Forkhead-box series expression network is associated with outcome of clear-cell renal cell carcinoma

ZHONGWEI JIA[1,2*], FANGNING WAN[1,2*], YAO ZHU[1,2], GUOHAI SHI[1,2], HAILIANG ZHANG[1,2], BO DAI[1,2] and DINGWEI YE[1,2]

[1]Department of Urology, Fudan University Shanghai Cancer Center; [2]Department of Oncology, Fudan University Shanghai Medical College, Shanghai 200032, P.R. China

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Abstract. Previous studies have demonstrated that several members of the Forkhead-box (FOX) family of genes are associated with tumor progression and metastasis. The objective of the current study was to screen candidate FOX family genes identified from analysis of molecular networks in clear cell renal cell carcinoma (ccRCC). The expression of FOX family genes as well as FOX family-associated genes was examined, and Kaplan-Meier survival analysis was performed in The Cancer Genome Atlas (TCGA) cohort (n=525). Patient characteristics, including sex, age, tumor diameter, laterality, tumor-node-metastasis, tumor grade, stage, white blood cell count, platelet count, the levels of hemoglobin, overall survival (OS) and disease-free survival (DFS), were collected for univariate and multivariate Cox proportional hazards ratio analyses. A total of seven candidate FOX family genes were selected from the TCGA database subsequent to univariate and multivariate Cox proportional hazards ratio analyses. *FOXA1*, *FOXA2*, *FOXD1*, *FOXD4L2*, *FOXK2* and *FOXL1* were associated with poor OS time, while *FOXA1*, *FOXA2*, *FOXD1* and *FOXK2* were associated with poor DFS time (P<0.05). *FOXN2* was associated with favorable outcomes for overall and disease-free survival (P<0.05). In the gene cluster network analysis, the expression of FOX family-associated genes, including nuclear receptor coactivator (*NCOA*)*1*, NADH-ubiquinone oxidoreductase flavoprotein 3 (*NDUFV3*), phosphatidylserine decarboxylase (*PISD*) and pyruvate kinase liver and red blood cell (*PKLR*), were independent prognostic factors for OS in patients with ccRCC. Results of the present study revealed that the expression of FOX family genes, including *FOXA1*, *FOXA2*, *FOXD1*, *FOXD4L2*, *FOXK2* and *FOXL1*, and FOX family-associated genes, including *NCOA1*, *NDUFV3*, *PISD* and *PKLR*, are independent prognostic factors for patients with ccRCC.

Correspondence to: Dr Bo Dai or Dr Dingwei Ye, Department of Urology, Fudan University Shanghai Cancer Center, 270 Dongan Road, Shanghai 200032, P.R. China
E-mail: bodai1978@126.com
E-mail: dwyeli@163.com

*Contributed equally

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Introduction

Renal cell carcinoma (RCC), which accounts for 2-3% of all adult malignancies, is a relatively common malignancy with an incidence rate that is increasing at a rate of 2% each year (1). Clear cell renal cell carcinoma (ccRCC), which accounts for ~90% of RCC cases (2), is the most common histological subtype of RCC and exhibits a 5-year disease-specific survival rate of 50-69% (3). RCC is notoriously refractory to radiation therapy and standard chemotherapy. If detected at an early stage, ccRCC can be cured by surgery. However, ~25% of patients with RCC are identified with lymph node metastasis or distant metastasis at first diagnosis, and 30-40% of patients experience recurrence or metastasis even following surgery (4). Currently, the primary prognostic index for ccRCC is the Fuhrman nuclear grade and disease staging at the time of surgery (5). Thus, it is important to develop new biomarkers to screen out high-risk patients for additional appropriate postoperative therapy and surveillance.

Forkhead-box (FOX) family proteins are involved in the regulation of cell growth and differentiation as well as embryogenesis and tissue development. These proteins are characterized by a conserved FOX domain and extra-FOX protein-protein interaction domains (6). The FOX domain is ~100 amino acids in length and is involved in DNA binding (6-8). The extra-FOX regions are involved in interactions with transcriptional activators, transcriptional repressors or DNA repair complexes (6,7). Previous studies have demonstrated an association between the expression of FOX family genes and the prognosis of different types of cancer, including lung cancer, basal cell carcinoma, esophageal cancer, pancreatic cancer, rhabdomyosarcoma, acute myeloblastic leukemia and acute lymphocytic leukemia (9). However, the role of FOX family genes in ccRCC has not been described.

The present study examined the expression of FOX family genes in 525 ccRCC cases from The Cancer

Genome Atlas (TCGA) database with the aim of potentially identifying a prognostic marker for ccRCC. The associations between FOX family-related gene expression and clinicopathological characteristics were also investigated.

Materials and methods

Patients and data. The expression levels of FOX family genes, FOX family-related genes, and associated clinical data were downloaded from the TCGA data portal, which is available from the Cancer Genomics Browser of the University of California Santa Cruz (https://genome-cancer.ucsc.edu/). A total of 51 gene members of the FOX family were studied in 525 primary ccRCC tumors from patients with detailed FOX family gene expression data, and related clinical follow-up data was selected from the updated TCGA data portal. Patients included a total of 184 females and 341 males (age range, 26-90 years; median age, 61 years). All patients had received partial or radical nephrectomy. The enrolled patients had not received pretreatment and had fully characterized tumors, complete RNA sequencing information and intact overall survival (OS) and disease-free survival (DFS) information. Appropriate genes were selected to construct gene networks according to the standards described in a previous study (10). Furthermore, clinicopathological characteristics, including sex, age, tumor diameter, laterality, tumor-node-metastasis, tumor grade, American Joint Committee on Cancer (AJCC) stage (11), levels of white blood cells, platelets and hemoglobin, OS and DFS, were also collected. A network of prognostic FOX genes was obtained from the cBioPortal (http://www.cbioportal.org), and the following criteria were used to construct the network: 'In the same complex', 'interacted with each other' and 'more than 12% changes'. Unigene accession numbers were obtained from https://www.ncbi.nlm.nih.gov/unigene.

Statistical analysis. Duration of DFS was calculated from the date of diagnosis to the date of first recurrence or mortality. Duration of OS was calculated from the date of diagnosis to the date of mortality or last follow-up which undertaken for a median of 35.95 months. Patients without recurrence or did not succumb to disease were marked as censored at the time of the last follow-up. The Kaplan-Meier method was used for survival analysis, and the log-rank test was used for comparing cumulative survival. The association between overall survival and FOX gene expression was analyzed by performing univariate and multivariate analysis using Cox proportional-hazards regression. All the statistical tests were performed using SPSS (version 22.0; IBM SPSS, Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of patients with ccRCC in the TCGA cohort. A total of 525 patients were enrolled in the present study. The patients included 184 females and 341 males with a range of 26-90 years and a median of 61 years. Among the 525 patients, 45.7% of the patients had low-grade (grade 1 and 2) ccRCC, 52.8% had high-grade ccRCC and only 8 cases were of undetermined grade. The clinicopathological characteristics

Table I. Clinical characteristics of 525 patients with clear cell renal cell carcinoma in The Cancer Genome Atlas cohort.

Variables	Patients
Age, median (range)	61 (26.0-90.0)
Sex, n (%)	
Male	341 (65.0)
Female	184 (35.0)
Grade, n (%)	
1	12 (2.3)
2	228 (43.4)
3	202 (38.5)
4	75 (14.3)
Gx	8 (1.5)
Tumor diameter, mean (range)	1.67 (0.4-4.0)
pT, n (%)	
T1	266 (50.7)
T2	68 (13.0)
T3	179 (34.1)
T4	11 (2.1)
N, n (%)	
N0	237 (45.1)
N1	17 (3.2)
Nx	271 (51.6)
M, n (%)	
M0	406 (77.3)
M1	78 (14.9)
Mx	25 (4.8)
Stage[a], n (%)	
I	262 (49.9)
II	56 (10.7)
III	126 (24)
IV	81 (15.4)
Laterality, n (%)	
Left	247 (47.0)
Right	277 (52.8)
Bilateral	1 (0.2)
Hb, n (%)	
Low	258 (49.1)
Normal	181 (34.5)
Elevated	5 (1.0)
Unavailable	81 (15.4)
WBC, n (%)	
Low	45 (8.6)
Normal	261 (49.7)
Elevated	162 (30.9)
Unavailable	94 (17.9)
PLT, n (%)	
Low	45 (8.6)
Normal	352 (67.0)
Elevated	37 (7.0)
Unavailable	91 (17.3)

[a]American Joint Committee on Cancer stage. Hb, hemoglobin; WBC, white blood cell; PLT, platelet; pT, pathological T stage; N, node; M, metastasis.

Table II. Univariate and multivariate Cox proportional hazards analysis of FOX gene expression and overall survival of patients with clear cell renal cell carcinoma in The Cancer Genome Atlas cohort.

	Univariate		Multivariate	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
Age	1.03 (1.01-1.04)	<0.001	1.03 (1.02-1.05)	<0.01
Sex	0.95 (0.69-1.30)	0.75	1.00 (0.64-1.58)	0.99
Stage[a]	1.95 (1.71-2.24)	<0.001	1.29 (0.65-2.57)	0.47
Grade[b]	2.40 (1.94-2.97)	<0.001	1.23 (0.86-1.75)	0.25
Hb	0.56 (0.40-0.79)	<0.001	0.79 (0.51-1.23)	1.29
WBC	0.67 (0.48-0.92)	0.01	0.92 (0.59-1.45)	0.73
PLT	1.71 (1.16-2.53)	0.01	1.12 (0.73-1.71)	0.60
Tumor diameter	1.22 (0.98-1.50)	0.07	0.72 (0.52-0.99)	0.04
Position[c]	0.70 (0.51-0.94)	0.02	0.85 (0.56-1.27)	0.42
TNM stage				
Tumor	2.00 (1.69-2.36)	<0.001	1.12 (0.58-2.16)	0.75
Node	1.00 (0.56-1.75)	0.98	0.57 (0.28-1.19)	0.13
Metastasis	4.55 (3.31-6.26)	<0.001	3.13 (1.15-8.48)	0.03
FOX family of genes				
FOXL2	1.25 (1.08-1.45)	<0.001	1.27 (0.95-1.70)	0.11
FOXL1	1.22 (1.07-1.39)	<0.001	1.26 (1.01-1.57)	0.04
FOXS1	1.16 (1.03-1.30)	0.01	0.93 (0.71-1.21)	0.57
FOXN1	1.20 (0.99-1.45)	0.06		
FOXN2	0.78 (0.61-0.99)	0.03	0.62 (0.39-0.98)	0.04
FOXN3	0.59 (0.47-0.74)	<0.001	1.07 (0.63-1.80)	0.81
FOXH1	1.29 (1.16-1.44)	<0.001	0.85 (0.68-1.05)	0.13
FOXG1	1.22 (1.11-1.34)	<0.001	1.00 (0.84-1.18)	0.96
FOXP2	1.01 (0.94-1.07)	0.88		
FOXD1	1.26 (1.14-1.39)	<0.001	0.83 (0.70-0.99)	0.04
FOXC2	0.95 (0.86-1.05)	0.30		
FOXC1	1.10 (0.93-1.30)	0.29		
FOXF1	0.94 (0.82-1.08)	0.41		
FOXF2	1.13 (1.00-1.27)	0.05	0.94 (0.79-1.14)	0.54
FOXE1	1.21 (1.12-1.31)	<0.001	1.13 (0.99-1.30)	0.08
FOXO3B	0.77 (0.59-1.01)	0.06		
FOXB2	0.76 (0.51-1.13)	0.18		
FOXR1	1.16 (0.60-2.23)	0.66		
FOXN4	1.20 (1.06-1.36)	<0.001	0.82 (0.61-1.10)	0.18
FOXM1	1.62 (1.43-1.83)	<0.001	0.87 (0.66-1.15)	0.32
FOXP3	1.25 (1.14-1.38)	<0.001	0.99 (0.86-1.15)	0.94
FOXP1	1.34 (1.09 -1.64)	0.01	1.20 (0.78-1.85)	0.41
FOXP4	2.02 (1.57-2.59)	<0.001	1.49 (0.91-2.44)	0.11
FOXO3	0.73 (0.56-0.94)	0.01	0.64 (0.39-1.07)	0.09
FOXO1	0.67 (0.53-0.85)	<0.001	0.99 (0.62-1.57)	0.96
FOXO4	0.62 (0.46-0.84)	<0.001	0.73 (0.40-1.37)	0.33
FOXR2	1.31 (0.80-2.16)	0.29		
FOXI1	0.98 (0.92-1.03)	0.41		
FOXI3	1.25 (0.76-2.07)	0.38		
FOXI2	0.85 (0.77-0.93)	<0.001	1.07 (0.92-1.25)	0.37
FOXRED2	1.12 (0.91-1.38)	0.28		
FOXRED1	1.23 (0.95-1.59)	0.12		
FOXD4L5	0.99 (0.61-1.61)	0.98		
FOXD4L6	1.31 (1.10-1.57)	<0.001	0.91 (0.66-1.26)	0.57

Table II. Continued.

	Univariate		Multivariate	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
FOXD4L1	1.35 (1.16-1.58)	<0.001	0.79 (0.56-1.13)	0.20
FOXD4L2	1.26 (1.08-1.46)	<0.001	1.40 (1.10-1.79)	0.01
FOXD4L3	1.37 (0.92-2.04)	0.13		
FOXB1	1.18 (1.02-1.37)	0.03	0.97 (0.73-1.28)	0.80
FOXK2	2.90 (2.02-4.14)	<0.001	2.71 (1.29-5.71)	0.01
FOXK1	0.98 (0.77-1.26)	0.89		
FOXD3	1.29 (1.05-1.58)	0.02	1.01 (0.74-1.39)	0.93
FOXA1	1.19 (1.13-1.27)	<0.001	1.12 (1.01-1.24)	0.03
FOXA3	1.05 (0.96-1.14)	0.29		
FOXA2	1.18 (1.10-1.25)	<0.001	1.13 (1.02-1.26)	0.02
FOXJ1	1.13 (1.05-1.21)	<0.001	0.98 (0.86-1.112)	0.71
FOXJ2	1.02 (0.69-1.52)	0.91		
FOXJ3	0.96 (0.84-1.11)	0.59		
FOXE3	1.72 (1.41-2.09)	<0.001	1.34 (0.97-1.86)	0.07
FOXQ1	1.01 (0.91-1.12)	0.93		
FOXD4	1.16 (1.00-1.34)	0.04	1.17 (0.87-1.57)	0.30
FOXD2	1.21 (1.01-1.44)	0.04	0.87 (0.58-1.30)	0.49

[a]American Joint Committee on Cancer stage; [b]Fuhrman grade; [c]Tumors on the left kidney set as 0 and tumors on the right kidney set as 1. CI, confidence interval; HR, hazards ratio; FOX, forkhead-box; Hb, preoperative hemoglobin count; WBC, preoperative white blood cell count; PLT, preoperative platelet count; TNM, tumor-node-metastasis.

Table III. Multivariate logistic regression analysis of factors that may affect the expression of *FOXA1* and *FOXA2* in The Cancer Genome Atlas cohort with clear cell renal cell carcinoma.

A, *FOXA1*

Variables	OR (95% CI)	P-value
Age	1.01 (0.99-1.02)	0.57
Sex	1.02 (0.68-1.53)	0.93
Stage[b]	1.32 (1.10-1.59)	<0.001[a]
Grade[c]	1.51 (1.12-2.04)	0.01[a]
Tumor diameter	0.96 (0.71-1.29)	0.78
Position	1.06 (0.72-1.55)	0.77

B, *FOXA2*

Variable	OR (95% CI)	P-value
Age	1.00 (0.98-1.01)	0.68
Sex	0.66 (0.44-0.99)	0.04[a]
Stage[b]	1.25 (1.03-1.50)	0.02[a]
Grade[c]	1.18 (0.88-1.58)	0.28
Tumor diameter	1.10 (0.82-1.48)	0.54
Position	1.13 (0.78-1.65)	0.51

[a]P<0.05 was considered statistically significant. [b]American Joint Committee on Cancer stage; [c]Fuhrman grade. CI, confidence interval; OR, odds ratio; FOX, forkhead-box.

of the enrolled patients are summarized in Table I. Follow-up was undertaken for a median of 35.95 months. At the end of the follow-up, 31.6% of patients had succumbed to disease (166/525).

Selection of independent prognostic factors for OS in the TCGA cohort among FOX gene family members. The median follow-up duration of the patients was 35.95 months, and 166 patients succumbed to disease during the follow-up period. The results of univariate analysis and multivariate analysis of the potential prognostic factors are shown in Table II. Age, AJCC stage and Fuhrman grade and 37 FOX genes were determined to be potential prognostic factors for OS according to univariate Cox proportional hazards ratio analysis (P<0.05; Table II).

These factors were then analyzed by the multivariate Cox proportional hazards ratio model for analysis of OS (Table II). Following adjustment for all potential prognostic factors, the results indicated that age [odds ratio (OR)=1.034, 95% confidence interval (CI), 1.015-1.052], tumor diameter (OR, 0.718; 95% CI, 0.523-0.986), metastasis stage (OR, 3.129; 95% CI, 1.154-8.484), *FOXA1* (OR, 1.120; 95% CI, 1.014-1.236), *FOXA2* (OR, 1.131; 95% CI, 1.018-1.256), *FOXD1* (OR, 0.829; 95% CI, 0.695-0.987), *FOXD4L2* (OR, 1.404; 95% CI, 1.104-1.786), *FOXK2* (OR, 2.712; 95% CI, 1.288-5.713), *FOXL1* (OR, 1.260; 95% CI, 1.008-1.574) and *FOXN2* (OR, 0.621; 95% CI, 0.393-0.981) were independent prognostic factors for OS (all P<0.05; Table II).

Kaplan-Meier analysis was performed with the cut-off set at the median expression level of each FOX family gene. The

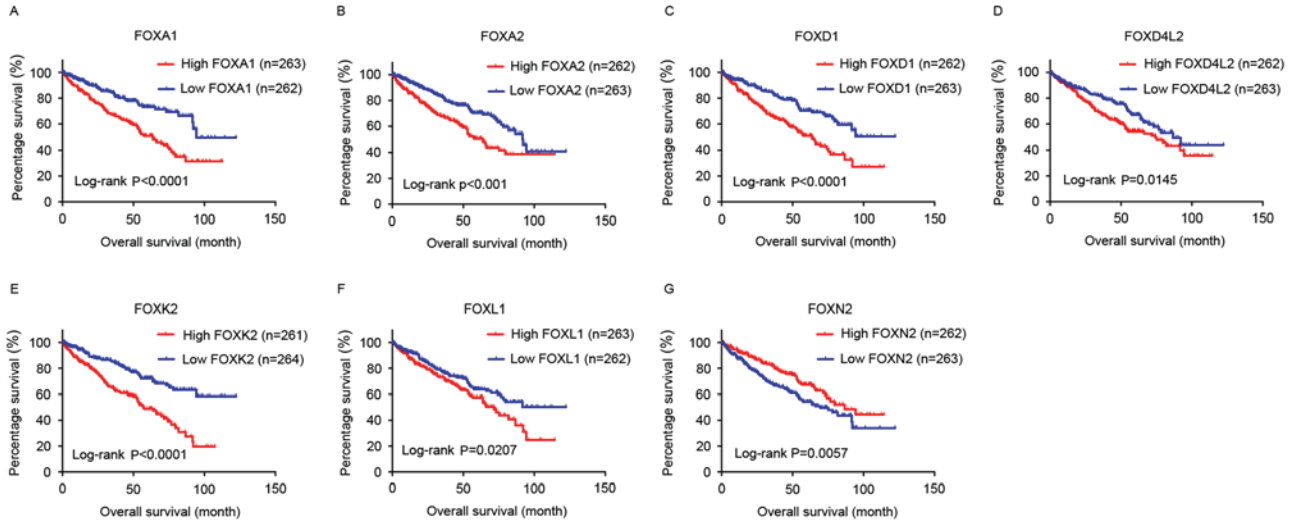

Figure 1. Kaplan-Meier survival curves according to the expression level of FOX family genes and OS in the The Cancer Genome Atlas cohort. (A-G) Kaplan-Meier estimates of OS according to the expression level of (A) *FOXA1*, (B) *FOXA2*, (C) *FOXD1*, (D) *FOXD4L2*, (E) *FOXK2*, (F) *FOXL1* and (G) *FOXN2*, respectively. FOX, forkhead-box. OS, overall survival.

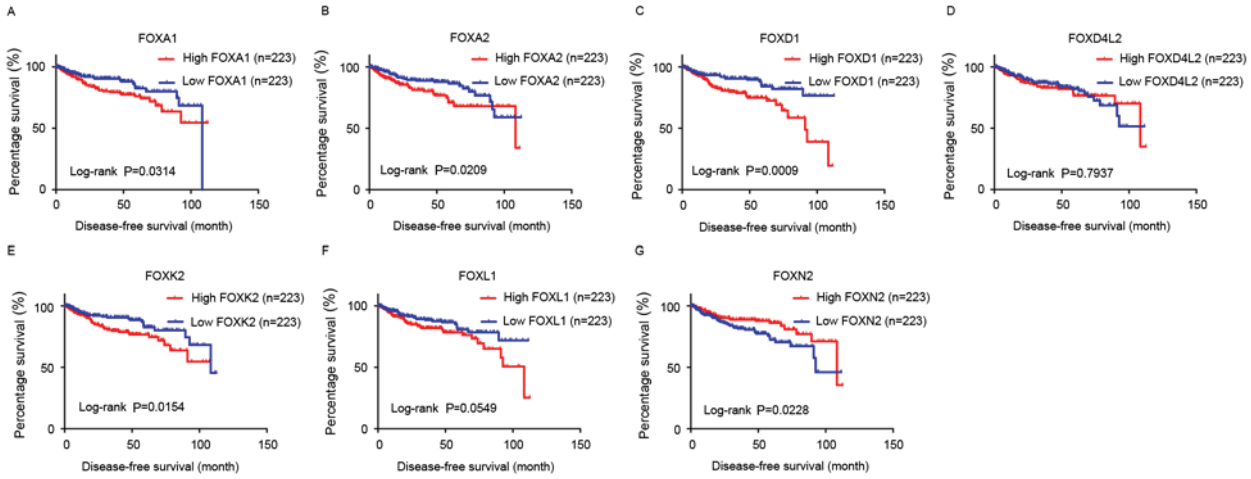

Figure 2. Kaplan-Meier survival curves according to the expression level of FOX family genes and DFS in The Cancer Genome Atlas cohort. Kaplan-Meier estimates of DFS according to the expression level of (A) *FOXA1*, (B) *FOXA2*, (C) *FOXD1*, (D) *FOXD4L2*, (E) *FOXK2*, (F) *FOXL1* and (G) *FOXN2*, respectively. FOX, forkhead-box. DFS, disease-free survival.

results revealed that low levels of *FOXA1*, *FOXA2*, *FOXD1* and *FOXK2* were associated with longer OS and DFS (P<0.05), while a high level of *FOXN2* was associated with longer OS and DFS (P<0.05; Figs. 1 and 2). A low level of *FOXL1* and *FOXD4L2* was only associated with longer OS and not DFS (P<0.05; Figs. 1 and 2).

To investigate the association between FOX gene expression and clinical factors, multivariate logistic regression analysis was performed. The results indicated that *FOXA1* expression was significantly associated with tumor stage (P<0.001, OR, 1.32; 95% CI, 1.10-1.59) and grade (P=0.01, OR, 1.51; 95% CI, 1.12-2.04) (Table III). FOXA2 was associated with gender (P=0.04, OR, 0.66; 95% CI, 0.44-0.99) and stage (P=0.02, OR, 1.25, 95% CI, 1.03-1.50) (Table III). *FOXD1* was only associated with grade (P<0.001, OR, 1.65; 95% CI, 1.22-2.23). *FOXK2* was associated with gender (P<0.001, OR, 0.54; 95% CI, 0.36-0.81) and tumor grade (P=0.02, OR, 1.44; 95% CI, 1.07-1.94). *FOXL1* was associated with gender (P=0.04, OR, 1.53; 95% CI, 1.03-2.26). However, no significant association was observed between *FOXD4L2*, *FOXN2* and clinical variables (Tables IV and V).

FOX gene network revealed nuclear receptor coactivator (NCOA)1, NADH dehydrogenase (ubiquinone) flavoprotein (NDUFV)3, phosphatidylserine decarboxylase (PISD) and pyruvate kinase, liver and red blood cell (PKLR) are independent prognostic factors for OS in the TCGA cohort. It was investigated whether the expression level of FOX family-associated genes had an effect on patient OS in TCGA cohort. The gene network is shown in Fig. 3, and details of the genes in the network are shown in Table VI. The data from univariate Cox proportional hazards ratio analysis indicated that the expression levels of acyl-coenzyme A dehydrogenase, C-4 to C-12 straight chain, androgen receptor, α-fetoprotein, bone morphogenetic protein receptor type II, CCAAT/enhancer-binding protein β, engrailed homeobox 2, hydroxyacyl-coenzyme A

Table IV. Multivariate logistic regression analysis of factors that may affect the expression of *FOXD1* and *FOXD4L1* in The Cancer Genome Atlas cohort with clear cell renal cell carcinoma.

A, *FOXD1*

Variable	OR (95% CI)	P-value
Age	1.01 (0.99-1.02)	0.32
Sex	1.43 (0.96-2.15)	0.08
Stage[b]	1.12 (0.93-1.34)	0.25
Grade[c]	1.65 (1.22-2.23)	<0.001[a]
Tumor diameter	1.02 (0.76-1.38)	0.89
Position	1.15 (0.79-1.67)	0.48

B, *FOXD4L1*

Variable	OR (95% CI)	P-value
Age	1.01 (0.99-1.02)	0.37
Sex	0.75 (0.50-1.11)	0.15
Stage[b]	1.20 (1.00-1.44)	0.05
Grade[c]	1.11 (0.83-1.49)	0.48
Tumor diameter	1.18 (0.88-1.58)	0.28
Position	0.72 (0.49-1.04)	0.08

[a]P<0.05 was considered statistically significant; [b]American Joint Committee on Cancer stage; [c]Fuhrman grade. CI, confidence interval; OR, odds ratio; FOX, forkhead-box.

Table V. Multivariate logistic regression analysis of factors that may affect the expression of *FOXK2*, *FOXL1* and *FOXN2* in The Cancer Genome Atlas cohort with clear cell renal cell carcinoma.

A, *FOXK2*

Variable	OR (95% CI)	P-value
Age	1.00 (0.98-1.01)	0.56
Sex	0.54 (0.36-0.81)	<0.001[a]
Stage[b]	1.10 (0.92-1.33)	0.30
Grade[c]	1.44 (1.07-1.94)	0.02[a]
Tumor diameter	1.03 (0.77-1.39)	0.82
Position	1.00 (0.69-1.45)	0.99

B, *FOXL1*

Variable	OR (95% CI)	P-value
Age	1.00 (0.99-1.02)	0.97
Sex	1.53 (1.03-2.26)	0.04[a]
Stage[b]	1.13 (0.94-1.35)	0.20
Grade[c]	1.00 (0.75-1.34)	0.99
Tumor diameter	0.87 (0.65-1.16)	0.34
Position	0.84 (0.58-1.22)	0.35

C, *FOXN2*

Variable	OR (95% CI)	P-value
Age	0.99 (0.97-1.00)	0.13
Sex	1.20 (0.80-1.79)	0.38
Stage[b]	0.85 (0.71-1.03)	0.09
Grade[c]	0.89 (0.67-1.20)	0.46
Tumor diameter	0.79 (0.59-1.06)	0.12
Position	1.46 (1.01-2.12)	0.05

[a]P<0.05 was considered statistically significant; [b]American Joint Committee on Cancer stage; [c]Fuhrman grade. CI, confidence interval; OR, odds ratio; FOX, forkhead-box.

dehydrogenase, 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1, hepatocyte nuclear factor 4α, insulin-like growth factor binding protein 1, interleukin-2 (*IL-2*), potassium inwardly-rectifying channel subfamily J member 11, *NCOA1*, *NCOA3*, *NDUFV3*, *PISD*, *PKLR* and uncoupling protein 2 were associated with OS. Multivariate analysis for prognostic factors was performed by the Cox proportional hazards ratio analysis and revealed that the expression of *NCOA1*, *NDUFV3*, *PISD* and *PKLR* were independent prognostic factors for OS in the TCGA cohort (Table VII).

Discussion

In the present study, it was revealed that *FOXA1*, *FOXA2*, *FOXD1*, *FOXD4L2*, *FOXK2* and *FOXL1* genes were risk factors for clinical outcome of ccRCC. However, high expression of the *FOXN2* gene was associated with longer survival in the TCGA cohort. Furthermore, in a network of FOX family-related genes, *NCOA1*, *NDUFV3*, *PISD* and *PKLR* were identified as independent prognostic factors for OS in patients with ccRCC.

FOXA1 and FOXA2 are two members of the FOXA transcription factor family. FOXA1, also termed HNF-3, has an important role in the progression of bladder, prostate and breast cancer (12-15). A previous study has demonstrated that downregulation of FOXA1 is associated with poor OS in human bladder cancer (12). FOXA1 may also be a potential treatment target of breast and prostate cancer due to its effects on chromatin remodeling via androgen and estrogen receptors (16). FOXA2 is involved in proliferation, differentiation and maintenance of cancer stem cells (17-19). However, FOXA2 may have different roles in different tissues. FOXA2 is associated with the prognosis of human gastric cancer, and patients with high FOXA2 expression level had longer OS compared with patients with low FOXA2 expression (19). However, one study conducted in breast carcinoma revealed that FOXA2 promotes the development of triple-negative/basal-like tumors (18).

FOXD1 performs an essential role in numerous biological processes, including proliferation, differentiation and tumorigenesis (20,21). Upregulation of FOXD1 is associated with the development of resistance to chemotherapy

Table VI. List of FOX family-associated genes as revealed by gene network analysis.

Gene	Full gene name	UniGene[a]
FOXK2	Forkhead box K2	Hs.591140
XBP1	X-box binding protein 1	Hs.437638
FOXL1	Forkhead box L1	Hs.533830
EN2	Engrailed homeobox 2	Hs.134989
FOXA2	Forkhead box A2	Hs.155651
FOXF1	Forkhead box F1	Hs.155591
CEBPB	CCAAT/enhancer-binding protein β	Hs.517106, Hs.716248
FOXA1	Forkhead box A1	Hs.163484
HADH	Hydroxyacyl-coenzyme A dehydrogenase	Hs.438289
BDH1	3-hydroxybutyrate dehydrogenase, type 1	Hs.274539
ACADM	Acyl-coenzyme A dehydrogenase, C-4 to C-12 straight chain	Hs.445040
ACADVL	Acyl-coenzyme A dehydrogenase, very long chain	Hs.463928, Hs.437178
AP1B1	Adaptor-related protein complex 1, β1 subunit	Hs.368794
HMGCS1	3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (soluble)	Hs.397729
BMPR2	Bone morphogenetic protein receptor type II (serine/threonine kinase)	Hs.471119
NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	Hs.122926
KCNJ11	Potassium inwardly-rectifying channel subfamily J member 11	Hs.248141
SHH	Sonic hedgehog homolog (Drosophila)	Hs.164537
AKT1	V-Akt murine thymoma viral oncogene homolog 1	Hs.525622
APOB	Apolipoprotein B (including Ag(x) antigen)	Hs.120759
HOXA5	Homeobox A5	Hs.655218
SLC2A2	Solute carrier family 2 (facilitated glucose transporter), member 2	Hs.167584
SERPINA1	Serpin peptidase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 1	Hs.525557
NR2F2	Nuclear receptor subfamily 2, group F, member 2	Hs.657455, Hs.347991
DSCAM	Down syndrome cell adhesion molecule	Hs.397800
COL18A1	Collagen, type XVIII, α1	Hs.517356
AR	Androgen receptor	Hs.496240
KLK3	Kallikrein-related peptidase 3	Hs.171995
OTX2	Orthodenticle homeobox 2	Hs.288655
PISD	Phosphatidylserine decarboxylase	Hs.420559
SOD1	Superoxide dismutase 1, soluble	Hs.443914
NRIP1	Nuclear receptor interacting protein 1	Hs.155017
NDUFV3	NADH dehydrogenase (ubiquinone) flavoprotein 3, 10 kDa	Hs.473937
AFP	α-fetoprotein	Hs.518808
NCOA1	Nuclear receptor coactivator 1	Hs.596314
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	Hs.238990
NCOA3	Nuclear receptor coactivator 3	Hs.592142
HNF4A	Hepatocyte nuclear factor 4α	Hs.116462
UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	Hs.80658
PKLR	Pyruvate kinase, liver and red blood cell	Hs.95990
IL-2	Interleukin 2	Hs.89679

[a]Accession numbers (https://www.ncbi.nlm.nih.gov/unigene). Hs, Homo sapiens.

in patients with prostate and ovarian cancer (22). Another study reported that FOXD1 is upregulated in breast cancer, and the depletion or overexpression of FOXD1 may cause changes in proliferation and chemoresistance (20). FOXK2, also termed ILF or ILF1, was first identified as a regulator of IL-2 transcription. FOXK2 upregulates activator protein-1 (AP-1)-dependent gene expression through its interaction with AP-1 and accelerates the binding of AP-1 to chromatin (23). FOXL1 is associated with pancreatic carcinoma and has an important inhibitory role in pancreatic tumor progression (24). However, to the best of our knowledge, no study has examined FOXD4L2 to date, and the findings of the present study suggest that FOXD4L2 should be investigated further in future studies. In addition, it was observed

Table VII. Cox proportional hazards analysis of FOX family genes, related gene network, clinical parameters and overall survival for The Cancer Genome Atlas clear cell renal cell carcinoma cohort.

	Univariate		Multivariate	
Variables	HR (95% CI)	P-value	HR (95% CI)	P-value
Demographic parameters				
Age	1.028 (1.015-1.041)	<0.0001	1.046 (1.024-1.068)	<0.0001
Sex (male vs. female)	0.950 (0.693-1.302)	0.752	0.722 (0.390-1.336)	0.300
Clinical parameters				
Stage[a] (I-IV)	1.954 (1.707-2.236)	<0.0001		
Grade[b] (I-IV)	2.399 (1.941-2.965)	<0.0001	1.717 (1.104-2.672)	0.016
Tumor diameter	1.215 (0.983-1.502)	0.071	0.600 (0.412-0.873)	0.008
Laterality (left vs. right)	0.695 (0.512-0.944)	0.020	0.665 (0.412-1.074)	0.096
pT (T1/T2/T3)	1.992 (1.685-2.355)	<0.0001	1.337 (0.954-1.874)	0.092
pN (N1 vs. N2)	0.992 (0.562-1.752)	0.978	0.552 (0.236-1.292)	0.171
pM (M0 vs. M1)	4.548 (3.305-6.257)	<0.0001	6.362 (3.172-12.757)	<0.0001
Hb (low/normal/elevated)	0.563 (0.400-0.792)	0.001	0.665 (0.391-1.131)	0.132
WBC (low/normal/elevated)	0.668 (0.483-0.923)	0.014	0.766 (0.434-1.354)	0.360
PLT (low/normal/elevated)	1.709 (1.156-2.526)	0.007	1.443 (0.912-2.285)	0.117
FOX family genes				
FOXL2	1.250 (1.076-1.452)	0.004	1.882 (1.283-2.760)	0.001
FOXL1	1.219 (1.071-1.387)	0.003	1.418 (1.056-1.904)	0.020
FOXS1	1.160 (1.031-1.303)	0.013	0.648 (0.436-0.964)	0.032
FOXN1	1.200 (0.992-1.451)	0.060		
FOXN2	0.775 (0.614-0.978)	0.032	0.253 (0.103-0.618)	0.003
FOXN3	0.589 (0.470-0.740)	<0.0001	0.515 (0.228-1.163)	0.110
FOXH1	1.292 (1.156-1.444)	<0.0001	0.825 (0.603-1.130)	0.231
FOXG1	1.217 (1.110-1.335)	<0.0001	0.825 (0.658-1.034)	0.095
FOXP2	1.005 (0.942-1.072)	0.875		
FOXD1	1.261 (1.142-1.392)	<0.0001	0.703 (0.554-0.891)	0.004
FOXC2	0.948 (0.855-1.050)	0.304		
FOXC1	1.095 (0.926-1.296)	0.288		
FOXF1	0.942 (0.819-1.084)	0.406		
FOXF2	1.129 (1.000-1.274)	0.050	0.942 (0.741-1.197)	0.625
FOXE1	1.212 (1.120-1.312)	<0.0001	1.175 (0.983-1.405)	0.077
FOXO3B	0.774 (0.591-1.012)	0.061		
FOXB2	0.762 (0.513-1.130)	0.177		
FOXR1	1.160 (0.604-2.228)	0.655		
FOXN4	1.200 (1.062-1.357)	0.003	0.615 (0.421-0.899)	0.012
FOXM1	1.618 (1.433-1.827)	<0.0001	0.950 (0.638-1.414)	0.800
FOXP3	1.252 (1.141-1.375)	<0.0001	0.926 (0.713-1.203)	0.565
FOXP1	1.336 (1.089-1.639)	0.006	1.761 (0.820-3.781)	0.147
FOXP4	2.018 (1.572-2.591)	0.000	1.695 (0.900-3.191)	0.102
FOXO3	0.726 (0.562-0.938)	0.014	0.393 (0.199-0.779)	0.007
FOXO1	0.671 (0.529-0.851)	0.001	1.978 (0.973-4.021)	0.059
FOXO4	0.624 (0.464-0.840)	0.002	0.521 (0.228-1.194)	0.123
FOXR2	1.310 (0.795-2.160)	0.290		
FOXI1	0.976 (0.920-1.034)	0.409		
FOXI3	1.253 (0.757-2.074)	0.379		
FOXI2	0.848 (0.772-0.930)	0.001	0.999 (0.816-1.222)	0.990
FOXRED2	1.121 (0.909-1.383)	0.284		
FOXRED1	1.231 (0.950-1.594)	0.115		
FOXD4L5	0.994 (0.612-1.614)	0.980		
FOXD4L6	1.312 (1.100-1.566)	0.003	0.909 (0.607-1.362)	0.644

Table VII. Continued.

Variables	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
FOXD4L1	1.352 (1.158-1.578)	<0.0001	0.841 (0.546-1.296)	0.433
FOXD4L2	1.256 (1.082-1.458)	0.003	1.450 (1.059-1.986)	0.021
FOXD4L3	1.366 (0.91-2.036)	0.126		
FOXB1	1.183 (1.017-1.375)	0.029	0.954 (0.652-1.394)	0.806
FOXK2	2.895 (2.023-4.142)	<0.0001	1.164 (0.356-3.798)	0.802
FOXK1	0.982 (0.765-1.260)	0.885		
FOXD3	1.286 (1.049-1.577)	0.016	0.930 (0.637-1.358)	0.708
FOXA1	1.194 (1.127-1.266)	<0.0001	1.224 (1.066-1.405)	0.004
FOXA3	1.047 (0.962-1.140)	0.290		
FOXA2	1.175 (1.103-1.253)	<0.0001	1.153 (1.010-1.316)	0.035
FOXJ1	1.126 (1.046-1.213)	0.002	1.074 (0.914-1.316)	0.383
FOXJ2	1.024 (0.688-1.524)	0.907		
FOXJ3	0.963 (0.838-1.106)	0.591		
FOXE3	1.715 (1.409-2.088)	<0.0001	1.374 (0.923-2.045)	0.118
FOXQ1	1.005 (0.905-1.115)	0.929		
FOXD4	1.159 (1.004-1.337)	0.044	1.063 (0.735-1.538)	0.744
FOXD2	1.206 (1.011-1.440)	0.038	0.960 (0.543-1.697)	0.889
Network genes				
BDH1	1.047 (0.955-1.148)	0.331	0.945 (0.777-1.149)	0.570
EN2	1.258 (1.173-1.350)	<0.0001	0.930 (0.800-1.080)	0.341
PKLR	0.898 (0.852-0.947)	<0.0001	0.794 (0.659-0.955)	0.015
ACADVL	1.211 (0.949-1.546)	0.123	0.774 (0.388-1.546)	0.468
UCP2	1.292 (1.108-1.508)	0.001	1.217 (0.791-1.871)	0.372
KCNJ11	1.108 (1.000-1.226)	0.049	0.936 (0.698-1.256)	0.661
HOXA5	0.985 (0.842-1.153)	0.854	1.748 (1.214-2.518)	0.003
OTX2	1.263 (0.970-1.646)	0.083	0.740 (0.466-1.176)	0.202
NR3C1	0.823 (0.642-1.054)	0.123	0.861 (0.421-1.763)	0.683
HNF4A	0.916 (0.866-0.969)	0.002	1.011 (0.831-1.229)	0.916
IL2	1.339 (1.100-1.631)	0.004	0.874 (0.589-1.298)	0.506
CDKN1B	0.750 (0.557-1.010)	0.058	2.720 (1.215-6.093)	0.015
AR	0.798 (0.744-0.857)	<0.0001	1.023 (0.795-1.317)	0.860
NCOA1	0.523 (0.385-0.711)	<0.0001	3.901 (1.399-10.875)	0.009
COL18A1	1.143 (0.933-1.400)	0.196	1.024 (0.611-1.715)	0.929
HMGCS1	0.524 (0.386-0.711)	<0.0001	0.962 (0.519-1.783)	0.903
ACADM	0.537 (0.454-0.634)	<0.0001	1.011 (0.561-1.822)	0.971
SLC2A2	0.926 (0.879-0.976)	0.004	1.045 (0.882-1.237)	0.614
AKT1	1.442 (0.943-2.204)	0.091	1.924 (0.612-6.044)	0.263
BMPR2	0.608 (0.475-0.777)	<0.0001	0.929 (0.369-2.337)	0.875
XBP1	1.032 (0.826-1.289)	0.783	0.647 (0.410-1.023)	0.062
DSCAM	1.076 (0.971-1.191)	0.162	1.262 (1.047-1.520)	0.014
NDUFV3	1.560 (1.144-2.126)	0.005	2.021 (1.080-3.785)	0.028
SOD1	1.280 (0.954-1.718)	0.100	1.191 (0.494-2.871)	0.697
CEBPB	1.525 (1.336-1.741)	<0.0001	0.905 (0.627-1.305)	0.592
NCOA3	0.701 (0.534-0.920)	0.011	1.128 (0.407-3.126)	0.816
AP1B1	1.023 (0.728-1.436)	0.897	0.502 (0.203-1.305)	0.134
KLK3	0.990 (0.892-1.099)	0.853	0.895 (0.630-1.272)	0.537
SHH	0.990 (0.893-1.098)	0.855	0.930 (0.765-1.130)	0.463
PISD	2.048 (1.617-2.595)	<0.0001	3.389 (1.722-6.667)	<0.0001
AFP	1.124 (1.035-1.221)	0.006	0.974 (0.822-1.155)	0.765
IGFBP1	1.095 (1.052-1.141)	<0.0001	0.970 (0.872-1.080)	0.581

Table VII. Continued.

Variables	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
APOB	1.001 (0.948-1.057)	0.970	1.087 (0.962-1.228)	0.183
NR2F2	0.985 (0.765-1.266)	0.903	0.558 (0.317-0.983)	0.043
SERPINA1	1.027 (0.940-1.122)	0.552	1.139 (0.899-1.442)	0.281
HADH	0.375 (0.264-0.533)	<0.0001	0.560 (0.235-1.335)	0.191

[a]American Joint Committee on Cancer stage; [b]Fuhrman grade. FOX, forkhead-box; Hb, hemoglobin; WBC, white blood cell; PLT, platelet; pN, pathological node stage; pM, pathological metastasis stage; pT, pathological T stage; HR, hazard ratio; CI, confidence interval.

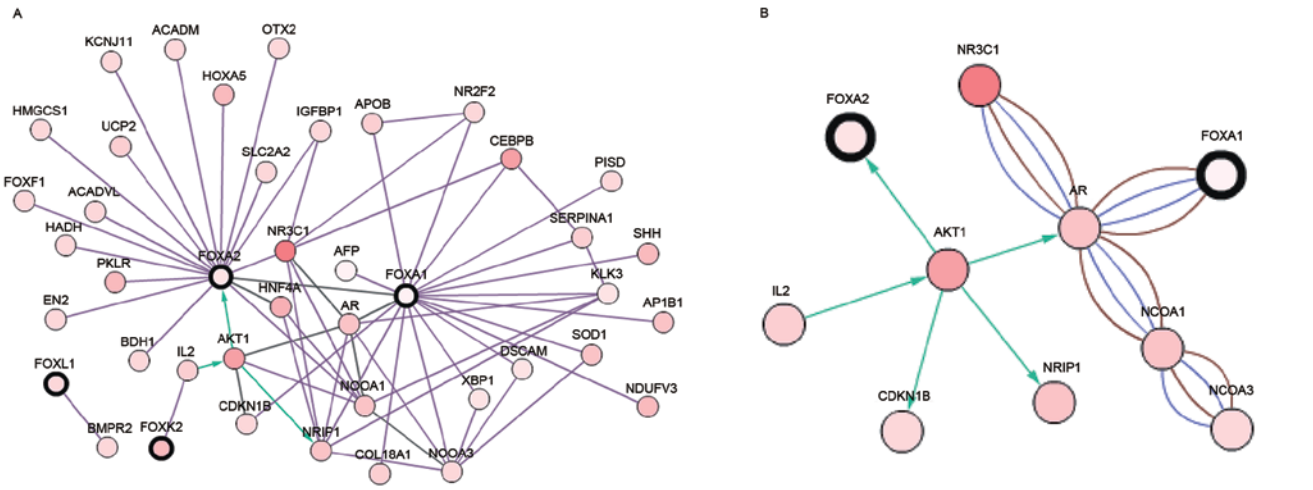

Figure 3. Gene network of prognosis-associated FOX genes. Gene network was drawn with the independent prognosis predictors *FOXA1*, *FOXA2*, *FOXD1*, *FOXD4L2*, *FOXK2*, *FOXL1* and *FOXN2* genes. A total of three criteria were selected for construction of the network: 'Interacts with', 'state change' and 'in the same component'. The threshold of state change was set as 12%. The network was plotted using the cBioPortal website (www.cbioportal.org). (A) *FOXN2*, *FOXD1* and *FOXD4L2* were absent in the network since they were not connected with any genes. A network involving FOXA1, FOXA2, FOXK2 and FOXL1 was constructed with the aforementioned conditions. Connecting lines meant an association between the two connected genes. (B) Most significant associations between *FOXA1* and *FOXA2* were drawn. Brown line, genes in in the same component, blue line, gene interactions; green line, co-expression.

in the present study that high *FOXN2* mRNA expression was associated with longer OS, and this is consistent with previous results in glioblastoma multiforme (25).

Previous studies have shown that FOX genes have an essential role in the progression of several types of tumors, including ccRCC (9,12-14,19,21). However, to the best of our knowledge, the present study is the first to comprehensively examine the association between the outcome of ccRCC and the gene expression of the entire FOX gene family. The detailed mechanisms remain unknown and would need to be investigated in future studies.

The present study also investigated the association between FOX-related genes and prognosis of patients with ccRCC. The results indicated that the FOX-associated genes *NCOA1*, *NDUFV3*, *PISD* and *PKLR* are associated with OS of patients with ccRCC. It has been previously demonstrated that the nuclear co-activator NCOA1 (SRC-1) is able to promote breast cancer metastasis through directly targeting macrophage colony-stimulating factor 1 expression (26). Overexpression of NCOA1 is associated with resistance to endocrine therapy and disease recurrence (26). The *NDUFV3* gene is located at chromosome 21q22.3 and may be associated with the occurrence of Down syndrome (27). Although limited information is known about PISD, one study reported that PISD was associated with tumorigenesis and tumor growth (28). The *PKLR* gene is considered to be involved in pyruvate kinase-deficient hemolytic anemia (29).

The major strength of the present study is that it is the first comprehensive evaluation of the association between FOX genes and the prognosis of patients with ccRCC. The study involved a large cohort, and the clinical follow-up was long. These findings will help provide the foundation to elucidate the mechanisms of FOX genes and their function in ccRCC.

However, the present study also has a number of limitations. Firstly, only data from TCGA database was analyzed and further validation is required. Secondly, the present study did not investigate the specific mechanisms of action of FOX genes in patients with ccRCC. Additionally, the *FOX* gene signature may not be sufficient to predict the prognosis of ccRCC, since other factors (tumor stage, surgical procedures, state of nutrition, economic issues, response to sunitinib, comorbidities and lifestyle factors), can also affect

the prognosis of ccRCC (5,9,11,30). Therefore, additional study is required to examine the association between FOX genes and ccRCC.

Findings of the present study suggest that the expression of FOX family genes *FOXA1*, *FOXA2*, *FOXD1*, *FOXD4L2*, *FOXK2*, *FOXL1* and *FOXN2* and FOX family-related genes *NCOA1*, *NDUFV3*, *PISD* and *PKLR* are associated with survival in patients with ccRCC. Findings of the present study and the specific underlying require further investigation.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the Cancer Genomics Browser of University of California Santa Cruz (https://genome-cancer.ucsc.edu/), and the datasets are available on reasonable request from the corresponding author.

Authors' contributions

ZWJ and FNW conceived the present study, collected and analyzed the clinical data, and drafted the manuscript. BD and DWY designed and supervised this study. YZ and GHS contributed to the collection of the clinical data, and HLZ helped to analyze the data. All authors reviewed and approved the manuscript.

Ethics approval and consent to participate

The datasets we used in this article were generated and analyzed from The Cancer Genome Atlas Group, which had been approved by Memorial Sloan-Kettering Cancer Center institutional review board (31), and the informed consent was provided at the same time.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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