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ORIGINAL ARTICLE

Bioinformatics Analysis Suggests the Combined Expression of AURKB and KIF18B Being an Important Event in the Development of Clear Cell Renal Cell Carcinoma

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Abstract

Clear cell renal cell carcinoma (ccRCC) is the most common type of renal cell carcinoma with high metastatic rate and high mortality rate, needing to find potential therapeutic targets and develop new therapy methods. The bioinformatics analysis was used in this study to find the targets. Firstly, the expression profile of ccRCC obtained from The Cancer Genome Atlas (TCGA) database and GSE53757 dataset were used to identify the significant up-regulated genes. IL20RB, AURKB and KIF18B with the top efficiency of capable of diagnosis ccRCC from para cancer tissue, were over-expressed in ccRCC samples, and expressed increasedly with the development of ccRCC. There was the closest correlation between AURKB and KIF18B in these three over-expressed genes. AURKB (high) or KIF18B (high) were all significantly correlated with higher T, N, M stage, G grade and shorter overall survival (OS) of ccRCC patients. Furthermore, the ccRCC patients with AURKB (high) + KIF18B (high) showed worse clinical characteristics and prognosis. Multivariate COX regression analysis indicated AURKB (high) and KIF18B (high) were all the independent prognostic risk factor without considering the interaction of AURKB and KIF18B. Moreover, considering the combination of each other, only AURKB (high) + KIF18B (high) expression was an independent prognostic risk factor for ccRCC patients, but not other situations. Collectively, AURKB was closely associated with KIF18B, and the combined expression of AURKB and KIF18B may be of great significance in ccRCC.

Keywords AURKB · KIF18B · Clear cell renal cell carcinoma · Differentially expressed genes · Combined expression

Introduction

In 2018, new cases of kidney tumors accounted for approximately 2% of all new tumors and 1.8% of death cases [1]. Renal cell carcinoma (RCC) is the most common type of cancers in the kidney, and the most common histological subtype is clear cell renal cell carcinoma (ccRCC), which accounts for about 75% of RCC [2–4]. With the advancement of surgical methods and the application of targeted drugs, ccRCC in the early stages usually could be cured [5]. However, it is still difficult to correctly diagnose ccRCC in early stage. About one third of patients have metastasized at the time of diagnosis, and they are usually unable to be cured [6, 7]. In summary, we still need to find new treatments for ccRCC patients. The molecular biological mechanisms of ccRCC occurrence and progression have not been fully elucidated until now. It is of great significance for the diagnosis and treatment of ccRCC patients to explore the mechanism of the development of ccRCC and to find new markers of ccRCC as well as corresponding targeted therapies.

Nowadays, many biomedical databases can be used by researchers with the popularity of the Internet and the completion of the Human Genome Project. For example, the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) have accumulated rich gene expression profiles [8,

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9]. By processing and refining the contents of these databases, we can sort out useful information to gain a profound understanding of the mechanisms by which disease progresses, or to find new therapeutic targets for specific diseases. For instance, analysis of bioinformatics database suggests that EPIC1 is an important lncRNA that promotes cell-cycle progression in cancer [10], and miR-148a-3p is a potential target for the treatment of cisplatin-resistant gastric cancer [11]. In the bioinformatics field of ccRCC, Chen L et al. found that ANLN and CDK1, etc. may be biomarkers related to the progression and prognosis of ccRCC [12]; Song J et al. found that two novel lncRNAs (DNM1P35 and MIR155HG) may act as prognostic biomarkers for predicting the survival of ccRCC patients [13]. Our study comprehensively used the latest updated bioinformatics databases to analyze the gene expression profile of ccRCC samples for finding new potential therapeutic targets.

In the present study, we identified differentially expressed genes (DEGs) that were up-regulated in ccRCC samples compared to adjacent cancer samples, and which were significantly up-regulated with the progression of ccRCC. Meanwhile, we validated the above results using GSE53757 dataset. We also analyzed the correlation between up-regulated DEGs as well as the relationship between up-regulated DEGs with the clinicopathological features and prognosis of ccRCC patients. Through the bioinformatics analysis, we can identify key genes that are expected to be potential prognostic markers or to be new therapeutic targets of ccRCC patients.

Materials and Methods

Data Collection

RNA-Seq data were downloaded from TCGA database (https://cancergenome.nih.gov/), including 539 ccRCC samples and 72 paired adjacent cancer samples. These ccRCC samples included 331 cancer samples with AJCC stage I/II and 205 stage III/IV samples. Moreover, an independent dataset of GSE53757 obtained from GEO database (https://www.ncbi.nlm.nih.gov/geo/) was as a test set to validate our results.

Identification of Differentially Expressed Genes

The "edgeR" package of R software was used to screen the DEGs meeting the cutoff criteria with $|\log_2 FC \geq 1|$ and P value<0.01, which were considered statistically significant. First, DEGs that differentially expressed in ccRCC samples and adjacent samples were screened out. Then, DEGs that differentially expressed in stage III/IV compared to stage I/II cancer samples were selected. The genes up-regulated in both groups served as candidate genes associated with the development of ccRCC.

The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficiency of candidate genes involving in the differential diagnosis of cancer and para cancer tissue. These genes with the area under the curve (AUC) > 0. 95 being considered have high diagnostic value, which were regard as important candidate genes related to the development of ccRCC. Spearman correlation analysis was used to explore the relationship of candidate key genes.

GEO Dataset Validation

GSE53757 dataset included 72 ccRCC samples and 72 adjacent cancer samples, of which cancer samples including 43 cases of stage I/II and 29 stage III/IV cases. GSE53757 dataset was used to verify the differential expression and correlation of candidate key genes in ccRCC samples.

Clinical Value and Prognostic of Candidate Key Genes in ccRCC

According to the median value of candidate key genes, ccRCC samples were divided into high expression group and low expression group. Meanwhile, clinical information of 537 ccRCC patients was also downloaded from TCGA database. We analyzed the clinical value of candidate key genes in ccRCC patients. Clinical factors considered including age, T stage, N stage, M stage and G grade. Kaplan-Meier survival curves were plotted using the Log-rank method to evaluate the prognostic value of candidate key genes in ccRCC patients. Univariate and multivariate COX regression analysis were performed on ccRCC patients.

KEGG Pathway Enrichment Analysis

The co-expression tool in cBioPortal (http://www.cbioportal.org/) was used to analyze the co-expression genes of candidate key genes in ccRCC, Spearman correlation coefficient ≥ 0.5 were considered positive co-expression genes. DAVID (https://david.ncifcrf.gov/) was used to perform KEGG pathway enrichment analysis of these co-expression genes.

Statistical Analysis

Statistical analysis was performed using SPSS 23.0 and GraphPad Prism 7.0. ROC curve was used to assess the diagnostic efficiency of DEGs in ccRCC patients. The differentially expressed analysis of candidate key genes was evaluated by Mann-Whitney U test. P values for differential analysis of the paired samples were derived from Wilcoxon signed rank test. Spearman analysis was performed on the correlation between candidate key genes. Chi-square test or Fisher's exact probability test was used to evaluate the association between the expression of candidate key genes and clinicopathological

features in ccRCC. Log-rank method was used to draw Kaplan-Meier curves to evaluate overall survival (OS) of ccRCC patients, univariate and multivariate Cox regression analysis was used to assess prognostic risk factors for ccRCC patients. $P < 0.05$ was considered statistically significant.

Results

AURKB and KF18B are Candidate Key Genes of ccRCC

In order to identify candidate key genes related to the development of ccRCC, the "edgeR" package was used to analyze the DEGs of ccRCC samples, and up-regulated DEGs were selected. A total of 5778 up-regulated genes were identified by differential analysis of ccRCC tumor samples and adjacent samples, and the differential expression analysis between stage III/IV samples and stage I/II samples were screened out 488 up-regulated genes. After comparing the up-regulated genes, 254 genes were selected as they intersected in the two groups of up-regulated genes, these intersection genes were considered to be important candidate genes involved in the development of ccRCC (Fig. 1a).

The ROC curve was used to obtain genes which were capable of diagnosis ccRCC from para cancer tissue efficiently. IL20RB, AURKB and KIF18B showed high predictive accuracy to distinguish ccRCC from adjacent cancer samples with AUC value greater than 0.95 (Fig. 1b). Further comparing the expression of the above 3 genes in ccRCC samples and adjacent cancer samples, we found that the expression levels of them were significantly up-regulated ($p < 0.0001$) in ccRCC samples compared with all adjacent samples (Fig. 1c) or matched adjacent samples (Fig. 1d). Comparing the differential expression of the above 3 genes in ccRCC samples with different clinical stages, it was found that patients with stage III/IV were significantly higher than those in stage I/II ($p < 0.0001$) (Fig. 1e).

Spearman correlation test was performed on the expression values of IL20RB, KIF18B and AURKB in ccRCC, and the closest positive correlation between AURKB and KIF18B was found (Fig. 1f, AURKB&KIF18B, $r = 0.913$, $p < 0.0001$; IL20RB&AURKB, $r = 0.547$, $p < 0.0001$; IL20RB&KIF18B, $r = 0.520$, $p < 0.0001$). This result suggested that the interaction of AURKB with KIF18B may be an important event in the development of ccRCC.

GSE53757 Dataset Verified the Correlation between AURKB and KIF18B in ccRCC

GSE53757 dataset was used as a test set to verify the expression and correlation of candidate key genes in ccRCC. We found that AURKB expression level in ccRCC samples was significantly higher than adjacent cancer samples (Fig. 2a, $p < 0.0001$) and the AURKB expression level of stage III/IV ccRCC samples were significantly up-regulated than that of stage I/II samples (Fig. 2b, $p = 0.0138$). The expression level of KIF18B had a significant difference in ccRCC samples versus adjacent samples (Fig. 2c, $p < 0.0001$), meanwhile, KIF18B expression was significantly increased as the pathological stage of ccRCC progressing (Fig. 2d, $p = 0.0002$). GSE53757 dataset also verified the close correlation between AURKB and KIF18B in ccRCC samples (Fig. 2e, $r = 0.572$, $p < 0.0001$).

The Relationship between AURKB and KIF18B Expression and Clinical Characteristics of ccRCC Patients

To further explore the relationship between the expression of AURKB and KIF18B and clinical features, clinical data of ccRCC including 537 samples were downloaded from the TCGA database. The expression levels of AURKB and KIF18B were divided into high expression group and low expression group according to the median expression value. Table 1 summarized the correlation between AURKB, KIF18B expression levels and various clinical characteristics of ccRCC patients. The high expression levels of AURKB and KIF18B were found to be significantly correlated with T stage ($p < 0.0001$, $p < 0.0001$), N stage ($p = 0.008$, $p = 0.005$), M stage ($p < 0.0001$, $p < 0.0001$) and G grade ($p < 0.001$, $p < 0.0001$). These results revealed that AURKB and KIF18B playing important roles in the progression of ccRCC.

The significant correlation between the expression of AURKB and KIF18B in ccRCC has been demonstrated by the above research results. We continued to investigate the correlation between the combined expression of AURKB and KIF18B with different clinical characteristics of ccRCC patients (Table 2). The different subgroups of the combined expression of AURKB and KIF18B in ccRCC samples includes 235 cases of AURKB (high) + KIF18B(high) expression, 235 cases of AURKB (low) + KIF18B (low) expression, 30 cases of AURKB (high) + KIF18B (low) expression, and 30 cases of AURKB (low) + KIF18B (high) expression. T, N, M stage and G grade were not significantly different among AURKB (high) + KIF18B (low) expression samples or AURKB (low) + KIF18B (high) expression samples comparing with AURKB (low) + KIF18B (low) expression samples. In addition, there was no significant difference found in various clinical characteristics, except G grade (p=0.020), in the comparison of samples with AURKB (high) + KIF18B (low) expression and samples with AURKB (low)+KIF18B (high) expression. However, T stage (p<0.0001), N stage (p=0.001), M stage (p=0.0001) and G grade (p<0.0001) of AURKB

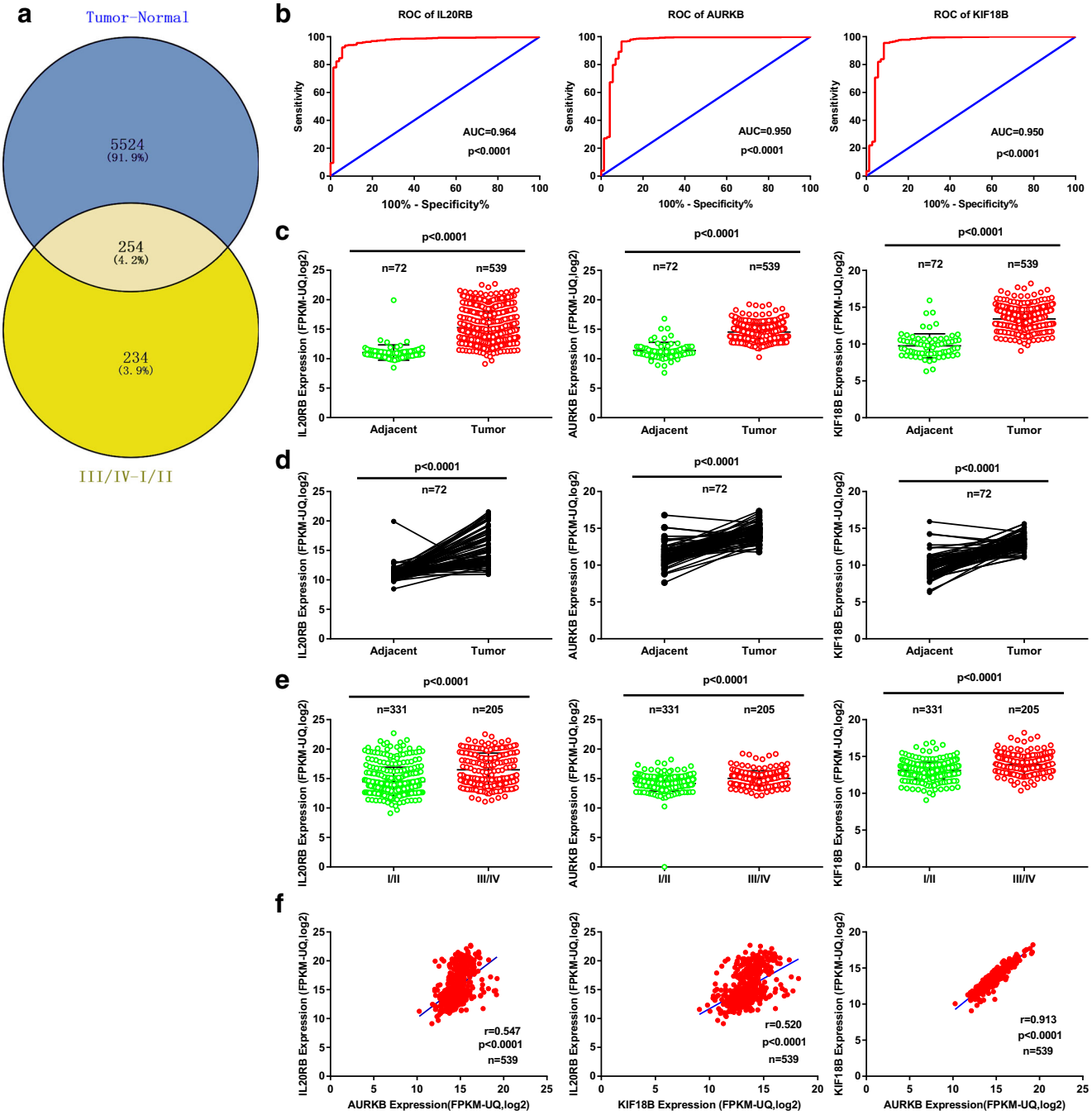

Fig. 1 The close relationship between DEGs AURKB and KIF18B in ccRCC based on TCGA database (**a**) Venn diagram of up-regulated genes differentially expressed in ccRCC samples vs adjacent samples and stage III/IV ccRCC samples vs stage I/II samples. **b** Receiver operating characteristic (ROC) curves of IL20RB, AURKB and KIF18B sorted by area under the curve (AUC) in ccRCC. **c** Differential expression of IL20RB, AURKB, KIF18B in adjacent samples compared with ccRCC samples. **d** Differential expression of IL20RB, AURKB, KIF18B in adjacent samples compared with matched ccRCC samples. **e** Differential expression of IL20RB, AURKB, KIF18B in stage I/II ccRCC samples vs stage III/IV samples. **f** The correlation of IL20RB, AURKB, KIF18B in ccRCC samples. The *p* value of the differential expression scatter plots was derived from the Mann Whitney U test, the differential expression of paired samples was calculated using Wilcoxon signed rank test to calculate p value and the correlation coefficient was calculated by Spearman

(high) + KIF18B(high) expression samples increased significantly compared with non-(AURKB (high) + KIF18B (high)) expression samples. These results further described that only when AURKB and KIF18B were highly expressed simultaneously, can they play important roles in the development of ccRCC.

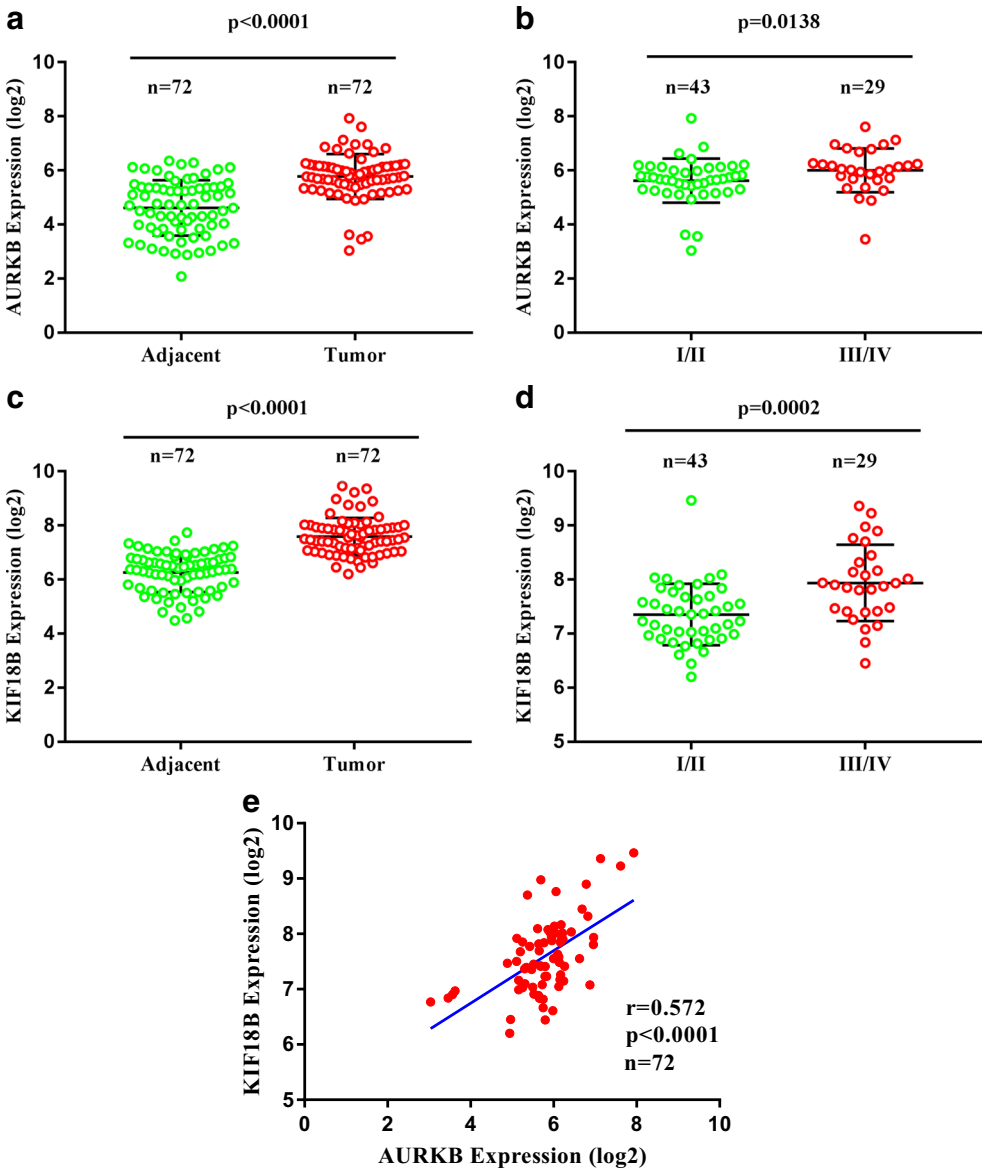

Fig. 2 The correlation of AURKB and KIF18B in ccRCC samples verified by GSE53757 dataset (**a**) Differential expression scatter plot of AURKB between ccRCC samples and adjacent samples. **b** Differential expression scatter plot of AURKB between stage III/IV ccRCC samples vs stage I/II samples. **c** Differential expression scatter plot of KIF18B between ccRCC samples and adjacent samples. **d** Differential expression scatter plot of KIF18B between stage III/IV ccRCC samples vs stage I/II samples. **e** The correlation between AURKB and KIF18B in ccRCC samples. The *p* values of A, B, C and D pictures were derived from the Mann-Whitney U test; The statistical results of the E picture were calculated using Spearman's correlation coefficient

The Expression Levels of AURKB and KIF18B are Associated with Overall Survival (OS) in ccRCC Patients

The expression of AURKB and KIF18B were divided into high expression group and low expression group according to the median expression value. K-M survival curves were plotted in order to investigate the prognostic value of AURKB and KIF18B expression in ccRCC patients. We found that high expression of AURKB (Fig. 3a, $p < 0.0001$) and KIF18B (Fig. 3b, $p < 0.0001$) were negatively related to OS of ccRCC patients.

We also analyzed the association of the combined expression of AURKB and KIF18B with the OS in ccRCC patients. It was found that there was no significant difference in OS between ccRCC patients with AURKB (low) + KIF18B (low) expression, AURKB (high) + KIF18B (low) expression and AURKB (low) + KIF18B (high) expression (Fig. 3c, $p = 0.955$); Compared with AURKB (high) + KIF18B (high) expression samples, the additional samples of combined expression were associated with longer OS in ccRCC patients ($p < 0.0001$, Fig. 3d. AURKB (high) + KIF18B (high) vs AURKB (low) + KIF18B (low), $p < 0.0001$, Fig. 3e; AURKB (high) + KIF18B (high) vs AURKB (high) + KIF18B (low), $p = 0.015$, Fig. 3f; AURKB (high) + KIF18B (high) vs AURKB (low) + KIF18B (high), $p = 0.015$, Fig. 3g; AURKB (high) + KIF18B (high) vs non-(AURKB (high) + KIF18B (high)), Fig. 3h, $p < 0.0001$).

Table 1 Correlation of AURKB and KIF18B expression with clinicopathological features in ccRCC patients

Characteristics	KIF18B			AURKB		
	low	high	p value	low	high	p value
Age (years)						
<60	125	139	0.224	129	135	0.602
≥60	140	126		136	130	
T Stage						
T1 + T2	203	137	<0.0001	206	134	<0.0001
T3 + T4	62	128		59	131	
N Stage						
N0	117	122	0.005	111	128	0.008
N1	2	14		2	14	
M Stage						
M0	226	194	<0.0001	227	193	<0.0001
M1	18	60		18	60	
Grade						
G1 + G2	146	95	<0.0001	155	86	<0.0001
G3 + G4	113	168		104	177	

P value was derived from Pearson's chi-square test

The univariate and multivariate Cox proportional hazard models were used to calculate the influence of each clinicopathological features on the OS in ccRCC patients. The univariate Cox regression analysis showed age (HR, 1.748, 95%CI, 1.286–2.376), T stage (HR, 3.152, 95%CI, 2.326–4.272), N stage (HR, 3.411, 95%CI, 1.810–6.428), M stage (HR, 4.323, 95%CI, 3.163–5.908), G grade (HR, 2.668, 95%CI, 1.893–3.759), AURKB expression (HR,2. 459, 95%CI, 1.779–3.398) and KIF18B expression (HR, 2.434, 95%CI, 1.768–3.351) were all associated with shorter OS of ccRCC patients (Table 3). Moreover, we investigated the association of the combined expression of AURKB and KIF18B with the OS in ccRCC patients. Compared with AURKB (low) + KIF18B (low) expression samples (HR, 1.383, 95%CI, 1.236–1. 549), AURKB (high) + KIF18B (low) expression (HR, 1.539, 95%CI, 1.075–2.205), AURKB (low) + KIF18B (high) expression (HR, 2.882, 95%CI, 1.176–7.064) and non-AURKB (high) + KIF18B (high) expression (HR, 1.384, 95%CI, 1.247–1.537), samples with AURKB (high) + KIF18B (high) expression have significantly shorter OS in ccRCC patients.

The multivariate Cox regression analysis included four different models: Besides age, T stage, N stage, M stage, G grade, Model a adopted AURKB expression levels alone; Model b only adopted KIF18B expression levels; Model c included AURKB expression levels and KIF18B expression levels simultaneously; Model d included the combined expression of AURKB and KIF18B (Table 4). In Model a and Model b, AURKB expression (HR = 1.955, 95%CI, 1.203–3.176) and KIF18B expression (HR = 2.077, 95%CI, 1.282–3.365) were important independent prognostic risk factors of shorter OS in ccRCC patients respectively. In model c,

Table 2 Association of the combined expression of AURKB and KIF18B with clinical features in ccRCC patients

Characteristics	Combination of AURKB and KIF18B				p value[#]			
	1[*]	2[*]	3[*]	4[*]	p^a	p^b	p^c	p^d
Age(years)								
<60	108	17	21	118	0.269	0.013	0.284	0.869
≥60	127	13	9	117				
T Stage								
T1 + T2	181	22	25	112	0.653	0.434	0.347	<0.0001
T3 + T4	54	8	5	123				
N stage								
N0	99	18	12	110	1	1	–	0.001
N1	2	0	0	14				
M Stage								
M0	202	24	25	169	0.431	0.442	1	0.0001
M1	15	3	3	57				
Grade								
G1 + G2	134	12	21	74	0.055	0.228	0.020	<0.0001
G3 + G4	95	18	9	159				

[*] 1, AURKB (low) + KIF18B(low); 2, AURKB (high) + KIF18B (low); 3, AURKB (low) + KIF18B (high); 4, AURKB (high) + KIF18B (high);

#P value obtained from Pearson's chi-square test or Fisher's exact probability test; p^a, 1 vs 2; p^b, 1 vs 3; p^c, 2 vs 3; p^d, 4 vs 1 + 2 + 3

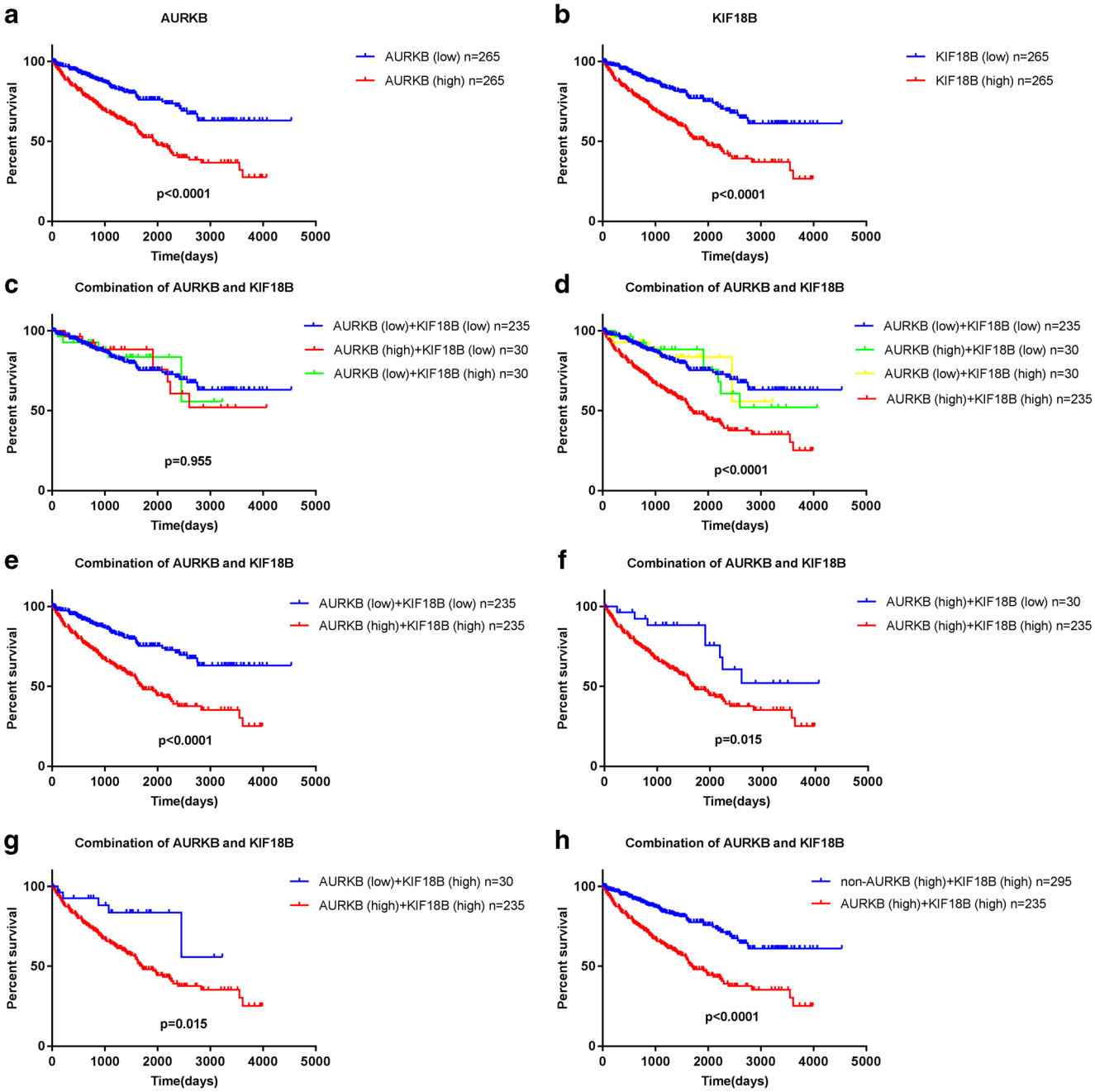

Fig. 3 Log-rank method to draw Kaplan-Meier curves to evaluate the prognostic value of AURKB, KIF18B in ccRCC patients (**a**) The influence of AURKB expression on the survival of ccRCC patients; **b** The influence of KIF18B expression on the survival of ccRCC patients; **c** No influence on the survival of ccRCC patients between patients with AURKB (low) + KIF18B (low) expression, AURKB (high) + KIF18B (low) expression and AURKB (low) + KIF18B (high) expression; **d** The influence of **combination** of AURKB and KIF18B on the survival of ccRCC patients; **e** The influence of AURKB (high) + KIF18B (high) expression on the survival of ccRCC patients compared with AURKB (low) + KIF18B(low) expression; **f** The influence of AURKB (high) + KIF18B (high) expression on the survival of ccRCC patients compared with AURKB (high) + KIF18B (low) expression; **g** The influence of AURKB (high) + KIF18B (high) expression on the survival of ccRCC patients compared with AURKB (low) + KIF18B (high) expression; **h** The influence of AURKB (high) + KIF18B (high) expression on the survival of ccRCC patients compared with non-AURKB (high) + KIF18B (high) expression

neither AURKB expression nor KIF18B expression was independent prognostic risk factors of shorter OS in ccRCC patients. Model d showed that the combined expression of AURKB and KIF18B were independent prognostic risk factors of shorter OS in ccRCC patients, and AURKB (high) + KIF18B (high) expression (HR = 2.094, 95% CI,1.297–3.379) significantly shortened the OS of ccRCCC patients.

Table 3 Univariate Cox regression analysis of the association between clinicopathological parameters and overall survival (OS) in ccRCC patients

Characteristics	All cases	HR (95%CI)	p value
Age (years) (≥60/<60)	530	1.748 (1.286–2.376)	<0.0001
T stage (T3 + T4/T1 + T2)	530	3.152 (2.326–4.272)	<0.0001
N stage (N1/N0)	255	3.411 (1.810–6.42)	<0.0001
M stage (M1/M0)	498	4.323 (3.163–5.908)	<0.0001
Grade (G3 + G4/G1 + G2)	522	2.668 (1.893–3.759)	<0.0001
AURKB (high/low)	530	2.459 (1.779–3.398)	<0.0001
KIF18B (high/low)	530	2.434 (1.768–3.351)	<0.0001
Combination of AURKB and KIF18B			
*4/1	470	1.383 (1.236–1.549)	<0.0001
*4/2	265	1.539 (1.075–2.205)	0.019
*4/3	265	2.882 (1.176–7.064)	0.021
*2/1	265	1.090 (0.515–2.306)	0.822
*3/1	265	0.943 (0.595–1.497)	0.805
*4/1 + 2 + 3	530	1.384 (1.247–1.537)	<0.0001

* 1, AURKB (low) + KIF18B (low); 2, AURKB (high) + KIF18B (low); 3, AURKB (low) + KIF18B (high); 4, AURKB (high) + KIF18B (high)

KEGG Pathway Enrichment Analysis

In ccRCC, we found 598 genes positively co-expressed with AURKB and 430 genes with KIF18B through the co-expression function of cBioPortal. KEGG analysis (Fig. 4) demonstrated that the enrichment pathway of positive co-expression genes of AURKB and KIF18B in ccRCC including cell cycle, HTLV-I infection, oocyte meiosis, p53 signalling pathway, progesterone-mediated oocyte maturation, Fanconi anemia pathway, homologous recombination, etc. These pathways were closely related to cell mitosis. The functional analysis supported that AURKB and KIF18B are closely related to the progression of ccRCC.

Discussion

The cure rate of early-stage ccRCC patients without metastasis is high, conversely, 5-year survival for patients with distant metastatic decreases to 12% [14]. comparing to other subtypes of renal cell carcinoma, ccRCC has a higher rate of metastasis [15], about 20–30% of ccRCC patients have advanced disease at the time of diagnosis [4]. Therefore, searching for a biomarker of the progression related to invasion and metastasis of ccRCC possess great significance for the treatment of ccRCC patients. In our study, we performed differential expression analysis of the ccRCC RNA expression profile from TCGA database to find intersection genes that were up-regulated in

Table 4 Multivariate Cox regression analysis of the association between clinicopathological parameters and overall survival (OS) in ccRCC patients

Characteristics	Model a#		Model b#		Model c#		Model d#	
	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value	HR (95%CI)	p value
Age (years) (≥60/<60)	1.627(1.063–2.492)	0.025	1.590(1.037–2.438)	0.034	1.581(1.031–2.426)	0.036	1.537(1.000–2.362)	0.05
T stage (T3 + T4/T1 + T2)	1.704(1.056–2.749)	0.029	1.673(1.030–2.716)	0.038	1.658(1.022–2.692)	0.041	1.641(1.013–2.659)	0.044
N stage (N1/N0)	1.449(0.719–2.920)	0.3	1.414(0.703–2.845)	0.331	1.385(0.687–2.792)	0.363	1.367(0.677–2.762)	0.383
M stage (M1/M0)	3.026(1.882–4.867)	<0.0001	2.973(1.833–4.821)	<0.0001	3.014(1.860–4.881)	<0.0001	3.040(1.878–4.924)	<0.0001
Grade (G3 + G4/G1 + G2)	1.512(0.905–2.527)	0.115	1.472(0.877–2.468)	0.143	1.453(0.865–2.442)	0.158	1.446(0.863–2.424)	0.162
AURKB (high/low)	1.955(1.203–3.176)	0.007	–	–	1.329(0.646–2.736)	0.44	–	–
KIF18B (high/low)	–	–	2.077(1.282–3.365)	0.003	1.684(0.824–3.442)	0.153	–	–
Combination of AURKB and KIF18B (4/1 + 2 + 3)*	–	–	–	–	–	–	2.094(1.297–3.379)	0.002

* 1, AURKB (low) + KIF18B (low); 2, AURKB (high) + KIF18B (low); 3, AURKB (low) + KIF18B (high); 4, AURKB (high) + KIF18B (high)

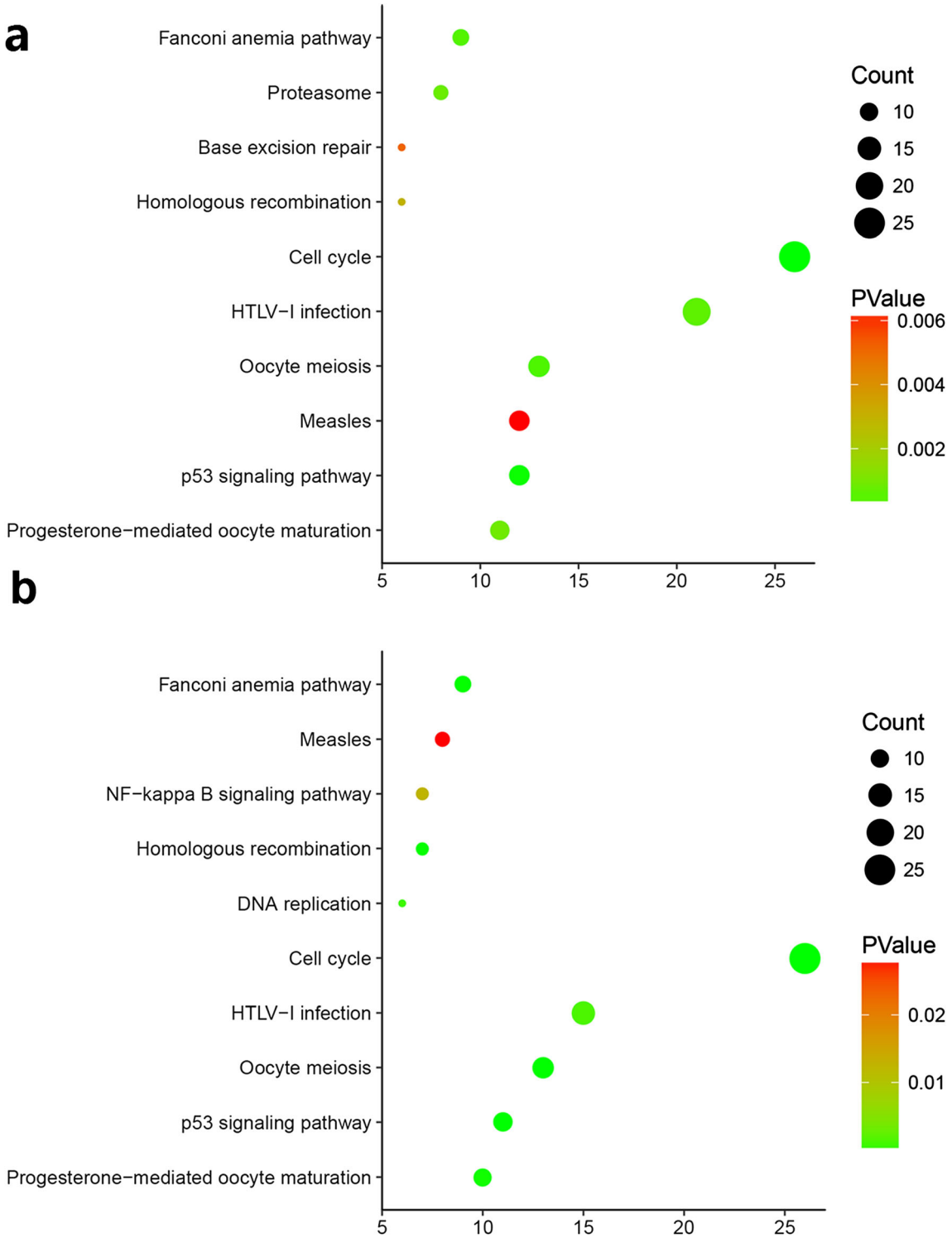

Fig. 4 Top 10 KEGG enrichment pathway for positive co-expression genes of AURKB and KIF18B in ccRCC samples (**a**) Top 10 enrichment pathways of positive co-expression genes of AURKB in ccRCC. **b** Top 10 enrichment pathways of positive co-expression genes of KIF18B in ccRCC

cancer samples compared with adjacent cancer samples and up-regulated in stage III/IV ccRCC samples compared with stage II/II ccRCC samples as well. Finally, two genes AURKB and KIF18B which were closely related in ccRCC samples were obtained. GSE53757 dataset verified the differential expression and correlation of the genes AURKB, KIF18B in ccRCC samples.

Aurora kinase is a serine/threonine kinase that is a key regulator of the mitotic process. Aurora kinase family is comprised of AURKA, AURKB, AURKC [16]. It has been

reported that AURKB is highly expressed in a variety of malignant tumors, such as lung cancer [17], gastric cancer [18] and liver cancer [19]. And the high expression of AURKB is associated with tumor invasion and metastasis [20, 21], high AURKB expression is also associated with poor prognosis in cancer patients [22, 23]. In this study, we examined the expression levels of AURKB in 539 ccRCC samples and 72 adjacent cancer samples that obtained from the TCGA database. we found that the expression of AURKB was increased in ccRCC samples compared with adjacent samples. Our finding that AURKB was up-regulated in ccRCC is consistent with previous findings showing AURKB over-expression in multiple tumors. Similar to previous reports, our results indicated that the expression of AURKB increased with the progression of ccRCC. High AURKB expression was significantly correlated with T stage, N stage, M stage and shorter OS of ccRCC patients. AURKB has been considered a target for cancer therapeutics [24]. It has been studied that several AURKB inhibitors like AZD1152 have done clinical trials in different stages of advanced solid tumors [25–27]. These results suggested that AURKB is a potential therapeutic target for ccRCC patients.

The kinesin superfamily is a class of microtubule-dependent motor proteins that play an essential role in mitosis [28]. The alterations in the expression and function of kinesins make it possible for the emergence of cancer so that specific kinesins probably become key proteins of cancer therapeutic targets [28]. KIF18B is a member of the kinesin 8 families [29]. In the present study, we found that the expression of KIF18B was significantly increased in ccRCC samples compared with adjacent cancer samples. At the same time, the expression of KIF18B up-regulated with the development of ccRCC clinical stage, and high KIF18B expression was significantly negatively associated with T stage, N stage, M stage and prognosis of ccRCC samples. Previous studies have shown that KIF18B expression is also significantly increased in a variety of tumors such as cervical cancer, breast cancer, lung cancer, ovarian cancer and kidney cancer [30, 31]. In addition, the results of this study are consistent with the finding that the high expression of KIF18B promotes tumor proliferation and migration [30]. Our research supported using KIF18B as a new target for cancer therapy.

In addition, we found that the expression levels of AURKB and KIF18B were significantly increased, and both showed a significant positive correlation in ccRCC samples. Reports on the correlation between AURKB and KIF18B in tumors have not been seen currently. In a report mentioning the association between AURKB and KIF18B, it is mentioned that Aurora kinase (AURKA and AURKB) can negatively regulate KIF18B-MCAK complex by phosphorylation of MCAK, achieving normal spindle assembly and cell division [32]. The negative correlation between AURKB and KIF18B found in this study occurred at the level of protein expression, which was different from the mRNA expression levels of AURKB and KIF18B in our study. We speculated that AURKB and KIF18B playing roles in different stages of mitosis, so the intrinsic mechanism of cells precisely regulates the protein translation levels of both. Therefore, when examining a specific link in the process of mitosis, there is a negative correlation between the protein expression of AURKB and KIF18B. Both AURKB and KIF18B are important regulators involved in cell mitosis. Accordingly, mRNA expression of AURKB and KIF18B can be increased simultaneously and there is a close positive correlation in the tissue with strong proliferation, which is not inconsistent with the negative correlation between the expression of the AURKB and KIF18B proteins in cells at specific stages of cell division.

By further analysis of the clinical significance of the combined expression of AURKB and KIF18B in the progression of ccRCC, we found that AURKB (high) + KIF18B (high) expression was associated with a significant increase in T stage, N stage, M stage and G grade in ccRCC patients compared to other subgroups of ccRCC patients. However, the clinical pathological features of AURKB (low) + KIF18B (low) expression, AURKB (high) + KIF18B (low) expression, or AURKB (low) + KIF18B (high) expression were not significantly different in ccRCC patients. Furthermore, analyzing the effect of the combined expression of AURKB and KIF18B on the prognosis of ccRCC patients, we found that there were no significant differences in OS between the other subgroups of ccRCC patients, except the subgroup of patients with AURKB (high) + KIF18B (high) expression. Compared with the different subgroups of ccRCC patients described above, the OS of ccRCC patients with AURKB (high) + KIF18B (high) expression were significantly shortened. Moreover, COX regression model analysis found that except age, TNM stage, G grade, when we examined AURKB expression or KIF18B expression alone, both were independent risk factors for prognosis in patients with ccRCC, and when we examined the effects of AURKB expression and KIF18B expression on the prognosis of ccRCC patients meantime, neither was an independent prognostic factor for shorter OS. We investigated the combined expression of AURKB and KIF18B on the prognosis of ccRCC patients and found that AURKB (high) + KIF18B (high) expression was an independent risk factor for shorter OS in ccRCC patients. The above findings indicated that although the progression of ccRCC patients can be affected by AURKB and KIF18B alone, however, they have strong dependence on each other due to the close correlation between AURKB expression and KIF18B expression. Only when AURKB and KIF18B work simultaneously (simultaneous high expression) can the progression of ccRCC patients be promoted and the prognosis of ccRCC patients be affected.

In previous studies, there were also many attempts to use bioinformatics methods to finding DEGs to predict cancer therapeutic targets. Our study used a more stringent AUC value to screen for the top DEGs, focusing on DEGs which associated with the development of ccRCC. It was found that AURKB and KIF18B, which are closely related in the ccRCC samples, can promote the progression of ccRCC and lead to poor prognosis when both are active. In summary, the combined expression between the DEGs AURKB and KIF18B is an important event in the development of ccRCC, providing a potential new target for clinical treatment of ccRCC patients.

Conclusion

Our study revealed the expression of closely related genes AURKB and KIF18B were all up-regulated in ccRCC samples, and over-expressed with the development of ccRCC. AURKB (high) or KIF18B (high) were all significantly correlated with shorter OS of ccRCC patients. Moreover, AURKB (high) + KIF18B (high) expression was an independent prognostic risk factor for ccRCC patients considering the combined effect of them. In summary, the combined effect of AURKB and KIF18B in ccRCC may be of great significance for cancer patients.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations *ccRCC*, Clear cell renal cell carcinoma; *TCGA*, The Cancer Genome Atlas; *GEO*, Gene Expression Omnibus; *DEGs*, Differentially expressed genes; *ROC*, Receiver operating characteristic; *AUC*, Area under the curve; *OS*, Overall survival

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424
2. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L et al (2017) Renal cell carcinoma. Nat Rev Dis Primers 3(17009):9
3. Hsieh JJ, Le V, Cao D, Cheng EH, Creighton CJ (2018) Genomic classifications of renal cell carcinoma: a critical step towards the future application of personalized kidney cancer care with pan-omics precision. J Pathol 244(5):525–537
4. Li QK, Pavlovich CP, Zhang H, Kinsinger CR, Chan DW (2019) Challenges and opportunities in the proteomic characterization of clear cell renal cell carcinoma (ccRCC): a critical step towards the personalized care of renal cancers. Semin Cancer Biol 55:8–15
5. Capitanio U, Montorsi F (2016) Renal cancer. Lancet 387:894–906
6. Keefe SM, Nathanson KL, Rathmell WK (2013) The molecular biology of renal cell carcinoma. Semin Oncol 40(4):421–428
7. Fan Y, Ma X, Li H, Gao Y, Huang Q, Zhang Y, Bao X, du Q, Luo G, Liu K, Meng Q, Zhao C, Zhang X (2018) miR-122 promotes metastasis of clear-cell renal cell carcinoma by downregulating dicer. Int J Cancer 142(3):547–560
8. Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, Mills GB, Shaw KR et al (2013) The cancer genome atlas Pan-Cancer analysis project. Nat Genet 45(10):1113–1120
9. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF et al (2013) NCBI GEO: archive for functional genomics datasets-update. Nucleic Acids Res 41:D991–D995
10. Wang Z, Yang B, Zhang M, Guo W, Wu Z et al (2018) lncRNA epigenetic landscape analysis identifies EPIC1 as an oncogenic lncRNA that interacts with MYC and promotes cell-cycle progression in cancer. Cancer Cell 33(4):706–720
11. Li B, Wang W, Li Z, Chen Z, Zhi X, Xu J, Li Q, Wang L, Huang X, Wang L, Wei S, Sun G, Zhang X, He Z, Zhang L, Zhang D, Xu H, el-Rifai W, Xu Z (2017) MicroRNA-148a-3p enhances cisplatin cytotoxicity in gastric cancer through mitochondrial fission induction and cyto-protective autophagy suppression. Cancer Lett 410: 212–227
12. Chen L, Yuan L, Qian K, Qian G, Zhu Y, Wu CL, Dan HC, Xiao Y, Wang X (2018) Identification of biomarkers associated with pathological stage and prognosis of clear cell renal cell carcinoma by co-expression network analysis. Front Physiol 9:399
13. Song J, Peng J, Zhu C, Bai G, Liu Y, Zhu J, Liu J (2018) Identification and validation of two novel prognostic lncRNAs in kidney renal clear cell carcinoma. Cell Physiol Biochem 48(6): 2549–2562
14. Atkins MB, Tannir NM (2018) Current and emerging therapies for first-line treatment of metastatic clear cell renal cell carcinoma. Cancer Treat Rev 70:127–137
15. Shuch B, Amin A, Armstrong AJ, Eble JN, Ficarra V, Lopez-Beltran A, Martignoni G, Rini BI, Kutikov A (2015) Understanding pathologic variants of renal cell carcinoma: distilling therapeutic opportunities from biologic complexity. Eur Urol 67(1):85–97
16. Yan M, Wang C, He B, Yang M, Tong M et al (2016) Aurora-a kinase: a potent oncogene and target for cancer therapy. Med Res Rev 36(6):1036–1079
17. Al-Khafaji AS, Davies MP, Risk JM, Marcus MW, Koffa M et al (2017) Aurora B expression modulates paclitaxel response in non-small cell lung cancer. Br J Cancer 116(5):592–599
18. Enjoji M, Iida S, Sugita H, Ishikawa T, Uetake H et al (2009) BubR1 and AURKB overexpression are associated with a favorable prognosis in gastric cancer. Mol Med Rep 2(4):589–596
19. Lin ZZ, Jeng YM, Hu FC, Pan HW, Tsao HW, Lai PL, Lee PH, Cheng AL, Hsu HC (2010) Significance of Aurora B overexpression in hepatocellular carcinoma. Aurora B overexpression in HCC. BMC Cancer 10:461
20. ZhangY JC, Li H, Lv F, Li X et al (2015) Elevated Aurora B expression contributes to chemoresistance and poor prognosis in breast cancer. Int J Clin Exp Pathol 8(1):751–757
21. Chen YJ, Chen CM, Twu NF, Yen MS, Lai CR, Wu HH, Wang PH, Yuan CC (2009) Overexpression of Aurora B is associated with

poor prognosis in epithelial ovarian cancer patients. Virchows Arch 455(5):431–440
22. Lens SM, Voest EE, Medema RH (2010) Shared and separate functions of polo-like kinases and aurora kinases in cancer. Nat Rev Cancer 10(12):825–841
23. Tang A, Gao K, Chu L, Zhang R, Yang J et al (2017) Aurora kinases: novel therapy targets in cancers. Oncotarget 8(14): 23937–23954
24. Lok W, Klein RQ, Saif MW (2010) Aurora kinase inhibitors as anticancer therapy. Anti-Cancer Drugs 21(4):339–350
25. Sehdev V, Peng D, Soutto M, Washington MK, Revetta F, Ecsedy J, Zaika A, Rau TT, Schneider-Stock R, Belkhiri A, el-Rifai W (2012) The aurora kinase A inhibitor MLN8237 enhances cisplatin-induced cell death in esophageal adenocarcinoma cells. Mol Cancer Ther 11(3):763–774
26. Cheung CH, Sarvagalla S, Lee JY, Huang YC, Coumar MS (2014) Aurora kinase inhibitor patents and agents in clinical testing: an update (2011-2013). Expert Opin Ther Pat 24(9): 1021–1038
27. Schwartz GK, Carvajal RD, Midgley R, Rodig SJ, Stockman PK, Ataman O, Wilson D, Das S, Shapiro GI (2013) Phase I study of barasertib (AZD1152), a selective inhibitor of Aurora B kinase, in patients with advanced solid tumors. Investig New Drugs 31(2): 370–380
28. Yu Y, Feng YM (2010) The role of kinesin family proteins in tumorigenesis and progression: potential biomarkers and molecular targets for cancer therapy. Cancer 116(22):5150–5160
29. Lee YM, Kim E, Park M, Moon E, Ahn SM, Kim W, Hwang KB, Kim YK, Choi W, Kim W (2010) Cell cycle-regulated expression and subcellular localization of a kinesin-8 member human KIF18B. Gene 466(1–2):16–25
30. Wu Y, Wang A, Zhu B, Huang J, Lu E (2018) KIF18B promotes tumor progression through activating the Wnt/β-catenin pathway in cervical cancer. Onco Targets Ther 11:1707–1720
31. Itzel T, Scholz P, Maass T, Krupp M, Marquardt JU, Strand S, Becker D, Staib F, Binder H, Roessler S, Wang XW, Thorgeirsson S, Müller M, Galle PR, Teufel A (2015) Translating bioinformatics in oncology: guilt-by-profiling analysis and identification of KIF18B and CDCA3 as novel driver genes in carcinogenesis. Bioinformatics 31(2):216–224
32. Tanenbaum ME, Macurek L, van der Vaart B, Galli M, Akhmanova A, Medema RH (2011) A complex of Kif18b and MCAK promotes microtubule depolymerization and is negatively regulated by Aurora kinases. Curr Biol 21(16):1356–1365