Original Article

High PRAS40 mRNA expression and its role in prognosis of clear cell renal cell carcinoma

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Background: Clear cell renal cell carcinoma (ccRCC) is one of the most common type of kidney malignancy. The proline-rich Akt substrate of 40 kDa (PRAS40) plays an important role in tumor growth. The present study aimed to analysis the prognostic value of PRAS40 mRNA expression in ccRCC.

Methods: We analyzed the PRAS40 mRNA expression using the data from TCGA-KIRC cohort. A receiver operating characteristic (ROC) curve was performed to assessed the diagnostic value of PRAS40 mRNA expression in ccRCC. Chi-square test was used to analyzed the correlation between clinical characteristics and PRAS40 mRNA expression. Kaplan-Meier analysis and Cox analysis were performed to determine the prognostic value of PRAS40 mRNA expression in ccRCC. Gene set enrichment analysis (GSEA) was conducted using TCGA database.

Results: Our results revealed that PRAS40 mRNA expression was higher in ccRCC tissues than in normal tissues. PRAS40 presented a moderate diagnostic value in ccRCC. High PRAS40 mRNA expression was correlated with histological grade, clinical stage, T classification, distant metastasis and vital status of ccRCC. High PRAS40 mRNA expression was associated with poor overall survival. Furthermore, Multivariate analysis revealed that PRAS40 was an independent risk factor for ccRCC patients. Myc targets, DNA repair, oxidative phosphorylation, glycolysis, adipogenesis, p53 pathway, reactive oxygen species pathway, myogenesis were differentially enriched in the phenotype that positively correlated with PRAS40.

Conclusions: In conclusion, our results suggest that PRAS40 was a promising diagnostic and prognostic biomarker for ccRCC.

Keywords: PRAS40; TCGA; bioinformatics; diagnosis; prognosis; clear cell renal cell carcinoma (ccRCC)

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Introduction

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney malignancy tumor, representing about 80% of all primary malignant kidney tumors (1-5). In most cases, ccRCC is resistant to radiation, cytotoxic and hormone therapies, and surgery is the main treatment for ccRCC (5,6). 60% ccRCC patients die in the first 2–3 years, and 30% of ccRCC patients diagnosed with metastases (2). Therefore, searching for new sensitive biomarkers are important for early diagnosis of ccRCC.

PRAS40 also known as AKT1S1 (AKT serine/threonine kinase 1 substrate 1) was initially identified as a 14-3-3 binding protein (7). PRAS40 has two proline-enriched stretches at the N-terminal region (8). PRAS40 dynamically shifts between the nucleus and the cytoplasm. In the nucleus, PRAS40 forms a nuclear-specific complex with PRL11 (ribosomal protein L11) and contributes to senescence

Table 1 Clinical characteristics of the ccRCC patients

Clinical characteristics	Variable	Total (N=533)	%
Age	<55 years	173	32.50
	≥55 years	360	67.50
Gender	Male	345	64.70
	Female	188	35.30
Grade	G1	14	2.60
	G2	229	42.96
	G3	206	38.64
	G4	76	15.80
Stage	I	267	50.00
	II	57	10.69
	III	123	23.07
	IV	83	15.57
	Unknow	3	0.67
Distant metastasis	M0	422	79.47
	M1	79	14.87
	Mx	30	5.66
Lymph nodes	N0	240	45.19
	N1	16	3.01
	NX	275	51.78
Survival status	Death	173	32.45
	Survival	360	67.54
T classification	T1	271	51.04
	T2	69	13.00
	T3	180	33.89
	T4	11	2.07

and radio resistance (9). In the cytoplasm, PRAS40 is an important interacting partner of mTORC1 (the mechanistic target of rapamycin complex 1) and negatively regulates mTORC1 signaling (10,11). PRAS40 is a substrate of Akt and mediates the PI3K (phosphatidylinositol 3-kinase)/Akt signaling pathway (12). PRAS40 has many phosphorylation sites, and its function was determined by its phosphorylation (13,14). PRAS40 was phosphorylated at T246 by Akt and at S183 by mTORC1 (15). It has been reported that PRAS40 was upregulated in many tumors, including gastric cancer, prostate cancer, *etc*. (16,17). However, the correlations between clinical characteristics and PRAS40 mRNA expression of ccRCC patients remains to be investigated.

In this study, we compared the PRAS40 mRNA expression between ccRCC tissues and normal tissues. We evaluated the correlations between clinical characteristics and PRAS40 mRNA expression of ccRCC patients. We investigated the biological pathways that related to PRAS40 using GSEA analysis. Our results suggest that PRAS40 was a promising diagnostic and prognostic biomarker for ccRCC.

Methods

Gene expression and clinical characteristics in TCGA

The relevant data provided by TCGA are publicly available and open-ended, and do not require the approval of the local ethics committee. TCGA_KIRC HiSeqV2_PANCAN data were obtained from TCGA database (https://tcga.xenahubs.net). The levels of PRAS40 mRNA, clinicopathological details and general information of ccRCC were collected.

GSEA

In this study, we analyzed the correlations between PRAS40 mRNA expression and all genes by R (v.3.6.1), then performed GSEA analysis using the cluster Profiler package in R. |ES|>1, P<0.05, and FDR <0.25 were considered to be statistically significant.

Statistical analysis

All statistical analysis was conducted by R. ROC curve was generated using the pROC package. We analyzed the correlations between clinical characteristics and PRAS40 mRNA expression using chi-square test. Kaplan-Meier analysis and Multivariate cox analysis was used to analyzed the prognostic value of PRAS40 mRNA expression. P<0.05 was considered statistically significant.

Results

Clinical characteristics of the ccRCC patients

Clinical and gene expression data of 533 primary tumors and 73 normal samples were download from TCGA database, including patients' age, gender, grade, overall survival, stage, distant metastasis, survival status, T classification and lymph nodes (*Table 1*).

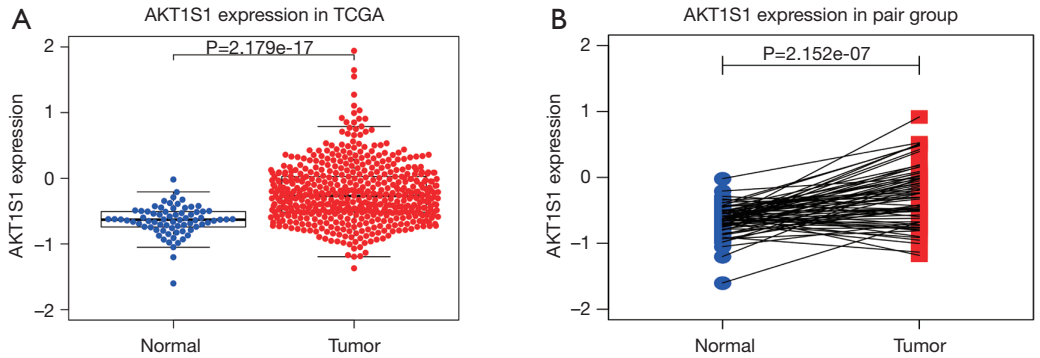

Figure 1 PRAS40 mRNA expression in ccRCC patients. (A) PRAS40 mRNA expression in ccRCC tissues and normal tissues (P=2.179e-17). (B) PRAS40 mRNA expression in ccRCC tissues and adjacent tissues (P=2.15e-07).

High PRAS40 expression in ccRCC patients

We analyzed the transcription levels of PRAS40 based on TCGA database. We found that PRAS40 mRNA expression was significantly higher in ccRCC tissues than that in normal tissues (P=2.179e-17, P=2.152e-07, respectively) (*Figure 1*). Furthermore, different PRAS40 mRNA expression were observed in groups based on T classification, clinical stage, distant metastasis and histological grade. The PRAS40 mRNA expression of patients with T3/T4 classification were higher than that of patients with T1/T2 classification (P=6.139e-04). Patients who were in high histological stage had higher PRAS40 mRNA expression than patients who were in low histological stage (P=8.262e-06). Patients with a positive distant metastasis had higher PRAS40 mRNA expression than patients with a negative status. High grade groups (G3/G4) had higher PRAS40 mRNA expression than low grade groups (G1/G2) (P=1.86e-06) (*Figure 2*).

Diagnostic value of PRAS40 mRNA expression in ccRCC

In this study, we evaluated the diagnostic value of PRAS40 mRNA expression by ROC curve. The results showed that the Area Under the Curve (AUC) of PRAS40 was 0.808. We also analyzed the diagnostic capability of PRAS40 mRNA expression in different stages, and the results showed similar diagnostic value with AUC values of 0.791, 0.714, 0.831 and 0.884 for stage I, II, III and IV, respectively (*Figure 3*).

Correlation between clinical characteristics and PRAS40 mRNA expression of ccRCC

In this study, correlations between clinical characteristics and PRAS40 mRNA expression in ccRCC were analyzed by chi-square test. Based on medium PRAS40 mRNA expression, we divided into high or low group. Our results showed that high PRAS40 mRNA expression was associated with T classification (P=0.0033), survival status (P=2.391e-05), histological grade (P=0.004), distant metastasis (P=5.24e-05) and clinical stage (P=0.0001), respectively (*Table 2*).

High PRAS40 mRNA expression is an independent risk factor for overall survival in ccRCC patients

Kaplan-Meier analysis revealed that higher PRAS40 mRNA expression was correlated with poor overall survival (P=4.825e-05). Subgroup analysis indicated that PRAS40 mRNA overexpression was significantly affected the overall survival in ccRCC cases of M0 (P=6.155e-04), histological grade G3/G4 (P=0.002), N0 (P=0.026), T1/T2 (P=0.025), T3/T4 (P=0.033) and clinical stage III/IV (P=0.015), respectively (*Figure 4*). The univariate analysis revealed that higher PRAS40 mRNA expression, advanced stage, high grade, distant metastasis and positive lymph nodes were correlated with overall survival (*Table 3*). Multivariate analysis showed that PRAS40 mRNA expression was an independent risk factor for overall survival in ccRCC (*Table 3*).

GSEA identifies a PRAS40-related signaling pathway

GSEA was performed to identify the signaling pathways that activated in ccRCC. The results showed that myc targets, DNA repair, oxidative phosphorylation, glycolysis, adipogenesis, p53 pathway, reactive oxygen species pathway and myogenesis were differentially enriched in the positively

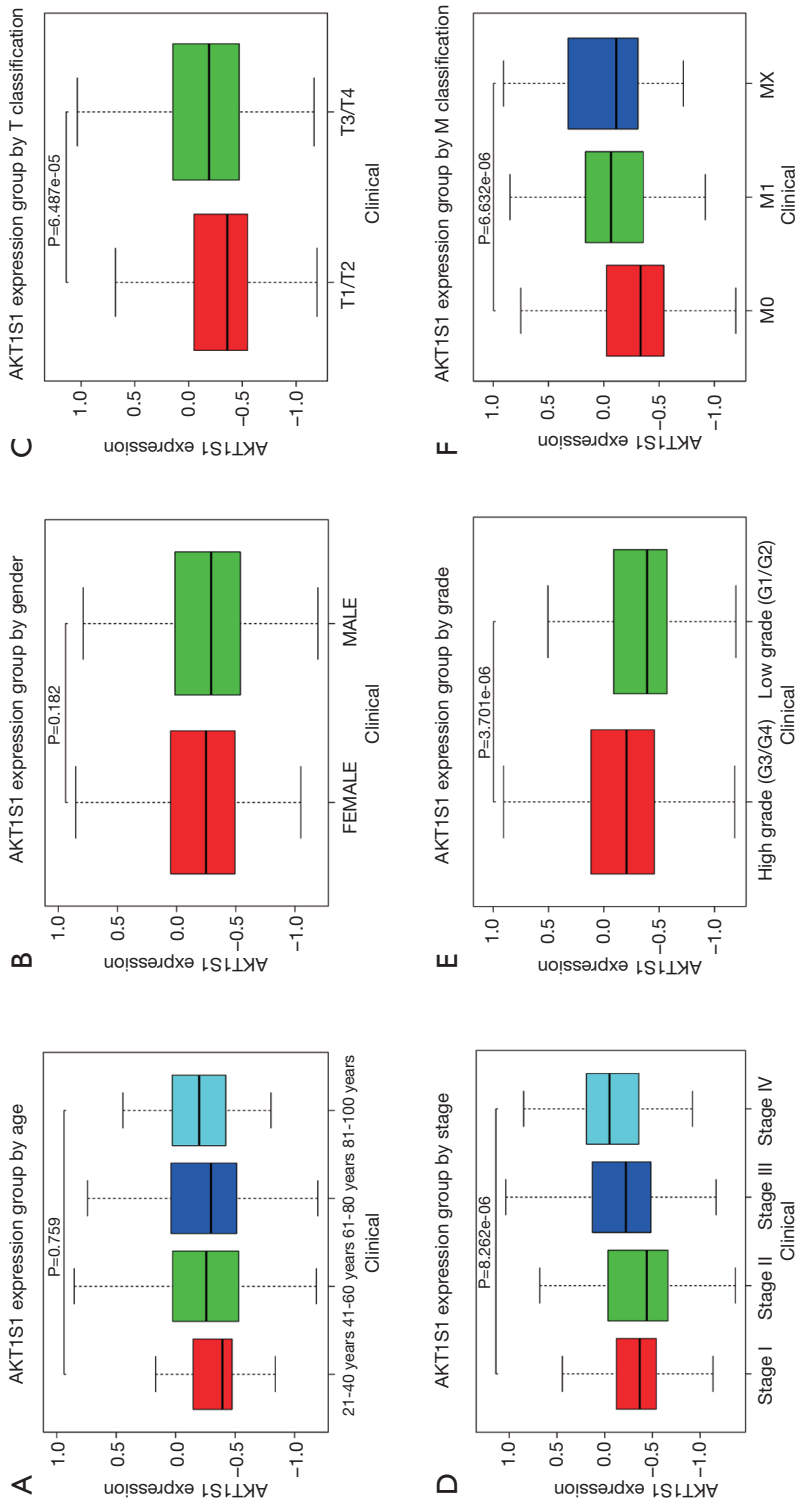

Figure 2 Association with PRAS40 mRNA expression and clinicopathologic characteristics. (A) Age (P=0.75), (B) Gender (P=0.182), (C) T classification (P=6.139e-04), (D) Clinical stage (P=8.262e-06), (E) Histologic grade (P=1.86e-06), (F) Distant metastasis (P=6.32e-06).

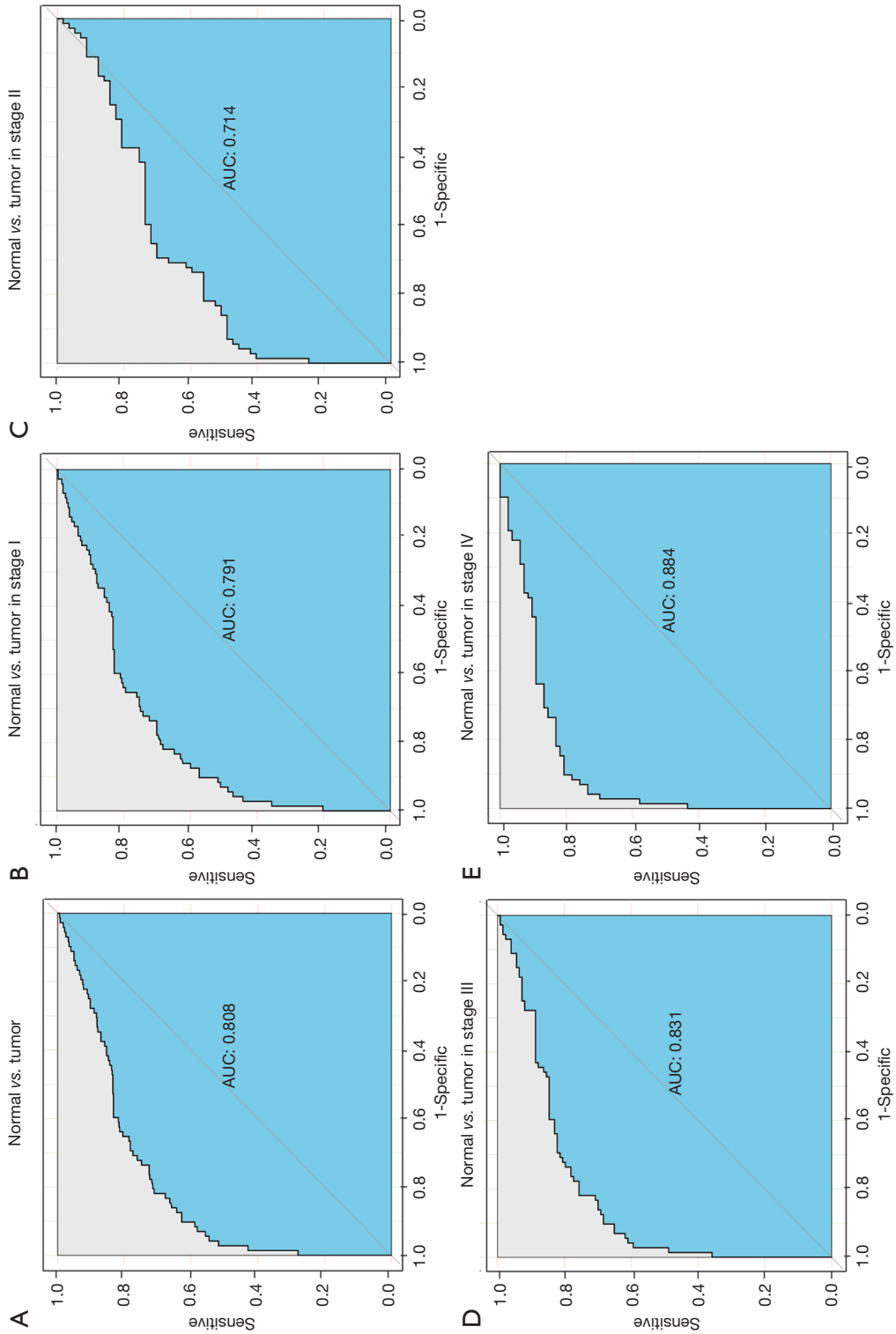

Figure 3 ROC curve of PRAS40 mRNA expression in ccRCC cohort. (A) ROC curve of PRAS40 mRNA expression in normal and tumor, (B-E) Subgroup analysis for stage I, II, III and IV, respectively.

Table 2 Relationship between PRAS40 mRNA expression and clinical characteristics in ccRCC

Clinical characteristics	Variable	N	PRAS40 mRNA		χ^2	P
			High (%)	Low (%)		
Age	<55 years	173	81 (46.8)	92 (53.2)	0.801	0.370
	≥55 years	360	185 (53.6)	175 (46.4)		
Gender	Male	345	165 (47.8)	180 (52.2)	1.465	0.226
	Female	188	101 (53.7)	87 (46.3)		
Grade	G1	14	5 (35.7)	9 (64.3)	17.065	0.004
	G2	229	99 (43.2)	130 (56.8)		
	G3	206	109 (52.9)	97 (47.1)		
	G4	76	51 (67.1)	25 (32.9)		
	Gx	8	2 (25.0)	6 (75.0)		
Stage	I	267	114 (42.7)	153 (57.3)	22.078	0.0001
	II	57	24 (42.1)	33 (57.9)		
	III	123	69 (56.1)	54 (43.9)		
	IV	83	56 (67.4)	27 (32.6)		
	Unknow	3	3 (100.0)	0		
Distant metastasis	M0	422	189 (44.8)	233 (55.2)	22.347	5.24e-05
	M1	79	53 (67.1)	26 (32.9)		
	Mx	2	2 (100.0)	0		
Lymph nodes	N0	240	115 (47.9)	125 (52.1)	1.505	0.471
	N1	16	10 (62.5)	6 (37.5)		
	NX	277	141 (50.9)	136 (49.1)		
T classification	T1	273	117 (42.9)	156 (57.1)	13.697	0.0033
	T2	69	34 (49.3)	35 (50.7)		
	T3	180	109 (60.5)	71 (39.5)		
	T4	11	6 (54.5)	5 (45.5)		
Survival status	Death	173	107 (61.8)	61 (38.2)	17.849	2.391e-05
	Survival	360	159 (44.2)	206 (55.8)		

correlated with PRAS40 mRNA expression phenotype (*Table 4*, *Figure 5*).

Discussion

In this study, our results confirmed that PRAS40 mRNA expression was higher in ccRCC tissues than in normal tissues. High PRAS40 mRNA expression was associated with survival status, distant metastasis, clinical stage, T classification and histological grade, respectively. Moreover, high PRAS40 mRNA expression correlated with poor overall survival. In addition, PRAS40 was an independent prognostic factor of ccRCC.

PRAS40 was aberrantly expressed or hyperphosphorylated in a variety of tumors (16-18). The most common type of PRAS40 alteration is overexpression, PRAS40 was overexpressed in breast cancer, melanoma, colon cancer, etc., but not found in ccRCC yet (19-21). Yuan *et al*. reported

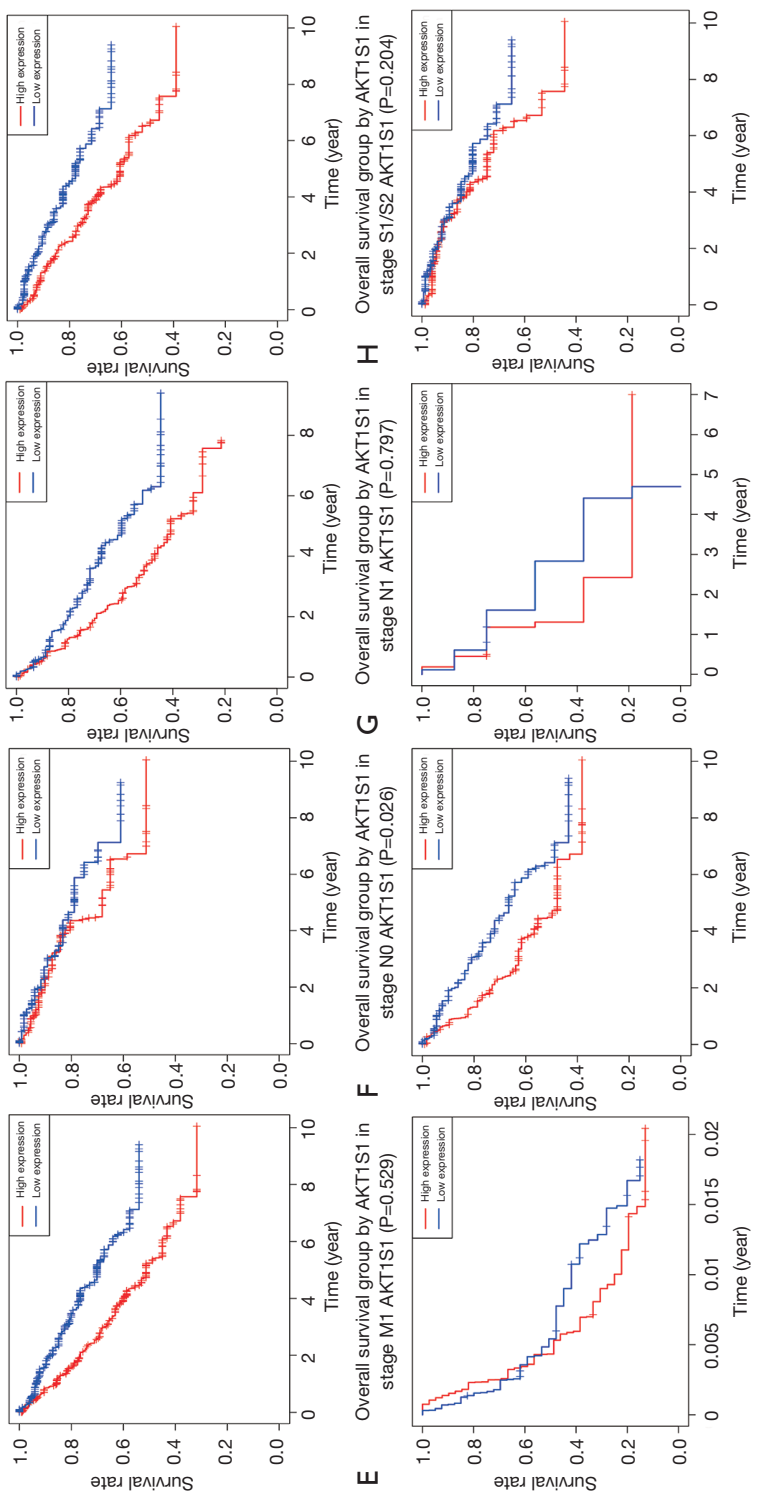

Figure 4 Overall survival analysis with PRAS40 mRNA expression. (A) Kaplan-Meier curves for overall survival in ccRCC for all cases, (B) G1/G2, (C) G3/G4, (D) M0, (E) M, (F) N0, (G) N1, (H) clinical stage I/II, (I) clinical stage III/IV, (J) T1/T2, (K) T3/T4.

Table 3 Correlations between overall survival and mRNA expression of PRAS40 analyzed by univariate and multivariate Cox regression

Clinical characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age	1.02	1.0–1.046	0.01	1.02	1.00–1.04	5.60e-04
Gender	1.02	0.67–1.55	0.90			
Grade	2.19	1.65–2.92	6.05e-08			
Stage	1.83	1.52–2.21	2.18e-10			
T classification	1.90	1.51–2.40	4.54e-08			
M classification	4.03	2.61–6.22	3.31e-10			
N classification	2.90	1.50–5.62	1.40e-03	3.16	1.62–6.16	6.80e-04
AKT1S1	1.62	1.10–2.39	0.014	1.53	1.04–2.26	0.03

Table 4 Gene sets enriched in positively correlated with PRAS40 mRNA expression phenotype

Gene set name	NES	MOM p-val	FDR q-val
HALLMARK_MYC_TARGETS_V2	2.192	0.001	0.008
HALLMARK_DNA_REPAIR	2.099	0.001	0.008
HALLMARK_OXIDATIVE_PHOSPHORYLATION	2.117	0.001	0.008
HALLMARK_GLYCOLYSIS	1.660	0.001	0.008
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	1.595	0.006	0.022
HALLMARK_ADIPOGENESIS	1.532	0.003	0.008
HALLMARK_P53_PATHWAY	1.530	0.003	0.008
HALLMARK_MYC_TARGETS_V1	1.486	0.004	0.011
HALLMARK_MYOGENESIS	1.471	0.008	0.015

that approximately 40% of trastuzumab resistant HER2-positive patients with p-PRAS40 Thr246 overexpression, and increased risk of tumor progression (22). Ma *et al*. found that PRAS40 was upregulated and reduction of PRAS40 expression inhibit the proliferation of HCC (23). PRAS40 was overexpressed in Ewing sarcoma family tumors (ESFT), and silence PRAS40 inhibited the proliferation of ESFT cell (24). Consistently, our results revealed that higher PRAS40 mRNA expression in ccRCC. ROC analysis revealed that PRAS40 present a moderate diagnostic value, providing evidence that PRAS40 was a promising biomarker for the diagnosis of ccRCC.

High PRAS40 mRNA expression was significantly correlated with poor prognosis in different tumors (7). Phospho-PRAS40 Thr246 was overexpressed in gastric cancers (17). PRAS40 deletions suppresses the invasion and migration of ESFT and hepatocellular carcinoma (HCC) cells (24,25). Consistently, we found that PRAS40 mRNA expression was significantly associated with poor overall survival, we further investigated the relation between clinical characteristics and PRAS40 mRNA expression in ccRCC patients, we found that histological grade, clinical stage, T classification were highly correlated with PRAS40 mRNA expression. The potential mechanism may involve in myc targets, DNA repair, oxidative phosphorylation, glycolysis, adipogenesis, p53 pathway, reactive oxygen species pathway and myogenesis.

Overall, our study verified the value of PRAS40 mRNA expression in diagnosis and prognosis of ccRCC patients. However, there are some limitations. First, we only conducted bioinformatics mining, our results should be verified in clinical samples. The second limitation is that the

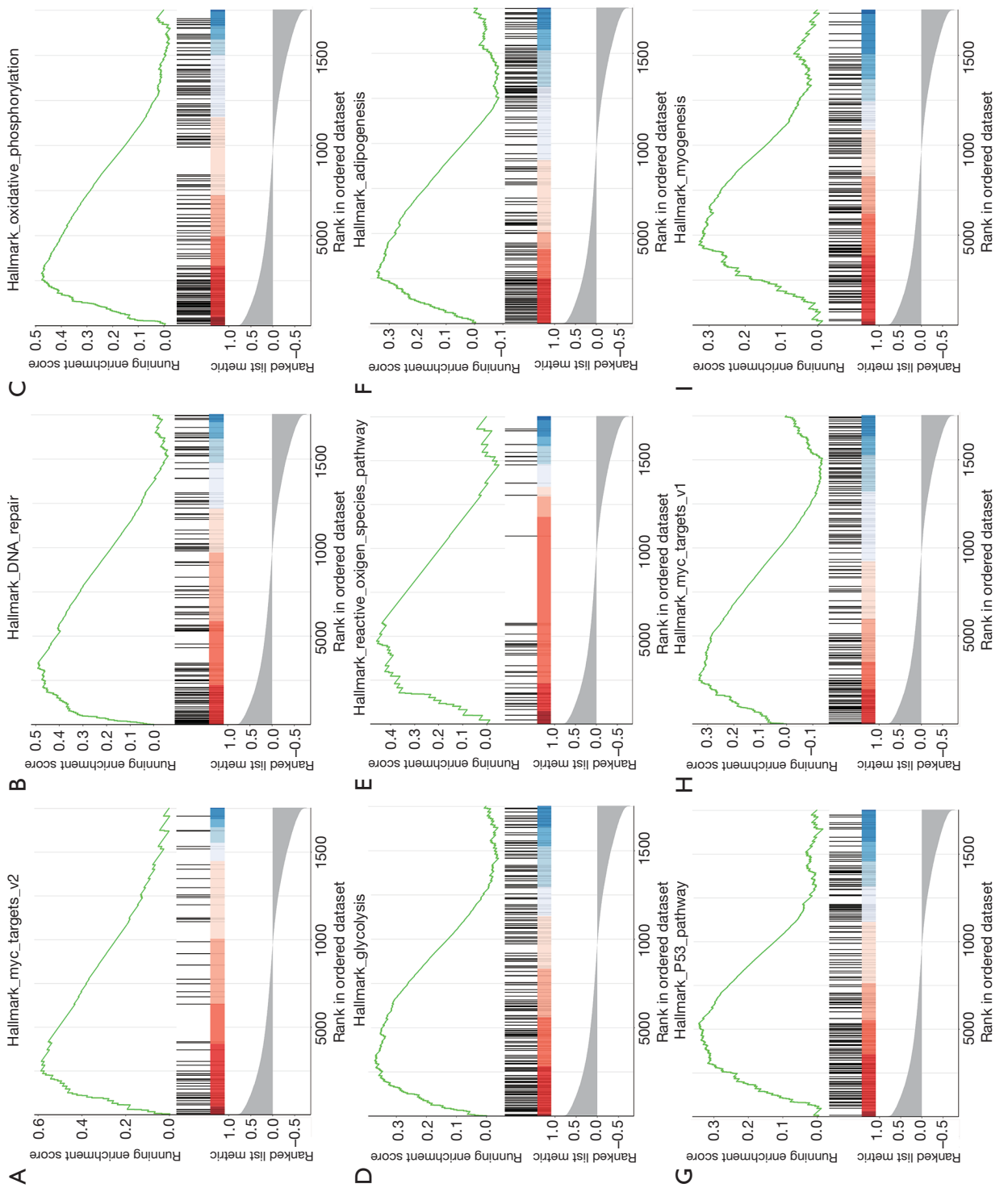

Figure 5 Enrichment plots by GSEA. (A) Myc targets_v2, (B) DNA repair, (C) oxidative phosphorylation, (D) glycolysis, (E) oxygen species pathway, (F) adipogenesis, (G) P53 pathway, and (H) Myc target_v1, (I) myogenesis.

current study focuses on the transcription level of PRAS40 but not protein level, the specific mechanism of PRAS40 in ccRCC should be further investigated.

Conclusions

Our results confirmed that PRAS40 mRNA expression was upregulated in ccRCC tissues. Moreover, higher PRAS40 mRNA expression was correlated with poor overall survival of ccRCC patients. In addition, PRAS40 mRNA expression was an independent prognostic factor of ccRCC, making it a promising biomarker with great potential in the future.

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Footnote

Data Sharing Statement: Available at http://dx.doi.org/10.21037/tau-20-741

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Conflict of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tau-20-741). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The relevant data provided by TCGA are publicly available and open-ended, and do not require the approval of the local ethics committee.

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