



Increased Expression of *TICRR* Predicts Poor Clinical Outcomes: A Potential Therapeutic Target for Papillary Renal Cell Carcinoma

Shuang Xia^{1†}, Yan Lin^{2†}, Jiaqiong Lin³, Xiaoyong Li⁴, Xuexian Tan⁵ and Zena Huang^{6*}

¹ Department of Cardiology, Guangdong Cardiovascular Institute, Guangdong Provincial Key Laboratory of Coronary Heart Disease Prevention, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China, ² Department of Nephrology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, ³ Department of Medical Genetics, School of Basic Medical Sciences, Southern Medical University, Guangzhou, China, ⁴ Department of Surgery, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, ⁵ Department of Surgery, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, ⁶ Department of Surgery, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, ⁶ Department of Pathology, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, ⁶ Department of Medical Sciences, Guangzhou, China, ⁶ Depart

OPEN ACCESS

Edited by:

Yanni Sun, City University of Hong Kong, Hong Kong

Reviewed by:

Xiangqian Guo, Henan University, China Ka-Chun Wong, City University of Hong Kong, Hong Kong

*Correspondence: Zena Huang 506647168@qq.com †These authors have contributed

equally to this work

Specialty section:

This article was submitted to Computational Genomics, a section of the journal Frontiers in Genetics

Received: 12 September 2020 Accepted: 07 December 2020 Published: 11 January 2021

Citation:

Xia S, Lin Y, Lin J, Li X, Tan X and Huang Z (2021) Increased Expression of TICRR Predicts Poor Clinical Outcomes: A Potential Therapeutic Target for Papillary Renal Cell Carcinoma. Front. Genet. 11:605378. doi: 10.3389/fgene.2020.605378 **Background:** Papillary renal cell carcinoma (PRCC), although the second-most common type of renal cell carcinoma, still lacks specific biomarkers for diagnosis, treatment, and prognosis. TopBP1-interacting checkpoint and replication regulator (*TICRR*) is a DNA replication initiation regulator upregulated in various cancers. We aimed to evaluate the role of *TICRR* in PRCC tumorigenesis and prognosis.

Methods: Based on the Kidney Renal Papillary cell carcinoma Project (KIRP) on The Cancer Genome Atlas (TCGA) database, we determined the expression of *TICRR* using the Wilcoxon rank sum test. The biological functions of *TICRR* were evaluated using the Metascape database and Gene Set Enrichment Analysis (GSEA). The association between *TICRR* and immune cell infiltration was investigated by single sample GSEA. Logistic analysis was applied to study the correlation between *TICRR* expression and clinicopathological characteristics. Finally, Cox regression analysis, Kaplan–Meier analysis, and nomograms were used to determine the predictive value of *TICRR* on clinical outcomes in PRCC patients.

Results: *TICRR* expression was significantly elevated in PRCC tumors (P < 0.001). Functional annotation indicated enrichment with negative regulation of cell division, cell cycle, and corresponding pathways in the high *TICRR* expression phenotype. High *TICRR* expression in PRCC was associated with female sex, younger age, and worse clinical stages. Cox regression analysis revealed that *TICRR* was a risk factor for overall survival [hazard ratio (HR): 2.80, P = 0.002], progression-free interval

1

(HR: 2.86, P < 0.001), and disease-specific survival (HR: 7.03, P < 0.001), especially in patients with male sex, age below 60 years, clinical stages II–IV and clinical T stage T1–T2.

Conclusion: Increased *TICRR* expression in PRCC might play a role in tumorigenesis by regulating the cell cycle and has prognostic value for clinical outcomes.

Keywords: TICRR, papillary renal cell carcinoma, clinical outcome, Treslin, immune infiltration

INTRODUCTION

Renal cell carcinoma (RCC) is a life-threatening cancer worldwide, ranking sixth among the most commonly diagnosed cancers in men and 10th in women (Siegel et al., 2018). Papillary RCC (PRCC) is the second most common type of RCC, accounting for nearly 18% of RCC (Srigley et al., 2013). In addition, it is the most common histological subtype in pediatric RCC and has been reported in 18% of dialysis patients (Morabito et al., 2010). However, diagnosis, treatment, and prognosis of PRCC are now mostly based on histological features, whose subtyping remains unsatisfactory (Fernandes and Lopes, 2015). Recent studies have introduced several novel biomarkers for RCC diagnosis and prognosis, such as urine aquaporin-1 and perilipin-2 (Farber et al., 2017; Cao et al., 2018; Song et al., 2019). However, these studies were mostly carried out in patients with rough RCC or clear cell RCC, lacking specific result for PRCC. An immunohistochemical marker α -methylacyl coenzyme A racemase was used for identifying PRCC (Alshenawy, 2015), while it was unrelated with PRCC prognosis. Several mutated genes were proved to be associated with PRCC diagnosis and treatment, including MET, NF2, SETD2, and Nrf2 pathway genes. Unfortunately, they were not sensitive enough, as they were only found in about 10 to 15% of PRCC (Akhtar et al., 2019). Therefore, it is urgent to search for a more convincing and suitable biomarker for PRCC.

TopBP1-interacting checkpoint and replication regulator (*TICRR*), also known as Treslin, is a critical DNA replication initiation regulator mediated by cyclin-dependent kinases and a DNA damage checkpoint (Boos et al., 2011). Biologically, *TICRR* regulates the cell cycle via determining S-phase progression from the expression level to epigenetic control (Charrasse et al., 2017; Maya-Mendoza et al., 2018) and thus promotes DNA replication. Overexpression of *TICRR* has been observed in several cancers, such as breast invasive carcinoma and liver hepatocellular carcinoma (Yu et al., 2019). It is associated with tumorigenesis, resistance to chemotherapy, and poor clinical outcomes (Yu et al., 2019). However, the potential role and underlying mechanism of *TICRR* in PRCC is not clear yet.

Using the RNA sequencing and clinical data of PRCC patients retrieved from The Cancer Genome Atlas (TCGA) database, we carried out a bioinformatics analysis to identify the significance of *TICRR* in PRCC tumorigenesis and prognosis. We observed an overexpression of *TICRR* in PRCC and investigated its potential role in PRCC tumorigenesis. Next, we performed a correlation analysis between *TICRR* and several clinicopathological characteristics. Finally, we identified

the diagnostic and prognostic values of *TICRR*. This study provides novel insight into the underlying mechanisms of PRCC tumorigenesis and revealed *TICRR* as a potential diagnostic and prognostic biomarker in PRCC.

MATERIALS AND METHODS

Data Processing and Ethics Statement

We downloaded high-throughput sequencing RNA data [fragments per kilobase per million (FPKM) format] and corresponding clinicopathological information from the Kidney Renal Papillary cell carcinoma Project (KIRP) on the TCGA database¹. Excluding three patients with incomplete clinicopathological information, a total of 288 PRCC patients were enrolled. RNA sequencing data were transformed from FPKM format to transcripts per million reads for this study. As the TCGA database is open to the public under specific guidelines, it confirms that all written informed consents were obtained before data collection.

Differentially Expressed Genes in Papillary Renal Cell Carcinoma Tumors

In total, 288 PRCC patients were separated into high- and low-*TICRR* expression groups according to *TICRR* median value. The R package "DESeq2"(Love et al., 2014) was used to identify differentially expressed genes (DEGs) between the two groups by a two-tailed hypothetical test based on the negative binomial generalized linear models, where the log-fold change larger than 1.5 and an adjusted *P*-value less than 0.05 were set as thresholds. The R packages "pheatmap" (Kolde, 2019) and "EnhancedVolcano" (Blighe, 2019) were applied to present results as heatmaps and volcano plots.

Functional Annotation of *TICRR*-Associated Differentially Expressed Genes in Papillary Renal Cell Carcinoma Tumors

The identified DEGs were then processed for functional annotation on the Metascape database² and online tool (Zhou et al., 2019). Minimum counts larger than 3, enrichment factors larger than 1.5, and a *P*-value less than 0.01 were set as analysis thresholds. Further, the R package "clusterProfiler" (Yu et al.,

¹https://portal.gdc.cancer.gov/

²http://metascape.org

2012) was utilized for the Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) of the DEGs in the two groups. In GSEA, C2: curated gene sets from MSigDB collections were selected as reference gene sets. In total, 404 clusters were identified; clusters with a false discovery rate (FDR) less than 0.25 and *P*-value less than 0.05 were identified as significant. Protein-protein interaction (PPI) networks were investigated based on STRING database³ (Szklarczyk et al., 2019) and visualized using Cytoscape software (v3.7.1) (Shannon et al., 2003).

Association of *TICRR* and Immune Cell Infiltration in Papillary Renal Cell Carcinoma Tumors

First, we used the single sample GSEA method from the R package "GSVA" (Hänzelmann et al., 2013) to present infiltration enrichment of 24 common immune cells, including dendritic cells (DCs), immature DCs (iDCs), activated DCs (aDCs),

plasmacytoid DCs (pDCs), T cells, T helper (Th) cells, type 1 Th cells (Th1), type 2 Th cells (Th2), type 17 Th cells (Th17), regulatory T cells (Treg), T gamma delta (Tgd), T central memory (Tcm), T effector memory (Tem), T follicular helper (Tfh), CD8 + T cells, B cells, neutrophils, macrophages, cytotoxic cells, mast cells, eosinophils, natural killer (NK) cells, NK 56- cells, and NK 56 + cells. Next, the association between *TICRR* expression and immune cell infiltration was evaluated by Spearman's analysis, and the infiltration levels of immune cells were compared for high- and low-*TICRR* expression groups by Wilcoxon rank sum test.

Correlation Analyses for *TICRR* Expression and Clinicopathological Characteristics of Papillary Renal Cell Carcinoma Patients

Clinicopathological characteristics were compared for highand low-*TICRR* expression groups using the Wilcoxon rank sum test (continuous variables) or Pearson's chi-square test



FIGURE 1 Differential mRNA expression profiles in papillary renal cell carcinoma (PRCC) patients stratified by *TICRR* levels. (A) The comparison of *TICRR* expression between tumor and pericarcinous tissue in different types of cancers based on TCGA database. ns, $P \ge 0.05$; *P < 0.05; *P < 0.01; ***P < 0.01; ***P < 0.01; (B) *TICRR* expression is higher in PRCC tumors than pericarcinous tissue. Based on the median *TICRR* level, 288 PRCC patients from The Cancer Genome Atlas –Kidney Renal Papillary cell carcinoma Project (TCGA-KIRP) were stratified into high- and low-*TICRR* expression groups. Shown are expression profiles of mRNA in two groups; and data are presented by volcano plots (C) and heatmaps (D).

³http://string-db.org



FIGURE 2 | Functional annotation of differentially expressed genes (DEGs) in papillary renal cell carcinoma (PRCC) patients with distinct *TICRR* levels. According to the Metascape database, 691 differentially expressed mRNAs between high- and low-*TICRR* expression groups were used for functional annotation. All statistically enriched terms were identified and then hierarchically clustered into a tree (A) based on the threshold of kappa score as 0.3. Representative terms from the cluster were converted into a network layout (B). The size of a node is proportional to the number of input genes that fall into that term, and the respective color represents its cluster identity. Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score). The same enrichment network presents nodes colored by the *P*-value (C). (D–O) Representative Gene Set Enrichment Analysis of differentially expressed mRNAs between high- and low-*TICRR* expression groups.



FIGURE 3 | Correlation of immune cell infiltration and *TICRR* expression in papillary renal cell carcinoma (PRCC) patients. (A) Relationships among infiltration levels of 24 immune cell types and *TICRR* expression profiles by Spearman's analysis. Shown is the comparison of infiltration levels of most correlated immune cells, including dendritic cells (B), neutrophils (C), macrophages (D), type 2 T helper cells (Th2) cells (E), Th cells (F), and Tcm memory cells (G) between high- and low-*TICRR* expression groups. DCs, dendritic cells; aDCs, activated DCs; iDCs, immature DCs; pDCs, plasmacytoid DCs; Th, T helper cells; Th1, type 1 Th cells; Th2, type 2 Th cells; Th17, type 17 Th cells; Treg, regulatory T cells; Tgd, T gamma delta; Tcm, T central memory; Tem, T effector memory; Tfh, T follicular helper; NK, natural killer.

(rank variables). The correlation between *TICRR* expression and clinicopathological characteristics was evaluated by logistic analysis.

Clinical Significance of *TICRR* Expression in Papillary Renal Cell Carcinoma

TICRR expression was compared between PRCC tumors and pericarcinous tissues by receiver operating characteristic (ROC)

analysis to test the predictive value of *TICRR* for PRCC diagnosis. Information on PRCC patients' clinical outcome was obtained from a published study (Liu et al., 2018), including overall survival, progression-free interval, and disease-specific survival. Kaplan–Meier (K-M) analysis, univariate, and multivariate Cox regression analysis were employed for prognosis analysis. The R package "randomForest" (Svetnik et al., 2003) was used for random forest regression. The R package "rms" (Harrell, 2020) was used to construct nomograms and calibration plots. The R package "forestplot" (Max Gordon, 2020) was applied for the clinicopathological subgroup study. The above statistical analyses were all carried out by R (v4.0.0), with *P*-values less than 0.05 considered significant.

RESULTS

Expression Profiles of *TICRR* in Different Cancers and Related Differentially Expressed Genes in Papillary Renal Cell Carcinoma

Based on TCGA database, we determined the expression of *TICRR* mRNA in different cancers. As shown in **Figure 1A**, among 33 cancer types, the *TICRR* was significantly highly expressed in 19 cancers, especially in tumors located in gastrointestinal and urogenital tracts. More specifically, *TICRR* expression was much higher in PRCC tumors than in pericarcinous tissues (P < 0.001, **Figure 1B**). Interestingly, in none of the investigated cancer profiles was *TICRR* expression significantly decreased.

 $\label{eq:table_$

Characteristic	Level	Low- <i>TICRR</i> group (n = 144)	High- <i>TICRR</i> group (n = 144)
Sex (%)*	Female	24 (16.7%)	52 (36.1%)
Age (median [IQR])*		64.00 [57.00, 71.00]	59.00 [51.00, 69.00]
Race (%)	Asian	1 (0.7%)	5 (3.6%)
	Black or African American	30 (22.4%)	30 (21.9%)
	White	103 (76.9%)	102 (74.5%)
Smoker (%)		65 (52.0%)	65 (53.7%)
Clinical T stage (%)*	T1	80 (77.7%)	59 (60.2%)
	T2	13 (12.6%)	13 (13.3%)
	ТЗ	10 (9.7%)	25 (25.5%)
	T4	0 (0.0%)	1 (1.0%)
Clinical N stage (%)*	NO	72 (93.5%)	60 (78.9%)
	N1	5 (6.5%)	14 (18.4%)
	N2	0 (0.0%)	2 (2.6%)
Clinical M stage (%)	MO	101 (96.2%)	98 (95.1%)
	M1	4 (3.8%)	5 (4.9%)
Clinical stage (%)*	Stage I	80 (78.4%)	58 (60.4%)
	Stage II	12 (11.8%)	9 (9.4%)
	Stage III	7 (6.9%)	22 (22.9%)
	Stage IV	3 (2.9%)	7 (7.3%)
Serum calcium (%)	Normal	69 (75.0%)	64 (72.7%)
	Elevated	2 (2.2%)	4 (4.5%)
	Low	21 (22.8%)	20 (22.7%)
Hemoglobin (%)	Normal	64 (61.0%)	48 (46.6%)
	Elevated	0 (0.0%)	1 (1.0%)
	Low	41 (39.0%)	54 (52.4%)
MET status (%)	Mut	6 (4.3%)	14 (10.1%)

IQR, interquartile range; PRCC, papillary renal cell carcinoma. *p < 0.05.

Based on the median *TICRR* expression in PRCC tumors, 288 PRCC patients were stratified into two groups, highand low-*TICRR* expression groups. We next compared mRNA, miRNA, and lncRNA expression between the two groups. Finally, 667 mRNAs (619 upregulated and 48 downregulated, **Figure 1C**), 2 miRNAs (2 upregulated, **Supplementary Figure 1**), and 341 lncRNAs (316 upregulated and 25 downregulated, **Supplementary Figure 1**) were recognized as DEGs (absolute value of fold change >1.5, P < 0.05) in the high-*TICRR* group. Representative DEGs were also illustrated by heatmaps (**Figure 1D** and **Supplementary Figure 1**).

Functional Annotation of *TICRR*-Associated Differentially Expressed Genes in Papillary Renal Cell Carcinoma Tumors

In order to evaluate the function of TICRR-associated DEGs in PRCC patients, the software "Metascape" was applied. As presented in Figures 2A-C and Supplementary Table 1, we found that several PRCC-related pathways were enriched, including epithelial cell differentiation (GO: 0030855, P < 0.001, enrichment factor = 2.654, FDR = 0.037), urogenital system development (GO: 0001655, P < 0.001, enrichment factor = 3.448, FDR = 0.141), and negative regulation of cell division (GO: 0051782, P = 0.001, enrichment factor = 15.802, FDR = 0.266). Moreover, the GSEA showed TICRR-associated DEGs significantly enriched in cell proliferation related clusters (Figures 2D-K), such as mitotic cell cycle [normalized enrichment score (NES) = 1.510, adjusted P = 0.022, FDR = 0.018], cyclin events during G2 to M transition (NES = 1.912, adjusted P = 0.022, FDR = 0.018), mitotic metaphase and anaphase (NES = 1.524, adjusted P = 0.022, FDR = 0.018), and mitotic prometaphase (NES = 1.576, adjusted P = 0.022, FDR = 0.018). TICRR-associated DEGs were also enriched in cancer pathways (Figure 2L), especially the cell cycle-related Hedgehog signaling pathway (Figure 2M). More interestingly, TICRR-associated DEGs were associated with the activity of the MET gene (Figures 2N,O), which is usually involved in oncogenesis. We also constructed a PPI network for DEGs (Supplementary Figure 2), where the TICRR served as the hub gene related to another eight genes.

Association of *TICRR* and Immune Cell Infiltration in Papillary Renal Cell Carcinoma Tumors

Infiltration of 24 immune cell types in PRCC was determined using the ssGSEA method first, and subsequently the association between *TICRR* and immune cell infiltration was investigated by Spearman's analysis. As shown in **Figure 3A**, Tcm (R = 0.317, P < 0.001), Th cells (R = 0.317, P < 0.001), and NK cells (R = 0.180, P = 0.002) were all positively correlated with *TICRR* expression. However, DCs (R = -0.231, P < 0.001), macrophages (R = -0.233, P < 0.001), neutrophils (R = -0.235, P < 0.001), and B cells (R = -0.160, P = 0.007) showed a negative association with *TICRR*. More specifically, we evaluated the infiltration levels of six most relevant immune cells—DCs (**Figure 3B**),



FIGURE 4 | *TICRR* expression is associated with clinicopathological characteristics in papillary renal cell carcinoma (PRCC) patients. The Wilcoxon rank sum test was applied to analyze the association of *TICRR* expression with sex (A), age (B), hemoglobin level (C), clinical stage (D), clinical T stage (E), clinical N stage (F), clinical M stage (G), and MET status (H).

 TABLE 2 | Logistic regression analysis of association between clinicopathological characteristics and *TICRR* expression in PRCC patients.

Characteristic	Odds ratio (OR)	P-value
Clinical T stage (T3–T4 vs. T1–T2)	2.13 (1.29–3.79)	0.006
Clinical N stage (N1–N2 vs. N0)	2.64 (1.51–5.11)	0.002
Clinical M stage (M1 vs. M0)	1.60 (0.77-2.78)	0.115
Clinical stage (stage II-IV vs. stage I)	2.87 (1.61–5.72)	0.001
Serum calcium (abnormal vs. normal)	0.93 (0.48-1.59)	0.808
Hemoglobin (abnormal vs. normal)	1.74 (1.09–3.08)	0.032
MET status (Mut vs. WT)	0.71 (0.19–1.47)	0.501
Sex (Female vs. male)	2.22 (1.47-3.62)	< 0.001
Age (>60 vs. ≤60)	0.66 (0.41–0.98)	0.054

PRCC, papillary renal cell carcinoma; WT, wild type; Mut, mutation.

neutrophils (Figure 3C), macrophages (Figure 3D), Th2 cells (Figure 3E), Th cells (Figure 3F), and Tcm (Figure 3G)—in distinct *TICRR* groups, which showed results consistent with those in Figure 3A.

Association of *TICRR* Expression and Clinicopathological Characteristics in Papillary Renal Cell Carcinoma Patients

We investigated the clinicopathological characteristics of PRCC patients with differential *TICRR* expression, as shown in **Table 1**. Compared with the low-*TICRR* group, patients in the high-*TICRR* group manifested a higher proportion of female sex, younger age, worse clinical stages, and more severe T and M stages. However, there was no significant

difference in the distribution of clinical T stages, serum calcium concentration, hemoglobin level, or MET gene mutational status between two groups.

Further, we analyzed *TICRR* expression in patients with different clinicopathological characteristics. *TICRR* expression was significantly elevated in patients of female sex (Figure 4A), age below 60 years (Figure 4B), abnormal hemoglobin level (Figure 4C), clinical stages III and IV (Figure 4D), T stages T3 and T4 (Figure 4E), and N stages N1 and N2 (Figure 4F). As shown in Table 1, patients with different M stages (Figure 4G) and MET mutational status (Figure 4H) both shared similar *TICRR* expression levels. We also utilized logistics analysis to determine the correlation between *TICRR* expression and clinicopathological characteristics (Table 2). We found prominently positive correlations of *TICRR* expression with clinical stage (including T and N stages), hemoglobin, and female sex.

Predictive Value of *TICRR* for Papillary Renal Cell Carcinoma Diagnosis and Prognosis

In order to explore the clinical benefits of *TICRR* evaluation, we used a ROC curve to demonstrate its value on discriminating PRCC diagnosis. As the area under the curve (AUC) was 0.807, *TICRR* showed significant high sensitivity and specificity for PRCC diagnosis (**Figure 5A**). Next, K-M analyses were applied to verify the prediction of *TICRR* on clinical outcomes. As shown in **Figures 5B–D**, overall survival [hazard ratio (HR): 2.80, P = 0.002), progression-free interval (HR: 2.86, P < 0.001),



TABLE 3	Cox regression	analysis for	clinical	outcomes	in PRCC	patients.
---------	----------------	--------------	----------	----------	---------	-----------

Characteristics	HR for	HR for overall survival (95% CI)		HR for progression-free interval (95% CI)		HR for disease-specific survival (95% CI)	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate	
Clinical T stage (T3–T4 vs. T1–T2)	4.687***	0.546	7.383***	1.923	8.926***	0.513	
Clinical N stage (N1–N2 vs. N0)	10.637***	8.683*	17.022***	6.790 * *	19.162***	7.111*	
Clinical M stage (M1 vs. M0)	38.111***	16.622 * *	10.324***	1.089	40.575***	20.996**	
Clinical stage (stage II-IV vs. stage I)	5.123***	3.686	6.983***	1.976	27.918***	12.037*	
Smoker (yes vs. no)	0.564		1.230		0.610		
Age (>60 vs. ≤60 years)	0.956		0.820		0.447*	1.321	
Sex (male vs. female)	0.617		0.528		0.544		
Serum calcium (abnormal vs. normal)	1.659		1.180		1.749		
Hemoglobin (abnormal vs. normal)	4.381***	2.141	1.976*	2.137	3.174*	2.172	
MET status (Mut vs. WT)	1.025		1.158		0.508		
Race (White vs. Black or African American and Asian) 0.921		0.863		0.891		
TICRR (high vs. low)	2.801**	3.862*	2.859***	2.496	7.029***	4.705*	

HR, hazard ratio; PRCC, papillary renal cell carcinoma; WT, wild type; Mut, mutation; CI, confidence interval. *P < 0.05; **P < 0.01; ***P < 0.001.

and disease-specific survival (HR: 7.03, P < 0.001) for high-*TICRR* groups were all statistically worse than those for the low-*TICRR* group.

Moreover, we performed a multivariate Cox regression analysis to further evaluate the predictive value of *TICRR* on clinical outcomes. As shown in **Table 3**, *TICRR* expression was an independent risk factor for overall survival (HR: 3.862, P = 0.036) and disease-specific survival (HR: 4.705, P = 0.039) in multivariate Cox regression, although it did not provide any significant predictive ability for progression-free interval. Conversely, clinical stage, especially clinical N and M stages, also showed predictive advantages for clinical outcomes in multivariate Cox regression analyses. In order to evaluate the importance of each predictive factor for clinical outcomes, we carried out a random forest analysis to predict overall survival. The random forest model reached an overall percentage accuracy of 86.8%. As shown in **Supplementary Figure 3**, *TICRR* expression ranked second among the most important predictors of overall survival in PRCC patients.

All the statistically significant prognostic factors in each multivariate Cox regression analysis were then used to construct a prognostic nomogram, and a calibration curve was drawn to test the efficiency of the nomogram. Clinical N and M stages, as well as *TICRR*, were included in the nomogram to predict



FIGURE 6 | Construction and validation of nomograms based on *TICRR* expression. Shown are the nomograms constructed to establish *TICRR* expression-based risk scoring models for 1-, 3-, and 5-year overall survival (A), progression-free interval (C), and disease-specific survival (E). Calibration plots validating the efficiency of nomograms for overall survival (B), progression-free interval (D), and disease-specific survival (F). OS, overall survival; PFI, progression-free interval; DSS, disease-specific survival.

overall survival, which had a C-index of 0.892 (Figure 6A). Clinical N and *TICRR* were included in a nomogram constructed to predict progression-free interval, which had a C-index of 0.787 (Figure 6C). Clinical stage, clinical N and M stages, and *TICRR* were used to construct a predictive nomogram for diseasespecific survival, which had a C-index of 0.931 (Figure 6E). The calibration curves all presented desirable prediction of the three nomograms for the 1-, 3-, and 5-year clinical outcomes, with the exception of the 1-year prediction for overall survival, which was slightly underestimated (Figures 6B,D,F).

Prognostic Performance of *TICRR* in the Papillary Renal Cell Carcinoma Clinicopathological Subgroups

Next, we attempted to determine the predictive value of *TICRR* for clinical outcomes in several clinicopathological subgroups. We carried out Cox regression analyses in specific subgroups (**Table 4**). The results were also presented as forest plots (**Figure 7**). As shown in the forest plot in **Figure 7A**, *TICRR* was a significant risk factor for overall survival in patients of male sex (HR = 2.386, P = 0.019), age below 60 years (HR = 12.615, P = 0.014), clinical stage II–IV (HR = 3.740, P = 0.019), clinical T stages T1 and T2 (HR = 4.038, P = 0.009), clinical N0 stage (HR = 3.030, P = 0.048), clinical M0 stage (HR = 3.795, P = 0.002), and wild-type MET gene status (HR = 2.892, P = 0.002). Similar observations occurred for progression-free

interval (**Figure 7B**) and disease-specific survival (**Figure 7C**). As there were few patients with clinical M1 stage (9 patients, occupying 4% of the sample) and MET mutation (20 patients, 7% of the sample), the subgroup analyses for clinical M1 stage and MET mutational status could not be performed. We also presented K-M analyses for clinical outcomes (overall survival, progression-free interval, and disease-specific survival) in the following four representative subgroups: male sex, age below 60 years, clinical stages II–IV, and T stages T1 and T2 (**Figure 8**). All the results demonstrated significantly better clinical outcomes in the low-*TICRR* expression groups.

DISCUSSION

In the present study, we focused on expression profiles, clinicopathological associations, and the clinical significance of a DNA replication initiation regulator, *TICRR*, in PRCC by analyzing datasets from the TCGA-KIRP. We observed prominent increased *TICRR* expression in PRCC tumors. DEGs related to higher *TICRR* levels were specifically enriched in cell cycle- and MET-associated pathways. We also revealed a marked association of *TICRR* expression with sex, age, and clinical stages in PRCC patients. Finally, we determined the predictive value of *TICRR* for overall survival, progression-free interval, and disease-specific survival in PRCC patients, especially in those of male sex, age below 60 years, and clinical stages II–IV and T stages T1–T2.

TABLE 4 | Prognostic performance of TICRR on clinical outcomes in PRCC patient subgroups by Cox regression analysis.

Characteristics	N (%)	HR for overall survival (95% CI)	HR for progression-free interval (95% CI)	HR for disease-specific survival (95% CI)
Sex				
Female	76 (26)	3.653 (0.805–16.585)	5.541 (1.275–24.081)*	N.A.
Male	211 (74)	2.386 (1.157-4.922)*	2.131 (1.110-4.090)*	4.831 (1.590–14.681)**
Age				
≤60	133 (47)	12.615 (1.682–94.637)*	2.732 (1.102–6.778)*	12.081 (1.606–90.905)*
>60	152 (53)	2.212 (0.983-4.980)	3.182 (1.469–6.892)**	3.846 (0.993–14.899)
Clinical stage				
Stage I	138 (70)	2.406 (0.675-8.577)	1.932 (0.648–5.759)	N.A.
Stage II–IV	60 (30)	3.740 (1.245–11.236)*	2.449 (1.012–5.927)*	3.740 (1.245–11.236)*
Clinical T stage				
T1-T2	165 (82)	4.038 (1.414–11.527)**	2.938 (1.226–7.037)*	12.189 (1.522–97.619)*
T3–T4	36 (18)	2.322 (0.637-8.461)	1.594 (0.556–4.575)	2.322 (0.637-8.461)
Clinical N stage				
NO	132 (86)	3.030 (1.012–9.076)*	2.531 (0.979–6.545)	9.620 (1.156–80.079)*
N1-N2	21 (14)	2.065 (0.570-7.477)	2.069 (0.632-6.775)	2.065 (0.570-7.477)
Clinical M stage				
MO	199 (96)	3.795 (1.602–8.992)**	2.979 (1.481–5.991)**	10.397 (2.389–45.253)**
M1	9 (4)	N.A.	N.A.	N.A.
MET status				
WT	257 (93)	2.892 (1.473–5.676)**	2.999 (1.638–5.488)***	6.904 (2.367–20.137)***
Mut	20 (7)	0.354 (0.022–5.659)	N.A.	N.A.
Hemoglobin				
Normal	112 (54)	4.097 (0.794–21.126)	2.346 (0.870-6.327)	8.275 (0.966–70.858)
Abnormal	96 (46)	1.587 (0.677–3.715)	1.887 (0.768–4.636)	2.470 (0.679–8.986)

HR, hazard ratio; Cl, confidence interval; WT, wild type; Mut, mutation. *P < 0.05; **P < 0.01; ***P < 0.001.

A Characteristics N (%) Hazard Rate (26% C) P value Gendar Frame 76 (20) 2.85(3) 650-16.850 0.00 A Adv 2.11 (74) 2.86(3) 650-16.850 0.013 A Ape 2.11 (74) 2.86(3) 650-16.850 0.014 A Ape 2.11 (74) 2.86(1) 652-6577) 0.014 Standorfinder 156 (50) 2.42(0) 657-6577) 0.017 0.019 Clinical tage 156 (160) 2.82(1) 672-6578) 0.022 0.002 National 152 (160) 2.882(1,473-5578) 0.002 0.002 Mod 199 (160) 3.76(1,602-6892) 0.014 0.022 0.022 Mod 199 (160) 3.76(1,602-6892) 0.02 0.022 0.022 Mod 199 (160) 3.76(1,602-6892) 0.02 0.022 0.022 Mod 197 (0.7) 2.54(1,475-5278) 0.022 0.022 Momal 192 (07) 5.64(1,1275-24.81) 0.022 0.023 Momal 192 (A					
Centoric 0003 Permale 76 (26) 3.63(0.650-16.56) Male 211 (74) 2.384(1.157-4.822) 0.019 Age 0.019 0.019 0.019 -e00 13.3 (7) 12.615(1.682-64.637) 0.019 Stape I 0.013 0.019 0.019 Stape I 0.013 (47) 12.615(1.682-64.637) 0.019 Clinical stage 0.019 0.019 0.019 Stape I 0.018 (3.010 (11-2.057)) 0.019 0.022 T137 4 3.0 (18) 2.322(0.637-64.71) 0.022 N163k2 21 (14) 2.080(1.672-676) 0.022 WT 2.57 (95) 2.882(1.472-5.670) 0.022 WT 12.654 4.067(0.754-21.126) 0.022 Abcommal 96 (46) 1.587(0.677-3.719) 0.022 VT Tacade Ratio (696: C) P.visig P.visig Female 76 (27) 5.541(1.275-24.091) 0.023 More 12.073 2.131(1.10-4000) 4.023 0.		Characteristics	N (%)	Hazard Ratio (95% CI)		P value
Fermine 76 (26) 3.653(0.605-06.85) 0.033 Age 0.011 0.239(1.157-4.622) 0.019 -e-60 1.35 (47) 1.2.615(1.82-04.87) 0.019 -e-60 1.35 (47) 1.2.615(1.82-04.87) 0.019 -e-60 1.35 (47) 1.2.615(1.82-04.87) 0.019 Singe II.85age III.85age IV 0.09 3.74(1.424-11.827) 0.059 TATZ 1.66 (62) 4.039(1.414-11.627) 0.021 TATZ 1.66 (62) 4.039(1.414-11.627) 0.022 TATZ 1.66 (62) 4.039(1.414-11.627) 0.022 TATZ 1.66 (62) 4.039(1.412-41.122) 0.022 Nitat 1.2 (40) 2.059(0.570-74.77) 0.042 Mitat 1.2 (40) 2.059(0.570-74.77) 0.022 Mitat 1.2 (40) 2.059(0.570-74.77) 0.022 Mitat 1.2 (40) 0.022 (40-6.020) 0.022 Mitat 1.2 (47) 2.21 (102-6.77) 0.022 Mitat 1.2 (40) 2.21 (102-6.77) 0.022 </td <td></td> <td>Gender</td> <td></td> <td></td> <td></td> <td></td>		Gender				
Male 211 (74) 2.38(11 157-4522) 0.019 -e60 133 (77) 12.65(1.622-64.637) 0.055 Stage I 138 (75) 2.21 (20 88-4.697) 0.055 Stage I 0.060 3.76(1.267-6.577) 0.075 Stage I 0.060 3.76(1.267-1.285) 0.019 TAT2 155 (622) 4.039(1.414-11.527) 0.020 TAT2 156 (622) 4.039(1.474-56.77) 0.276 TAT2 12.(69) 3.030(1.072-0.076) 0.022 WT 2.77 (59) 2.862(1.477-56.767) 0.022 WT 2.77 (59) 2.862(1.477-56.767) 0.022 WT 2.77 (59) 2.862(1.477-56.767) 0.022 Mo 19.6 (49) 1.587(0.677-3.715) 0.022 Mo 15.87(0.677-3.715) 0.022 0.023 Ancrmal 96 (49) 1.587(0.677-3.715) 0.023 Mota 2.01 (73) 2.131(1.107-4.690) 0.023 Ancrmal 96 (49) 1.587(0.677-5.759) 0.237 Stat<		Female	76 (26)	3.653(0.805-16.585)	•	0.093
Age ===0 133 (47) 12 (55) 2.2 (2) (882-64.637) 0.014 Stage 11 Stage 1 133 (70) 2.408(0.675-677) 0.178 Stage 11 Stage 1 153 (25) 2.2 (2) (882-64.637) 0.019 T14572 105 (82) 4.008(1.414-11.627) 0.029 T14572 105 (82) 3.030(1.012-40.75) 0.022 No 132 (85) 3.030(1.012-40.75) 0.022 MET status 199 (96) 3.786(1.602-8.992) 0.002 MET status 199 (96) 3.786(1.602-8.992) 0.022 Memoglobin 12 (57) 3.282(1.472-6.676) 9.0282 Memoglobin 102 (57) 3.182(1.469-6.882) 0.023 Male 2.10 (73) 2.241 (12-2.677) 0.228 Chincia tsige 138 (70) 1.332 (1.492-5.78) 0.027 Male 2.10 (73) <td></td> <td>Male</td> <td>211 (74)</td> <td>2.386(1.157-4.922)</td> <td>•</td> <td>0.019</td>		Male	211 (74)	2.386(1.157-4.922)	•	0.019
e=60 133 (47) 12 (51) (122-6437) 0.014 >66 152 (53) 2.210 (83-2.6837) 0.055 Stage I 138 (70) 2.4080 675-8.577) 0.019 Chincat Stage 0.019 0.019 0.019 Ti AT 2 156 (82) 4.038 (144-11.527) 0.019 Ti AT 1 152 (68) 3.030 (1.02-6.076) 0.022 N0 152 (68) 3.030 (1.02-6.076) 0.022 N0 152 (68) 3.030 (1.02-6.076) 0.022 MCT status 2.11 (107 (207 -21.128)) 0.022 WT 2.57 (13) 2.882 (1.473-5.676) 0.022 WT status 12 (51) 4.007 (27-3.176) 0.022 Mot stage 76 (27) 5.541 (127-2.408) 0.023 Accommal 12 (53) 3.182 (1.407-5.677) 0.033 Male 210 (73) 2.131 (110-4.009) 0.022 Male 20 (13) 2.131 (110-4.009) 0.022 Male 20 (13) 2.231 (110-4.091) 0.023 Stage I <		Age			-	
>00 12 (15) 2.212(0.88-4.880) 0.055 Stage II (Stage IIV) 138 (70) 2.406(0.67-8.577) 0.079 TiAT2 155 (82) 4.008(1.41-11.527) 0.090 TiAT2 155 (82) 3.000(1.01-8.076) 0.048 N16M2 122 (86) 3.050(1.67-8.767) 0.022 M0 199 (96) 3.756(1.602-6.592) 0.022 WT 257 (05) 2.892(1.472-5.676) 0.022 Normal 12 (54) 4.007(0.754-21.126) 0.023 Abcormal 96 (40) 1.587(0.677-3.716) 0.023 Normal 12 (57) 2.531(0.717-2.76.061) 0.030 Singe II 133 (47) 2.732(1.102-6.778) 0.030 Normal 13 (67) 1.582(0.648-5.789) 0.030 Singe II 133 (47) 2.283(0.678-6.479) 0.047 <td></td> <td><=60</td> <td>133 (47)</td> <td>12.615(1.682-94.637)</td> <td>•</td> <td>0.014</td>		<=60	133 (47)	12.615(1.682-94.637)	•	0.014
Clinical Hage 33 (7) 2.406(0.675-6.577) 9 9 176 Shape II & Shage III & Shage I		>60	152 (53)	2.212(0.983-4.980)	b •	0.055
Stage I 138 (70) 2.408(0.679-6.577) 0.176 Stage II.Stage II.Stag		Clinical stage				
Stage ILISSinge ILISSinge IV 60 (30) 3.740(1.245-11.250) 0.019 Clinical Trage 156 (62) 4.039(1.41-15.27) 0.009 T1374 39 (18) 2.322(0.637-6.461) 0.222 Clinical Narge 0.019 0.021 0.021 N0 132 (66) 3.030(1.012-6.076) 0.048 N18/2 21 (14) 2.065(0.570-7.477) 0.270 Clinical Marge 0.002 0.002 0.002 MET status VT 257 (93) 2.882(1.473-5.675) 0.002 Normal 112 (24) 4.097(0.754-21.128) 0.002 0.022 Memoralitics N (%) Nazard Ratio (6%: C1) P value 0.022 Gender 76 (27) 5.541(1.275-24.081) 0.022 0.023 Age		Stage I	138 (70)	2.406(0.675-8.577)	↓	0.176
Clinical Triage 165 (42) 4.030(1.414-11.527) 0.009 Ti374 36 (16) 2.32(0.637-6.461) 0.242 Nin Nin Ninge 132 (66) 3.030(1.072-6.076) 0.048 Nin Nin Ninge 21 (14) 2.66(0.570-7.477) 0.270 Clinical M stage 109 (66) 3.756(1.602-6.076) 0.002 M0 196 (66) 3.756(1.602-6.076) 0.002 M0 196 (66) 3.756(1.602-6.076) 0.002 Momal 112 (24) 4.007(0.764-21.128) 0.002 Abnormal 96 (46) 1.587(0.677-3.715) 0.022 Male 210 (73) 2.131(1.110-4.069) 0.033 Age 2.00 445 165 467 175 467 160 0.033 Age 2.00 (13) 3.182(1.486-6.982) 0.033 Clinical trape 133 (47) 2.732(1.02-6.775) 0.47 Shage II Stage IIX Stage IX 0.033 0.032 0.033 Shage IIX Stage IIX Stage IX 0.041(0.12-5.627) 0.47 Clinical trape 133 (47) 2.399(1.28-6.775) 0.23 </td <td></td> <td>Stage II&Stage III&Stage IV</td> <td>60 (30)</td> <td>3.740(1.245-11.236)</td> <td>•</td> <td>0.019</td>		Stage II&Stage III&Stage IV	60 (30)	3.740(1.245-11.236)	•	0.019
T 1812 165 (62) 4.038(144)-11527) Image 0.056 T 1814 36 (16) 2.32(0.637-6.461) 0.232 N0 132 (66) 3.030(1.012-076) 0.046 N1 Max 2.1 (14) 2.063(0.570-7.477) 0.247 Clinical Misage MG 199 (66) 3.755(1.602-6.592) 0.002 MCT status WT 2.57 (33) 2.852(1.472-5.676) 0.002 Normal 112 (24) 4.097(0.744-21.128) 0.002 Abromal 96 (69) 1.587(0.677-3.715) 0.022 Gender Female 76 (27) 5.541(1.275-24.081) 0.022 Male 2.10 (73) 2.131(1.102-6.078) 0.033 -800 138 (70) 1.582(1.480-6.862) 0.033 Clinical Tage 138 (70) 1.582(1.480-6.862) 0.032 Clinical Tage 138 (70) 1.582(1.480-6.862) 0.033 Clinical Tage 138 (70) 1.582(1.480-6.862) 0.033 Clinical Misage 138 (71) 2.534(1.287-7.037) 0.346 N0 132 (87) 2.538(1.287-7.037) 0.346		Clinical T stage				
T344 38 (16) 2.322(0.637-8.461) 0.202 Ninexal Misage 132 (66) 3.030(1.072-0.767) 0.488 N13N2 21 (14) 2.065(0.570-7.477) 0.270 M0 199 (66) 3.756(1.602-8.092) 0.002 WT 2.57 (53) 2.852(1.472-5.676) 0.002 Normal 112 (26) 4.067(0.707-8-71.126) 0.002 Abnormal 192 (66) 1.587(0.677-3.715) 0.022 B Characteristics N (%) Hazard Ratic (6% C1) P value Gender 76 (27) 5.541(1.27-8.081) 0.022 Male 2107(3) 2.132(1.02-6.775) 0.033 -60 133 (47) 2.732(1.02-6.776) 0.047 Stage II.85ting IV 96 (30) 2.339(0.566-3.769) 0.033 Stage II.85ting IV 96 (30) 2.339(0.696-3.769) 0.047 Stage II.85ting IV 2.090(0.32-6.775) 0.47 0.287 Stage II.85ting IV 2.090(0.32-6.775) 0.231 0.47 Not 132 (67) 2.33(0.677-6.546) 0.045 N134 2 20 (13)		T1&T2	165 (82)	4.038(1.414-11.527)	→	0.009
Clinical N stage N0 122 (6) 300(102-070) Clinical M stage 122 (6) 300(102-070) Clinical M stage MET status VT Henopobin 122 (6) 4007(734-21126) Anormal 66 (46) 1557(67-3715) 005 Characteristics N (%) Hazard Ratio (6% Cl) Clinical stage Singe II Stage IV 2011 22 (8) (200-2773) Clinical stage Singe II Stage IV 2011 22 (8) (200-2773) Clinical stage N1 452 Clinical M		T3&T4	36 (18)	2.322(0.637-8.461)	, <u> </u>	0.202
No. 132 (26) 3 030(1012-6 076) 0.048 N1 8N2 21 (14) 2.068(0.570-7.477) 0.270 MG 199 (96) 3.795(1602-8.992) 0.002 MT 122 (83) 2.892(1.473-5.576) 0.002 MT 112 (54) 4.097(0.794-21.126) 0.002 Anormal 56 (46) 1.597(0.577-3.716) 0.002 Anormal 76 (27) 5.541(1.275-24.081) 0.002 Male 210 (73) 2.131(1.110-4.090) 0.023 Age 0.013 13(47) 2.732(1.102-6.778) 0.030 Singe I 138 (70) 1.393(0.048-5.759) 0.047 Chincal Hage 133 (47) 2.732(1.102-6.778) 0.047 Singe I 138 (70) 1.393(0.048-5.759) 0.047 Chincal M Singe IIIAStage IV 59 (30) 2.448(1012-5.527) 0.047 Chincal M Singe IIIAStage IIIAStage IIIAStage IIIAStage IV 59 (10) 2.098(1.032-6.775) 0.055 N16N2 20 (13) 2.098(1.032-6.775) 0.056 0.056 Nikal <		Clinical N stage		, , , , , , , , , , , , , , , , , , ,		
N 18A2 21 (14) 2.058(0.570-7.477) 0.270 MC Testus 99 (96) 3.795(1.602-6.592) 0.002 MC Testus 0.002 0.002 MC Testus 0.002 MC Dotation 112 (54) 4.097(0.794-21.126) 0.002 Momal 66 (46) 1.587(0.677-3.715) 0.002 B Characteristics N (%) Hazard Ratio (95% C1) P value Gender 76 (27) 5.541(1.275-24.081) 0.022 Male 210 (73) 2.131(1.110-4.090) 0.023 Age 0.002 0.003 0.0030 Stape II 138 (7) 1.092(0.484-5759) 0.023 Stape III Stape IIII Stape IIII Stape IIII Stape IIII Stape IIII Stape IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		NO	132 (86)	3.030(1.012-9.076)		0.048
Clinical M stage 199 (96) 3.796(1 602 - 6.992) 0.002 MM 199 (96) 3.796(1 602 - 6.992) 0.002 VT 257 (93) 2.892(1 473 - 6.76) 0.002 Memoglobin 112 (54) 4.097(0 734 - 2.1 126) 0.002 Anormal 96 (46) 1.587(0 577 - 3.7 16) 0.002 B Characteristics N (%) Hazard Ratio (65% CI) P value Gender 76 (27) 5.541(1.275 - 24.081) 0.022 Male 210 (73) 2.131(1.10 - 4.096) 0.022 Age		N1&N2	21 (14)	2.065(0.570-7.477)	—	0.270
Mo 199 (98) 3.795(1 602–6.992) 0.002 WET status 257 (93) 2.892(1 473–6.676) 0.002 Mormal 112 (54) 4.097(0.794–21.126) 0.002 Anormal 56 (46) 1.557(0.677–3.716) 0.002 B Characteristics N (%) Hazard Ratio (26% CI) P value Gender 76 (27) 5.541(1.275–24.081) 0.022 0.032 Age 0.033 0.032 0.033 0.033 -e60 133 (47) 2.732(1.102–6.778) 0.022 0.033 Stage I IStage IIStage IIIStage IIIStage II 133 (70) 1.532(0.484–5.578) 0.237 Stage I IStage IIIStage IIIStage IIIStage III 1.59(0.872–6.575) 0.237 0.047 Stage I IStage IIIStage I		Clinical M stage				
MET Image I		MO	199 (96)	3.795(1.602-8.992)	• ••	0.002
Vit Hemoglobin 257 (93) 2.692(1473-5.676) 0.002 Hemoglobin 112 (54) 4.0970.794-21.126) 0.092 Anormal 96 (46) 1.587(0.677-3.715) 0.092 B Tharacteristics N (%) Hazard Ratio (95% Cl) P value Gender 78 (27) 5.541(1.275-24.081) 0.022 Age 0.022 0.033 0.032 Age 0.022 0.033 0.033 -e60 153 (47) 2.732(1.102-6.778) 0.033 Stage ILStage INStage		MET status			•	
Hemaploin Normal 112 (5) 15 (2) 35 (4) (5) (5) (5) (5) (5) (5) (5) (5) (5) (5		WT	257 (93)	2.892(1.473-5.676)	•	0.002
Normal 112 (54) 4.0970.794-21.26) 0 <th0< th=""> 0 0 <th0<< td=""><td></td><td>Hemoglobin</td><td></td><td></td><td>*</td><td></td></th0<<></th0<>		Hemoglobin			*	
Abnormal 96 (46) 1.587(0.677-3.715) 0.288 0 5 11 5 28 35 45 56 7.75 85 95 0<		Normal	112 (54)	4.097(0.794-21.126)	•	0.092
B Characteristics N (%) Hazard Ratio (95% CI) P value Gender 76 (27) 5.541(1.275-24.081) 0.022 Age - 0.030 0.022 Age - 0.030 0.023 Stage I 133 (47) 2.732(1.102-6.778) 0.030 >60 151 (53) 3.182(1.469-6.862) 0.003 Clinical stage 138 (70) 1.932(0.646-5.759) 0.237 Stage II&Stage III.Stage IV 59 (30) 2.448(1.012-5.927) 0.047 Clinical T stage 1.934 (70) 1.932(0.646-5.759) 0.337 Clinical N stage 0.047 0.047 0.047 T1812 1.95 (8) 2.981(1.287-037) 0.047 Clinical N stage 0.001 1.98 (96) 2.979(1.481-5.991) 0.002 VT 2.56 (93) 2.999(1.638-5.486) -0.001 0.022 Mo 1.98 (96) 2.999(1.638-5.486) -0.001 0.922 VT 2.56 (93) 2.999(1.638-5.486) -0.016 0.022		Abnormal	96 (46)	1.587(0.677-3.715)	- b i	0.288
B Characteristics N (%) Hazard Ratic (95% Cl) P value Gender 76 (27) 5.541(1.275-24.081) 0.022 Male 210 (73) 2.131(1.110-4.090) 0.022 Age 0.030 0.030 ==60 133 (47) 2.732(1.122-6.778) 0.030 Stage II 515 ///////////////////////////////////						
B Characteristics N (%) Hazard Ratio (95% C1) P value Gender Female 76 (27) 5.541(1.275-24.081) 0.022 Male 210 (73) 2.131(1.10-4.090) 0.023 Age					0 5 15 25 35 45 55 65 75	85 95
Cender 76 (27) 5.541(1.275-24.081) 0.022 Male 210 (73) 2.131(1.110-4.090) 0.023 Age 0.039 0.039 c=60 151 (53) 3.182(1.469-6.892) 0.039 Clinical stage 0.038 0.039 Stage IIS Stage IIS Stage IV 0.930 (2.449(1.012-5.927) 0.047 Clinical T stage 0.052 0.047 0.047 T1817 165 (62) 2.938(1.256-4.575) 0.047 Clinical N stage 0.047 0.047 0.047 Nomal 152 (67) 2.531(0.576-6.545) 0.055 Ni RAV 20 (13) 2.069(0.632-6.775) 0.230 Clinical M stage 0.001 198 (96) 2.979(1.481-5.991) 0.0022 WT 256 (93) 2.999(1.638-5.488) -0.001 -0.001 Mormal 95 (46) 1.887(0.768-4.636) 0.166 0.092 Abrormal 95 (46) 1.887(0.768-4.636) 0.166 0.092 Abrormal 95 (46) 1.887(0.768-4.636) 0.16	в	Characteristics	N (%)	Hazard Ratio (95% CI)		P value
Female 76 (27) 5.541(1.275-24.081) 0.022 Male 210 (73) 2.131(1.110-4.090) 0.023 Age -e00 133 (47) 2.732(1.102-6.778) 0.030 Stage I 151 (53) 3.132(1.496-6.892) 0.033 Stage II 138 (70) 1.932(0.648-5.759) 0.237 Stage III.Stage III.Stage IV 59 (30) 2.449(1.012-5.927) 0.047 Clinical T stage 0.038 0.038 0.038 Clinical N stage 0.031 (2.077) 2.531(0.579-6.545) 0.035 No 132 (07) 2.059(0.632-6.775) 0.047 Clinical M stage 0.002 0.055 0.230 MO 198 (96) 2.979(1.481-5.991) 0.002 VT 256 (93) 2.999(1.638-5.488) - WT 256 (93) 2.999(1.638-5.488) - Male 207 (73) 4.831(1.590-14.681) 0.005 Ape - 0.018 0.051 Gender 0.014 0.019 0.016 Male 207 (73) 4.831(1.500-14.681) 0.018 <t< td=""><td></td><td>Gender</td><td></td><td></td><td></td><td></td></t<>		Gender				
Male 210 (73) 2.131(1.110-4.090) 0.023 Age 0.030 0.030 Clinical stage 0.030 0.030 Stage II Scage IV 0.930 (24490-5789) 0.037 Stage II Scage IV 0.930 (24490-5789) 0.037 Stage II Scage IV 0.930 (24490-5789) 0.037 Stage II Scage IV 0.930 (24490-5789) 0.038 Clinical T stage 0.047 0.047 Ti Stat 35 (16 0.936 (255-4575) 0.038 Clinical N stage 0.047 0.047 No 132 (87) 2.531(0.576-5455) 0.055 Ni N2 20 (13) 2.069(0.632-6775) 0.237 Old 158 (69) 2.979(1.481-5.991) 0.002 M0 198 (69) 2.979(1.481-5.991) 0.002 VT 256 (93) 2.999(1.638-5.488) -0.011 Normal 112 (54) 2.346(0.870-6.327) 0.092 Abormal 95 (46) 1.887(0.768-4.636) 0.168 UT 2.56 7.5 10 12.5 15 17.5 20 22.5 25 0.016 C Characteristics N (%)		Female	76 (27)	5.541(1.275-24.081)	•	0.022
Age -=00 133 (47) 2.732(1.102-6.778) 0.030 >-60 151 (53) 3.182(1.469-6.862) 0.003 Clinical stage 0.030 2.449(1.012-5.927) 0.047 Clinical T stage 151 (52) 2.938(1.226-7.037) 0.016 T18T2 165 (62) 2.938(1.226-7.037) 0.016 Clinical N stage 0.030 1.954(0.556-4.575) 0.388 Clinical M stage 0.030 1.92 (87) 2.531(0.979-6.545) 0.055 N0 132 (87) 2.059(0.632-6.775) 0.230 Clinical M stage 0.032 0.022 0.032 MCT status 74 25 (63) 2.999(1.633-5.488) - -0.001 WT 256 (9) 2.999(1.633-5.488) - -0.001 Normal 112 (54) 2.346(0.870-6.327) 0.0052 - - - Mot 198 (96) 1.837(0.768-4.636) 0.016 - - - - - - - - - - - - - - - - - - - <t< td=""><td></td><td>Male</td><td>210 (73)</td><td>2.131(1.110-4.090)</td><td>₩-1</td><td>0.023</td></t<>		Male	210 (73)	2.131(1.110-4.090)	₩ -1	0.023
-=60 133 (47) 2.732(1102-6.778) 0.030 >=60 151 (53) 3.182(1.469-6.892) 0.003 Stage II SStage IV 59 (30) 2.449(1.012-5.927) 0.237 Stage II SStage IV SStage IV 59 (30) 2.449(1.012-5.927) 0.016 T1 & T2 165 (62) 2.938(1.225-7.037) 0.016 T1 & T2 165 (62) 2.938(1.225-7.037) 0.016 T1 & T2 165 (62) 2.938(1.225-7.037) 0.055 N0 152 (87) 2.531(0.979-6.545) 0.055 N6 152 (87) 2.531(0.979-6.545) 0.002 WT 256 (93) 2.999(1.638-5.488)		Age				
>60 151 (53) 3.182(1.469-6.882) 0.003 Clinical stage 138 (70) 1.932(0.648-5.759) 0.237 Stage I & Stage II&Stage IV 59 (30) 2.449(1.012-5.927) 0.047 Clinical stage 151 (62) 2.938(1.226-7.037) 0.016 T1&T2 165 (62) 2.938(1.226-7.037) 0.016 Clinical N stage 0.003 0.002 N0 152 (67) 2.531(0.979-6.545) 0.239 Clinical N stage 0.001 198 (96) 2.979(1.481-5.991) 0.002 MET status 112 (54) 2.346(0.870-6.327) 0.001 Abnormal 112 (54) 2.346(0.870-6.327) 0.005 Abnormal 112 (54) 2.346(0.970-6.327) 0.005 Abnormal 12 (57) 2.31(1.500-14.681) 0.005 Abnormal 12 (57) 1.25 (15) 17.5 20 22.5 25 25 C Characteristics N (%) Hazard Ratio (95% C1) P value Cender 0.005 3.846(0.930-14.899) 0.061 Clinical stage 112 (54) 2.322(0.637-8.461) 0.202 Clinical stage<		<=60	133 (47)	2.732(1.102-6.778)		0.030
Clinical stage Stage II & Stage II & Stage IV Stage II & Stage IV Stage II & Stage IV Clinical T stage T1 & T2 Clinical N stage M0 132 (87) 2 (87) 2 (97) 2		>60	151 (53)	3.182(1.469-6.892)		0.003
Stage I 138 (70) 1.932(0.648-5.759) 0.047 Stage IISStage IISStage IV 59 (30) 2.449(1.012-5.927) 0.047 Clinical T stage 0.047 0.047 T18T2 165 (82) 2.938(1.267-0.37) 0.047 Clinical N stage 0.047 0.047 N0 132 (87) 2.531(0.979-6.545) 0.386 Clinical N stage 0.097 (1.481-5.991) 0.002 MET status 20(13) 2.069(6.632-6.775) 0.336 V/T 256 (93) 2.999(1.638-5.488) -0.001 Normal 112 (54) 2.346(0.870-6.327) 0.062 Abnormal 95 (46) 1.887(0.768-4.636) 0.168 V/T 256 (93) 3.999(1.638-6.488) -0.001 Normal 112 (54) 2.346(0.870-6.327) 0.0052 Abnormal 95 (46) 1.887(0.768-4.638) 0.168 V/T 256 (93) 3.946(0.993-14.889) 0.051 Clinical stage 0.033 3.740(1.245-11.236) 0.016 Clinical stage 60 (30) 3.740(1.245-11.236) 0.016 Clinical stage<		Clinical stage				
Stage II&Stage II&Stage IV 59 (30) 2.449(1.012-5.927) 0.047 Clinical T stage 165 (62) 2.938(1.226-7.037) 0.016 T3&T4 35 (19) 1.594(0.556-4.575) 0.386 Clinical N stage 0.021 0.021 0.0230 NO 132 (87) 2.531(0.979-6.545) 0.055 NI&N2 20 (13) 2.069(0.632-6.775) 0.230 Clinical M stage 0.002 0.021 0.022 MO 198 (96) 2.979(1.481-5.991) 0.002 WT 256 (93) 2.999(1.638-5.488) -0.001 Hemoglobin 0.092 0.166 0.092 Normal 95 (45) 1.887(0.768-4.636) 0.166 C Characteristics N (%) Hazard Ratio (95% CI) P value Gender 0.055 0.166 0.055 0.016 Age 0.051 0.016 0.051 0.016 Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.018 0.051 Clinical stage 0.018 3.246(0.970-7.477) 0.018 0.022 0.018 0		Stage I	138 (70)	1.932(0.648-5.759)	• • •	0.237
Clinical T stage T1&T2 145 (82) 2.938(1.226-7.037) T3&T4 35 (18) 1.594(0.556-4.575) 0.056 N1&N2 2.0 (13) 2.069(0.632-6.775) 0.230 Clinical M stage M0 198 (96) 2.979(1.481-5.991) 0.002 MET status VT 256 (93) 2.999(1.638-5.488) 0.002 MT 2 status 0.002 Clinical M stage M1 4 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 25 C Characteristics N (%) Hazard Ratio (95% CI) P value Clinical stage 1.834(1.590-14.681) 0.005 Age -c=60 133 (47) 12.081(1.606-90.905) 0.016 Clinical stage 1.834(51) 3.846(0.993-14.889) 0.051 Clinical stage 1.837(4) 3.646(0.993-14.889) 0.051 Clinical stage 1.837(4) 12.081(1.522-67.619) 1.337(4) 2.326(0.677-6.461) 0.018 T3&T4 36 (18) 2.322(0.637-8.461) 0.022 Clinical N stage N0 132 (86) 9.620(1.156-80.079) 0.038 N1&N2 21 (14) 2.069(0.570-7.477) 0.277 Clinical M stage M0 198 (96) 10.397(2.389-45.253) 0.002 MET status VT 254 (93) 6.504(2.367-20.137) 0.038 MET status VT 254 (93) 6.504(2.367-20.137) 0.038 Abnormal 93 (45) 2.470(0.679-8.898) 0.051 Abnormal 93 (45) 2.470(0.679-8.898) 0.051 Clinical M stage N1 Normal 112 (55) 8.275(0.966-70.658) 0.016 Clinical M stage N1 Normal 112 (55) 8.275(0.966-70.658) 0.016 Clinical M stage N1 Normal 112 (55) 8.275(0.966-70.658) 0.054 Abnormal 93 (45) 2.470(0.679-8.988) 0.054 Clinical M stage N1 Normal 0.055 Clinical M stage N1 Normal 0.055		Stage II&Stage III&Stage IV	59 (30)	2.449(1.012-5.927)		0.047
T18T2 165 (62) 2.938(1,226-7.037) 0.016 T38T4 35 (18) 1.594(0,556-4.575) 0.386 Clinical N stage 0.013 2.069(0,632-6.775) 0.230 NN 132 (67) 2.531(0,979-6.545) 0.230 Clinical M stage 0.002 0.022 0.023 MET status WT 256 (93) 2.999(1.638-5.488) - -0.001 WT 256 (93) 2.999(1.638-5.488) - -0.001 Normal 112 (54) 2.346(0,870-6.327) 0.092 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 0.166 VT 256 0 133 (47) 12.081(1.606-90.905) - 0.016 -<60		Clinical T stage				
T3R14 35 (18) 1.594(0.556-4.575) 0.386 Clinical N stage 0 0 132 (67) 2.531(0.979-6.545) 0.065 N18N2 20 (13) 2.069(0.632-6.775) 0.230 0.002 Clinical M stage 0 0.002 0.002 0.002 MM 198 (96) 2.979(1.481-5.991) 0.002 0.002 WT 256 (93) 2.999(1.638-5.488) -0.001 Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 VT 25 s 7.5 10 12.5 15 17.5 20 22.5 25 25 C Characteristics N (%) Hazard Ratio (95% C1) P value Gender 0.005 0.051 0.016 Male 207 (73) 4.831(1.590-14.681) 0.005 Age 0.051 0.016 0.051 Clinical stage 133 (47) 12.081(1.606-90.905) 0.056 >60 133 (47) 12.081(1.52-97.619) 0.018 T38.74 36 (18) 2.322(0.637-8.461) 0.019 Clinical		T1&T2	165 (82)	2.938(1.226-7.037)	⊢♦ −−−1	0.016
Clinical N stage 0 132 (87) 2.531(0.979-6.545) 0.055 N15N2 20 (13) 2.069(0.632-6.775) 0.002 M0 198 (96) 2.979(1.481-5.991) 0.002 MET status 0 0.065 0.002 WT 256 (93) 2.999(1.638-5.488) - - Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 VT 255 5 7.5 10 12.5 15 17.5 20 22.5 25 25 C Characteristics N (%) Hazard Ratio (95% CI) P value Gender 0.005 - 0.005 - Male 207 (73) 4.831(1.590-14.681) 0.005 - Age - - 0.016 - 0.016 Clinical stage 133 (47) 12.081(1.606-90.905) - 0.016 - 0.019 Clinical stage 138 (48) 2.322(0.637-8.461) 0.016 - 0.018 - 0.018 T38 T4 36 (18) 2.322(0.637-8.461) 0.202 0.018 0.036		T3&T4	35 (18)	1.594(0.556-4.575)	+	0.386
NO 132 (87) 2.531(0.979-6.545) 0.065 N1&N2 20 (13) 2.069(0.632-6.775) 0.230 Clinical M stage 0.002 0.002 MO 198 (96) 2.979(1.481-5.991) 0.002 WT 256 (93) 2.999(1.638-5.488) -0.001 WT 256 (93) 2.999(1.638-5.488) -0.002 WT 256 (93) 2.999(1.638-6.488) -0.002 WT 122 (54) 1.887(0.768-4.636) 0.166 Characteristics N (%) Hazard Ratio (95% C1) P value Gender Male 207 (73) 4.831(1.590-14.681) 0.005 Age		Clinical N stage				
M1&N2 20 (13) 2.069(0.632-6.775) 0.230 Clinical M stage M0 198 (96) 2.979(1.481-5.991) 0.002 MET status V/T 256 (93) 2.999(1.638-5.488) - - Normal 112 (54) 2.346(0.870-6.327) 0.092 0.016 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 0.166 V ralue Gender Male 207 (73) 4.831(1.590-14.681) 0.005 Age - 0.011 0.051 0.016 Stage II&Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.019 Clinical stage 132 (47) 12.081(1.50-90.005) 0.019 Stage II&Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.019 Clinical stage 0.0113 0.018 0.202 T1&T2 164 (82) 12.169(1.52-97.619) 0.018 T1&T2 164 (82) 12.169(1.52-97.619) 0.018 T1&T2 164 (82) 12.169(1.52-97.619) 0.022 T1&T2 164 (82) 12.169(0.57-7.477) 0.270 <td></td> <td>N0</td> <td>132 (87)</td> <td>2.531(0.979-6.545)</td> <td> ♦—</td> <td>0.055</td>		N0	132 (87)	2.531(0.979-6.545)	 ♦—	0.055
Clinical M stage 0 198 (96) 2.979(1.481-5.991) 0.002 MET status WT 256 (93) 2.999(1.638-5.488) <0.001		N1&N2	20 (13)	2.069(0.632-6.775)	† i	0.230
M0 198 (96) 2.979(1.481-5.991) 0.002 MET status 256 (93) 2.999(1.638-5.488) 0.001 WT 256 (93) 2.999(1.638-5.488) 0.002 Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 V 0.25 5 7.5 10 12.5 15 17.5 20 22.5 25 0.166 C Characteristics N (%) Hazard Ratio (95% Cl) P value Gender 0.005 0.005 0.005 Age 0.005 0.016 0.0051 Stage II&Stage III&Stage IV 60 (30) 3.740(1.245-11.236) 0.018 Clinical stage 12.184 (52) 12.189(1.522-97.619) 0.018 Ti&T2 164 (82) 12.189(1.522-97.619) 0.018 Ti&T2 164 (82) 12.189(1.522-97.619) 0.018 Ti&T4 0.0132 (86) 9.620(1.165-80.079) 0.036 N0 132 (86) 9.620(1.165-80.079) 0.036 N18AV 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage M0 198 (96) 10.397		Clinical M stage				
MET status VT 256 (93) 2.999(1.638-5.488) -0.001 Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 C Characteristics N (%) Hazard Ratio (95% Cl) P value Gender P value 0.005 9005 0.005 Age 0.005 0.005 0.005 0.005 >60 133 (47) 12.081(1.606-90.905) 0.016 0.005 >60 148 (53) 3.846(0.993-14.899) 0.051 0.019 Clinical stage Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.018 Clinical N stage 0 0.232(0.637-8.461) 0.022 0.036 N0 132 (86) 9.620(1.165-80.079) 0.036 0.036 N18N2 21 (14) 2.065(0.570-7.477) 0.270 0.036 Clinical M stage M0 198 (96) 10.397(2.389-45.253) 0.002 0.021 WT 254 (93) 6.904(2.367-20.137) -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 <td></td> <td>MO</td> <td>198 (96)</td> <td>2.979(1.481-5.991)</td> <td>⊢●−−1</td> <td>0.002</td>		MO	198 (96)	2.979(1.481-5.991)	⊢● −−1	0.002
WT 256 (93) 2 999(1638-5488) < <td></td> <td>MET status</td> <td></td> <td></td> <td></td> <td></td>		MET status				
Hemoglobin Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 C Characteristics N (%) Hazard Ratio (95% Cl) P value Gender 0.005 0.005 Male 207 (73) 4.831(1.590-14.681) 0.005 Age 0.005 0.016 0.005 C Contracteristics N (%) Hazard Ratio (95% Cl) P value Gender 0.005 0.016 0.005 Age 0.005 0.016 0.005 C Clinical stage 0.005 0.016 Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.018 Clinical stage 0.013 2.322(0.637-8.461) 0.022 Clinical N stage 0 0.336 (18) 2.322(0.637-2.0137) 0.036 N0 132 (86) 9.620(1.156-80.079) 0.036 0.036 N18N2 21 (14) 2.065(0.570-7.477) 0.270 0.036 Clinical M stage 0.002 0.022 0.021 0.022 0.022 0.022		WT	256 (93)	2.999(1.638-5.488)	+ 	<0.001
Normal 112 (54) 2.346(0.870-6.327) 0.092 Abnormal 95 (46) 1.887(0.768-4.636) 0.166 C C C C C C P value Gender 0.052 5 7.5 10 12.5 15 17.5 20 22.5		Hemoglobin				
Abnormal 95 (46) 1.887(0.768-4.636) 0.166 0 2.5 5 7.5 10 12.5 15 17.5 20 22.5 25 C Characteristics N (%) Hazard Ratio (95% Cl) P value P value Gender Male 207 (73) 4.831(1.590-14.681) 0.005 0.005 Age - 0 0.016 0.005 0.016 >60 133 (47) 12.081(1.606-90.905) 0.016 0.051 Clinical stage Stage II&Stage II&Stage IV 60 (30) 3.740(1.245-11.236) 0.019 Clinical Tstage 11.887 (2.997.619) 0.018 0.019 0.018 Ti&T2 164 (82) 12.189(1.522-97.619) 0.018 0.202 Clinical N stage N0 132 (86) 9.620(1.156-80.079) 0.036 N1&N2 21 (14) 2.065(0.570-7.477) 0.270 0.002 Clinical M stage M0 198 (96) 10.397(2.389-45.253) 0.002 MT 254 (93) 6.904(2.367-20.137) <0.001		Normal	112 (54)	2.346(0.870-6.327)	t	0.092
C Characteristics N (%) Hazard Ratio (95% Cl) P value Gender Male 207 (73) 4.831(1.590-14.681) 0.005 Age -60 133 (47) 12.081(1.606-90.905) 0.016 >60 148 (53) 3.846(0.993-14.899) 0.051 Clinical stage Stage II&Stage III&Stage IV 60 (30) 3.740(1.245-11.236) 0.018 Clinical stage T18T2 164 (82) 12.189(1.522-97.619) 0.018 0.202 Clinical N stage N0 132 (86) 9.620(1.156-80.079) 0.036 0.022 Nt &Nz 21 (14) 2.065(0.570-7.477) 0.270 0.002 0.021 WT 254 (93) 6.904(2.367-20.137) - - 0.054 Normal 112 (55) 8.275(0.966-70.858) 0.054 0.054		Abnormal	95 (46)	1.887(0.768-4.636)	†− −	0.166
C Characteristics N (%) Hazard Ratio (95% Cl) P value Gender Male 207 (73) 4.831(1.590–14.681) 0.005 Age 0.005 0.016 >60 133 (47) 12.081(1.606–90.905) 0.016 >60 148 (53) 3.846(0.993–14.899) 0.051 Clinical stage 0.019 0.011 Stage II&Stage II&Stage II&Stage IV 60 (30) 3.740(1.245–11.236) 0.019 Clinical stage 0.013 0.018 0.022 Ti & T2 164 (82) 12.189(1.522–97.619) 0.018 Ti & T3 132 (86) 9.620(1.156–80.079) 0.036 Ni & Nu 132 (86) 9.620(1.156–80.079) 0.036 Nt & Nu 132 (86) 9.620(1.156–80.079) 0.036 Ni & Nu 132 (86) 9.620(1.156–80.079) 0.036 Ni & Nu 132 (86) 9.620(1.156–80.079) 0.036 WT 254 (93) 6.904(2.367–20.137) 4.3276 4.001 Memoglobin Mormal 112 (55) 8.275(0.966–70.858) 4.054 Abnormal						
• Unitatiziensus N (%) Hazaro Ratio (95% Cl) P Value Gender Male 207 (73) 4.831(1.590–14.681) • • 0.005 Age - 0.005 • • 0.005 • 0.005 >=60 133 (47) 12.081(1.606–90.905) • • 0.016 >=60 148 (53) 3.846(0.993–14.899) 0.051 0.016 Clinical stage Stage II&Stage II&Stage II 60 (30) 3.740(1.245–11.236) • 0.019 Clinical T stage 0.018 2.322(0.637–8.461) 0.202 0.018 Ti&RT2 164 (82) 12.189(1.522–97.619) • 0.036 Ti&RT2 164 (82) 12.289(1.56–80.079) • 0.036 N0 132 (86) 9.620(1.156–80.079) • 0.036 Nt&RV2 21 (14) 2.085(0.570–7.477) 0.270 0.036 Clinical M stage M0 198 (96) 10.397(2.389–45.253) • • • • • •		Characteristics	N (9/)	Hanned Datic (05%) ON	- 1.0 0 7.0 10 12.0 10 17.5 20 .	
Male 207 (73) 4.831(1.590-14.681) 0.005 Age 133 (47) 12.081(1.606-90.905) 0.016 >50 148 (53) 3.846(0.993-14.899) 0.051 Clinical stage 148 (53) 3.740(1.245-11.236) 0.019 Clinical T stage 0.019 0.018 0.022 Clinical T stage 12.189(1.522-97.619) 0.036 0.0202 T1&T2 164 (62) 12.189(1.522-97.619) 0.036 T3&T4 36 (18) 2.322(0.637-8.461) 0.202 Clinical N stage 0.001 0.036 0.036 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&NZ 21 (14) 2.065(0.570-7.477) 0.0270 Clinical M stage 0.002 0.0270 0.036 MET status 198 (96) 10.397(2.389-45.253) 0.002 WT 254 (93) 6.904(2.367-20.137) - - Hemoglobin 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.986) 0.170	c	Onaracteristics	IN (%)	nazaru Kalio (95% CI)		
Age <=60	с	Gender				1 Value
<=60	с	Male	207 (73)	4.831(1.590-14.681)	•	0.005
>60 148 (53) 3.846(0.993-14.899) 0.051 Clinical stage 0.019 0.019 Clinical T stage 112.189(1.245-11.236) 0.019 T1&T2 164 (82) 12.189(1.522-97.619) 0.0202 Clinical N stage 0.013 0.021 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&NZ 21 (14) 2.055(0.570-7.477) 0.270 Clinical M stage 0.002 0.002 0.002 M0 198 (96) 10.397(2.389-45.253) 0.002 WT 254 (93) 6.904(2.367-20.137)	с	Gender Male Age	207 (73)	4.831(1.590-14.681)	•	0.005
Clinical stage 5tage III&Stage III&Stage IV 60 (30) 3.740(1.245-11.236) 0.019 Clinical T stage 0.019 T1&T2 164 (82) 12.189(1.522-97.619) 0.018 T3&T4 36 (18) 2.322(0.637-8.461) 0.202 Clinical N stage 0.019 0.036 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&NZ 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.002 0.022 MO 198 (96) 10.397(2.389-45.253) 0.002 WT 254 (93) 6.904(2.367-20.137) - - Hemoglobin 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.886) 0.170	с	Age <=60	207 (73) 133 (47)	4.831(1.590-14.681) 12.081(1.606-90.905)	♦	0.005 0.016
Stage II&Stage III&Stage III 60 (30) 3.740(1.245-11.236) 0.019 Clinical T stage 12.189(1.522-97.619) 0.018 T1&T2 164 (82) 12.189(1.522-97.619) 0.012 Clinical N stage 0.019 0.036 0.019 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.019 0.036 0.022 M0 198 (96) 10.397(2.389-45.253) 0.002 MET status WT 254 (93) 6.904(2.367-20.137) - Hemoglobin 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 112 (55) 8.275(0.967-70.858) 0.170	с	Gender Male Age <=60 >60	207 (73) 133 (47) 148 (53)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899)	∲ 1 ∲ 1	0.005 0.016 0.051
Clinical T stage 164 (62) 12.189(1.522-97.619) 0.018 T38T4 36 (18) 2.322(0.637-8.461) 0.202 Clinical N stage 0.018 0.028 N0 132 (66) 9.620(1.156-80.079) 0.036 N18N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.002 0.028 0.002 M0 198 (96) 10.397(2.389-45.253) 0.002 WT 254 (93) 6.904(2.367-20.137) - - WT 254 (93) 6.904(2.367-20.137) - - - - Normal 112 (55) 8.275(0.966-70.858) 0.054 0.170 0.170	с	Geneer Male Age <=60 >60 Clinical stage	207 (73) 133 (47) 148 (53)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899)	♦	0.005 0.016 0.051
T1&T2 164 (82) 12.189(1.522-97.619) 0.018 T3&T4 36 (18) 2.322(0.637-8.461) 0.202 Clinical N stage 0 0.036 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0 0.002 M0 198 (96) 10.397(2.389-45.253) 0.002 MET status WT 254 (93) 6.904(2.367-20.137) - Hemoglobin Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.96) - 0.170	С	Genoer Male Age <=60 >60 Clinical stage Stage II&Stage IV	207 (73) 133 (47) 148 (53) 60 (30)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236)	◆-1	0.005 0.016 0.051 0.019
T3&T4 36 (18) 2.322(0.637-8.461) 0.202 Clinical N stage 0 0.036 N0 132 (86) 9.620(1.156-80.079) 0.036 N1&N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.002 0.036 M0 198 (96) 10.397(2.389-45.253) 0.002 MET status 0.001 0.036 WT 254 (93) 6.904(2.367-20.137) Hemoglobin 0.054 0.054 Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.986) 0.170	С	Gender Male Age <=60 >60 Clinical stage Stage II&Stage II/&Stage IV Clinical T stage	207 (73) 133 (47) 148 (53) 60 (30)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236)	 ↓ ↓	0.005 0.016 0.051 0.019
Clinical N stage 132 (86) 9 620(1.156-80.079) 0.036 N18N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.002 0.002 MO 198 (96) 10.397(2.389-45.253) 0.002 MET status 0.001 0.001 WT 254 (93) 6.904(2.367-20.137) - Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.886) 0.170	с	Gender Male Age <=60 >60 Clinical stage Stage II&Stage III&Stage IV Clinical T stage T1&T2	207 (73) 133 (47) 148 (53) 60 (30) 164 (82)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619)		0.005 0.016 0.051 0.019 0.018
NO 132 (86) 9.620(1.156-80.079) 0.036 N1&N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0.002 0.002 MO 198 (96) 10.397(2.389-45.253) 0.002 MET status 0.001 0.001 0.001 WT 254 (93) 6.904(2.367-20.137) 0.001 Hemoglobin 0.0054 0.0054 Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.96) 0.170	с	Gender Male Age <=60 Clinical stage Stage II&Stage III&Stage IV Clinical T stage T1&T2 T3&T4	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461)		0.005 0.016 0.051 0.019 0.018 0.202
N1&N2 21 (14) 2.065(0.570-7.477) 0.270 Clinical M stage 0 0 198 (96) 10.397(2.389-45.253) 0.002 MET status WT 254 (93) 6.904(2.367-20.137) 0.001 Hemoglobin Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.966) 0.170	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461)		0.005 0.016 0.051 0.019 0.018 0.202
Clinical M stage 0.002 M0 198 (96) 10.397(2.389-45.253) 0.002 MET status	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079)		0.005 0.016 0.019 0.018 0.202 0.036
M0 198 (96) 10.397(2.389-45.253) 0.002 MET status	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0 N1&N2	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477)		0.005 0.016 0.051 0.019 0.018 0.202 0.036 0.270
MET status <	с	Gender Male Age <=60 Clinical stage Stage II&Stage III&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical M stage	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477)		0.005 0.016 0.051 0.019 0.018 0.202 0.036 0.270
WT 254 (93) 6.904(2.367-20.137) <0.001 Hemoglobin Normal 112 (55) 8.275(0.966-70.858) • • 0.054 Abnormal 93 (45) 2.470(0.679-8.986) • 0.170	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical M stage M0	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253)		0.005 0.016 0.019 0.018 0.202 0.036 0.270 0.002
Hemoglobin Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.986) 0.170	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage TI&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical M stage M0 MET status	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253)		0.005 0.016 0.019 0.019 0.018 0.202 0.036 0.270 0.002
Normal 112 (55) 8.275(0.966-70.858) 0.054 Abnormal 93 (45) 2.470(0.679-8.986) 0.170	С	Gender Male Age <=60 Clinical stage Stage II&Stage III&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical M stage M0 MET status WT	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96) 254 (93)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253) 6.904(2.367-20.137)		0.005 0.016 0.051 0.019 0.018 0.202 0.036 0.270 0.002 <0.001
Abnormal 93 (45) 2.470(0.679-8.986) 0.170	с	Gender Male Age <=60 Clinical stage Stage II&Stage III&Stage IV Clinical T stage T1&T2 T3&T4 Clinical T stage N0 N1&N2 Clinical N stage N0 Clinical M stage M0 MET status WT Hemoglobin	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96) 254 (93)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253) 6.904(2.367-20.137)		0.005 0.016 0.019 0.018 0.202 0.036 0.270 0.002 <0.001
	с	Gender Male Age <=60 Clinical stage Stage II&Stage II Stage II&Stage IV Clinical T stage TI&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical M stage M0 MET status WT Hemoglobin Normal	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96) 254 (93) 112 (55)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253) 6.904(2.367-20.137) 8.275(0.966-70.858)		0.005 0.016 0.019 0.019 0.018 0.202 0.036 0.270 0.002 <0.001 0.054
	с	Gender Male Age <=60 Clinical stage Stage II&Stage II&Stage IV Clinical T stage T1&T2 T3&T4 Clinical N stage N0 N1&N2 Clinical N stage M0 MET status WT Hemoglobin Normal Abnormal	207 (73) 133 (47) 148 (53) 60 (30) 164 (82) 36 (18) 132 (86) 21 (14) 198 (96) 254 (93) 112 (55) 93 (45)	4.831(1.590-14.681) 12.081(1.606-90.905) 3.846(0.993-14.899) 3.740(1.245-11.236) 12.189(1.522-97.619) 2.322(0.637-8.461) 9.620(1.156-80.079) 2.065(0.570-7.477) 10.397(2.389-45.253) 6.904(2.367-20.137) 8.275(0.966-70.858) 2.470(0.679-8.986)		0.005 0.016 0.019 0.019 0.018 0.202 0.036 0.270 0.002 <0.001 0.054 0.170

0 5 15 25 35 45 55 65 75 85 95

FIGURE 7 | Prognostic performance of *TICRR* on clinical outcomes in different papillary renal cell carcinoma (PRCC) patient subgroups. Patients were divided into different subgroups according to sex, age, clinical stage, clinical TNM stage, MET status, and hemoglobin level. For each subgroup, the prognostic performance of *TICRR* on overall survival (A), progression-free interval (B), and disease-specific survival (C) were evaluated by Cox regression, and the results are presented as hazard ratio. The bar represents the 95% confidence interval of hazard ratio, the diamond's size represents the significance of *TICRR*'s performance.





12

Uncontrollable DNA replication and thus cell proliferation are an essential mechanism in tumorigenesis. As a critical DNA replication regulator, *TICRR* plays an important role in several solid cancers (Yu et al., 2019). In our study, we found that *TICRR* was significantly elevated in several urogenital cancers, including PRCC, chromophobe renal carcinoma, renal clear cell carcinoma, bladder urothelial carcinoma, cervical squamous cell carcinoma, and in endocervical adenocarcinoma. Moreover, *TICRR* was also upregulated in tumors of other organs, such as breast invasive carcinoma, colon adenocarcinoma, and glioblastoma multiforme. Thus, *TICRR* may be a crucial hub gene in tumorigenesis.

Further, we attempted to describe the potential functions and mechanisms involving TICRR in PRCC. Based on results from previous studies (Bruck and Kaplan, 2015; Bruck et al., 2015), TICRR coordinates the assembly and activation of the eukaryotic replication fork helicase, which further unwinds double-stranded DNA and initiates DNA replication. In our study, based on functional annotation of TICRR-associated DEGs, epithelial cell differentiation and urogenital system development were closely associated with TICRR expression. Moreover, TICRR was associated with negative regulation of cell division. Based on additional GSEA, several cell cycle-related events were enriched in the high-TICRR group. The above data all provided evidence that TICRR functions as a critical DNA replication initiation regulator in PRCC. In a different study focusing on breast cancer, TICRR showed a similar effect on tumorigenesis, as silencing of TICRR significantly inhibited DNA replication, arrested cell cycle progression, and activated DNA damage (Yu et al., 2019). More interestingly, we found that patients in the high-TICRR group more frequently harbored MET mutations, which represents an appealing drug target given its prevalence in PRCC. The functional annotation analysis revealed that higher TICRR levels were associated with increased pathophysiological activity of the MET gene. Therefore, TICRR expression might be of great importance in PRCC tumorigenesis by affecting MET status and function.

We also revealed an underlying relationship between TICRR expression and immune cell infiltration. TICRR expression was negatively correlated with DCs, macrophages, and neutrophils. As the most effective antigen presenting cells, DCs activate CD 8 + T cells by cross-priming and further initiate antitumor immunity (Fu and Jiang, 2018). In the following immune response, neutrophils and macrophages work together against tumors (Qu et al., 2018). Moreover, neutrophils proved to be associated with better prognosis in different cancers (Donskov, 2013). Therefore, overexpressed TICRR seemed to dampen tumor immunity, help cancer cells escape from elimination, and finally accelerate tumorigenesis. On the other hand, we found a significantly positive correlation between TICRR expression and Tcm infiltration. Tumor-infiltrated Tcm cells have been reported in multiple cancers (Beckhove et al., 2004), which often exhibit dysfunctional phenotypes correlating with cancer progression (Reading et al., 2018). It can be explained that excessive neoantigen exposure caused functionally altered Tcm cells that skewed the anti-tumor response toward non-responsiveness (Merad et al., 2013).

Another issue of interest was the clinical significance of TICRR in PRCC. The ROC curve for TICRR discrimination of PRCC diagnosis had an AUC of 0.807, strongly suggesting that TICRR was a convincing biomarker for PRCC diagnosis. Moreover, we demonstrated that higher TICRR expression was correlated with several clinicopathological characteristics: female sex, younger age, abnormal hemoglobin, and worse clinical stages. As most of the above characteristics were risk factors for survival in PRCC patients (Fernandes and Lopes, 2015; Peng et al., 2018), we proposed TICRR as a marker for poor survival in PRCC. According to further Cox regression analyses and nomograms, TICRR presented satisfactory performance on clinical outcomes in PRCC. Patients with higher TICRR levels showed strikingly worse overall survival, progression-free interval, and diseasespecific survival. This prognostic value of TICRR seemed to be more prominent in patients with specific features: male sex, age below 60 years, and clinical stages II-IV and T stages T1-T2. Using an online tool LOGpc (Long-term Outcome and Gene Expression Profiling Database of pan-cancers)⁴, TICRR was proved to be associated with lower overall survival in other tumors, such as renal clear cell carcinoma (Xie et al., 2019a), adrenocortical carcinoma (Xie et al., 2019b), breast invasive carcinoma (Yan et al., 2019), and lung cancer (Yan et al., 2020). The universal upregulation and predictive performance of TICRR indicated a possibility that it could represent a common prognostic biomarker for these cancer types.

Although we uncovered a potential mechanism for *TICRR* activity in PRCC tumorigenesis and its predictive value in PRCC clinical outcomes, our study still presented several limitations. First, because of the incomplete information about treatments and corresponding responses, we could not evaluate a specific role for *TICRR* in PRCC treatment. Second, we mainly focused on the RNA sequencing data from the TCGA database; thus, we were unable to provide information on relative protein levels or downstream pathways involving *TICRR*. Thus, these will remain areas for further *in vivo* and *in vitro* studies concentrating on the direct mechanism of *TICRR* activity in PRCC.

CONCLUSION

Increased *TICRR* expression in PRCC might play a role in tumorigenesis by regulating cell cycle and exhibiting prognostic value for clinical outcomes. This study sheds light on *TICRR* as a potential therapeutic target for PRCC.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://portal.gdc.cancer.gov/.

ETHICS STATEMENT

All the data were collected and downloaded from TCGA database. As TCGA database is open to the public under specific

⁴http://bioinfo.henu.edu.cn/DatabaseList.jsp

guidelines, it is confirmed that all written informed consents were achieved. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SX and YL: project investigation. XT: methodology. JL: writing-original draft. XL: writing-review and editing. ZH: project administration and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Natural Science Foundation of Guangdong Province (No. 2018A030310016), Guangdong Provincial Medical Science Foundation (No. A2018110), and the Lab Opening-up Project for college students of Guangzhou Medical University.

REFERENCES

- Akhtar, M., Al-Bozom, I. A., and Al Hussain, T. (2019). Papillary renal cell carcinoma (PRCC): an Update. Adv. Anat. Pathol. 26, 124–132.doi: 10.1097/ pap.00000000000220
- Alshenawy, H. A. (2015). Immunohistochemical panel for differentiating renal cell carcinoma with clear and papillary features. *Pathol. Oncol. Res.* 21, 893–899.doi: 10.1007/s12253-015-9898-7
- Beckhove, P., Feuerer, M., Dolenc, M., Schuetz, F., Choi, C., Sommerfeldt, N., et al. (2004). Specifically activated memory T cell subsets from cancer patients recognize and reject xenotransplanted autologous tumors. *J. Clin. Invest.* 114, 67–76.doi: 10.1172/jci20278
- Blighe, K. (2019). EnhancedVolcano: Publication-Ready Volcano Plots With Enhanced Colouring and Labeling.
- Boos, D., Sanchez-Pulido, L., Rappas, M., Pearl, L. H., Oliver, A. W., Ponting, C. P., et al. (2011). Regulation of DNA replication through Sld3-Dpb11 interaction is conserved from yeast to humans. *Curr. Biol.* 21, 1152–1157.doi: 10.1016/j.cub. 2011.05.057
- Bruck, I., and Kaplan, D. L. (2015). Conserved mechanism for coordinating replication fork helicase assembly with phosphorylation of the helicase. *Proc. Natl. Acad. Sci. U.S.A.* 112, 11223–11228.doi: 10.1073/pnas.1509608112
- Bruck, I., Perez-Arnaiz, P., Colbert, M. K., and Kaplan, D. L. (2015). Insights into the initiation of eukaryotic DNA replication. *Nucleus* 6, 449–454.doi: 10.1080/ 19491034.2015.1115938
- Cao, Q., Ruan, H., Wang, K., Song, Z., Bao, L., Xu, T., et al. (2018). Overexpression of PLIN2 is a prognostic marker and attenuates tumor progression in clear cell renal cell carcinoma. *Int. J. Oncol.* 53, 137–147. doi: 10.3892/ijo.2018.4384
- Charrasse, S., Gharbi-Ayachi, A., Burgess, A., Vera, J., Hached, K., Raynaud, P., et al. (2017). Ensa controls S-phase length by modulating Treslin levels. *Nat. Commun.* 8:206. doi: 10.1038/s41467-017-00339-4
- Donskov, F. (2013). Immunomonitoring and prognostic relevance of neutrophils in clinical trials. Semin. Cancer Biol. 23, 200–207.doi: 10.1016/j.semcancer.2013. 02.001
- Farber, N. J., Kim, C. J., Modi, P. K., Hon, J. D., Sadimin, E. T., and Singer, E. A. (2017). Renal cell carcinoma: the search for a reliable biomarker. *Transl. Cancer Res.* 6, 620–632. doi: 10.21037/tcr.2017.05.19
- Fernandes, D. S., and Lopes, J. M. (2015). Pathology, therapy and prognosis of papillary renal carcinoma. *Future Oncol.* 11, 121–132.doi: 10.2217/fon.14.133
- Fu, C., and Jiang, A. (2018). Dendritic cells and CD8 T Cell immunity in tumor microenvironment. Front. Immunol. 9:3059. doi: 10.3389/fimmu.2018.03059

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene. 2020.605378/full#supplementary-material

Supplementary Figure 1 | Differential non-coding RNA expression profiles in PRCC patients stratified by *TICRR* levels. Expression profiles of miRNAs (A,C) and IncRNAs (B,D) in two groups are presented by volcano plots (A,B) and heatmaps (C,D).

Supplementary Figure 2 | Protein-protein interaction networks of DEGs in PRCC patients with high- and low-*TICRR* expression levels. Based on the 691 differentially expressed mRNAs between high- and low-*TICRR* expression groups, we analyzed interactions using the STRING database, where the interaction threshold was set as 0.4. The line represents protein-protein interactions. The darker the filling color, the more mRNA interactions.

Supplementary Figure 3 | Mean decrease Gini plot for important indexes associated with overall survival in PRCC patients. The random forest model was used to rank significant indexes, enrolling age, sex, smoking history, serum calcium level, hemoglobin level, TMN stage, clinical stage, and *TICRR* expression.

Supplementary Table 1 | Top 20 clusters in pathway and process enrichment analysis of DEGs in PRCC patients with distinct *TICRR* levels.

- Hänzelmann, S., Castelo, R., and Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinformatics 14:7.doi: 10. 1186/1471-2105-14-7
- Harrell, F. E. Jr. (2020). rms: Regression Modeling Strategies.
- Kolde, R. (2019). *ptheatmap: Pretty Heatmaps*.
- Liu, J., Lichtenberg, T., Hoadley, K. A., Poisson, L. M., Lazar, A. J., Cherniack, A. D., et al. (2018). An integrated TCGA pan-cancer clinical data resource to drive high-quality survival outcome analytics. *Cell* 173, 400.e11–416.e11. doi: 10.1016/j.cell.2018.02.052
- Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8

Max Gordon, T. L. (2020). forestplot: Advanced Forest Plot Using 'grid' Graphics.

- Maya-Mendoza, A., Moudry, P., Merchut-Maya, J. M., Lee, M., Strauss, R., and Bartek, J. (2018). High speed of fork progression induces DNA replication stress and genomic instability. *Nature* 559, 279–284.doi: 10.1038/s41586-018-0261-5
- Merad, M., Sathe, P., Helft, J., Miller, J., and Mortha, A. (2013). The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev. Immunol.* 31, 563–604.doi: 10.1146/annurev-immunol-020711-074950
- Morabito, R. A., Talug, C., Zaslau, S., and Kandzari, S. (2010). Asymptomatic advanced pediatric papillary renal cell carcinoma presenting as a pulmonary embolus. Urology 76, 153–155.doi: 10.1016/j.urology.2009.09.016
- Peng, D., Zhang, C. J., Tang, Q., Zhang, L., Yang, K. W., Yu, X. T., et al. (2018). Prognostic significance of the combination of preoperative hemoglobin and albumin levels and lymphocyte and platelet counts (HALP) in patients with renal cell carcinoma after nephrectomy. *BMC Urol.* 18:20. doi: 10.1186/s12894-018-0333-8
- Qu, X., Tang, Y., and Hua, S. (2018). Immunological approaches towards cancer and inflammation: a cross talk. *Front. Immunol.* 9:563. doi: 10.3389/fimmu. 2018.00563
- Reading, J. L., Gálvez-Cancino, F., Swanton, C., Lladser, A., Peggs, K. S., and Quezada, S. A. (2018). The function and dysfunction of memory CD8(+) T cells in tumor immunity. *Immunol. Rev.* 283, 194–212.doi: 10.1111/imr.12657
- Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504.doi: 10.1101/ gr.1239303
- Siegel, R. L., Miller, K. D., and Jemal, A. (2018). Cancer statistics, 2018. CA Cancer J. Clin. 68, 7–30.doi: 10.3322/caac.21442

- Song, J., Liu, Y. D., Su, J., Yuan, D., Sun, F., and Zhu, J. (2019). Systematic analysis of alternative splicing signature unveils prognostic predictor for kidney renal clear cell carcinoma. *J. Cell. Physiol.* 234, 22753–22764. doi: 10.1002/jcp.28840
- Srigley, J. R., Delahunt, B., Eble, J. N., Egevad, L., Epstein, J. I., Grignon, D., et al. (2013). The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia. *Am. J. Surg. Pathol.* 37, 1469–1489.doi: 10. 1097/PAS.0b013e318299f2d1
- Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15545–15550.doi: 10.1073/pnas.0506580102
- Svetnik, V., Liaw, A., Tong, C., Culberson, J. C., Sheridan, R. P., and Feuston, B. P. (2003). Random forest: a classification and regression tool for compound classification and QSAR modeling. *J. Chem. Inf. Comput. Sci.* 43, 1947–1958.doi: 10.1021/ci034160g
- Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., et al. (2019). STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genomewide experimental datasets. *Nucleic Acids Res.* 47, D607–D613. doi: 10.1093/nar/gky1131
- Xie, L., Wang, Q., Dang, Y., Ge, L., Sun, X., Li, N., et al. (2019a). OSkirc: a web tool for identifying prognostic biomarkers in kidney renal clear cell carcinoma. *Future Oncol.* 15, 3103–3110.doi: 10.2217/fon-2019-0296
- Xie, L., Wang, Q., Nan, F., Ge, L., Dang, Y., Sun, X., et al. (2019b). OSacc: gene expression-based survival analysis web tool for adrenocortical carcinoma. *Cancer Manag. Res.* 11, 9145–9152.doi: 10.2147/cmar.S215586

- Yan, Z., Wang, Q., Lu, Z., Sun, X., Song, P., Dang, Y., et al. (2020). OSluca: an interactive web server to evaluate prognostic biomarkers for lung cancer. *Front. Genet.* 11:420. doi: 10.3389/fgene.2020.00420
- Yan, Z., Wang, Q., Sun, X., Ban, B., Lu, Z., Dang, Y., et al. (2019). OSbrca: a web server for breast cancer prognostic biomarker investigation with massive data from tens of cohorts. *Front. Oncol.* 9:1349. doi: 10.3389/fonc.2019.01349
- Yu, G., Wang, L. G., Han, Y., and He, Q. Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics* 16, 284–287.doi: 10.1089/omi.2011.0118
- Yu, Q., Pu, S. Y., Wu, H., Chen, X. Q., Jiang, J. J., Gu, K. S., et al. (2019). TICRR contributes to tumorigenesis through accelerating DNA replication in Cancers. *Front. Oncol.* 9:516. doi: 10.3389/fonc.2019.00516
- Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., et al. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* 10:1523. doi: 10.1038/s41467-019-09234-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Xia, Lin, Li, Tan and Huang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.