Austin Journal of Cancer and Clinical Research

Open Access

Austin Publishing Group

Research Article

PRPF8 Expression is an Independent Predictor for Poor Prognosis in Patients with Kidney Renalclear Cell Carcinoma

Zhu D[1#], Qiqi Z[2,3#], Qi J[2,4], Sima M[2,5], Li C[6], Li Y[2], Li Z[2,7], Wang T[2*] and Lv C[2,4*]

[1]Sichuan Agricultural University, Chengdu, China
[2]Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Changchun, PR China
[3]North Sichuan College of Preschool Education Teacher, Guangyuan, China
[4]The Key Laboratory of Molecular Epigenetic, Institute of Genetics and Cytology, Northeast Normal University, Changchun, China
[5]College of Basic Medicine, Changchun University of Chinese Medicine, Changchun, PR China
[6]Fuxin Higher Training College, Fuxin, China
[7]College of Animal Medicine, Jilin University, Changchun, PR China
[#]Contributed Equally to this work

***Corresponding author:** Tiecheng Wang, Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Changchun, 130122, PR China

Chaoxiang Lv, Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 130122, PR China; The Key Laboratory of Molecular Epigenetic, Institute of Genetics and Cytology, Northeast Normal University, Changchun, 130024, China

Received: February 21, 2022; **Accepted:** March 17, 2022; **Published:** March 24, 2022

Abstract

Background: Alternative splicing is an important process associated with disease including tumors. *PRPF8* is a conserved protein in splice some component U5 snRNP that plays an important role in tumor cell growth.

Materials and Methods: The data in The Cancer Genome Atlas was downloaded and analyzed by R Studio. The box plots showed the expression pattern of *PRPF8*. The chi-square test was used to manifest the association between *PRPF8* expression and clinical parameters. The diagnostic value was assessed using ROC curve. The Kaplan-Meier curves and the Cox regression analysis elucidated the difference of overall survival and relapse-free survival between high expression and low expression and the prognostic value.

Results: We downloaded the *PRPF8* expression and the clinical data of 50 healthy individuals and 537 patients from TCGA database. We found *PRPF8* expression is lower in tumor tissues than that in normal tissues and is related to age, histologic grade, pathologic stage, M classification, T classification and vital status. The Kaplan Meier curves showed the patients with lower *PRPF8* expression has a poorer overall survival and relapse-free survival. The Univariate and Multivariate analysis suggested *PRPF8* expression was an independent prognostic factor for overall survival and relapse-free survival in kidney renal clear cell carcinoma.

Conclusion: Low *PRPF8* expression is an independent predictor for overall survival and relapse-free survival in kidney renal clear cell carcinoma.

Keywords: *PRPF8*; Predictor; Kidney renal clear cell carcinoma; TCGA; Molecular marker; Diagnosis

Introduction

Kidney cancer is one of the most common malignancies, accounting for 2% of all cancers, and usually has a poor prognosis [1]. The most common type of kidney cancer is renal cell carcinoma accounting for 90% of all kidney cancers [2]. Renal cell carcinoma includes Kidney Renal Clear Cell Carcinoma (KIRC), Kidney Renal Papillary Cell Carcinoma (KIRP) and Kidney Chromophobe (KICG) [3-6]. Among them, KIRC accounts for 70-75% of all renal cell carcinomas [7]. Therapy for KIRC includes radiation therapy, chemotherapy, and surgery. Nephrectomy remains the primary treatment for clinically localized disease, but 20% to 30% of patients with localized disease relapse after nephrectomy, and the majority of these patients die from the disease [8]. Unfortunately, there are currently no approved treatments to reduce the risk of renal cancer recurrence, progression, or death after primary treatment for the localized disease. Therefore, it is very important to search for markers that indicate the prognosis of renal cell carcinoma.

Alternative splicing is an important process in eukaryotes and is also of great value in diseases, especially in tumors. Alternative splicing can lead to the appearance of various abnormally expressed proteins, which may be the key to the development of disease. It is mainly performed in spliceosomes, which are composed of U1, U2, U4/U6, U5 and more than 200 proteins. *PRPF8* is a key protein in the component U5 [9,10]. In eukaryotes, deletion of *PRPF8* leads to the development of many physiological or pathological diseases. For example, studies have reported that decreased expression of *PRPF8* leads to increased cellular proliferation in K542 and CD 34+ cells [11]. The *PRPF8* depletion disrupts Homology-Directed Repair (HDR), Single Strand Annealing (SSA), and end resection [12].In HCC caused by different causes, *PRPF8* over expression is related to the invasion of HCC cell lines *in vitro* [13].

In our study, we evaluated the expression pattern of *PRPF8* in kidney renal clear cell carcinoma patients and the relation between *PRPF8* expression and clinical pathological parameters. In addition, we also assessed the diagnostic value of *PRPF8* expression and prognostic significance of *PRPF8* expression for overall survival and relapse-free survival in kidney renal clear cell carcinoma patients.

Materials and Methods

Data downloading from the Cancer Genome Atlas (TCGA) database We downloaded *PRPF8* mRNA expression data of normal tissues and tumor tissues and obtained the clinical information of patients from TCGA database by RTCGA Tool box package in R

Citation: Zhu D, Qiqi Z, Qi J, Sima M, Li C, Li Y, et al. *PRPF8* Expression is an Independent Predictor for Poor Prognosis in Patients with Kidney Renalclear Cell Carcinoma. Austin J Cancer Clin Res. 2022; 9(1): 1100.

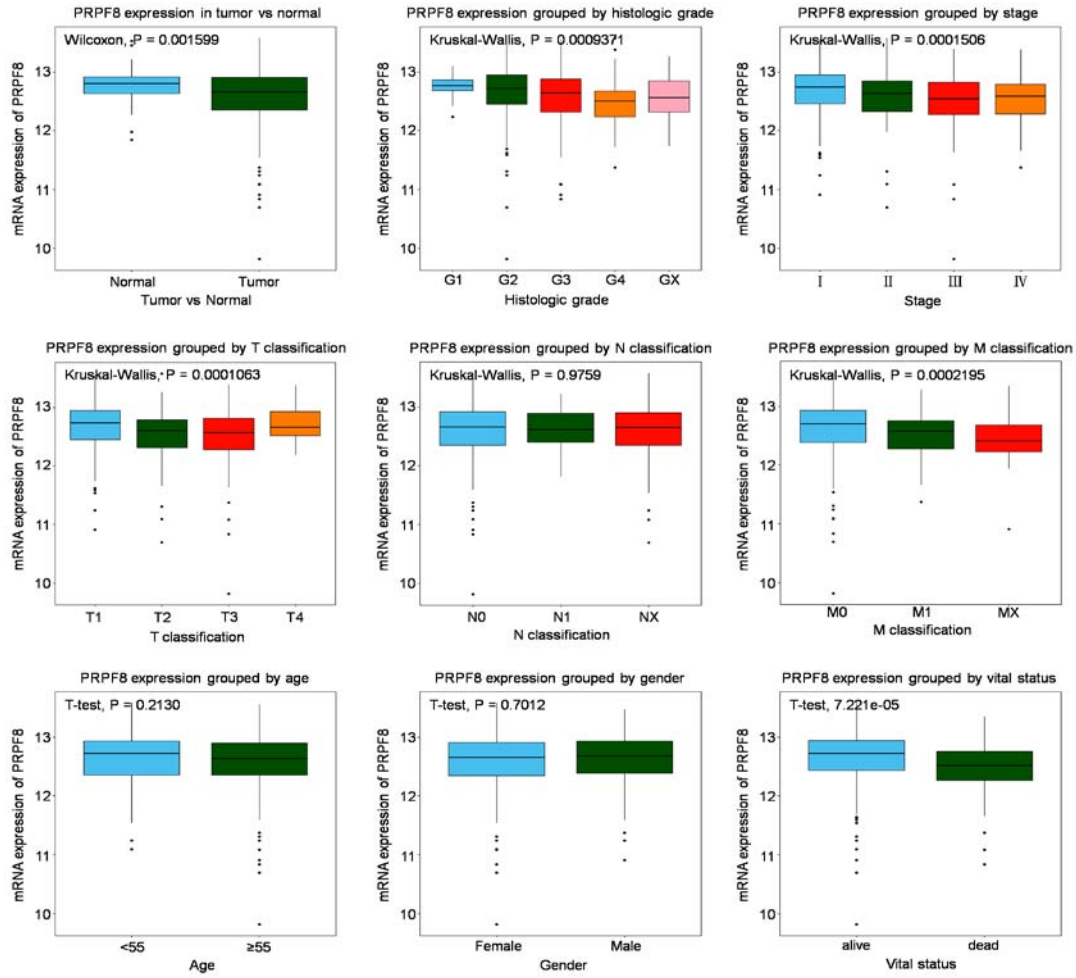

Figure 1: Expression of *PRPF8* in KIRC. Expression of *PRPF8* between tumor and normal tissue was compared. The expression of *PRPF8* was compared according to different age, gender, histologic grade, histological type, T/N/M classification, as well as radiation therapy, residual tumor, sample type, stage and vital status.

Studio [14,15].

Statistical analysis

Box plots were performed to analyze the expression pattern of *PRPF8* and the difference of *PRPF8* expression according to clinical variables by ggplot2 package in R. Chi-square test was used to evaluate the relationship of *PRPF8* expression with clinical characteristics. We generated ROC curves to predict the diagnostic value of *PRPF8* expression by pROC package in R [16]. Then according to ROC curve, we divided *PRPF8* expression into two groups, high *PRPF8* expression and low *PRPF8* expression. And Kaplan-Meier curve was plotted to describe the effect of high *PRPF8* expression and low *PRPF8* expression on overall survival and relapse-free survival by using survival package in R. Univariate Cox analysis was used to determine the factors affecting OS and RFS [17]. Multivariate Cox analysis was applied for to judge the influence of *PRPF8*expression on the overall survival and relapse-free survival of patients.

Results

Characteristics of patients in TCGA-KIRC database

We downloaded *PRPF8* expression and clinical information of 537 patients from TCGA-KIRC cohort. The clinical information included age, gender, histologic grade, pathologic stage, TNM stage, vital status, and recurrence of kidney renal clear cell carcinoma patients (Table 1).

The *PRPF8* expression and the relationship between its expression and clinical variables

The *PRPF8* expression is lower in tumor tissues than in normal tissues (P=0.001599). The box plots showed that significant difference of *PRPF8* expression was observed according to histologic grade, pathologic stage, T classification, M classification and vital status (Figure 1). Moreover, we analyzed the association of *PRPF8* expression with clinical variables by chi-square test. The results revealed *PRPF8* expression is closely related to age (P=0.011), histologic grade (P<0.001), pathologic stage (P<0.001), M classification (P<0.001), T classification (P<0.001) and vital status (P<0.001) (Table 2).

The diagnostic value of *PRPF8* expression in KIRC

To evaluate the diagnostic value of *PRPF8* expression, we made the ROC curve using the *PRPF8* expression and then the Area under the ROC Curve (AUC) was statistically calculated. The ROC curve of all patients indicated AUC was 0.614, which demonstrated the

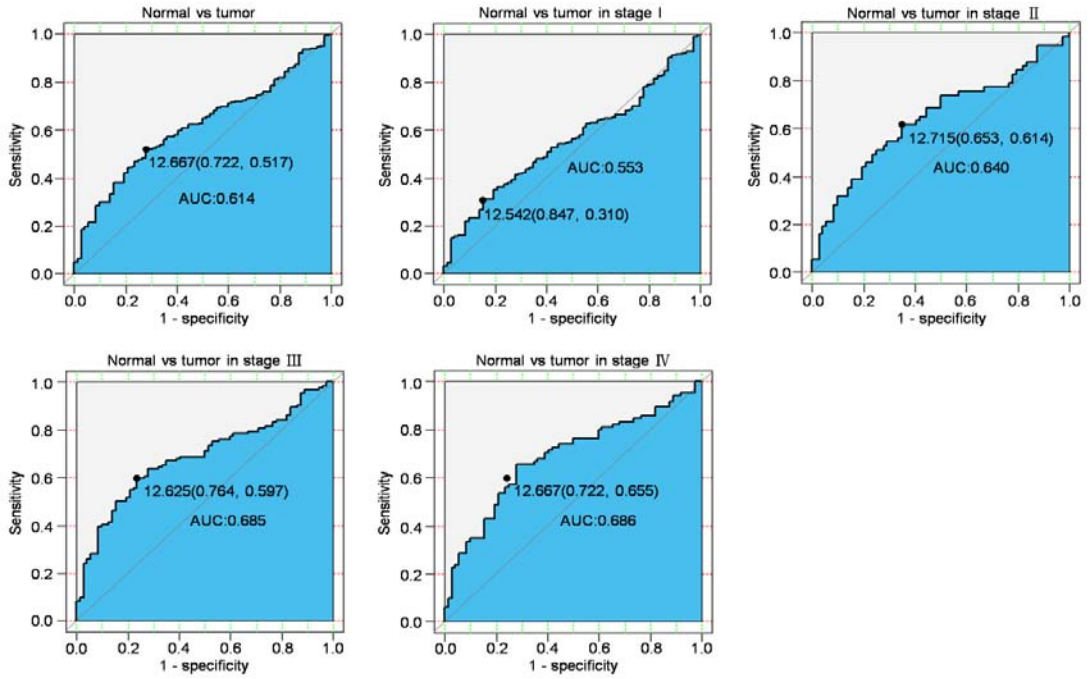

Figure 2: Diagnosis value of *PRPF8* expression in KIRC. The ROC curve of *PRPF8* expression in Cancerous *vs.* Normal liver tissues was generated. Cancerous *vs.* Normal liver tissues was analyzed in different stages of KIRC.

Table 1: The clinical characteristics of patients in the present study.

Parameters	Numbers (%)
Age	
≥55	365(67.97)
<55	172(32.03)
Gender	
Male	346(64.43)
Female	191(35.57)
Histologic grade	
NA	3(0.56)
G1	14(2.61)
G2	230(42.83)
G3	207(38.55)
G4	78(14.52)
GX	5(0.93)
Pathologic stage	
I	270(50.28)
II	57(10.61)
III	126(23.46)
IV	84 (15.64)
T classification	
T1	275(51.21)
T2	69(12.85)
T3	182(33.89)
T4	11(2.05)
N classification	
N0	240(44.69)
N1	17(3.17)
NX	280(52.14)
M classification	
NA	2(0.37)
M0	426 (79.33)
M1	79(14.71)
MX	30(5.59)
Vital status	
Dead	162(30.17)
Survival	375(69.83)
Relapse	
NA	28(5.21)
NO	364(67.78)
YES	145(27.01)
***PRPF8* expression**	
NA	4(0.74)
High	258(48.05)
Low	275(51.21)

NA: Not Available.

PRPF8 expression had a modest diagnostic value in all patients. Then we performed the subgroup analysis and the results showed that the AUC of patients in stage I stage II, stage III and stage IV was 0.553, 0.640, 0.685 and 0.686, respectively (Table 3). This suggested that the expression of *PRPF8* had different diagstic significance for patients at

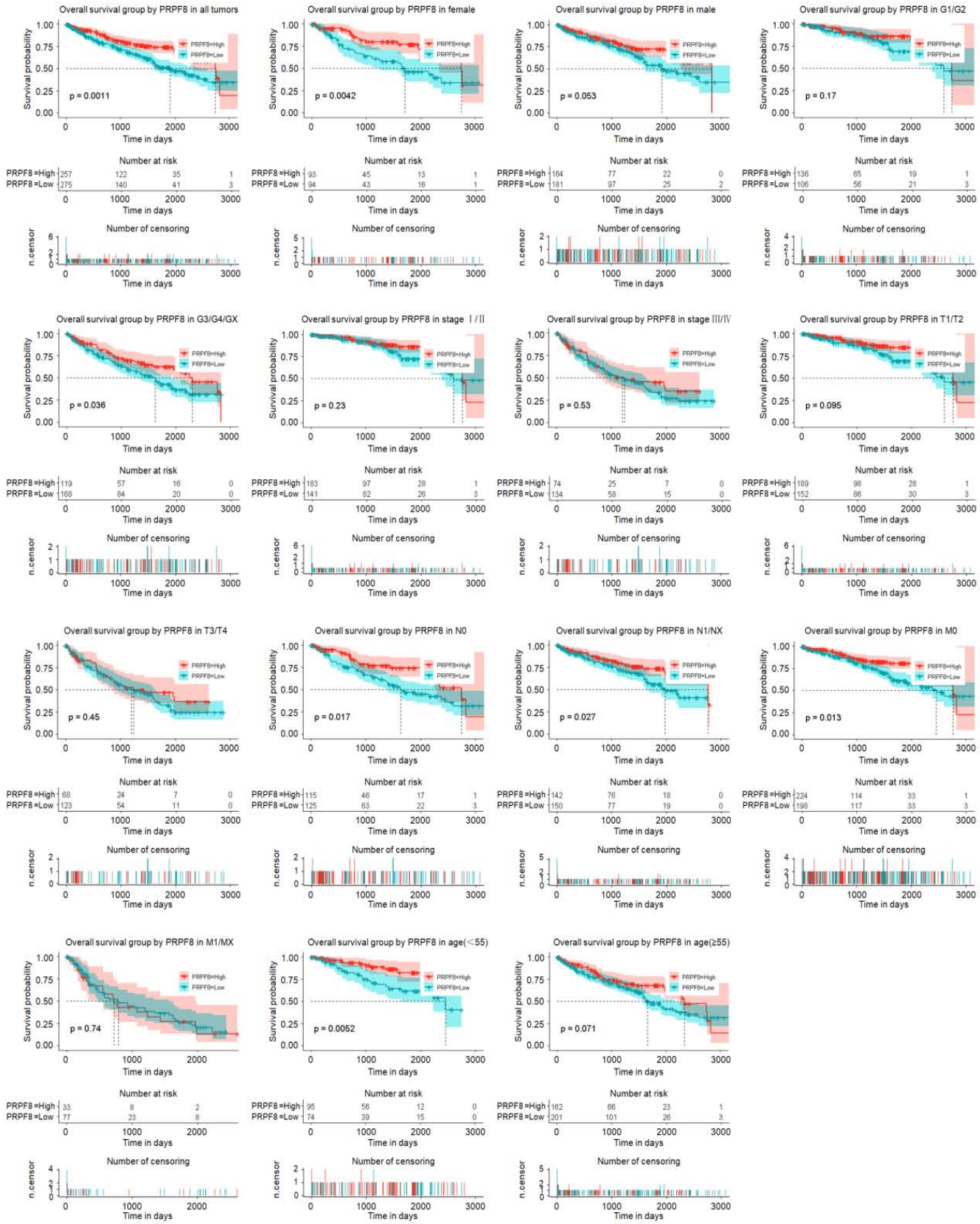

Figure 3: The effect of *PRPF8* expression on OS in KIRC. Kaplan-Meier curves of *PRPF8* expression in all patients with KIRC. Kaplan-Meier curves of *PRPF8* expression in subgroup.

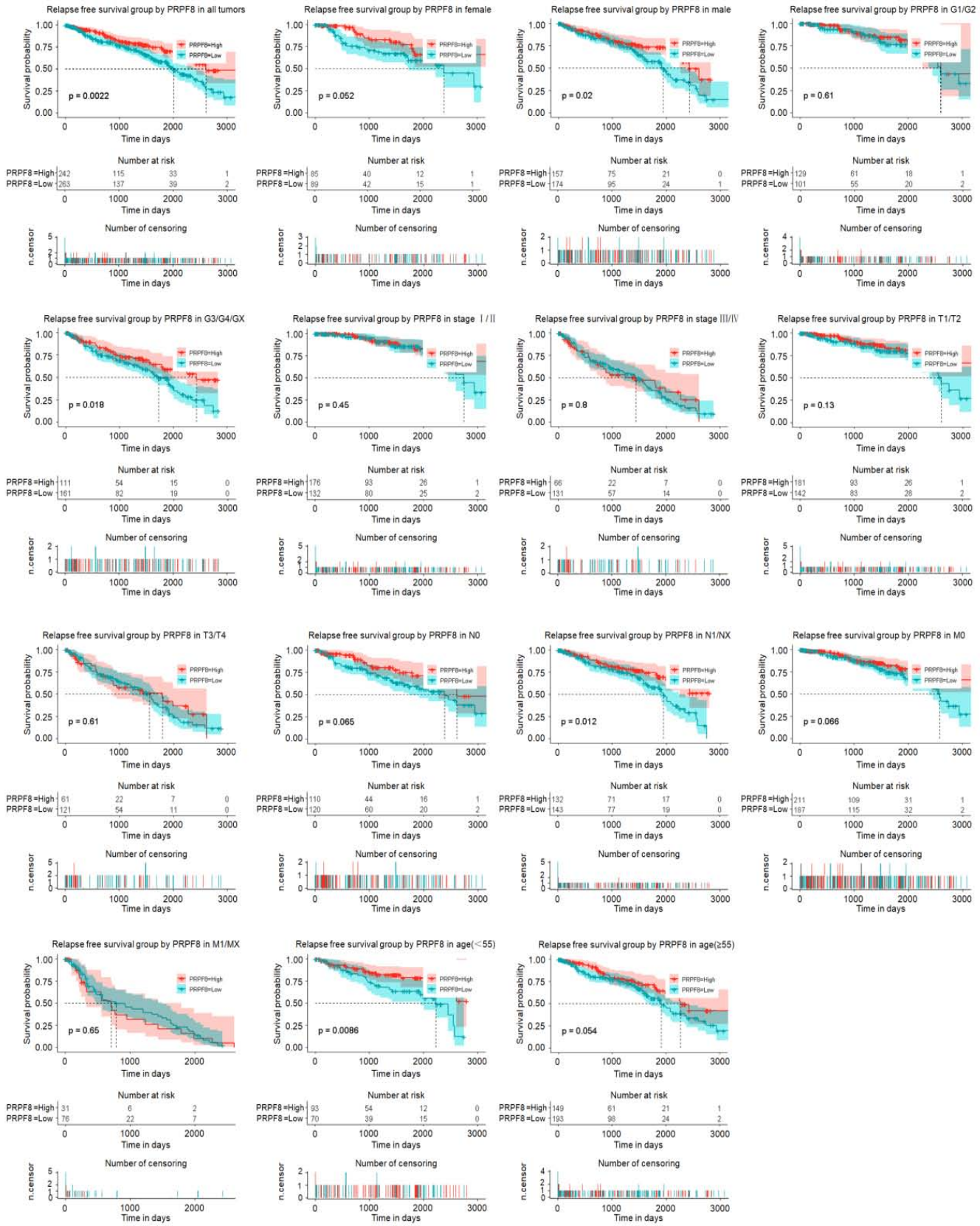

Figure 4: The effect of *PRPF8* expression on RFS in KIRC. Kaplan-Meier curves of *PRPF8* expression in all patients with KIRC. Kaplan-Meier curves of *PRPF8* expression in subgroup.

Table 2: Associations between the clinic pathologic variables and *PRPF8* expression in KIRC.

Parameters	Variables	Numbers	*PRPF8*				X^2	P-value
			high	Prop (%)	low	Prop (%)		
age	≥55	363	162	62.79	201	73.09	6.5015	0.011
	<55	170	96	37.21	74	26.91		
Gender	Male	345	164	63.57	181	65.82	0.2958	0.587
	Female	188	94	36.43	94	34.18		
Histologic grade	G1	14	11	4.30	3	1.09	25.9856	0.000
	G2	229	126	49.22	103	37.59		
	G3	206	98	38.28	108	39.42		
	G4	76	19	7.42	57	20.81		
	GX	5	2	0.78	3	1.09		
Pathologic stage	I	268	157	60.85	111	40.36	24.9067	0.000
	II	57	27	10.47	30	10.91		
	III	124	45	17.44	79	28.73		
	IV	84	29	11.24	55	20		
M classification	M0	422	224	86.82	198	72.53	16.9529	0.000
	M1	79	26	10.08	53	19.41		
	MX	30	8	3.10	22	8.06		
N classification	N0	240	115	44.57	125	45.45	0.0514	0.975
	N1	16	8	3.10	8	2.91		
	NX	277	135	52.33	142	51.64		
T classification	T1	273	160	62.01	113	41.09	25.0397	0.000
	T2	69	30	11.63	39	14.18		
	T3	180	63	24.42	117	42.55		
	T4	11	5	1.94	6	2.18		
Vital status	Dead	160	205	79.46	168	61.09	21.3748	0.000
	Survival	373	53	20.54	107	38.91		

Table 3: Univariate and Multivariate analysis of Over Survival in patients with KIRC.

	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI95	P value	Hazard Ratio	CI95	P value
Age	1.889	1.298-2.748	0.001	1.530	1.03-2.26	0.03
Gender	1.037	0.753-1.427	0.826	-	-	-
Histologic grade	2.056	1.711-2.471	0.000	1.520	1.226-1.884	0.000
Pathologic stage	1.958	1.708-2.245	0.000	2.051	1.406-2.992	0.000
M classification	2.500	1.949-3.207	0.000	1.005	0.620-1.623	0.982
N classification	0.861	0.736-1.008	0.063	-	-	-
T classification	2.067	1.741-2.455	0.000	0.872	0.590-1.289	0.260
PRPF8	1.779	1.278-2.475	0.001	1.435	1.034-2.734	0.037

different stages (Figure 2).

Low *PRPF8* expression serves as an independent predictor for poor OS in patients with KIRC

To assess the effect of *PRPF8*expression on Overall Survival (OS), we generated two Kaplan-Meier curves according to *PRPF8* expression. We found that low *PRPF8* expression was closely associated with worse OS in all tumor patients. Furthermore, the subgroup analysis proved that low *PRPF8* expression significantly correlated with poor OS of patients who was in female subgroup, histologic grade G3/G4/$G_X$subgroup, N0 subgroup, N1/Nx subgroup, M0 subgroup, and age (<55) sub group. Univariate Cox analysis showed that age, histologic group, pathologic stage, M classification, T classification and *PRPF8* expression was significantly associated with OS of patients with kidney renal clear cell carcinoma. Multivariate Cox analysis showed that *PRPF8* expression was an independent biomarker for OS of

Table 4: Univariate and Multivariate analysis of Relapse-Free Survival in patients with KIRC.

	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI 95	P value	Hazard Ratio	CI 95	P value
Age	1.906	1.304-2.786	0.001	1.535	1.042-2.263	0.03
Gender	1.049	0.760-1.448	0.769	-	-	-
Histologic grade	2.056	1.711-2.471	0.000	1.533	1.235-1.903	0.000
Pathologic stage	1.949	1.699-2.236	0.000	2.051	1.406-2.992	0.000
M classification	2.500	1.949-3.207	0.000	1.006	0.620-1.632	0.982
N classification	0.862	0.736-1.010	0.067	-	-	-
T classification	2.046	1.722-2.431	0.000	0.872	0.590-1.289	0.491
PRPF8	1.777	1.277-2.473	0.001	1.209	1.04-1.41	0.029

kidney renal clear cell carcinoma patients (Hazard Ratio (HR)=1.435, 95% Confidence Interval (CI): 1.034-2.734, P=0.037) (Figure 3).

Low *PRPF8* expression serves as an independent predictor for poor RFS in patients with KIRC

To assess the effect of *PRPF8* expression on relapse-free survival (RFS), we generated two Kaplan-Meier curves according to *PRPF8* expression. We found that low *PRPF8* expression was closely associated with worse RFS in all tumor patients. Furthermore, the subgroup analysis proved that low *PRPF8* expression significantly correlated with poor RFS of patients who was in male subgroup, histologic grade G3/G4/$G_X$subgroup, N1/Nx subgroup, and age (<55) subgroup. Univariate Cox analysis showed that age, histologic group, pathologic stage, M classification, T classification and *PRPF8* expression was significantly associated with RFS of patients with kidney renal clear cell carcinoma. Multivariate Cox analysis showed that *PRPF8* expression was an independent biomarker for RFS of kidney renal clear cell carcinoma patients (Hazard Ratio (HR)=1.209, 95% confidence interval (CI): 1.04-1.41, P=0.029) (Figure 4 and Table 4).

Discussion

In this study, it is demonstrated that *PRPF8* may be a potential biomarker in kidney renal clear cell carcinoma. By analyzing the TCGA-KIRC data, we revealed that *PRPF8* is down-regulated in KIRC. In addition, the expression of *PRPF8* varies according to histologic grade, pathologic stage, T classification, M classification and vital status. What's more, patients with low *PRPF8* expression are associated with poor prognosis. The low *PRPF8* expression has excellent clinical diagnostic value and can be used as a predictor of poor prognosis in patients with KIRC by Univariate and multivariate Cox regression analysis.

Many studies have reported the mutations of *PRPF8* in the alternative splice process can lead to many diseases. Mutations in *PRPF8* are known to be associated with human type 13 autosomal dominant mitochondrial retinal pigment [18,19]. In addition, the reduction of *PRPF8* expression led to increased cell proliferation in K562 cells and CD34+ cells [20]. This is consistent with our findings. It has been reported that the expression of *PRPF8* in a variety of breast cancer cells and found *PRPF8* was elevated in the breast cancer cells examined [21]. This is not consistent with our findings that *PRPF8* expression is lower in tissues of KIRC. The reason may be the cancer types and sample types studied were different, one is breast cancer cell, and the other one is kidney renal clear cell carcinoma tissues. In this study, we observed that the expression of *PRPF8* gradually decreased from G1 to G4, from I to IV, from T1 to T3, from M1 to Mx, which suggested *PRPF8* might affect the cancer progression and its expression could indicate the degree of disease progression. To our surprise, the expression of *PRPF8* increased from G4 to Gx, from I to IV, from T3 to T4. We assume that the *PRPF8* expression functions as different role in different stage of patients. In addition, we also found the expression of *PRPF8* was associated with the vital status, which revealed the *PRPF8* may associate with the survival of patients in kidney renal clear cell carcinoma.

The role of *PRPF8* in tumor development has been reported. The increased *PRPF8* expression promoted proliferation and inhibited apoptosis in ovarian cancer cells [22]. Silencing *PRPF8* resulted in cell death in both breast cancer cell lines (Cal 51 and HCC 1954) and colon cancer cell lines (HCT116 and DLD1) [23,24]. Moreover, the silencing of *PRPF8* leads to cancer cell apoptosis, including liver cancer [25], ovarian cancer [26] and breast cancer [27]. This is not consistent with what we have seen in renal cancer using TCGA. We need to verify it with experiments. To a certain extent, the role of *PRPF8* in the proliferation and apoptosis of kidney renal clear carcinoma is provided. In our study, we found the *PRPF8* expression was associated with the survival of patients and the patients with lower *PRPF8* expression had a poorer overall survival and the relapse-free survival, especially in female, histologic grade G3/G4/Gx, N classification N0/N1/Nx, M classification M0 and age≤55 subgroup of overall survival and in male, histologic grade G3/G4/Gx, N classification N1/Nx and age ≤55 subgroup of relapse-free survival. Clinically, this could be applied to more precise drug administration and personalized treatment according to the patient's clinical information.

Conclusion

We first use TCGA database to analyze the expression pattern of *PRPF8* in kidney renal clear cell carcinoma and the potential significance of *PRPF8* for diagnosis and prognosis of patients clinically. In the future, relevant verification will be carried out through clinical sample experiments that are what we're focusing on in the future.

Acknowledgements

This work was supported by grants from 2016YFD0501001

(National Key Research and Development Program of China). Thanks to TCGA database for providing the data for free.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 2018; 68: 394-424.
2. Hu F, Zeng W, Liu X. A Gene Signature of Survival Prediction for Kidney Renal Cell Carcinoma by Multi-Omic Data Analysis. 2019; 20: 5720.
3. Rathmell WK, Rathmell JC, Linehan WM. Metabolic Pathways in Kidney Cancer: Current Therapies and Future Directions. 2018: 36: 3540-3546.
4. Linehan WM, Ricketts CJ. The Cancer Genome Atlas of renal cell carcinoma: findings and clinical implications [J]. Nature Reviews Urology. 2019; 16: 539-552.
5. Moch H, Cubilla AL, Humphrey PA, et al. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. Eur Urol. 2016; 70: 93-105.
6. Hsieh JJ, Purdue MP, Signoretti S, et al. Renal cell carcinoma. 2017; 3: 17009.
7. Huang MJ, Zhang T, Yao ZY, et al. MicroRNA related prognosis biomarkers from high throughput sequencing data of kidney renal clear cell carcinoma. Bmc Medical Genomics. 2021; 14: 9.
8. Feng CY, Huang XL, Li XK, et al. The Roles of Base Modifications in Kidney Cancer. Front Oncol. 2020; 10: 6.
9. Yan C, Hang J, Wan R, et al. Structure of yeast spliceosome at 3.6-angstrom resolution. Science (New York, NY). 2015; 349: 1182-1191.
10. Bertram K, Agafonov DE, Liu WT, et al. Cryo-EM structure of a human spliceosome activated for step 2 of splicing. Nature, 2017; 542: 318-323.
11. Kurtovic-Kozaric A, Przychodzen B, Singh J, et al. *PRPF8* defects cause missplicing in myeloid malignancies. Leukemia. 2015; 29: 126-136.
12. Onyango DO, Lee G, Stark JM. *PRPF8* is important for BRCA1-mediated homologous recombination. Oncotarget. 2017; 8: 93319-93337.
13. Natalia H-S, Juan LL-C, Antonio CF-F, et al. *PRPF8* regulates FAK/AKT pathway and cytoskeleton remodeling through modulation of fibronectin 1 splicing in liver pathologies; proceedings of the Endocrine Abstracts F . Bioscientifica. 2021.
14. Lv C, Li Y, Zhang Q, et al. Low REST Expression Indicates a Biomarker of Poor Prognosis in Patients with Renal Cell Carcinoma. BioMed Research International. 2021: 2021.
15. Chen P, Zhang Q, Li Y, et al. CREBBP is a Novel Biomarker for Diagnostic and Prognostic of Patients with Renal Cell Carcinoma. Medical & Clinical Research. 2021; 6: 527-534.
16. Liu J, Li Y, Zhang Q, et al. PVT1 Expression Is a Predictor for Poor Survival of Prostate Cancer Patients. Technology in Cancer Research and Treatment. 2021; 20: 1533033820971610.
17. Jiao Y, Li Y, Ji B, et al. Clinical value of lncRNA LUCAT1 expression in liver cancer and its potential pathways. Journal of Gastrointestinal and Liver Diseases. 2019: 28; 439-447.
18. Maubaret CG, Vaclavik V, Mukhopadhyay R, et al. Autosomal dominant retinitis pigmentosa with intrafamilial variability and incomplete penetrance in two families carrying mutations in *PRPF8*. Investigative ophthalmology and visual science. 2011; 52: 9304-9309.
19. Xu G, Li T, Chen J, et al. Autosomal dominant retinitis pigmentosa-associated gene *PRPF8* is essential for hypoxia-induced mitophagy through regulating ULK1 mRNA splicing. Autophagy. 2018; 14: 1818-1830.
20. Kurtovic-Kozaric A, Przychodzen B, Singh J, et al. *PRPF8* defects cause missplicing in myeloid malignancies. Leukemia. 2015; 29: 126-136.
21. Cao Difei, Huang Guoqing, Xue Jiaying, et al. The Role of *PRPF8* Protein in Alternative Splicing of Eukaryotes. Chinese Herbivore Science. 2021; 41: 49-51.
22. Xu Q, Deng B, Li M, et al. circRNA-UBAP2 promotes the proliferation and inhibits apoptosis of ovarian cancer though miR-382-5p/*PRPF8* axis %J Journal of Ovarian Research. 2020; 13: 81.
23. Allende-Vega N, Dayal S, Agarwala U, et al. p53 is activated in response to disruption of the pre-mRNA splicing machinery. Oncogene. 2013; 32: 1-14.
24. Rines DR, Gomez-Ferreria MA, Zhou Y, et al. Whole genome functional analysis identifies novel components required for mitotic spindle integrity in human cells. Genome Biol. 2008; 9: 12.
25. Fan J, Zhang YQ, Li P, et al. Interaction between plasminogen activator inhibitor type-2 and pre-mRNA processing factor 8 . Acta Biochim Biophys Sin. 2004; 36: 623-628.
26. Xu Q, Deng B, Li M, et al. circRNA-UBAP2 promotes the proliferation and inhibits apoptosis of ovarian cancer though miR-382-5p/*PRPF8* axis. Journal of ovarian research. 2020; 13: 1-10.
27. Chan S, Sridhar P, Kirchner R, et al. Basal-A triple-negative breast cancer cells selectively rely on RNA splicing for survival. Molecular cancer therapeutics. 2017; 16: 2849-2861.