations and circulating dendritic cells. *Anticancer Res.* 2007;27(1a):165-173.
17. Reina-Campos M, Scharping NE, Goldrath AW. CD8(+) T cell metabolism in infection and cancer. *Nat Rev Immunol.* 2021;21(11):718-738.
doi: 10.1038/s41577-021-00537-8
18. Zhang L, Romero P. Metabolic Control of CD8(+) T Cell Fate Decisions and Antitumor Immunity. *Trends Mol Med.* 2018;24(1):30-48.
doi: 10.1016/j.molmed.2017.11.005
19. Ho PC, Bihuniak JD, Macintyre AN, *et al.* Phosphoenolpyruvate Is a Metabolic Checkpoint of Anti-tumor T Cell Responses. *Cell.* 2015;162(6):1217-1228.
doi: 10.1016/j.cell.2015.08.012
20. Nakaya M, Xiao Y, Zhou X, *et al.* Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity.* 2014;40(5):692-705.
doi: 10.1016/j.immuni.2014.04.007
21. Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat Immunol.* 2013;14(5):500-508.
doi: 10.1038/ni.2556
22. Wang R, Dillon CP, Shi LZ, *et al.* The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity.* 2011;35(6):871-882.
doi: 10.1016/j.immuni.2011.09.021
23. Gabriel SS, Tsui C, Chisanga D, *et al.* Transforming growth factor- β -regulated mTOR activity preserves cellular metabolism to maintain long-term T cell responses in chronic infection. *Immunity.* 2021;54(8):1698-1714.e5.
doi: 10.1016/j.immuni.2021.06.007
24. Yu YR, Imrichova H, Wang H, *et al.* Disturbed mitochondrial

- dynamics in CD8(+) TILs reinforce T cell exhaustion. *Nat Immunol.* 2020;21(12):1540-1551.
doi: 10.1038/s41590-020-0793-3
25. Scharping NE, Menk AV, Moreci RS, *et al.* The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. *Immunity.* 2016;45(2):374-388.
doi: 10.1016/j.immuni.2016.07.009
 26. Reinfeld BI, Madden MZ, Wolf MM, *et al.* Cell-programmed nutrient partitioning in the tumour microenvironment. *Nature.* 2021;593(7858):282-288.
doi: 10.1038/s41586-021-03442-1
 27. Xu S, Chaudhary O, Rodríguez-Morales P, *et al.* Uptake of oxidized lipids by the scavenger receptor CD36 promotes lipid peroxidation and dysfunction in CD8(+) T cells in tumors. *Immunity.* 2021;54(7):1561-1577.e7.
doi: 10.1016/j.immuni.2021.05.003
 28. Webb AE, Kundaje A, Brunet A. Characterization of the direct targets of FOXO transcription factors throughout evolution. *Aging Cell.* 2016;15(4):673-685.
doi: 10.1111/accel.12479
 29. Lin A, Yao J, Zhuang L, *et al.* The FoxO-BNIP3 axis exerts a unique regulation of mTORC1 and cell survival under energy stress. *Oncogene.* 2014;33(24):3183-3194.
doi: 10.1038/onc.2013.273
 30. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation, and transformation. *Cell.* 2004;117(4):421-426.
doi: 10.1016/s0092-8674(04)00452-0
 31. Doan AE, Mueller KP, Chen AY, *et al.* FOXO1 is a master regulator of memory programming in CAR T cells. *Nature.* 2024;629(8010):211-218.
doi: 10.1038/s41586-024-07300-8
 32. Marcel N, Hedrick SM. A key control point in the T cell response to chronic infection and neoplasia: FOXO1. *Curr Opin Immunol.* 2020;63:51-60.
doi: 10.1016/j.coi.2020.02.001
 33. Li J, Wang C, Xu X, Chen J, Guo H. An extensive analysis of the prognostic and immune role of FOXO1 in various types of cancer. *Braz J Med Biol Res.* 2024;57:e13378.
doi: 10.1590/1414-431X2024e13378
 34. Gan B, Lim C, Chu G, *et al.* FoxOs enforce a progression checkpoint to constrain mTORC1-activated renal tumorigenesis. *Cancer Cell.* 2010;18(5):472-484.
doi: 10.1016/j.ccr.2010.10.019
 35. Lin A, Piao HL, Zhuang L, Sarbassov dos D, Ma L, Gan B. FoxO transcription factors promote AKT Ser473 phosphorylation and renal tumor growth in response to pharmacologic inhibition of the PI3K-AKT pathway. *Cancer Res.* 2014;74(6):1682-1693.
doi: 10.1158/0008-5472.Can-13-1729
 36. Liu J, Lichtenberg T, Hoadley KA, *et al.* An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell.* 2018;173(2):400-416.e11.
doi: 10.1016/j.cell.2018.02.052
 37. Bindea G, Mlecnik B, Tosolini M, *et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity.* 2013;39(4):782-795.
doi: 10.1016/j.immuni.2013.10.003
 38. Ma S, Dahabieh MS, Mann TH, *et al.* Nutrient-driven histone code determines exhausted CD8(+) T cell fates. *Science.* 2025;387(6734):eadj3020.
doi: 10.1126/science.adj3020
 39. Kaymak I, Watson MJ, Oswald BM, *et al.* ACLY and ACS2 link nutrient-dependent chromatin accessibility to CD8 T cell effector responses. *J Exp Med.* 2024;221(9).
doi: 10.1084/jem.20231820
 40. Deng Y, Wang F, Hughes T, Yu J. FOXOs in cancer immunity: Knowns and unknowns. *Semin Cancer Biol.* 2018;50:53-64.
doi: 10.1016/j.semcancer.2018.01.005
 41. Fan W, Morinaga H, Kim JJ, *et al.* FoxO1 regulates Tlr4 inflammatory pathway signalling in macrophages. *EMBO J.* 2010;29(24):4223-4236.
doi: 10.1038/emboj.2010.268
 42. Xiao W, Dong G, Pacios S, *et al.* FOXO1 deletion reduces dendritic cell function and enhances susceptibility to periodontitis. *Am J Pathol.* 2015;185(4):1085-1093.
doi: 10.1016/j.ajpath.2014.12.006
 43. Dengler HS, Baracho GV, Omori SA, *et al.* Distinct functions for the transcription factor Foxo1 at various stages of B cell differentiation. *Nat Immunol.* 2008;9(12):1388-1398.
doi: 10.1038/ni.1667
 44. Chen J, Limon JJ, Blanc C, Peng SL, Fruman DA. Foxo1 regulates marginal zone B-cell development. *Eur J Immunol.* 2010;40(7):1890-1896.
doi: 10.1002/eji.200939817
 45. Kerdiles YM, Stone EL, Beisner DR, *et al.* Foxo transcription factors control regulatory T cell development and function. *Immunity.* 2010;33(6):890-904.
doi: 10.1016/j.immuni.2010.12.002
 46. Kim EH, Sullivan JA, Plisch EH, *et al.* Signal integration by Akt regulates CD8 T cell effector and memory differentiation. *J Immunol.* 2012;188(9):4305-4314.

- doi: 10.4049/jimmunol.1103568
47. Rao RR, Li Q, Gubbels Bupp MR, Shrikant PA. Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8(+) T cell differentiation. *Immunity*. 2012;36(3):374-387.
doi: 10.1016/j.immuni.2012.01.015
 48. Hess Michelini R, Doedens AL, Goldrath AW, Hedrick SM. Differentiation of CD8 memory T cells depends on Foxo1. *J Exp Med*. 2013;210(6):1189-1200.
doi: 10.1084/jem.20130392
 49. Vuong L, Kotecha RR, Voss MH, Hakimi AA. Tumor Microenvironment Dynamics in Clear-Cell Renal Cell Carcinoma. *Cancer Discov*. 2019;9(10):1349-1357.
doi: 10.1158/2159-8290.Cd-19-0499
 50. Şenbabaoğlu Y, Gejman RS, Winer AG, *et al*. Tumor immune microenvironment characterization in clear cell renal cell carcinoma identifies prognostic and immunotherapeutically relevant messenger RNA signatures. *Genome Biol*. 2016;17(1):231.
doi: 10.1186/s13059-016-1092-z
 51. Nakano O, Sato M, Naito Y, *et al*. Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. *Cancer Res*. 2001;61(13):5132-5136.
 52. Wang QJ, Hanada K, Robbins PF, Li YF, Yang JC. Distinctive features of the differentiated phenotype and infiltration of tumor-reactive lymphocytes in clear cell renal cell carcinoma. *Cancer Res*. 2012;72(23):6119-6129.
doi: 10.1158/0008-5472.Can-12-0588
 53. Lasorsa F, di Meo NA, Rutigliano M, *et al*. Immune Checkpoint Inhibitors in Renal Cell Carcinoma: Molecular Basis and Rationale for Their Use in Clinical Practice. *Biomedicines*. 2023;11(4):1071.
doi: 10.3390/biomedicines11041071
 54. Lasorsa F, Rutigliano M, Milella M, *et al*. Cellular and Molecular Players in the Tumor Microenvironment of Renal Cell Carcinoma. *J Clin Med*. 2023;12(12):3888.
doi: 10.3390/jcm12123888
 55. Boreddy SR, Pramanik KC, Srivastava SK. Pancreatic tumor suppression by benzyl isothiocyanate is associated with inhibition of PI3K/AKT/FOXO pathway. *Clin Cancer Res*. 2011;17(7):1784-1795.
doi: 10.1158/1078-0432.Ccr-10-1891
 56. Duan S, Huang W, Liu X, *et al*. IMPDH2 promotes colorectal cancer progression through activation of the PI3K/AKT/mTOR and PI3K/AKT/FOXO1 signaling pathways. *J Exp Clin Cancer Res*. 2018;37(1):304.
doi: 10.1186/s13046-018-0980-3
 57. Wang D, Yang L, Yu W, *et al*. Colorectal cancer cell-derived CCL20 recruits regulatory T cells to promote chemoresistance via FOXO1/CEBPB/NF-κB signaling. *J Immunother Cancer*. 2019;7(1):215.
doi: 10.1186/s40425-019-0701-2
 58. Lucarelli G, Loizzo D, Franzin R, *et al*. Metabolomic insights into pathophysiological mechanisms and biomarker discovery in clear cell renal cell carcinoma. *Expert Rev Mol Diagn*. 2019;19(5):397-407.
doi: 10.1080/14737159.2019.1607729
 59. di Meo NA, Lasorsa F, Rutigliano M, *et al*. Renal Cell Carcinoma as a Metabolic Disease: An Update on Main Pathways, Potential Biomarkers, and Therapeutic Targets. *Int J Mol Sci*. 2022;23(22):14360.
doi: 10.3390/ijms232214360
 60. Hu J, Wang SG, Hou Y, *et al*. Multi-omic profiling of clear cell renal cell carcinoma identifies metabolic reprogramming associated with disease progression. *Nat Genet*. 2024;56(3):442-457.
doi: 10.1038/s41588-024-01662-5
 61. Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. *Nat Rev Mol Cell Biol*. 2013;14(2):83-97.
doi: 10.1038/nrm3507
 62. Kousteni S. FoxO1: a molecule for all seasons. *J Bone Miner Res*. 2011;26(5):912-917.
doi: 10.1002/jbmr.306
 63. Fu X, Shi Y, Qi T, *et al*. Precise design strategies of nanomedicine for improving cancer therapeutic efficacy using subcellular targeting. *Signal Transduct Target Ther*. 2020;5(1):262.
doi: 10.1038/s41392-020-00342-0
 64. Bennie LA, McCarthy HO, Coulter JA. Enhanced nanoparticle delivery exploiting tumour-responsive formulations. *Cancer Nanotechnol*. 2018;9(1):10.
doi: 10.1186/s12645-018-0044-6
 65. Yamada Y, Sato Y, Nakamura T, Harashima H. Evolution of drug delivery system from viewpoint of controlled intracellular trafficking and selective tissue targeting toward future nanomedicine. *J Control Release*. 2020;327:533-545.
doi: 10.1016/j.jconrel.2020.09.007