A Novel Ferroptosis-Related Gene Signature Robustly Predicts Clinical Prognosis And Tumor Immunity in Patients With Kidney Renal Clear Cell Carcinoma

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Research

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Abstract

Objective: This study aimed to construct a ferroptosis-related gene signature to predict clinical prognosis and tumor immunity in patients with kidney renal clear cell carcinoma (KIRC).

Methods: The mRNA expression profiles and corresponding clinical data of KIRC patients were downloaded from The Cancer Genome Atlas (TCGA), which were randomly divided into training (398 patients) and validation set (132 patients). The iron death related (IDR) prediction model was constructed based on training set and 60 ferroptosis-related genes from previous literatures, followed by prognostic performance evaluation and verification using the validation set. Moreover, functional enrichment, immune cell infiltration, metagene clusters correlation, and TIDE scoring analyses were performed.

Results: In total, 23 ferroptosis-related genes were significantly associated with overall survival (OS). The IDR prediction model (a 10-gene signature) was then constructed to stratify patients into two risk groups. The OS of KIRC patients with high-risk scores was significantly shorter than those with low-risk scores. Moreover, the risk score was confirmed as an independent prognostic predictor for OS. The positive and negative correlated genes with this model were significantly enriched in p53 signaling pathway, and cGMP-PKG signaling pathway. The patients in the high-risk group had higher ratios of plasma cells, T cells CD8, and T cells regulatory Tregs. Furthermore, IgG, HCK, LCK, and Interferon metagenes were significantly correlated with risk score. By TIDE score analysis, patients in the high-risk group could benefit from immunotherapy.

Conclusions: The identified ferroptosis-related gene signature is significantly correlated with clinical prognosis and immune immunity in KIRC patients.

Highlights

1. The IDR prediction model (a 10-gene signature) was constructed to stratify patients into two risk groups.
2. The risk score was confirmed as an independent prognostic predictor for OS.
3. Patients in the high-risk group had higher ratios of plasma cells, T cells CD8, and T cells regulatory Tregs.
4. IgG, HCK, LCK, and Interferon metagenes was correlated with risk score.
5. Patients in the high-risk group could benefit from immunotherapy.

Introduction

Kidney renal clear cell carcinoma (KIRC), also known as clear cell renal cell carcinoma (ccRCC) is one of fatal malignancies, accounts for 75% of all renal cancers (1, 2). It is reported that more than 30% of KIRC patients eventually develop metastasis, and the five-year survival rate of metastatic KIRC is less than 10%, with a median survival of only 13 months (3). Due to the late diagnosis and resistance to

chemoradiotherapy (4), the prognosis of patients with metastatic KIRC is dismally poor (5). Therefore, early identification of KIRC metastatic potential and design of prognostic methods to identify patients at higher risk may be beneficial for the improvement of clinical outcomes. As human cancers are a consequence of genetic alterations, dysregulation of molecular signaling pathways, and epigenetic disorders (6), further investigations of molecular mechanisms underlying KIRC and identification of novel biomarkers are essential for early diagnosis, prognosis, and personalized treatment.

Programmed cell death (PCD) is a crucial terminal pathway for cells of multicellular organisms, whose dysregulation has been revealed to be related to tumorigenesis (7) and tumor progression (8). Ferroptosis is a newly discovered type of PCD that differs from traditional apoptosis, necrosis, or autophagic cell death, and is driven by iron-dependent lipid peroxide accumulation (9, 10). Recent research has revealed that ferroptosis is widely involved in the pathophysiological process of many diseases, such as blood diseases, neurological disorders, kidney injury, and various tumors (11). Moreover, accumulating evidence has confirmed that ferroptosis plays a pivotal role in tumor development and treatment (12–14), and may be a promising trigger option for cancer cell death, especially for malignancies that are resistant to traditional therapies (15, 16). Ferroptosis-related genes such as BRCA1-associated protein 1 (BAP1) (17), nuclear factor erythroid 2-related factor 2 (NRF2) (18, 19), and glutathione peroxidase 4 (GPX4) (20) are found to be implicated in tumorigenesis and progression. Although recent studies have revealed that some ferroptosis-related genes, such as Cystathionine beta-synthase (CBS), CD44, Fanconi anemia complementation group D2 (FANCD2), glutamate exchanger xCT (SLC7A11) are associated with high-risk patients with KIRC (21, 22), the key ferroptosis-related genes that could predict clinical prognosis and tumor immunity in KIRC have not been fully discovered.

In the present study, the mRNA expression profiles and corresponding clinical data of KIRC patients were downloaded from The Cancer Genome Atlas (TCGA). Based on training set (398 KIRC patients) and 60 ferroptosis-related genes retrieved from the previous literature (10, 15, 23, 24), the iron death related (IDR) prediction model was constructed, followed by performance evaluation for predicting prognosis of KIRC patients by survival analysis and verification using the validation set (398 KIRC patients). Moreover, functional enrichment analysis was conducted to explore the key functions of the IDR prediction model. Furthermore, immune cell infiltration analysis, gene set variation analysis (GSVA) for metagene clusters, tumor immune dysfunction and exclusion (TIDE) scoring analysis were carried out to analyze the association of the IDR prediction model with immune infiltration, inflammation, and immunotherapy response in KIRC, respectively. The flow chart of this study is shown in **Figure 1**. Our findings may help to improve the clinical outcomes of patients under personalized treatment.

Methods

Data collection

The RNA-sequencing (RNA-seq) data and clinical information of 602 KIRC patients in TCGA were downloaded from the public database UCSC Xena (<http://xena.ucsc.edu>). After excluding the control

samples and patients with incomplete clinical data, 530 KIRC patients were included in this study. Clinical indicators with a missing value of more than 50% were excluded in the clinical phenotypic study, and finally, 10 indicators were included, including hemoglobin, platelet, serum calcium, white cell count, M, N, T, TNM, age, and gender. Since the data was obtained from a public database, approval from the ethics committee or written informed consent from patients was not required. Moreover, 60 ferroptosis-related genes were retrieved from the previous literature (10, 15, 23, 24).

Construction of IDR prediction model

All data were randomly divided into training, testing and validation sets using R (4.0.2). Based on training set (398 KIRC patients) and 60 ferroptosis-related genes were retrieved from the previous literature, univariate Cox proportional hazards regression was performed to select candidate ferroptosis-related genes from 60 ferroptosis-related genes from previous literature, whose expression levels were significantly associated with the overall survival (OS) of the KIRC patient cohort ($P < 0.05$). The hazard ratios (HRs) were used to identify risk-related ferroptosis-related genes ($HR > 1$) and protective ferroptosis-related genes ($HR < 1$). Four-fold cross-validation for candidate genes was conducted and the area under the curve (AUC) of receiver operating characteristic (ROC) curves was calculated using R 4.0.2 ROCR (25) based on the testing set. Testing set was included in training set but was not used for model construction. In addition, another model construction method of non-cross-validation of all sample sets was used, and the AUC value was also calculated based on the testing set. By comparison of the AUC values obtained from the two model construction methods, the prediction model with the optimal AUC value was selected as the final IDR prediction model.

Survival analysis

Survival analysis was carried out using the Survival package in R (26), and log-rank tests were used to compare the differences in multiple subtypes (such as age, sex, and clinical stage) between high-risk and low-risk groups.

Functional enrichment analysis

The IDR prediction model-related genes with low expression value (50% of the gene expression value was 0) were filtered. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses for IDR-related genes were performed using clusterProfiler (27) in R. The false discovery rate (FDR) adjusted P value < 0.05 was set as the significance threshold.

Immune cell infiltration analysis

The IDR prediction model-related genes with low expression value (50% of the gene expression value was 0) were filtered. The proportion of human immune cell subpopulations, such as plasma cells, NK cells, mast cells, several T cell types, and macrophages were analyzed using Cell type identification by estimating relative subsets of known RNA transcripts (CIBERSORT, R 4.0.2) algorithm (28).

GSVA for metagene cluster

Seven metagene clusters including IgG, Interferon, lymphocyte specific kinase (LCK), major histocompatibility complex- I (MHC-I), MHC-II, signal transducer and activator of transcription 1 (STAT1), and hemopoietic cell kinase (HCK) metagenes have been revealed to act as surrogate markers for the amount of different immune cell types in the breast cancer sample (29). Herein, the expression value of 7 metagene clusters was analyzed using R GSVA (30), and then the association between metagene clusters and IDR risk scores were analyzed using Spearman analysis.

TIDE scoring analysis

The TIDE website (<http://tide.dfci.harvard.edu>) was used to analyze the immunotherapy biomarkers of all samples. They were classified into high-risk and low-risk groups using median risk score. The immune cell components and the differences of immunotherapy biomarkers between the two groups were analyzed by Mann-Whitney U test.

Results

Identification of prognostic ferroptosis-related genes in the training cohort

Based on the gene expression data and clinical data of training set (398 KIRC patients), the information of 60 ferroptosis-related genes was extracted. After excluding genes with a missing value of more than 50%, univariate Cox proportional hazards regression analysis was conducted. The results showed that 23 ferroptosis-related genes were significantly associated with OS. Among them, 15 ferroptosis-related genes were considered as risk factors ($HR > 1$), whereas the remaining 8 ferroptosis-related genes were considered as protective factors ($HR < 1$) (Table 1).

Table 1

Univariate Cox analysis revealed ferroptosis-related genes that were significantly associated with overall survival of patients with KIRC based on the training set obtained from TCGA

Ferroptosis-related genes	HR	95% CI		P value
		Low	High	
STEAP3	1.3154	1.1960	1.4468	2.96E-08***
CD44	1.5526	1.3101	1.8400	8.26E-07***
CHAC1	1.3550	1.1889	1.5443	1.00E-05***
FANCD2	1.6556	1.2903	2.1244	7.34E-05***
G6PD	1.7290	1.3024	2.2955	1.36E-04***
AKR1C1	0.8196	0.7394	0.9085	2.26E-04***
PHKG2	1.7489	1.2806	2.3885	2.95E-04***
CBS	1.2468	1.1098	1.4008	3.19E-04***
SLC1A5	1.5170	1.1967	1.9229	3.89E-04***
NOX1	1.5975	1.2026	2.1221	1.03E-03**
CARS	1.6816	1.2021	2.3522	1.65E-03**
GOT1	0.7728	0.6634	0.9002	1.70E-03**
HMGCR	0.7230	0.5894	0.8868	2.73E-03**
PTGS2	1.1540	1.0485	1.2701	4.58E-03**
NCOA4	0.7895	0.6766	0.9212	5.49E-03**
FADS2	1.2165	1.0541	1.4038	6.06E-03**
GCLC	0.7898	0.6636	0.9400	1.23E-02*
ALOX5	1.1582	1.0236	1.3106	1.73E-02*
DPP4	0.9010	0.8312	0.9767	1.74E-02*
SLC7A11	1.1864	1.0292	1.3676	1.85E-02*
SAT1	1.3081	1.0262	1.6676	2.62E-02*
PEBP1	0.7965	0.6540	0.9702	3.30E-02*
GLS2	0.8627	0.7463	0.9972	4.91E-02*
KIRC: kidney renal clear cell carcinoma; TCGA: The Cancer Genome Atlas (TCGA); HR: Hazard ratio; 95%CI: 95% confidence interval. ***P < 0.0001, **P < 0.01, and *P < 0.05.				

Results of IDR prediction model construction

Using the two model construction methods, the results of model construction were obtained (Table 2). Cvlist1 model with AUC = 0.74 was selected as the optimal IDR prediction model with the optimal AUC value was selected as the final IDR prediction model. This model contained 10 ferroptosis-related genes, including 6 risk factors (STEAP3, CD44, CHAC1, FANCD2, G6PD, and FADS2) and 4 protective factors (GOT1, NCOA4, PEBP1, and GLS2). The risk score was calculated using the following formula for the ferroptosis-related gene signature: risk score = (0.1002*STEAP3) + (0.3122*CD44) + (0.2719*CHAC1) + (0.6704*FANCD2) + (-0.3796*G6PD) + (-0.2123*GOT1) + (-0.5082*NCOA4) + (0.1419*FADS2) + (0.6165*PEBP1) + (-0.2321* GLS2).

Table 2
The result of model construction (4-fold cross validation)

Sample set	AUC	Median Y	Formula
Cvlist1	0.74	7.9775	Risk score = (0.1002*STEAP3) + (0.3122*CD44) + (0.2719*CHAC1) + (0.6704*FANCD2) + (-0.3796*G6PD) + (-0.2123*GOT1) + (-0.5082*NCOA4) + (0.1419*FADS2) + (0.6165*PEBP1) + (-0.2321*GLS2)
Cvlist2	0.72	4.7980	Risk score =(0.4685*CD44) + (0.2341*CHAC1) + (0.5161*FANCD2) + (-0.3309*G6PD) + (0.5498*PHKG2) + (0.2958*NOX1) + (-0.7670*HMGCR) + (0.1321*PTGS2) + (0.3151*GCLC) + (-0.4037*SAT1) + (-0.2334*GLS2)
Cvlist3	0.71	5.8744	Risk score = (0.1334*STEAP3) + (0.4218*FANCD2) + (-0.2042*AKR1C1) + (0.4552*NOX1) + (-0.6141*HMGCR) + (0.1253*PTGS2) + (0.2074*FADS2) + (0.2744*PEBP1)
Cvlist4	0.68	4.4162	Risk score = (0.1633*STEAP3) + (0.2252*CD44) + (0.1977*CHAC1) + (0.5708*FANCD2) + (0.6056*NOX1) + (-0.4688*HMGCR) + (-0.2986*SAT1)
Cvlist all	0.74	7.1970	Risk score = (0.1352*STEAP3) + (0.1807*CHAC1) + (0.5685*FANCD2) + (0.4214*NOX1) + (-0.3635*NCOA4) + (0.1307*FADS2) + (-0.1684*GLS2)
AUC: area under the curve.			

Prognostic value of the IDR prediction model in the training set

To reveal the prognostic value of the IDR prediction model, Kaplan–Meier (KM) survival analysis was performed on the training set. Based on the median risk score (= 7.9775) as the cut-off point, KIRC patients were categorized into high-risk (n = 199) and low-risk (n = 199) groups (P < 0.0001, Figure 2A). KM survival curve analysis showed that the OS of KIRC patients with high-risk scores was significantly shorter than those with low-risk scores (Figure 2B). Moreover, the patients were further grouped according to age (≥ 60 (n = 222) and < 60 (n = 176) years), gender (female (n = 135) and male (n = 263)), and AJCC stage (early (stages I and II, n = 247) and advanced (stages III and IV, n = 151) stages). KM survival curve analysis also showed that the OS rate was significantly shorter for the high-risk patients relative to the low-risk patients based on the prognostic signature among those with age ≥ 60 years (P < 0.001), those

with age < 60 years ($P < 0.001$), female ($P < 0.001$), male ($P < 0.001$), early stages ($P = 0.0033$), and advanced stages ($P < 0.001$) (**Figure 2C-E**). These results suggested that the IDR prediction model could determine the prognosis of KIRC patients.

We then analyzed whether the IDR prediction model was an independent prognostic predictor for OS. Univariate and multivariate Cox regression analyses were conducted. A total of 10 clinicopathological characteristics of the KIRC patients were also included, including hemoglobin, platelet, serum calcium, white cell count, M, N, T, TNM, age, and gender. Univariate analysis showed that age, tumor stage, serum calcium, T, M, platelet qualitative, white cell count, hemoglobin, and risk score were significantly associated with OS (all $P < 0.05$, **Table 3**). Further multivariate analysis revealed that age, tumor stage, serum calcium, T, and risk score were significantly associated with OS (all $P < 0.05$, **Table 3**). These data confirmed that the risk score was an independent prognostic factor for KIRC patients.

Table 3

Univariate and multivariate Cox regression analysis of the correlation between overall survival and risk score and various clinicopathological parameters

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
Risk Score	2.4646	2.0261–2.9981	7.27E-18***	1.8443	1.4773–2.3025	6.42E-08***
Tumor Stage (I,II,III,IV)	1.9260	1.6412–2.2602	1.10E-16***	2.3844	1.5850–3.5871	3.04E-05***
Age	1.0319	1.0154–1.0486	1.07E-04***	1.0335	1.0145–1.0529	5.21E-04***
Serum calcium	0.6650	2.3512 – 0.1094	9.59E-06***	1.2566	1.0509–1.5026	1.23E-02*
T (1,2,3,4)	1.9731	1.6219–2.4004	1.88E-12***	0.6328	0.4114–0.9734	3.73E-02*
M (0,1, X)	1.5982	1.2773–1.9997	3.35E-04***	0.8422	0.5144–1.3789	4.95E-01
N (0,1, X)	0.9705	0.8608–1.0942	6.25E-01	0.9573	0.8438–1.0861	4.98E-01
Platelet qualitative	0.8421	1.5163 – 0.1861	2.13E-05***	0.9289	0.7452–1.1578	5.12E-01
White cell count	1.5041	1.8760 – 0.7999	1.60E-02*	0.9398	0.7591–1.1635	5.69E-01
Gendermale	0.8752	0.6052–1.2658	4.82E-01	0.9470	0.6412–1.3987	7.84E-01
Hemoglobin	1.2109	1.6692 – 0.0585	1.33E-05***	0.9834	0.7136–1.3551	9.18E-01
***P < 0.001 and *P < 0.05.						

Validation of the IDR prediction model in the validation set

To test the robustness of the IDR prediction model constructed by training set, 132 KIRC patients in the validation set were stratified into high-risk (n = 68) and low-risk (n = 64) groups based on the median risk score (= 7.9775). Likewise, KM survival curve analysis revealed that KIRC patients in the high-risk group had a shorter OS compared with those in the low-risk group (P = 0.0087, **Figure 3A**). Then, the validation set were randomly divided into three cohorts, named Cvlist1, Cvlist2, and Cvlist3. According their respective optimal median cut-off value, KIRC patients were stratified into high-risk and low-risk groups, followed by KM survival curve analysis. Expect Cvlist1 set (**Figure 3B**), patients in the high-risk group in Cvlist2 and Cvlist3 sets had a significantly worse OS than those in the low-risk group (P = 0.027,

Figure 3C; $P = 0.045$, Figure 3D). Collectively, our results indicated that the predictive performance of the IDR prediction model was robust and reliable.

Functional analysis for the IDR prediction model

To elucidate the functions and pathways that were associated with the risk score, functional enrichment analysis was performed. The 398 samples (training set) and 132 samples (validation set) were all included in this part of the study. The genes with low expression were filtered out, and finally, the expression data of 32643 genes were analyzed. The genes that were significantly correlated with risk score was identified using Pearson analysis with the cut off value of $P < 0.05$ and coefficient $> |0.4|$. As results, 715 genes were found to be significantly correlated with risk score, among them, 668 genes were positive correlated while 47 were negative correlated. Heatmap for the top 100 positive correlated genes and 47 negative correlated genes is shown in **Figure 4A**. Further functional enrichment analysis showed that positive correlated genes were significantly enriched in several KEGG pathways, such as cell cycle and p53 signaling pathway, as well as GO functions associated with organelle fission and nuclear division (**Figure 4B**); the negative correlated genes were remarkably enriched in several KEGG pathways, such as Bile secretion and cGMP-PKG signaling pathway, as well as GO functions associated with apical plasma membrane and sodium ion transmembrane transporter activity (**Figure 4C**).

Analysis of the relationship between the IDR prediction model and immune infiltration or inflammatory activities

To investigate the relationship between the IDR prediction model and immune infiltration, the CIBERSORT algorithm was applied. The 398 samples (training set) and 132 samples (validation set) were all included in this part of the study. The genes with low expression were filtered out, and finally, the expression data of 32643 genes were analyzed. The landscape of immune cell infiltration of high-risk and low-risk groups was shown in **Figure 5A**. KIRC patients in the high-risk group had higher ratios of plasma cells, T cells CD8, and T cells regulatory Tregs ($P < 0.05$, **Figure 5B-C**).

Moreover, GSVA for 7 metagene clusters was conducted to reveal the relationship between the IDR prediction model and inflammatory activities. The duplicated genes and genes with 0 expression of more than 50% in 530 samples (398 samples in training set and 132 samples in validation set) were excluded, and finally the remaining 93 genes were included in the GSVA analysis. Heatmap for the expression value of 93 genes in the high-risk and low-risk groups is displayed in **Figure 5D**. By further Spearman correlation analysis, IgG metagenes (Coefficient = 0.2170, $P < 0.001$), HCK metagenes (Coefficient = 0.0977, $P = 0.0165$), and LCK metagenes (Coefficient = 0.0892, $P = 0.0286$) were found to be significantly positively correlated with risk score, and Interferon metagenes (Coefficient = -0.1333, $P = 0.0010$) was significantly negatively correlated with risk score (**Figure 5E**).

Association between the IDR prediction model and immunotherapy

Using TIDE website, 13 immunotherapy biomarkers of 530 samples (398 samples in training set and 132 samples in validation set) were obtained, including CAF, Responder, TIDE, T Cell Exclusion, CD8, Merck18, MDSC, T Cell Dysfunction, IFNG, CD274, CTL Flag, MSI.Expr.Sig, and TAM.M2. Except Responder, CTL Flag, and IFNG, there were significant differences in other biomarkers between high-risk and low-risk groups (**Table 4**). These data indicated that patients in the high-risk group could benefit from immunotherapy.

Table 4
The immunotherapy biomarkers that had significant difference between high-risk and low-risk groups

	High-risk group	Low-risk group	P value
TAM M2	-0.0201 ± 0.0143	-0.0156 ± 0.0108	0.00001***
CD8	0.6579 ± 0.4843	0.5295 ± 0.4267	0.00015***
Merck18	0.7282 ± 0.2588	0.6709 ± 0.2038	0.00032***
T Cell Dysfunction	-1.0197 ± 0.2324	-1.0772 ± 0.1958	0.00074***
CAF	0.1583 ± 0.0293	0.1511 ± 0.0243	0.00125**
MSI Expr Sig	0.8067 ± 0.0516	0.8181 ± 0.0475	0.00204**
MDSC	0.0573 ± 0.0227	0.0513 ± 0.0152	0.0037**
TIDE	1.4205 ± 0.2981	1.3677 ± 0.2396	0.02171*
T Cell Exclusion	1.4205 ± 0.2981	1.3677 ± 0.2396	0.02171*
CD274	0.5163 ± 0.2481	0.5484 ± 0.1907	0.04939*
HR: Hazard ratio; 95%CI: 95% confidence interval. ***P < 0.001, **P < 0.01, and *P < 0.05.			

Discussion

In the present study, we built a novel IDR prediction model integrating 10 ferroptosis-related genes to stratify KIRC patients into two risk groups. The overall survival (OS) of KIRC patients with high-risk scores was significantly shorter than those with low-risk scores in both training set and validation set. Moreover, the risk score was confirmed as an independent prognostic predictor for OS. In addition, the positive and negative correlated genes with this model were significantly enriched in p53 signaling pathway, and cGMP-PKG signaling pathway. Immune infiltration analysis revealed that KIRC patients in the high-risk group had higher ratios of plasma cells, T cells CD8, and T cells regulatory Tregs. Furthermore, IgG, HCK, and LCK metagenes were significantly positively correlated with risk score, and Interferon metagenes was negatively correlated. By TIDE score analysis, patients in the high-risk group could benefit from immunotherapy. These findings present future KIRC research with more information.

Increasing evidence has confirmed that ferroptosis plays a crucial role in diverse kidney diseases, including KIRC (31) and has anticancer functions that are useful in cancer treatment (10). Investigating the role of ferroptosis and identifying the ferroptosis-related genes will facilitate the development of effective treatment strategies for KIRC. Moreover, it is reported that multigene signatures could provide risk stratification and prognostic prediction in breast cancer, such as PAM50 signature (32). A multigene panel has exhibited the potent performance to predict poor prognosis in patients with KIRC (33). In this present study, the IDR prediction model was built by 10 ferroptosis-related genes, and the risk score was confirmed as an independent prognostic predictor for OS. Therefore, we conclude that our identified multigene signature can predict clinical prognosis and brings insight to molecular biologic characteristics of KIRC.

The identified IDR prediction model included 6 OS-related risk factors (STEAP3, CD44, CHAC1, FANCD2, G6PD, and FADS2) and 4 protective factors (GOT1, NCOA4, PEBP1, and GLS2). STEAP3 is shown to have a ability to catalyze the reduction of free ferric iron (Fe^{3+}) to divalent iron (Fe^{2+}) in endosome and then release Fe^{2+} to the cellular labile iron pool, thus triggering ferroptosis (34). CD44 is widely implicated in various malignant processes such as tumor growth, and angiogenesis (35), which has been observed to be associated with a poor survival rate in many human tumors (36). The P-selectin and the CD44 receptor interactions is revealed to promote the cellular uptake of Fe_3O_4 -SAS@PLT nanoparticles, resulting in higher cytotoxicity by ferroptosis (37). Upregulated CHAC1 can degrade intracellular glutathione and then contribute to ferroptosis in human triple negative breast cancer cells (38). FANCD2 is a nuclear protein that can protect against bone marrow injury from ferroptosis-mediated injury and represent an amenable target for the development of novel anticancer therapies (39). G6PD is a key enzyme that generates NADPH to maintain reduced glutathione, whose high expression is correlated with poor prognosis and poor outcome in several types of carcinoma (40, 41). FADS2 is a key enzyme involved in the biosynthesis of polyunsaturated fatty acids, whose expression is correlated with multiple tumor processes in human cancers, such as tumor proliferation, angiogenesis, ferroptosis, and prognosis (42). NCOA4-mediated ferritinophagy is shown to be implicated in the initiation of ferroptosis (43). PEBP1 is required to form the 15LOX/PEBP1 complex, and suppression of this complex can mediate the Ferrostatin-1 prevention of ferroptosis (44). GOT1 and GLS2 is found to take part in glutaminolysis to regulate ferroptosis process (45). It is reported that miR-9 can suppress ferroptosis via downregulating GOT1 expression in melanoma (46). In cancer cells, GLS2 upregulation can induce an antiproliferative response with cell cycle arrest (47). Notably, high expression of risk genes, such as CD44, FANCD2 and CHAC1 as well as low expression of NCOA4 were found to be correlated with worse OS in KIRC (21, 22), in line with our results. Taken together, these genes may be involved in KIRC via involved in ferroptosis. Moreover, the positive and negative correlated genes with this model were significantly enriched in p53 signaling pathway, and cGMP-PKG signaling pathway. The p53 signaling pathway has been confirmed to exert tumor suppressive function via regulating cellular processes, like anti-oxidant defense and ferroptosis (48). The cGMP/PKG signaling pathway is revealed to play a significant role in regulating the proliferation and survival of human renal carcinoma cells (49). Therefore, we speculate that p53 signaling pathway, and cGMP-PKG signaling pathway may participate in KIRC via regulating ferroptosis.

Although great efforts have been made to investigate the mechanisms underlying tumor susceptibility to ferroptosis, it remains elusive in the potential modulation between ferroptosis and tumor immunity. The immune infiltration analysis revealed that KIRC patients in the high-risk group had higher proportion of plasma cells, T cells CD8, and T cells regulatory Tregs. Tumor-infiltrating plasma cells are shown to play a positive role in antitumor immunity, suggesting that the potential of enhanced these response in the design of cancer immunotherapy (50). Lee et al. demonstrated that increased Tumor-infiltrating plasma cells are associated with worse survival in lung adenocarcinomas (51). CD8 + T cells can enhance ferroptosis-specific lipid peroxidation and, in turn, affect the anti-tumor efficacy of immunotherapy (52). Treg frequency in peripheral blood and in tumour-infiltrating lymphocytes is revealed associated with poor prognosis in renal cell carcinoma (53). Moreover, we found that IgG, HCK, and LCK metagenes were significantly positively correlated with risk score, and Interferon metagenes was negatively correlated. By TIDE score analysis, most of immunotherapy biomarkers, such as CAF, TIDE, T Cell Exclusion, CD8, Merck18, MDSC, T Cell Dysfunction, CD274, MSI.Expr.Sig, and TAM.M2 were significantly different between high-risk and low-risk groups. These data prompted that patients in the high-risk group were in an immunoactive state and benefit from immunotherapy, which will provide a more comprehensive view of cancer immunotherapy efficacy

Limitations of our study are as follows: first, our IDR prediction model was constructed and validated with data from TCGA database, therefore, the use of this model in a real clinical setting remains controversial. Second, the relationships between the risk score and immune immunity are not experimentally confirmed. More studies are still required to confirm our observation.

In conclusion, a novel gene signature consisting of 10 ferroptosis-related genes shows reliable ability in predicting clinical prognosis and is correlated with immune immunity in patients with KIRC. The patients at higher risk might be more suitable to benefit from immunotherapy. With further validation, this model can be explored as a predictive biomarker to develop personalized therapies for patients with KIRC.

Declarations

Ethics approval and consent to participate

Our study did not require an ethical board approval because it did not contain human or animal trials.

Availability of data and materials:

Not applicable. This study was only the primary research, and further study has been in progress.

Consent for publication

Not applicable.

Funding:

Competing Interests

The authors declare that they have no competing interests.

Author contributions

Qi Zhang carried out the Conception and design of the research, Wei Song participated in the Acquisition of data. Weiting Kang participated in the design of the study and performed the statistical analysis. Qi Zhang wrote and revised the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

The flow chart of this study.

Figure 2

The construction and validation of the IDR prediction model in the training set obtained from TCGA. A: the distribution of risk score, survival status, and the 10-gene expression panel. B-E: Kaplan–Meier (KM) survival analyses were conducted to estimate overall survival (OS) for the high- and low-risk groups based on the median risk score. B: 398 patients with KIRC. C: KIRC patients with age ≥ 60 and < 60 years. D: Male and female KIRC patients. E: Patients with early stages (stage I and II) and advanced stages (stage III and IV).

Figure 3

Validation of the IDR prediction model in the validation set obtained from TCGA. Kaplan–Meier (KM) survival analyses were conducted to estimate overall survival (OS) for the high- and low-risk groups based on the median risk score. A: 132 patients with KIRC. B-D: The validation set were randomly divided into three cohort, named Cvlist1 (B), Cvlist2 (C), and Cvlist3 (D).

Figure 4

Analysis of key functions and pathways that were associated with the IDR prediction model. A: Heatmap for the top 100 positive correlated genes and 47 negative correlated genes. B: Significant KEGG pathways and GO functions for positive correlated genes. C: Significant KEGG pathways and GO functions for negative correlated genes. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

Figure 5

Analysis of the relationship between the IDR prediction model and immune cell infiltration and inflammatory activities. A: The landscape of immune cell infiltration of high-risk and low-risk groups. B: Higher ratios of immune cells in the low-risk group. C: Higher ratios of immune cells in the high-risk group. D: Heatmap for the expression value of 93 genes in the high-risk and low-risk groups. E: The correlation between risk score and inflammatory metagenes.