Overexpression and Prognostic Significance of PTPN2 May be a Novel Immunotherapy Target in Renal Clear Cell Carcinoma

Peipei Liu*

Department of Histology and Embryology, Shenyang Medical College, Shenyang, China

*Corresponding author: Peipei Liu, Department of Histology and Embryology, Shenyang Medical College, Shenyang, China,

✉ E-mail: liupeipei@symc.edu.cn

Abstract

Immunotherapy has significantly advanced in Clear Cell Renal Cell Carcinoma (ccRCC). We aimed to find a new immune-related prognostic biomarker and immunotherapeutic target for ccRCC. We analyzed the expression, survival, and related immune gene marker sets data of PTPN2 in patients with ccRCC from TCGA. PTPN2 expression was increased in ccRCC compared to normal tissue. PTPN2 was closely related to T stage (P=0.008), TNM stage (P=0.017) and Grade (P=0.002). Overexpression of PTPN2 predicted a poor survival in ccRCC (P< 0.001). PTPN2 was also related to six types of tumor immune-infiltrating cells, including B cells, CD8+T cells, CD4+T cells, Macrophage, Neutrophils, Dendritic cells. PTPN2 was related CTLA-4 (P=5.645404E-26, r=0.4339333) and PDCD1 (P=5.645404E-26, r=0.4339333). Furthermore, the survival rate in patients with high PTPN2 and CTLA4 was significantly lower than that other three strata (P<0.0001). GSEA and GO biological analysis was conducted, which was indicated PTPN2 was involved in many immune and inflammatory pathways, including IL-4, IL-12, CD3 T-cell, CD4 T-cell, intestinal inflammation, systemic inflammatory regulation related factors, and so on. Our results implied that PTPN2 was considered as the potential immune therapeutic target and prognostic biomarker in ccRCC.

Keywords: ARenal cell; Carcinoma; Immunotherapy; Prognosis; Protein tyrosine phosphatase; Non-receptor type 2

Introduction

As the most common pathological subtype of Renal Cell Carcinoma (RCC), Clear Cell Renal Cell Carcinoma (ccRCC) is associated with high morbidity and poor prognosis. To date, surgery is also the primary treatment for most ccRCC; radiotherapy and chemotherapy are largely ineffective in the treatment of ccRCC. To improve the survival of ccRCC, many Immune Checkpoint Inhibitors (ICIs) have been approved by the FDA, such as nivolumab and ipilimumab. However, only a few patients with advanced renal cancer have responded to immunotherapy. So the new immunotherapy target may improve the naïve vision of single target-based immunotherapy [1-6].

PTPN2 was discovered in T-cells, which is also known as T-Cell Protein Tyrosine Phosphatases (TCPTP). PTPN2 negatively regulated the pro-inflammatory pathways, such as INF-γ induced Janus kinase (JAK)-signaling and STAT signaling et al. The importance of PTPN2 in regulating tumorigenicity pathways were also highlighted [7]. The study showed that PTPN2 was negatively associated with activation of AKT in breast cancer. Grohmedann et al. also demonstrated that depletion of PTPN2 in hepatocytes promoted Hepatocellular Carcinoma (HCC) in mice. PTPN2 may play a tumor-suppressive role in tumors based on the above studies. A fascinating new study published in Nature has reported that PTPN2 deletion markedly increased the response of tumors to immunotherapy by enhancing interferon-γ-mediated effects on antigen presentation and growth suppression. Besides, Wiede et al. reported that PTPN2 could regulate the production of exhausted CD8 positive T cell subsets and control tumor immunity [8]. Due to the role of PTPN2 in tumor immunity, it may become a new target of immunotherapy [7-12].

Therefore, in this study, we firstly investigated the expression profiling and prognostic value of PTPN2 in various solid tumors based on TCGA datasets. Then we focused on Kidney Renal Clear Cell Carcinoma (KIRC) and tried to pinpoint the meaningful finding for future cancer immunotherapy. In this study, ccRCC was used instead of KIRC.

Materials and Methods

Expression profiling of PTPN2 in human cancers

The Tumor Immunological Estimation Resource (TIMER) platform was used to explore the expression profiling of PTPN2 in pan-cancer and the correlation between infiltration levels of six immune cell types (B cells, CD4+T cells, CD8+T cells,

neutrophils, macrophages, and dendritic cells) in KIRC [23]. PTPN2 transcriptome data from 31 types of tumors in the TCGA dataset were obtained from the University of California, Santa Cruz Cancer. Gene expression level was presented as log2 RSEM (RNA-Seq by Expectation-Maximization). To determine the prognostic significance of PTPN2 in pan-cancer, the significance of Cox proportional hazards models were performed using the survival and survminer packages. The forest plot was performed with the forest plot package in the R language.

Correlations between clinic pathologic data and PTPN2 expression in KIRC

The clinic pathological information of Kidney Renal Clear Cell Carcinoma (KIRC), including gender, age, TNM stage, T stage, N stage, M stage. The correlations between clinicopathologic data and PTPN2 expression were analyzed by SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). Patients with KIRC were divided into high and low groups according to the median value of PTPN2 expression. The PTPN2 expression of different clinicopathologic groups was compared by Chiq test or student's t-test. Overall survival was estimated by the Kaplan–Meier method and compared by log-rank tests using survival and survminer package in R language. Univariate and multivariate Cox regression analyses were used to compare the effect of PTPN2 on prognosis, along with the included clinical variables. A value of $P<0:05$ were considered statistically significant. Based on Cox multivariate regression analysis for OS, a nomogram was formulated by the package of rms package in R language.

Functional annotation of co-expression gene network of PTPN2

To explore the unique roles of PTPN2, PTPN2 co-expression genes were identified in the TCGA KIRC database with R language by setting the Pearson coefficient >0.4. Gene ontology (GO)/KEGG terms, canonical pathways, hall mark gene sets enrichment among the co-expression genes of PTPN2 was performed using Metascape [24]. The immunologically relevant list of genes curated with functions and Gene Ontology terms was downloading from the immunology database and analysis portal (ImmPort) system [25]. ImmPort integrates across publicly available datasets related to immunology data and facilitates transparency and reproducibility in immunology research.

Functional gene set enrichment analysis (GSEA) of PTPN2

GSEA was carried out using the java-based graphical user interface GSEA v4.1.0. For our research, PTPN2 expression levels in KIRC were dichotomized into two groups to annotate phenotype. The: C7 immunologic signature gene sets were downloaded from the Broad Institute Molecular Signature Database (MSigDB v7.1). To characterize biologically relevant changes in molecular signaling pathways among two groups, we furthermore calculated the enrichment for each pathway to identify significantly enriched concepts. All other parameters were set to default values. A nominal $P<0.01$ and FDR<0.25 were used as thresholds for determining the significance of the Enrichment Score (ES) [26,27].

Results

The expression landscape of PTPN2 in pan-cancer TCGA data

Integrated analysis was performed on tumor patients in The Cancer Genome Atlas (TCGA) datasets, a comprehensive database containing 11,000 patients' samples. The expression profiling of PTPN2 was visualized by the TIMER platform based on pan-cancer TCGA data, including 31 different tumor types. Among 31 types of cancer, the result showed that PTPN2 was expressed in all kinds of cancer, but the expression levels were different. The highest expression was Thymoma (THYM), and the lowest was Liver Hepatocellular Carcinoma (LIHC). The expression level of other types of cancer is between these two types of cancer. Compared to normal tissue, PTPN2 was over-expressed in KIRC, LIHC, Lung Squamous Cell Carcinoma (LUSC), Stomach Adenocarcinoma (STAD), Head and Neck Squamous Cell Carcinoma (HNSC), Esophageal Carcinoma (ESCA), Bladder Urothelial Carcinoma (BLCA), and Cholangiocarcinoma (CHOL) based on TIMER pancancer analysis **(Figure 1A)**.

To further assess the prognostic role of PTPN2 in different cancers, we explored the relationship between mRNA expression of PTPN2 and patient survival using R. The relationship between PTPN2 and prognosis among different cancer types was summarized in **Figure 1B**. Among the 32 cancers examined, the high PTPN2 mRNA expression was found to be significantly correlated with decreased probability of survival for Adrenocortical Carcinoma (ACC) (HR=3.527; P=0.001), Glioblastoma Multiforme (GBM) (HR=1.820; P=0.003), KIRC (HR=2.331; P<0.001), Brain Lower Grade Glioma (LGG) (HR=2.559; P<0.001), Pancreatic Adenocarcinoma (PAAD) (HR=1.738; P=0.010), Pheochromocytoma and Paraganglioma (PCPG) (HR=9.665; P=0.007), Prostate adenocarcinoma (PRAD) (HR=10.477; P=0.025), Uterine Corpus Endometrial Carcinoma (UCEC) (HR=1.804; P= 0.007), and Uveal Melanoma (UVM) (HR=3.776; P=0.004). And high expression of PTPN2 correlated with better survival in patients with several types of cancer, including BLCA, Breast invasive carcinoma (BRCA), Ovarian serous cystadenocarcinoma (OV), Skin Cutaneous Melanoma (SKCM), and THYM.

The prognostic role of PTPN2 in KIRC

When we further investigate the potential clinical role of PTPN2 in KIRC patients, the results showed PTPN2 was closely related to the T stage (P=0.008), TNM stage (P=0.017), and Grade (P=0.002) as shown in **Table 1.** Overexpression of PTPN2 predicted poor survival in KIRC based on the TCGA cohort, which was revealed by the Kaplan-Meier method (P<0.001) **(Figure 2A)**. In the multiple Cox analysis, PTPN2, age, and Grade were independent risk factors for OS. The univariate and multivariate analyses are listed in **Table 2.** Furthermore, we established a nomogram to predict the probability of OS in KIRC patients (Figure 2B). In this model, the Grade stage, TNM stage, age, and PTPN2 expressions have important effects on KIRC overall survival prediction.
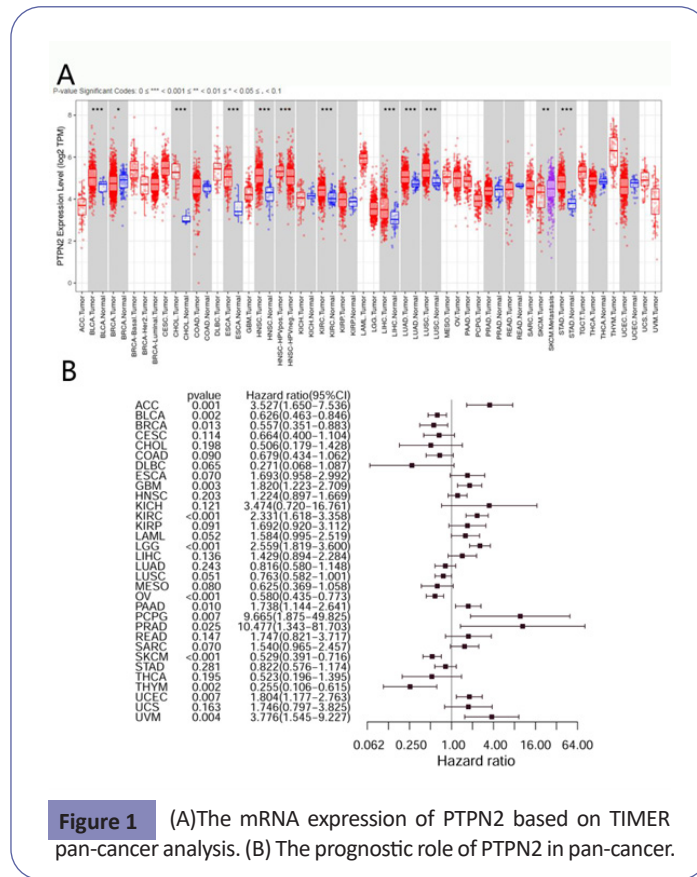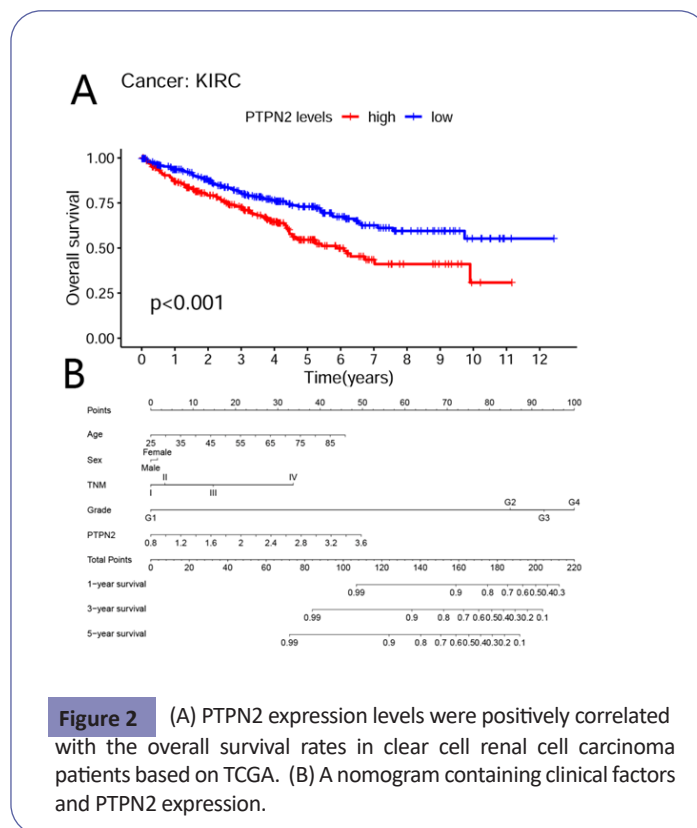
Figure 1 (A)The mRNA expression of PTPN2 based on TIMER pan-cancer analysis. (B) The prognostic role of PTPN2 in pan-cancer.

Table 1: Demographics and clinicopathologic characteristic for KIRC patients in the TCGA cohort.

	Total	PTPN2 low expression	PTPN2 high expression	P-value
Age	60.56 ± 12.14	60.59 ± 12.18	60.51 ± 12.10	0.939
Sex				
Female	186	99	87	0.372
Male	345	167	178	
T stage				0.008
T1	272	155	117	
T2	69	33	36	
T3	200	98	102	
T4	11	1	10	
TNM				0.017
Stage I	266	152	114	
Stage II	57	28	29	
Stage III	123	53	70	
Stage IV	82	32	50	
NA	3	1	2	
M stage				0.059
M0	420	222	198	
M1	78	31	47	

Mx	31	13	18	
NA	2	0	2	
N stage				
N0	239	113	126	0.213
N1	16	5	11	
Nx	276	148	128	
Grade				0.002
G1	14	9	5	
G2	227	116	111	
G3	207	114	93	
G4	75	23	52	
Gx	5	1	4	
NA	3	3	0	

Figure 2 (A) PTPN2 expression levels were positively correlated with the overall survival rates in clear cell renal cell carcinoma patients based on TCGA. (B) A nomogram containing clinical factors and PTPN2 expression.

Table 2: Univariate and multivariate analyses of prognostic factors for overall survival using Cox proportional hazards regression model (N=531).

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age	1.029	1.016-1.042	<0.001	1.027	1.009-1.046	0.003
T stage	1.892	1.606-2.230	<0.001	0.872	0.540-1.407	0.574
N stage	3.15	1.626-6.101	0.001	1.427	0.698-2.196	0.33
M stage	4.256	3.108-5.830	<0.001	1.521	0.676-3.419	0.311
TNM stage	1.857	1.625-2.123	<0.001	1.584	0.944-2.659	0.081
Grade	2.244	1.828-2.754	<0.001	1.48	1.067-2.053	0.019
PTPN2	2.389	1.659-3.439	<0.001	1.947	1.431-2.649	<0.001

PTPN2 and TIICs

The Tumour Immunological Estimation Resource (TIMER) platform was explored the correlation between tumor Immune-Infiltrating Cells (TIICs) and PTPN2, and the statistical results were based on Spearman correlation analysis. The correlation between PTPN2 and TIICs was very prominent. PTPN2 was related to all six types of TIICs (B cells, P=1.19e-8; CD8+T cells, P=2.27e-10; CD4+T cells, P=2.21e-15; Macrophage, 1.19e-19; Neutrophils, P=3.14e-33; Dendritic cells, P=1.09e-18) **(Figure 3)**.

## Gene co-expression net analysis and GSEA analysis

To further investigate the underlying mechanism of PTPN2 in KIRC, gene co-expression net analyses were performed by Metascape. These co-expression genes were significantly enriched in mitotic cell cycle phase transition, centrosome duplication; DNA-dependent DNA replication; DNA repair; regulation of T cell activation . GSEA was performed here to identify the biological gene sets or pathways for PTPN2. we found 560 immune-related terms, including GSE29615_CTRL_VS_DAY3_LAIV_IFLU_VACCINE_PBMC_UP; GSE1460_NAIVE_CD4_TCELL_CORD_BLOOD_VS_THYMIC_STROMAL_CELL_DN;

GSE12839_CTRL_VS_IL12_TREATED_PBMC_UP; SE21546_UNSTIM_VS_ANTI_CD3_STIM_SAP1A_KO_DP_THYMOCYTES_UP; GSE16385_UNTREATED_VS_12H_ROSIGLITAZONE_IL4_TREATED_MACROPHAGE_DN

For gene set enrichment analysis, a series of GO terms related to immune and inflammatory factors were also found, including CD4 T-cell differentiation-related factors, innate and adaptive responses to vaccination, regulating lipid metabolism and inflammatory response in macrophages and dendritic cells and systemic inflammatory regulation related factors.

PTPN2 and immune-related genes

We acquired the immune-related genes from the ImmPort database. Then we identified the correlation between PTPN2 and these immune-related genes. The results showed that PTPN2 was related to many immune-related genes. To our greatest interest, CTLA-4 (P=8.31E-39, r=0.495) and PDCD1 (P=1.22E-14, r=0.306) were included in these related many immune-related genes. Furthermore, when the patients with KIRC were grouped into four strata by the level of PTPN2 and CTLA-4, the survival rate in patients with high PTPN2 and CTLA4 was significantly lower than those other three strata **(Figure 4)**.
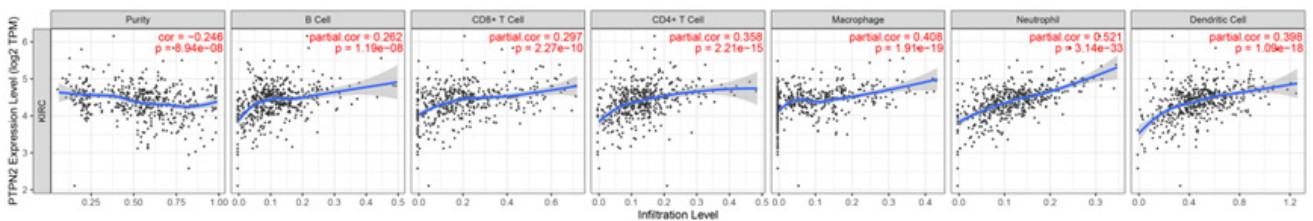
Figure 3   The correlation between tumor immune-infiltrating cells (TIICs) and PTPN2 expression levels based on TIMER analysis.
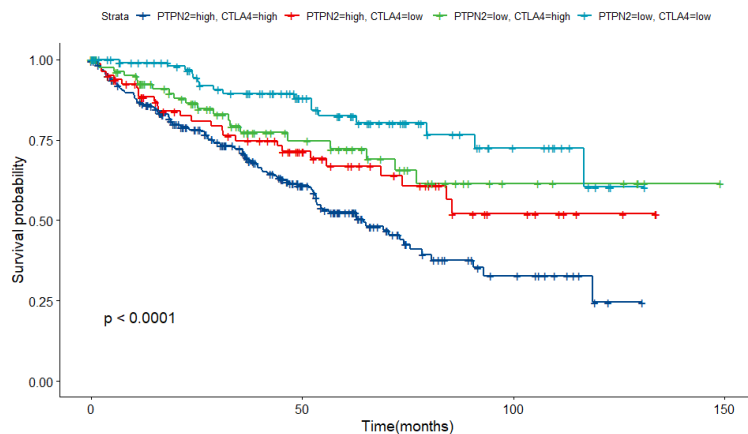
Figure 4   The Kaplan-Meier analysis comparing overall survival between PTPN2 and CTLA4 expressions.

Discussion

We performed an integrative analysis focused on the expression of PTPN2 based on TCGA clinical tumor cases and identified the over-expression of PTPN2 in KIRC. Next, we found PTPN2 was a prognostic marker in KIRC. Furthermore, the multivariate analysis demonstrated that age, Grade, and PTPN2 were independent prognostic factors in TCGA KIRC dataset. More importantly, using Timer platform analysis, we found PTPN2 correlated with B cell, CD8+T cell, CD4+T cell, Macrophage, Neutrophil, and Dendritic cell. Then, CTLA4 was screened from IMMPORT data due to the results of correlation analysis. So, PTPN2 combined with CTLA4 as a model to predict the prognosis of KIRC was constructed; this model could help clinicians predict the disease prognosis and select the suitable treatment for KIRC.

PTPN2 was a protein tyrosine phosphatase family member, which was associated with many key signaling in tumorigenesis. PTPN2 was deleted in 6% of all T cell acute lymphoblastic leukemia, and it was involved in JAK/STAT signaling and tumorigenesis. In chronic myeloid leukemia patients, high PTPN2 expression was associated with poor Major Molecular Response (MMR). Shilds et al. reported that PTPN2 was deficient in triple-negative primary breast cancer [11]. They also found that loss of PTPN2 in the human breast cancer cell lines could increase cell proliferation. In our study, PTPN2 was over-expressed in KIRC, LIHC, LUSC, STAD, HNSC, ESCA, BLCA, and CHOL based on TIMER pan-cancer analysis. Targeting on PTPN2 may be a novel candidate gene for personalized tumour treatment in these cancer types. The study from Wang et al. also found PTPN2 expression level was increased in glioblastomas and associated with gliomas of the IDH wild-type and mesenchymal subtype. In 2017, PTPN2 was identified as a cancer immunotherapy target through CRISPR screening, which was published in Nature [11]. They also found that the increased sensitivity to anti-PD-1 immunotherapy in PTPN2-deficient tumors dependent on IFN-γ signalling [11]. These findings redefine our understanding of the role of PTPN2 in the tumor [13-18].

Immune checkpoint inhibitors have been successfully used in various solid tumors, such as lung cancer and breast cancer. The combination of immunosuppressive agents has a good effect on these types of tumors, such as anti-CTLA-4 combine anti-PD-1. In our study, we not only found PTPN2 was correlated with many immune cells but also screened out CTLA-4, which was associated with PTPN2. Similarly, to sensitize anti-PD-1 immunotherapy, our findings suggested that if PTPN2 deletion may also sensitize tumors to anti-CTLA-4 immunotherapy [19-22].

Conclusion

In conclusion, high PTPN2 levels significantly correlated with poor survival in ccRCC. PTPN2 is extremely closely associated with many types of TILs and CTLA-4. Our comprehensive bioinformatics analysis results, PTPN2 may be a potential prognosis biomarker and a novel immunotherapeutic target for KIRC.

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Availability of Data and Materials

The raw data of this study are derived from the TCGA database, which are publicly available databases.

Ethics Approval and Consent to Participate

Not necessary.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

LP is the principle investigator. LP conducted statistical analysis and data management and edited and revised the manuscript.

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