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

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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...
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 database (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 (Thf), 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 high- and low-TICRR expression groups using the Wilcoxon rank sum test (continuous variables) or Pearson’s chi-square test.
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.
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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
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.

### 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),

**TABLE 1** | Clinicopathological characteristics of PRCC patients with differential TICRR expression.

| Characteristic   | Level | Low-TICRR group (n = 144) | High-TICRR 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 | 30 (22.4%) | 30 (21.9%) |
|                 | American | 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%)                |
|                 | T3    | 10 (9.7%)                 | 25 (25.5%)                |
|                 | T4    | 0 (0.0%)                  | 1 (1.0%)                  |
| Clinical N stage (%) | N0 | 72 (93.5%) | 60 (79.8%) |
|                 | N1    | 5 (6.5%)                  | 18 (18.4%)                |
|                 | N2    | 0 (0.0%)                  | 2 (2.6%)                  |
| Clinical M stage (%) | M0 | 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.
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),
FIGURE 5 | Predictive value of TICRR expression for diagnosis and clinical outcomes in papillary renal cell carcinoma (PRCC) patients. (A) Receiver operating characteristic (ROC) curve analysis evaluating the performance of TICRR for PRCC diagnosis. Shown are the Kaplan–Meier analyses comparing overall survival (B), progression-free interval (C), and disease-specific survival (D) between high- and low-TICRR expression groups.

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

| Characteristics | 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*** | 20.906** |
| Clinical stage (stage II–IV vs. stage I) | 5.123*** | 3.686 | 6.983*** | 1.976 | 27.918*** | 12.037* |
| Smoker (yes vs. no) | 0.584 | 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](https://www.frontiersin.org)

**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 (1.110–4.990)*                     | 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** |       |                                |                                           |                                        |
| N0              | 132 (86)| 3.030 (1.012–9.076)*           | 2.531 (0.979–6.545)                      | 9.620 (1.156–80.079)*                  |
| N1–N2           | 21 (14)| 2.066 (0.570–7.477)            | 2.069 (0.632–6.775)                      | 2.065 (0.570–7.477)                   |
| **Clinical M stage** |       |                                |                                           |                                        |
| M0              | 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 (1.473–6.767)**          | 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; CI, confidence interval; WT, wild type; Mut, mutation. *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \).
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.
FIGURE 8 | Distinct clinical outcomes based on TICRR expression in papillary renal cell carcinoma (PRCC) patient subgroups. Kaplan–Meier analysis showing the comparison of overall survival (A,D,G,J), progression-free interval (B,E,H,K), and disease-specific survival (C,F,I,L) between high- and low-TICRR expression groups in several PRCC patient subgroups, including male sex (A–C), age below 60 years (D–F), clinical stage II–IV (G–I), and T stages T1–T2 (J–L).
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 anti-tumor 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 disease-specific 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), 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

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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.

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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 mRNAs (A,C) and lncRNAs (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, TNM 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.
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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.

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