Research Article

# FDX1 is a Potential Prognostic Biomarker Related to Cuproptosis and Immune Infiltration for Patients with Kidney Clear Cell Carcinoma

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## Abstract

**Background:** Currently, the mechanisms of copper-induced cell death have attracted the attention of scientists. Since Cu homeostasis has a significant effect on angiogenesis response and immune function, cuproptosis is proposed as a new concept. FDX is a Cuproptosis-related gene because its dysfunction can suppress lipoic acid synthesis and regulate Cu-induced cell death.

**Objective:** To examine the association between the expression level of FDX1 and clinical characteristics, survival, and immune infiltration for kidney clear cell carcinoma patients.

**Method:** TCGA database followed the ethical policies of human subject protection. The sampling technique of TCGA was that samples were supposed to contain at least 80% tumor tissue and 20% necrotic normal tissue. Nucleic acids were extracted and genotype was determined to obtain genomic data. The data of 613 American patients from TCGA database was collected for this bioinformatics study based on the diagnostic criteria of KIRC. The study design consisted of Pan-cancer analysis, the Cox regression, the Kaplan-Meier model, Kyoto Encyclopedia of Genes and Genome analyses, Gene Ontology to analyze the progression and survival, immune infiltrate levels, and enrichment effect.

**Results:** The FDX1 level was significantly lower in KIRC analyzed by Wilcoxon test. In Pearson correlation analyses, the expression level of FDX1 was significantly positive related to the overall survival, while it was negatively associated with the immune infiltrate. The FDX1 expression levels and the infiltration level of T CD8+ cells, T regulatory cells, B memory cells, and NK cells had negative correlations, while it was negatively associated with the enrichment effects on inflammation and immune functions and signaling pathways.

**Conclusion:** FDX1 is a potential Cuproptosis-related prognostic indicator for the immunotherapy of KIRC, while correlations do not prove causations.

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## Introduction

Copper (Cu) is an essential micromineral for fundamental physiological processes. Cu acts as a prominent cofactor of various metabolic enzymes, critical polypeptides, and structural proteins such as superoxide

dismutase, cytochrome c oxidase, and the blood clotting proteins V and VIII.[1,2] Specifically, Cu homeostasis has a significant effect on angiogenesis response, immune function, etc. In recent decades, robust correlations between Cu homeostasis and many malignancies have been observed[2]. Florida Voli et al. reported that the anti-cancer effect on immune checkpoints could be improved by diminishing the intratumor levels of Cu. In their mouse model, Cu could regulate several important signaling pathways including proliferative immortality, angiogenesis, and metastasis to affect PD-L1-caused cancer immune evasion.[1,2]

Currently, the mechanisms of copper-induced cell death have attracted the attention of scientists. In March 2022, Tsvetkov et al. revealed that copper-related cell death was targeted by the TCA-cycle proteins and put forward a new concept, Cuproptosis. Cuproptosis indicates that excessive Cu selectively disturbs some lipoylation-related enzymes and then destroys the physiological function of mitochondria.[3]

FDX is a Cuproptosis-related gene. It can regulate Cu-induced cell death. FDX is one of the Iron-sulfur (Fe-S) proteins, which contains two homologs, FDX1 and FDX2. FDX 1 is located on chromosome 11q22 as a versatile electron donor to cytochrome P450 enzymes for the biosynthesis of Fe-S and adrenal steroid hormone. In this metabolic pathway, FDX1 plays a crucial role in the synthesis of pregnenolone, cortisol, corticosterone, or aldosterone, serving as a mitochondrial reductase. FDX1 is also a crucial regulatory protein of cuproptosis, since copper-related reductases can be encoded by FDX1, which converts toxic $Cu^{2+}$ ions to toxic $Cu^{1+}$ ions in the mitochondria. Then $Cu^{1+}$ binding to the mitochondrial protein can induce the aggregation of lipoylated proteins, which leads to the cuproptosis. Also, FDX1 can modify protein lipoylation which is a highly conserved lysine post-translational regulation. The deletion of FDX1 results in the depletion of succinate and the accumulation of pyruvate and α-ketoglutarate. Thus, the knock-out of FDX1 can lead to compromised protein lipoylation and rescue cells from copper toxicity. Furthermore, FDX1 has a promotional effect on the lipoylated oligomerization of dihydrolipoamide S-acetyltransferase owing to binding to lipoylated proteins of the TCA cycle. FDX1 is highly expressed in the adrenal cortex and medulla.[3–7] Therefore, it is predicted that FDX1 may be a potential cuproptosis-related biomarker for patients with kidney carcinoma. In general, kidney carcinoma can be commonly classified into four categories, KIRP, Sarcoma, KICH, and KIRC. Among them, KIRC accounts for around 80% of the total and it is one of the most immune-infiltrated tumors.[8]

Considering immune infiltrate, tumor tissue is commonly infiltrated by various immune cells that secrete cytokines and chemokines to impact the efficacy of clinical cancer treatment by regulating the tumor microenvironment. Hence, exploring the underlying mechanisms of immune infiltration is a promising strategy for KIRC treatment. In general, both T $CD^{8+}$ cells and Memory B cells are adaptive immunity. T $CD^{8+}$ cells can clear intracellular pathogens to provide long-term protection.[9] Memory B cells can provide protective immunity against infectious agents. They have a stronger affinity for immunoglobulin (Ig) genes, which are determinants for their malignant transformation levels in oncogenic events.[10] NK cells are cytotoxic lymphocytes, which are involved in early defense and tumor immune surveillance. NK cells directly exert anti-tumor effects by interacting with other immune cells to attack tumor cells.[11]

Taken together, KIRC may have particularly effective responses to immunotherapy and targeted-gene therapy.[12] The objective of this bioinformatics study using TCGA database is to examine the association between the expression level of FDX1 and the clinical characteristics, survival, and immune infiltration for KIRC therapy.

## Methods

The data of the present study came from TCGA database, which is a public database established by the NHGRI. TCGA strictly followed the ethical policies of human subject protection, which was developed by NHGRI and the National Cancer Institute. The sampling technique of TCGA was that samples were supposed to contain at least 80% tumor tissue and 20% necrotic normal tissue. Nucleic acids were extracted and genotype was determined to obtain genomic data. Study design consisted of Pan-cancer analysis, the Cox regression, the Kaplan-Meier model, Kyoto Encyclopedia of Genes and Genome analyses, Gene Ontology to analyze the progression and survival, immune infiltrate levels, and enrichment effect. The data of 613 patients from TCGA were involved in this study.

Pan-cancer analysis was conducted through TIMER, which is an online statistical tool. The CPHR model and the KMSE model were utilized to explore in which tumors the FDX1 expression levels and overall or progression-free survival were associated. The co-expression relationships between FDX1 and immune genes were profiled by TIP and IMMPORT online tools. CPHR model was used to evaluate statistical correlations of the FDX1 expression levels and the clinical factors. The immune genes were filtered by the Multi-Cox model. The overall survival of a patient was predicted from the Nomo plot. The corrections between the immune infiltrate levels and the expression levels of FDX1 were estimated by TIMER. GO and KEGG analyses were conducted to detect the enrichment effect on gene functions and pathways.

Unless stated otherwise, all correlation analyses, CPHR model, KMSE model, enrichment effect analyses, correction plots, forest plots, curves, the Nomo plot, and bubble plots were conducted by the R computing (4.1.2). The statistical significance of Pan-cancer analysis was computed by the Wilcoxon test. The statistical significance of the co-expression relationships between immune genes and FDX1 was computed by the Pearson correlation test. The screening criteria for genes with statistically significant co-expression relationships were p-value < 0.05 and |r| > 0.2.

This present bioinformatics research was conducted in Spring 2022 at Manhattan, Kansas, USA.

## Result

The data of 613 patients were involved in Pan-cancer analysis. The findings indicated that the FDX1 levels were lower in tumor tissues of BRCA, CHOL, COAD, KICH, KIRC, KIRP, LUAD, PCPG, READ, LUSC, SKCM, and THCA, but significantly increased in tumor of GBM, STAD (Figure 1).

The CPHR model and KMSE model exhibited that the FDX1 levels and survival of patients were significantly correlated only with KIRC (Fig 2,3,4,5).

In the heatmap and the PCA plot, 40 specific immune genes showed moderate negative co-expression relationships with FDX1 with Pearson values ranging from -0.2 to -0.5. They were Antimicrobials (TGFB1, CD40, CXCR4, VEGFA, CCR10, BIRC5, CCL5, CD40LG, KLRK1, CCL26, CXCL5), Cytokines (CSF1, TNFSF14, TNFSF9, CD70), Cytokine Receptors (TNFRSF25, TNFRSF18, TNFRSF14, TNFRSF8, TNFRSF4), Chemokines (TP53), Chemokine Receptors (CXCR3), Natural Killer (NK) Cell Cytotoxicity (ITGB2, Cd247), Antigen Processing and Presentation (ICAM1, MICB, HSPA6, MICA), TCR Signaling Pathway (CD3E), and Immune Checkpoints (LGALS9). Meanwhile, TNFSF14, TNFSF9, and CD70 have both cytokine functions and immune checkpoint functions. Additionally, LAIR1, EZH2, TLR9, NT5C, DNMT1, VSIR, SLAMF8, TLR6, SLAMF1, and TLR5 were not annotated with certain immune functions (Fig 6, 7).

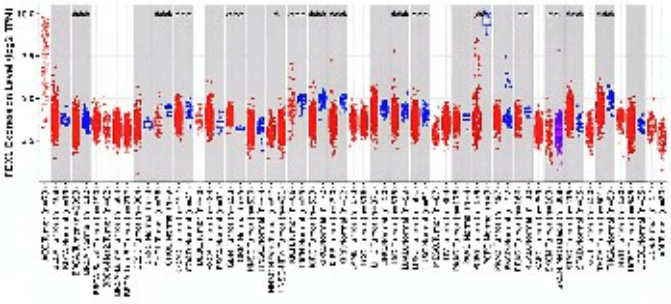


**Figure 1:** *The distributions of FDX1 mRNA levels were displayed by red box plots in tumor tissues and blue box plots in adjacent normal tissues, respectively. The statistical significances are annotated by the stars. *represented p-values < 0.05, ** represented p-values < 0.01, *** represented p-values < 0.001.*

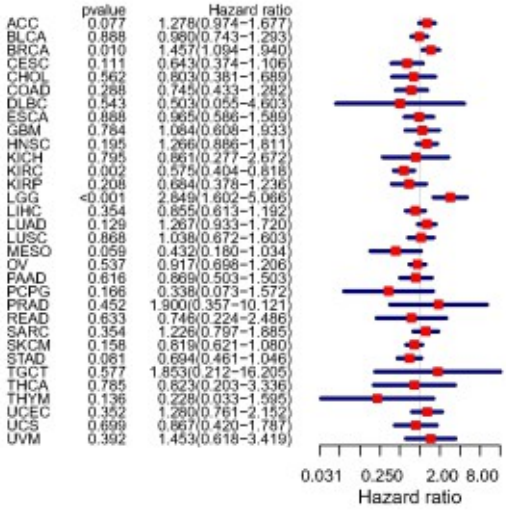



**Figure 2:** *Forest plot of the Cox proportional hazards regression model between the FDX1 levels and the overall survival*

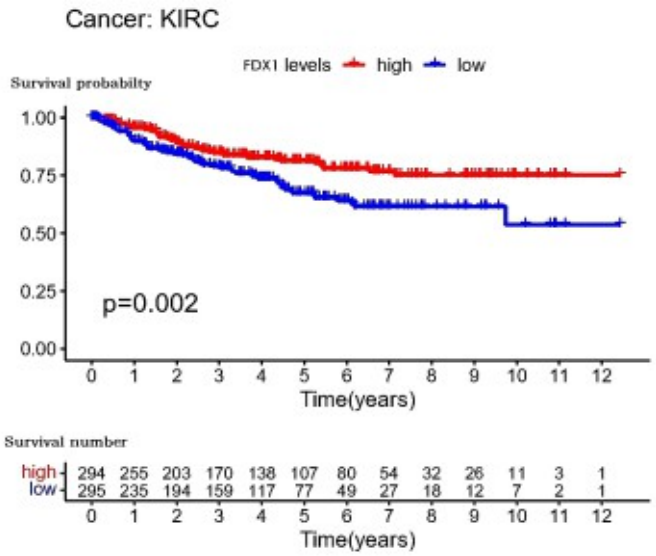



**Figure 3:** *Curves of the Kaplan-Meier survival estimation model between the FDX1 levels and the overall survival*

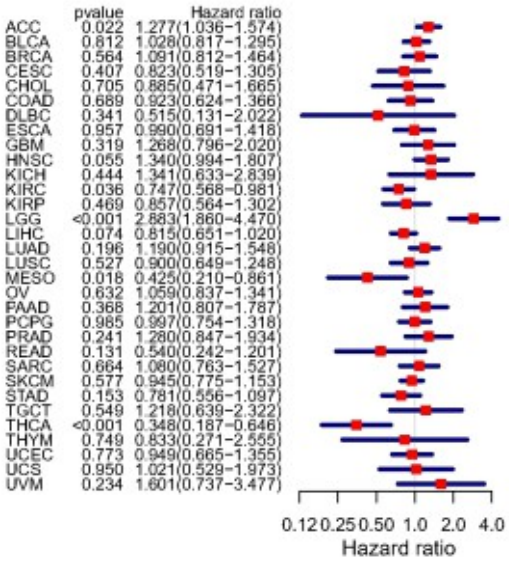



**Figure 4:** *Forest plot of the CPHR model between the FDX1 levels and the progression-free survival*

The findings of CPHR model demonstrated that age, gender, and stage (TNM) had significant correlations with the FDX1 expression levels. The HR values of age, gender and stage were 0.415, 0.397, 0.448, respectively. Next, the 40 immune genes were filtered by the Multi Cox model and then 13 genes remained. The overall survival of a patient with KIRC was predicted from the Nomo plot. Each clinical feature corresponded to a certain point (Figure 8).

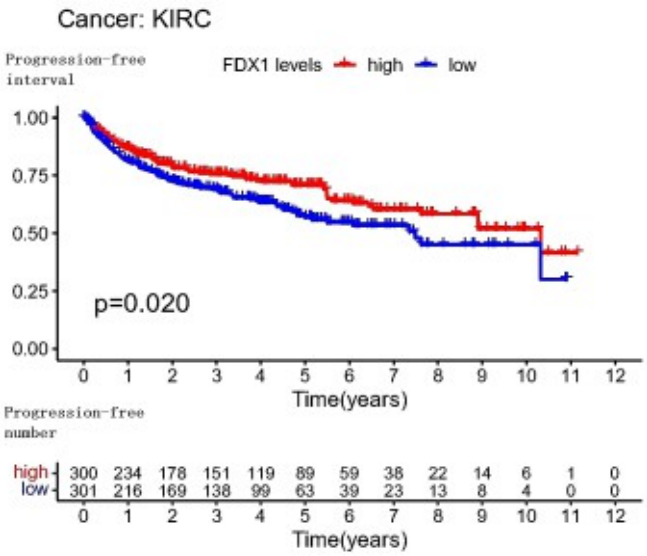



**Figure 5:** *Curves of the KMSE model between the FDX1 levels and the progression-free survival*

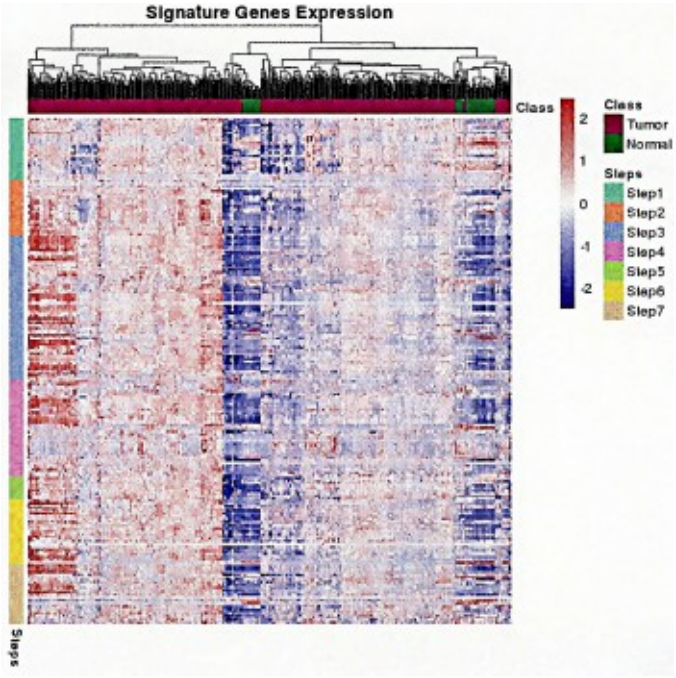



**Figure 6:** *The heatmap of the immune gene expression levels. The horizontal axle represented a single gene. The vertical axis represented one sample.*

The FDX1 expression levels and the infiltration level of T CD8+ cells, T regulatory cells, B memory cells, and NK cells had negative correlations (Fig 9A, B, C, D). In parallel, the FDX1 expression levels and the infiltration level of Macrophage M1s, Macrophage M2s, and Mast cells had positive correlations (Fig 9E, F, G).

In GO functions, the positive regulation of cell activation, lymphocyte activation, mononuclear cell proliferation, leukocyte activation, leukocyte cell-cell adhesion, and leukocyte proliferation have significantly enrichment

effects. (Figure 10).

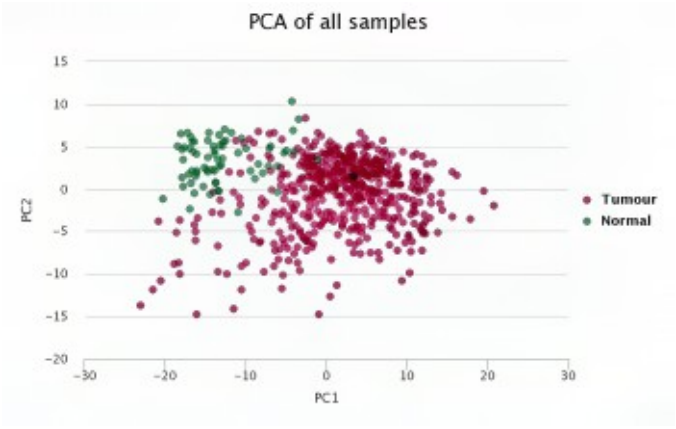



**Figure 7:** *The scores plot of the immune gene expression levels*

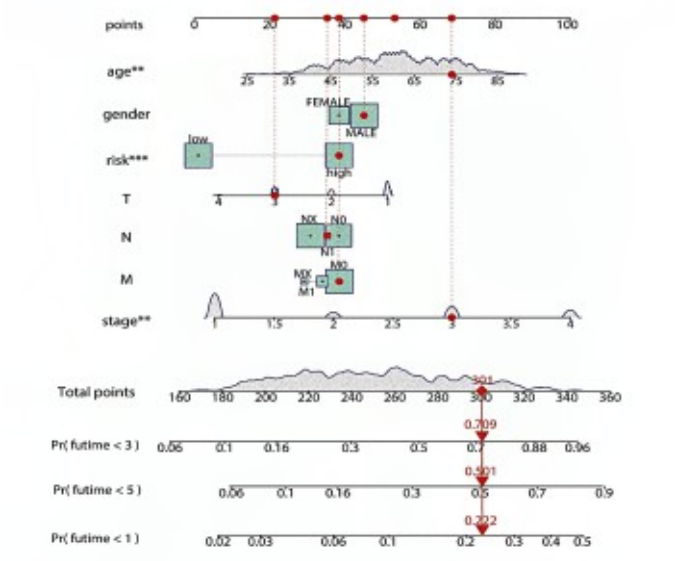



**Figure 8:** *The Nomo plot of the corrections between the clinical factors, risk scores, and overall survival. TX, MX, and NX represented unknown stages.*

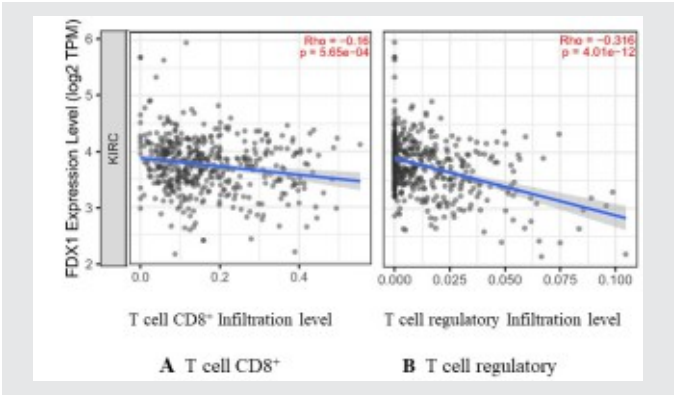
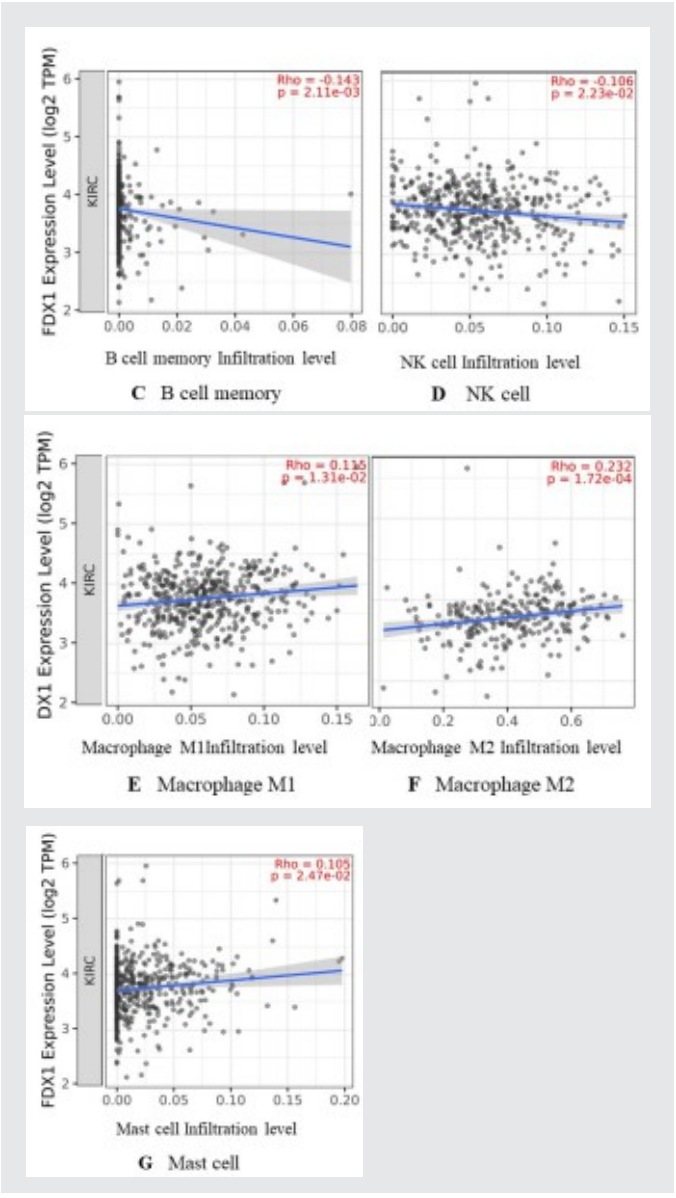



**Figure 9:** *The plot of the associations between the FDX1 levels and the immune infiltrate levels*



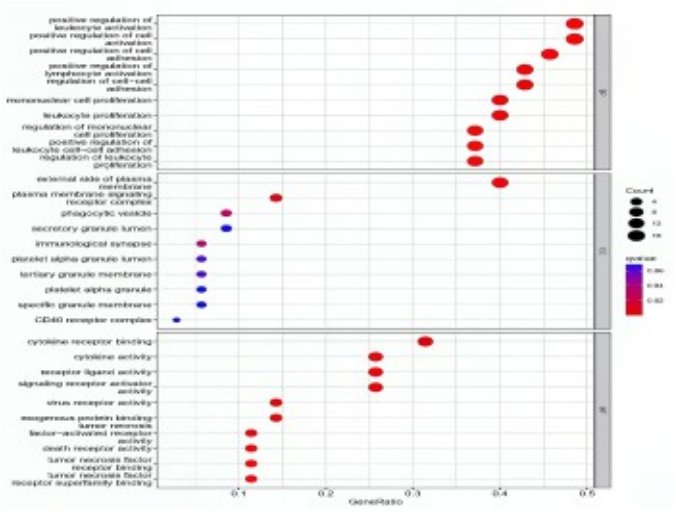



**Figure 10:** *GO enrichment plot. The horizontal axle represented gene ratios. The vertical axis represented*

*enrichment functions. The size of the dots represented the number of gene. The color of the dots represented P values.*

The KEGG pathway showed that FDX1 and its co-expressed immune genes were significantly enriched on the immune-relevant signal pathways including TCR, NK cell-mediated cytotoxicity, Th17 cell differentiation, NF-Kβ, Leukocyte transendothelial migration, TLR, Chemokine signaling pathway, Cytokine-cytokine receptor interaction, Viral protein interaction with cytokine and cytokine receptor, TNF, PD-1, and PDL-1 checkpoint signal pathways (Figure 11).

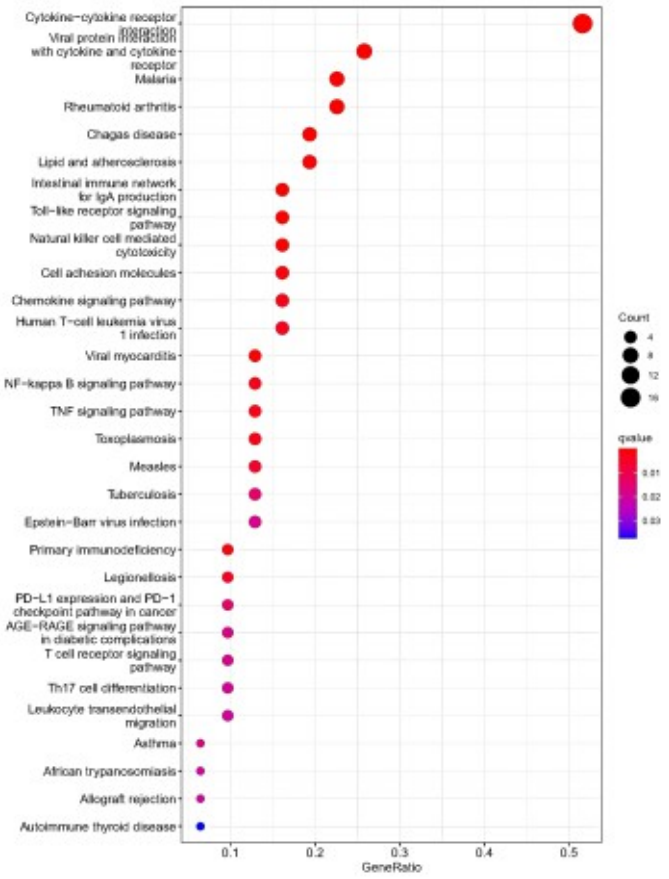



**Figure 11:** *KEGG enrichment analysis. The horizontal axle represented gene ratios. The vertical axis represented enrichment functions. The size of the dots represented the number of gene. The color of the dots represented P values.*

## Discussion

Since FDX1 might be an unrecognized biomarker of cancer, the Pan-cancer analysis (33 cancer types) was conducted due to these pivotal functions of FDX1 on Cuproptosis. The FDX1 expression level between normal tissues and tumor normal tissues was significantly different in 14 cancers. The FDX1 expression level was lower in tumor tissues of KIRC. Afterward, FDX1 was determined as an independent protective factor of survival in KIRC by Cox and Kaplan-Meier model. Furthermore, in KIRC tumor tissue, immune genes involving the gene of antimicrobials, cytokine, chemokine receptors, antigen processing and presentation, NK cell cytotoxicity, TCR pathway, antigen processing and presentation, and immune Checkpoints had a co-expression relationship with FDX1. They can work together with clinical factors including age, gender, stage, and risk scores to generate prognostic models through the Nomo plots. Similarly, in three recent studies, the authors also stated that FDX1 might be a prognostic factor for lung adenocarcinoma, hepatocellular carcinoma, and colon adenocarcinoma due to the mechanism of copper-induced cell death.[12-14] In another Pan-cancer analysis, the expression levels of FDX1 were down-regulated in KIRC, ACC, HNSC, THCA, and LGG. Since FDX1 was significant association with clinical characteristics, tumor mutational burden, microsatellite instability, immune-related signal pathways, immune cell infiltration, and antitumor drug susceptibility, it was considered to be a potential prognostic biomarker of immunotherapy.[15] These results were consistent with the findings of the present bioinformatics study.

Moreover, macrophage M1s and M2s, and Mast cells have positive correlations with the FDX1 expression levels. In chronic inflammatory conditions, macrophage cells generate cytokines, tissue repair factors, and angiogenic factors to promote tumor angiogenesis.[16,17] Similarly, mast cells can release vascular growth factors such as fibroblast growth factor 2.18 Normally, the infiltrated macrophages and mast cells in the tumor are conducive to the proliferation of tumor cells. Thus, these findings supported the previous view that the expression levels of FDX1 have a negative correlation with immune infiltrates. In alignment with the results of current study, Zilong Bian et al. found that the FDX1 level was positively correlated with the abundance of macrophages in KIRC tissue.[19] Zhen Zhang et al. also reported that hepatocellular carcinoma patients with FDX1-related high-risk gene scores showed high levels of protumor immune infiltration.[12]

However, FDX1 was negatively associated with T regulatory cells. It was inconsistent with the above view since Treg cells suppress immune function by releasing suppressive cytokines, leading to cell lysis and apoptosis of T cells, and cutting off the ATP supply of effector T cells.[20,21] One likely explanation presented by Winerdal et al. was that the low infiltration levels of Treg cells were related to a positive prognosis, since Tregs probably inhibited the macrophage expression levels and the invasive factor metalloproteinase 2, which reduced the invasiveness of tumor cells.[21] In light of these considerations, FDX1 probably affects the occurrence, progression, and metastasis of tumors.

The immune cell regulations on cancers are complex and most of their principles are not discovered yet. While it goes far beyond our understanding, intensive studies have revealed many fundamental mechanisms over the past decades. For example, the Th17 cell is the subclass of $CD^{4+}$ T cell. The differentiation of Th17 cells is regulated by the TCR signal pathway. Pathogenic Th17 cells and dysfunction of the TCR signal pathway result in multiorgan inflammatory diseases. It is clear that cancer progression and the response to immune therapy are impacted by inflammation.[22] In the present study, FDX1 was involved in multiple immune and inflammation functions, since FDX1 and its co-expressed immune genes were enriched in TCR and Th17 cell signal pathways, and other biological process responses, including Leukocyte transendothelial migration, NK cell-mediated cytotoxicity, cell-cell adhesion, phagocytic vesicle, cell proliferation of leukocyte and mononuclear, and plasma membrane. Particularly, FDX1 and its co-expressed immune genes had enrichment effects on the immunological synapse. It is a connection point connected by the CAR between a NK cell and a target cell. It enables a CAR-NK complex to recognize potential antigenic ligands, activate the immune response programs, and secrete some cytolytic molecules including secretory granule lumen and specific granules such as platelet alpha granule and tertiary granule membrane. Cell injury and tissue damage were eliminated by increasing cell number and cell life span.[22–25]

On the other hand, chronic inflammation can promote tumor cell growth and induce chemotherapy resistance. In the current study, FDX1 and its immune genes had enrichment effects on multiple signaling pathways of Cytokine and Chemokine such as NF-kβ, TLR, and TNF pathways. TNF normally exists in immune cells. It is a cytokine that can directly kill tumor cells due to a systemic inflammatory response. TNF and CD40 (one of the molecules of the TNF receptor family) can coactivate many molecular pathways involving the NF-kβ and MAPK.[26,27] TLR can also trigger NF-kβ and MAPK signal pathways through the interactions of the TLR and myeloid differentiation factor 88 structural domains.[28] Similarly, Morikawa T et al. reported that TLR was significantly overexpressed in tumor. Activating TLR and its ligand could increase interferon β expression and cell growth inhibition.[29] In addition, with the development of tumor immunotherapy, immune checkpoint inhibitors had already achieved encouraging successes over the last 10 years, particularly in the PD-1 signal pathway. PD-1 is an immune receptor in the CD28 series, when PD-1 binds to PDL-1, the cytotoxic T cells are suppressed and Treg cells are activated. However, if the bindings of PD-1 and PDL-1 are broken by immune checkpoint inhibitors, this immune escape mechanism is no long hijacked.[30] The present analysis of KEGG indicated that FDX1 and its co-expressed immune genes were enriched in the PD-1 signal pathway. In another Pan-cancer study, Cai et al. also illustrated that PD-1 was significantly upregulated in KIRC tumor tissue.[5] Collectively, FDX1 is likely an immune therapeutic target for KIRC.

Although the findings of this bioinformatic study provided useful clues on the correction between the FDX1 expression levels and the prognostic of KIRC due to the impact of Cuproptosis and immune infiltration, the underlying molecular mechanisms were not revealed clearly. More in vitro experiments, cancer cell-culture studies, animal tumor models, and human clinical trials are warranted to further explore and verify the molecular mechanisms of FDX1 on KIRC in detail. After all, correlations do not prove causations.

## Conclusion

The expression of FDX1 (Cuproptosis-related gene) was lower in KIRC tumor tissues. The high FDX1 expression level was a positive prognosis factor since it had a significantly positive correlation with the overall survival of patients with KIRC. Moreover, the FDX1 expression levels had a negative association with the

level of immune infiltrates and enriched effect on inflammation and immune signaling pathways. These findings suggested that FDX1 was a potential Cuproptosis-related prognostic biomarker for KIRC immunotherapy.

## Abbreviation

Programmed death 1 (PD-1)
Programmed death-ligand 1 (PD-L1)
Tricarboxylic acid (TCA)
Ferredoxin 1 (FDX1)
Kidney renal papillary cell carcinoma (KIRP)
Kidney Chromophobe (KICH)
Kidney clear cell carcinoma (KIRC)
The Cancer Genome Atlas (TCGA)
National (USA) Human Genome Research Institute (NHGRI)
University of California Santa Cruz (UCSC)
Tumor Immune Estimation Resource (TIMER)
Cox proportional hazards regression (CPHR)
Kaplan-Meier survival estimation (KMSE)
Tracking Tumor Immunophenotype (TIP)
The Immunology Database and Analysis Portal (IMMPORT)
Gene Ontology (GO)
Kyoto Encyclopedia of Genes and Genomes (KEGG)
Adrenocortical carcinoma (ACC)
Bladder Urothelial Carcinoma (BLCA)
Breast invasive carcinoma (BRCA)
Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC)
Cholangiocarcinoma (CHOL)
Colon adenocarcinoma (COAD)
Esophageal carcinoma (ESCA)
Glioblastoma multiforme (GBM)
Head and Neck squamous cell carcinoma (HNSC)
Acute Myeloid Leukemia (LAML)
Brain Lower Grade Glioma (LGG)
Liver hepatocellular carcinoma (LIHC)
Lung adenocarcinoma (LUAD)
Lung squamous cell carcinoma (LUSC)
Mesothelioma (MESO)
Ovarian serous cystadenocarcinoma (OV)
Pancreatic adenocarcinoma (PAAD)
Pheochromocytoma and Paraganglioma (PCPG)
Prostate adenocarcinoma (PRAD)
Rectum adenocarcinoma (READ)
Sarcoma (SARC)
Skin Cutaneous Melanoma (SKCM)
Stomach adenocarcinoma (STAD)
Testicular Germ Cell Tumors (TGCT)
Thyroid carcinoma (THCA)
Thymoma (THYM)
Uterine Corpus Endometrial Carcinoma (UCEC)
Uterine Carcinosarcoma (UCS)
Ocular melanomas (UVM).
Principal component analysis (PCA)
T cell receptor (TCR)
Natural killer (NK)
T helper 17 (Th17)
Nuclear factor kappa β (NF-Kβ),
Toll-like receptor (TLR)
Tumor necrosis factor (TNF)
Biological Process (BP)
Cellular Component (CC)
Molecular Function (MF)
Adenosine triphosphate (ATP)
Chimeric antigen receptor (CAR)
Mitogen-Activated Protein Kinase (MAPK)

**Ethical Approval:** The data of the present study is from TCGA database, which is a public database established by the National (USA) Human Genome Research Institute. Thus, its ethical approval and consent to participate from the patients with cancer have been addressed before the public access to data. Please check at https://www.cancer.gov/ccg/research/genome-sequencing/tcga/history/ethics-policies

**Conflict of Interest:** The author declare no conflict of interest.

**Funding Source:** None

**Authors' Contribution:**

**JZ:** Conception & design, analysis & interpretation of

data, drafting of article, critical revision for important intellectual content, final approval

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