

Tumor Mutational Burden and Survival in Papillary Renal Cell Carcinoma Patients

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Research Article

Keywords: papillary renal cell carcinoma, prognosis, tumor mutational burden, survival, immune cells, immune checkpoint genes

Posted Date: March 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-303671/v1>

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Abstract

Background: Papillary renal cell carcinoma (PRCC) is the second most prevalent subtype of renal cell carcinoma (RCC), accounting for 15% of all RCCs. Tumor mutational burden (TMB) is a promising prognostic factor in many types of cancers. The present study aimed to investigate the association between TMB and patient survival in PRCC patients.

Methods: Genomic and clinical data of 281 PRCC patients were collected from The Cancer Genome Atlas. Overall survival (OS) was compared between patients with high and low TMB using the Kaplan-Meier method with log-rank tests. Gene expression comparison and immune cell fraction comparison were performed using Student's t test or Wilcoxon's rank-sum test.

Results: Patients with high TMB tumors had longer OS than those with low TMB tumors. Among tumor-infiltrating immune cells, high TMB tumors were associated with high levels of CD4+ T-cell infiltration, which were further associated with better survival. Furthermore, low TMB tumors were associated with a high level of infiltration of regulatory T-cells and dendritic cells. Among immune checkpoint genes, low TMB tumors expressed high levels of CD274, PDCD1LG2, LAG3, and TGFB1, and these genes were associated with a poor prognosis in PRCC. Among cytokine-related genes, low TMB tumors were associated with a high expression of IL6, whereas high TMB tumors were associated with a high expression of IFNA1.

Conclusions: High TMB indicated better survival outcomes in PRCC patients. High TMB was associated with anti-tumor immune cell infiltration, whereas low TMB was associated with high expression of checkpoint genes that indicated a worse prognosis in RCC. Moreover, TMB was associated with the expression of immune cytokines. Thus, TMB may be a novel prognostic factor in PRCC.

Background

Renal cell carcinoma (RCC) has various subtypes, and each subtype has characteristic histologic features with corresponding genetic alterations along with a distinct clinical course and response to therapy[1, 2]. Papillary renal cell carcinoma (PRCC) is the second most prevalent RCC subtype, accounting for 15% of all RCCs. PRCC is a heterogeneous disease characterized by specific genetic alterations that influence disease progression and patient survival[3]. PRCC is commonly categorized into two main subtypes based on histology: type 1 with indolent outcomes and type 2 with more aggressive outcomes[2]. Previous studies have identified several mutated genes associated with PRCC. For instance, mutations in MET are significantly correlated with type 1 PRCC, whereas mutations in BAP1, SETD2, and PBRM1 genes are associated with type 2 PRCC. However, these mutations were found in at most 20% of PRCC tumors in these studies[1, 4, 5], and there are no reliable prognostic biomarkers for PRCC.

Tumor mutational burden (TMB), defined as the number of non-synonymous somatic coding, base substitution, and indel mutations per megabase (Mb) of the genome examined[6], is a promising prognostic factor in various cancers. TMB has varying survival effects across different cancer types[7].

On the one hand, a high TMB may represent a complex genetic profile that indicates a more aggressive and refractory disease, which may result in a worse prognosis. On the other hand, a high TMB may reflect the presence of mutation-associated neoantigens, which may lead to increased lymphocyte infiltration in the tumor microenvironment and a better prognosis[7, 8]. However, the prognostic effects of TMB in PRCC are yet to be clarified.

Immune surveillance of cancer is important to suppress tumor growth, progression, and metastasis. TMB is suggested to generate neoantigens which can attract immune cells. It has been shown that mutation-associated neoantigens elicit T-cell immunoreactivity and sensitivity to immune checkpoint blockade[9]. Many immune checkpoint genes, such as PD-L1, LAG3, and PD-L2, are associated with immune evasion. Numerous studies have shown that the expression of tumor-infiltrating immune cells and immune checkpoint genes profoundly affected the prognosis of RCC[10–14]. However, to our best knowledge, the relationship between TMB and the immunogenic landscape of PRCC has not been elucidated in a large cohort of patients. Thus, this study aimed to investigate the association between TMB and survival. To achieve this goal, we investigated the relationship of TMB with tumor-infiltrating immune cells and immune checkpoint genes in PRCC patients. In addition, we explored the prognostic value of tumor-infiltrating immune cells and immune checkpoint genes.

Methods

Study design and patients

The Cancer Genome Atlas (TCGA), supervised by the National Human Genome Research Institute, is a publicly available database that includes clinical and genomic data on PRCC patient samples collected worldwide[15]. Overall, the data of 283 patients with PRCC in the TCGA Provisional database on whole-exome somatic variants and gene expression were available; of them, TMB data for 281 patients were available[16]. The clinical data of the cohort, including the stage, age, type, sex, and overall survival (OS), were downloaded via cBioPortal[17, 18].

The primary endpoint of this study was OS, defined as the time from the date of diagnosis to death by any cause. TMB was defined as the number of non-synonymous somatic, coding, base substitution, and indel mutations per Mb of the genome examined[6]. We calculated TMB by counting all base substitutions and indels in the coding region, including silent alterations. Accordingly, the patients were divided into two groups as per the TMB value of their tumor samples. The high TMB group involved patients with TMB values higher than the higher quartile values, whereas the low TMB group involved patients with TMB values lower than the lower quartile values. The threshold of dichotomization of the high TMB and low TMB groups was determined by comparing differences in the OS between the two groups at multiple candidate cutoff points within the range of TMB, which is similar to the method previously published[19–22].

Statistical analysis

Kaplan-Meier curves were used to explore the prognostic value of immune cells, immune checkpoint genes, and cytokine-related genes via Timers2.0 (<http://timer.comp-genomics.org/>)[23–25]. We calculated the immune cell composition from their gene expression profiles to estimate the tumor-infiltrating cell composition using CIBERSORT.[26] Immune cell fraction data were downloaded using TCIA [27] (<http://tcia.at/home>). Each immune cell fraction was compared between the high TMB and low TMB groups in the TCGA cohort using the same cutoff used in the OS analysis. Gene expression comparison and immune cell fraction comparison were analyzed using the Student's t test or Wilcoxon's rank-sum test. Kaplan-Meier curves assessed using a stratified log-rank test were used to analyze the differences in OS between the high TMB and low TMB groups. All statistical analyses were performed using R v3.6.1 (<http://www.r-project.org/>) and SPSS software (SPSS version 19.0). In all analyses, a two-sided α value of < 0.05 was considered statistically significant.

Results

Patient and tumor characteristics

There was no significant difference in patient and tumor characteristics between the high TMB ($n = 140$) and low TMB ($n = 141$) groups. Most patients in both groups had stage 1 PRCC, with no significant difference in the subtype (type 1 and type 2) distribution between the two groups. Most patients in the two groups were male ($P = 0.008$) (Table 1). We found 15,208 putative somatic mutations in 281 tumors, with a median of 53 mutations. Furthermore, missense mutations, SNPs, and C > T mutations were most common (Fig. 1a). We identified six most significantly mutated genes, which included TTN, MUC16, KMT2C, MET, KIAA1109, and SETD2, with 38% of cases showing mutations in at least one of these genes (Fig. 1b).

Table 1
Patient demographics characterized by TMB level
in the TCGA PRCC cancer cohort

Factor	TMB High	TMB Low	P value
STAGE			
I	84	85	
II	10	11	
III	27	22	
IV	5	10	0.529
Unknown	14	13	
AGE			
> 60	102	49	
< 60	38	92	< 0.001
Unknown	0	0	
Type			
1	35	37	
2	47	36	0.391
Unknown	58	68	
Sex			
male	112	93	
female	28	48	0.008
Unknown	0	0	

TMB tumor mutational burden; *TCGA* The Cancer Genome Atlas; *PRCC* papillary renal cell carcinoma

Survival impact of high TMB

We found that the OS of patients with high TMB tumors (n = 86) was better than that of those with low TMB tumors (n = 80) (P = 0.044; Fig. 2).

Association of TMB and patient survival with immune cell infiltration in PRCC

Among various immune cells, we found that high TMB tumors were associated with significantly high expression of CD4 + T-cells (P = 0.011; Fig. 3a). Meanwhile, low TMB tumors were associated with

significantly high expression of dendritic cells ($P = 0.012$; Fig. 3b) and immunosuppressive regulatory T-cells (Tregs) ($P = 0.016$; Fig. 3c). Kaplan-Meier analysis of the prognostic value of these immune cells revealed that high expression of Tregs is significantly associated with poor OS in PRCC ($P = 0.0105$; Fig. 3c), whereas high expression of CD4 + T-cells is significantly associated with better survival ($P = 0.0375$; Fig. 3a). Meanwhile, the expression of dendritic cells showed no significant association with patient survival (Fig. 3c). These results indicated that tumors with high TMB have less immunosuppressive immune cells, and that this may lead to better survival than tumors with low TMB.

Association of TMB and patient survival with immune checkpoint gene expression

We found differences in the expression of several vital immune checkpoint genes between the high and low TMB groups. Low TMB tumors were associated with high expression of LAG3 ($P = 0.032$; Fig. 4a), CD274 ($P = 0.009$; Fig. 4b), PDCD1LG2 ($P = 0.037$; Fig. 4c), and TGFB1 ($P = 0.001$; Fig. 4d). Kaplan-Meier analysis of the prognostic value of these genes showed that high expression of LAG3 ($P = 0.00623$; Fig. 4a), PDCD1LG2 ($P = 0.0115$; Fig. 4c), and TGFB1 ($P = 0.0142$; Fig. 4d) was correlated with poor survival outcomes in PRCC. Meanwhile, CD274 expression showed no significant association with survival (Fig. 4b).

Association of TMB and patient survival with cytokine-related genes

We compared the expression of several cytokine-related genes and found that low TMB tumors were associated with high expression of IL6 ($P < 0.0001$; Fig. 5a). Meanwhile, high TMB tumors were associated with high expression of IFNA1 ($P = 0.005$; Fig. 5b). The results showed that cytokine-related genes were associated with survival in PRCC patients. High IL6 expression was associated with poor survival ($P = 0.00284$; Fig. 5a). Meanwhile, high IFNA1 expression was associated with prolonged OS ($P = 0.0134$; Fig. 5b).

Discussion

The relationship between TMB and the immunogenic landscape of PRCC is yet to be elucidated. Our results showed that high TMB is correlated with prolonged OS in patients with PRCC. We also found differences in immune cell infiltration and the expression of checkpoint genes and cytokine-related genes between the high and low TMB groups. Given that checkpoint genes and cytokine-related genes are potential prognostic factors, we considered that this may explain the mechanism for the association between TMB and prognosis in PRCC. Although the prognostic potential of TMB needs to be further validated, our results provide an initial basis for the potential of TMB as a prognostic marker in PRCC.

The association between TMB and immunotherapy response has been evaluated in many studies[28–32]. However, a relatively small number of studies on TMB as a promising prognostic factor in cancer has

been performed. There has been some research aiming to study the prognostic role of TMB in clear cell renal cell carcinoma (ccRCC). One study indicated that higher TMB correlated with poor survival outcomes and might inhibit the immune infiltrates in ccRCC according to the TCGA database[33]. Meanwhile, another study showed that higher TMB levels are correlated with poor survival outcomes in three independent populations[34]. However, TMB was not found to be associated with progression-free survival in a different arm of ccRCC patients in one large phase III ICI trial[35]. Nevertheless, similar research aiming to study the prognostic role of TMB in PRCC has not yet occurred. In this study, we inferred that high TMB promotes the production of mutation-associated neoantigens, which could lead to stronger immune responses in the tumor microenvironment and a better prognosis in PRCC. We observed differences in immune cell infiltration between the high and low TMB groups. The high TMB group showed increased levels of CD4 + T-cells, whereas the low TMB group showed significantly higher infiltration of Tregs and dendritic cells. CD4 + T-cells are critical anti-tumor effector cells that indicate active immune responses[36, 37]. A recent study found that a high level of CD4 + cells is associated with a better outcome in RCC[38], consistent with our results. We also found that the number of tumor-infiltrating Tregs was significantly higher in the low TMB group than that in the high TMB group. However, Tregs help in suppressing anti-tumor T-cell responses. They secrete immunosuppressive cytokines such as LAG3 to disrupt the activation of anti-tumor T-cells[39]. A previous study showed that Tregs can efficiently suppress the proliferation of effector T-cells in RCC[40]. In this study, Treg infiltration was associated with a poor prognosis and was significantly higher in the low TMB group. Meanwhile, although we found a higher infiltration of dendritic cells in the low TMB group, dendritic cells had no significant prognostic effect. Collectively, these findings indicate that high TMB may result in active immune responses and that, in turn, may lead to a better prognosis in PRCC.

Immune checkpoint molecules are vital for tumor immune evasion[41]. Accordingly, immune checkpoint blockade therapy has significantly improved the treatment prospects of advanced RCC. We further explored the expression of checkpoint genes in the high and low TMB groups. We found that low TMB is associated with high expression of various checkpoint genes, including LAG3, TGFB1, PDCD1LG2 (PD-L2), CD274 (PD-L1), and CD80. LAG3 is an immune checkpoint receptor expressed by T-cells[42–44]. LAG3 is also expressed on Tregs in the peripheral blood, tumor-involved lymph nodes, and within tumor tissues isolated from patients with advanced (stage III and IV) melanoma and colorectal cancer, which can contribute to tumor immune escape[45]. The expression levels of LAG3 in tumors have been reported to be associated with tumor progression, poor prognosis, and unfavorable clinical outcomes in various types of human tumors, such as RCC[13], colorectal cancer[46], head and neck squamous cell carcinoma[47], and non-small cell lung cancer[48]. In our research, we found that the expression of LAG3 in low TMB tumors is higher than that in high TMB tumors, whereas the infiltration level of Tregs in low TMB tumors is higher. These results indicate that the high infiltration of Tregs in low TMB tumors is associated with the high expression of LAG3, which may lead to the poor outcome in PRCC. Since LAG3 is now expected to be a highly promising target in cancer therapies[49], we believe that further research should be carried out to explore the value of LAG3 in the treatment of PRCC. It is known that an active immune response results in a better prognosis in PRCC. TGFB1 encodes a secreted ligand of the TGF-

beta. TGF-beta has been suggested to be the principal immune-suppressive factor secreted by tumor cells[50]. Moreover, TGF-beta secreted by dendritic cells contributes to the expression of Tregs, which actively inhibits the activity of anti-tumor T-cells. An increased level of plasma TGF-beta is associated with advanced-stage disease and can therefore be used to classify patients into prognostically high-risk populations[51]. The low TMB group in this study showed elevated levels of TGFB1, implying an association with a poor prognosis in PRCC, which is consistent with previous studies.

Previous studies have shown that PD-L1 expression is positively associated with treatment response to anti-PD-L1 immunotherapy[52]. However, the influence of PD-L1 expression on treatment response in RCC is yet to be clarified. One study has reported that PD-L1 expression and TMB are not associated with response to immunotherapy in metastatic RCC[53]. Meanwhile, PD-L1 expression has been found to be associated with a poor survival in ccRCC[54]. However, we did not find a significant association between PD-L1 expression and survival in PRCC. PD-L2 expression may be induced by tumor exosomes[55] or activated macrophages[56], and can inhibit T-cell-mediated anti-tumor response. Previous research has suggested that high PD-L2 expression in tumors is associated with a lower rate of cancer-free survival in RCC patients[57]. We found that PD-L2 expression may be associated with TMB and patient prognosis in PRCC. In brief, the expression of genes that were significantly increased in the low TMB group was mostly associated with a worse prognosis in PRCC. A high TMB could possibly promote the production of mutation-associated neoantigens, which leads to the activation of immune responses in the tumor microenvironment and suppression of the expression of these genes.

We also explored the association between TMB and cytokine-related genes in PRCC. Cytokines and chemokines, which are vital components of the tumor immune microenvironment, are widely involved in cancer cell progression[58]. We found that low TMB tumors were associated with high IL6 expression. IL6 recruits neutrophils and promotes the migration and proliferation of T lymphocytes into affected tissue[59]. In addition, IL6 promotes T-cell differentiation and activation[60]. Serum concentrations of IL6 have been found to be increased in RCC and seem to be associated with an advanced-stage disease and a negative prognosis[61]. In our research, high IL6 expression was associated with a poor prognosis in PRCC, with IL6 expression being significantly elevated in the low TMB group. Further, high TMB tumors were associated with high IFNA1 expression. IFNA1 encodes a type I membrane protein that forms one of the two chains of a receptor for interferons alpha and beta, and interferon alpha was previously a standard treatment modality for advanced RCC[62]. We found that IFNA1 expression is associated with high TMB and a better prognosis in PRCC.

It is important to acknowledge the following limitations of our study. First, although TCGA provides us with high-quality data, we still have some unaccounted confounders, such as cancer-specific survival, which is more appropriate to assess the prognostic impact of TMB, and also treatment information, the influence of which on our results cannot be ruled out. In addition, advanced cancers are relatively underrepresented in the TCGA cohort, which may lead to a potential bias. Second, the cutoff for defining high and low TMB was somewhat subjective. However, when we reanalyzed the data using a different cutoff to define the high and low TMB (upper third vs. lower third), consistent results were obtained. Third,

although there are various differences in tumor characteristics between type 1 and type 2 PRCC, we did not find a significantly different distribution of TMB according to different subtypes, which may result from limited data. Finally, we did not use the method of adjusting for multiple tests such as the false discovery rate[63] to define a more stringent significance threshold, considering that the numbers of tests was relatively small. At present, TMB can be estimated via targeted next-generation sequencing panels, which is simpler and cheaper[64]. Further prospective studies are needed to explore the molecular, genetic, and cellular changes influencing TMB to ultimately establish modalities for improving the prognosis of patients with PRCC.

Conclusion

High TMB is associated with anti-tumor immune cell infiltration and indicates better survival outcomes in PRCC. Meanwhile, low TMB is associated with high expression of checkpoint genes, which is further associated with a worse prognosis in RCC. Moreover, TMB influences the expression of immune cytokines. Therefore, TMB may be a novel prognostic factor of PRCC.

Abbreviations

PRCC, papillary renal cell carcinoma

Mb, megabase

OS, overall survival

Tregs, regulatory T-cells

RCC, renal cell carcinoma

ccRCC, clear cell renal cell carcinoma

TCGA, The Cancer Genome Atlas

TMB, tumor mutational burden

Declarations

Availability of data and materials

We downloaded gene somatic mutations (Level 3) and clinical data for PRCC from the TCGA data portal (<https://portal.gdc.cancer.gov/>). For survival analyses, we used clinical data from cBioPortal (<http://www.cbioportal.org/>) for the TCGA data. Immune cell fraction data were downloaded through TCIA (<https://tcia.at/home>).

Competing Interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Capital Health Research and Development of Special (2020-2-1111).

Acknowledgements

Not applicable.

Ethics approval and informed consent

Ethical approval was waived since we used only publicly available data and materials.

Consent for publication

Not applicable.

Authors' contributions

HD, SY, and HY performed the literature search, data extraction, and statistical analysis and drafted the manuscript. ZJ and PY designed and supervised the study. All authors have read and approved the final manuscript.

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Figures

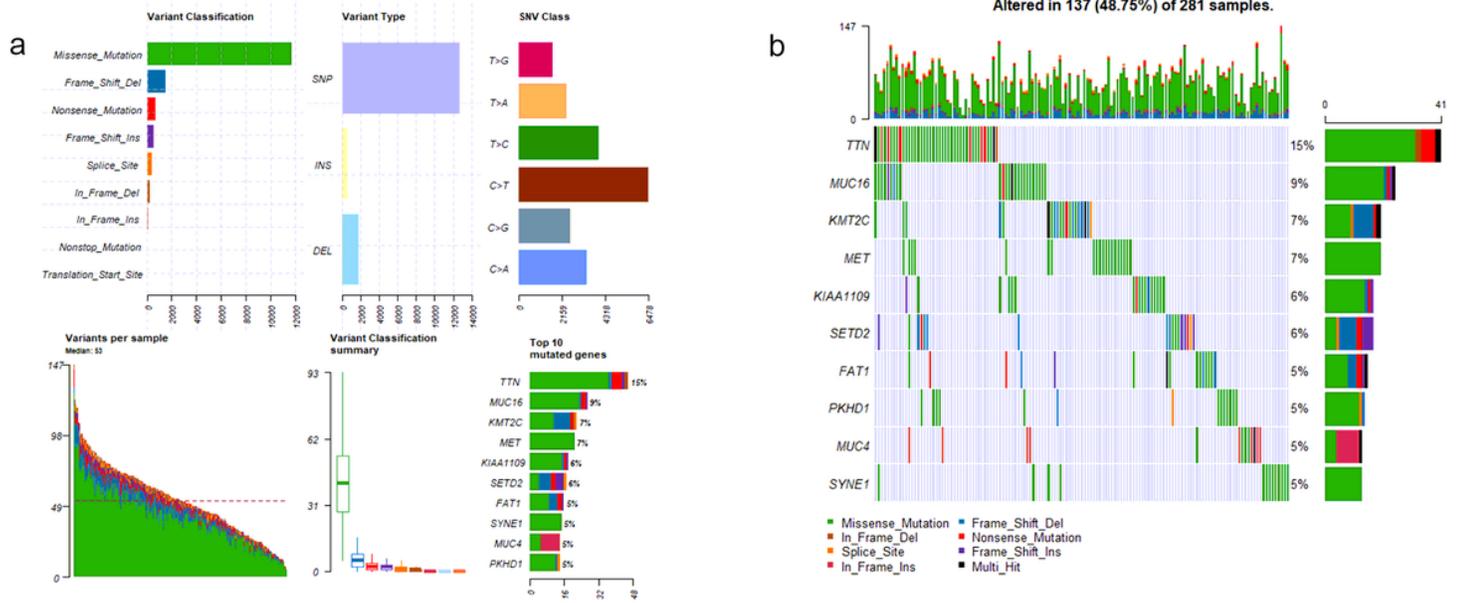


Figure 1

Overview of TCGA PRCC cohort mutations (a). Waterfall of the top 10 mutated genes in the TCGA PRCC cohort (b). TCGA The Cancer Genome Atlas; PRCC papillary renal cell carcinoma

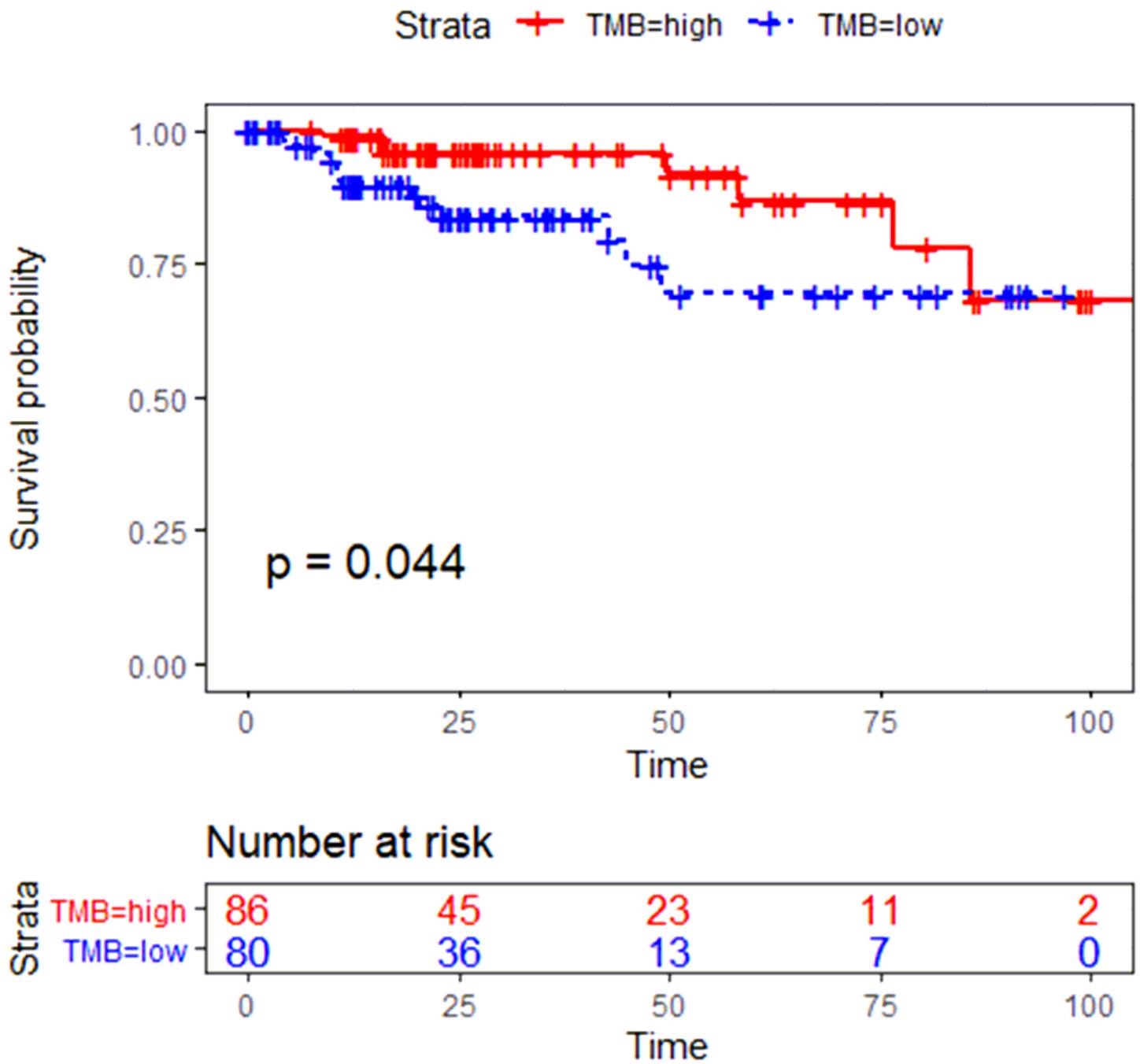


Figure 2

Tumor mutational burden and patient survival in the papillary renal cell carcinoma cohort of The Cancer Genome Atlas. TMB tumor mutational burden

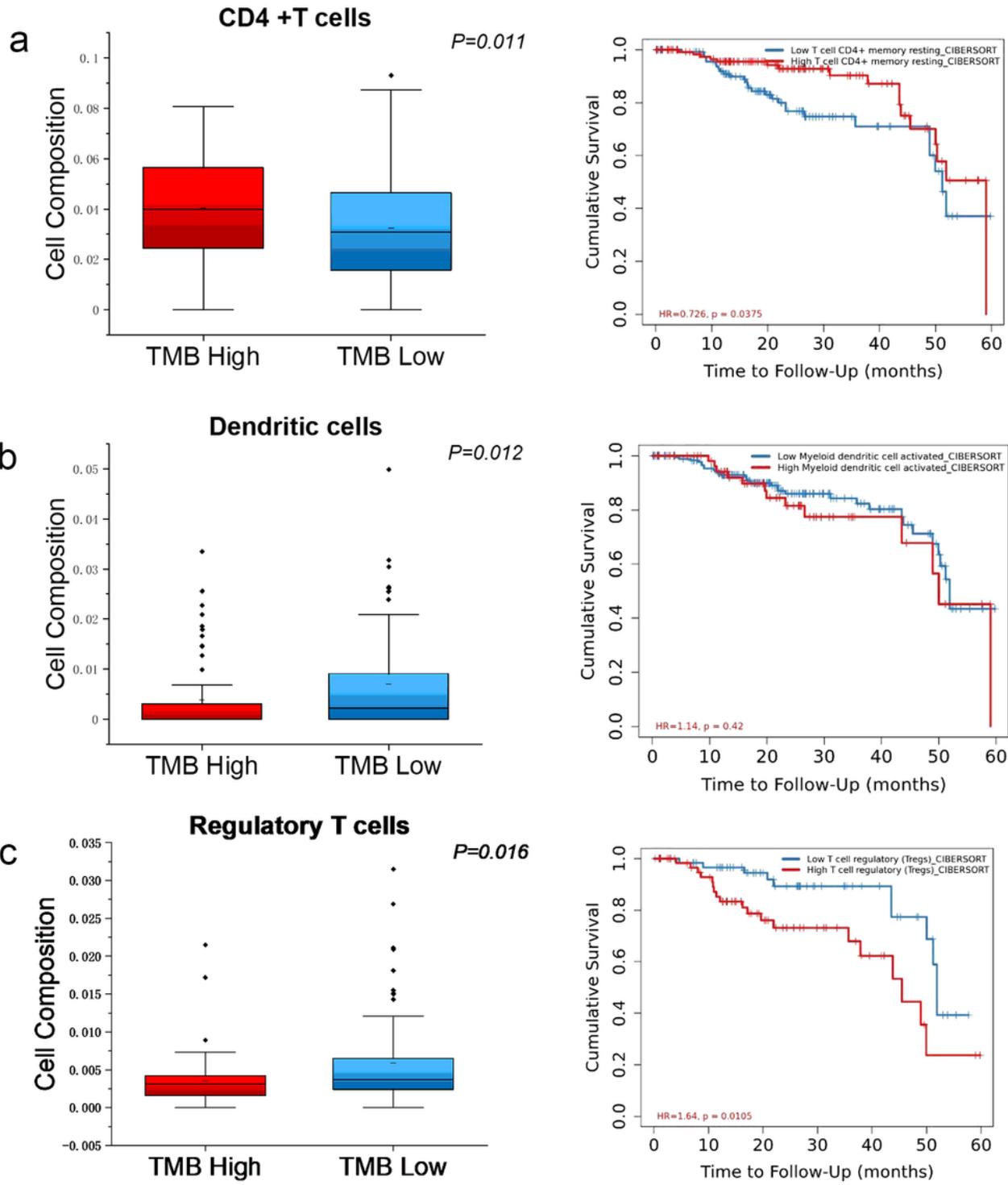


Figure 3

Association between TMB and immune cell composition and association between immune cell composition and OS in PRCC. CD4+ T-cells (a), dendritic cells (b), Treg cells (c). TCGA The Cancer Genome Atlas; Tregs regulatory T-cells; TMB tumor mutational burden; PRCC papillary renal cell carcinoma

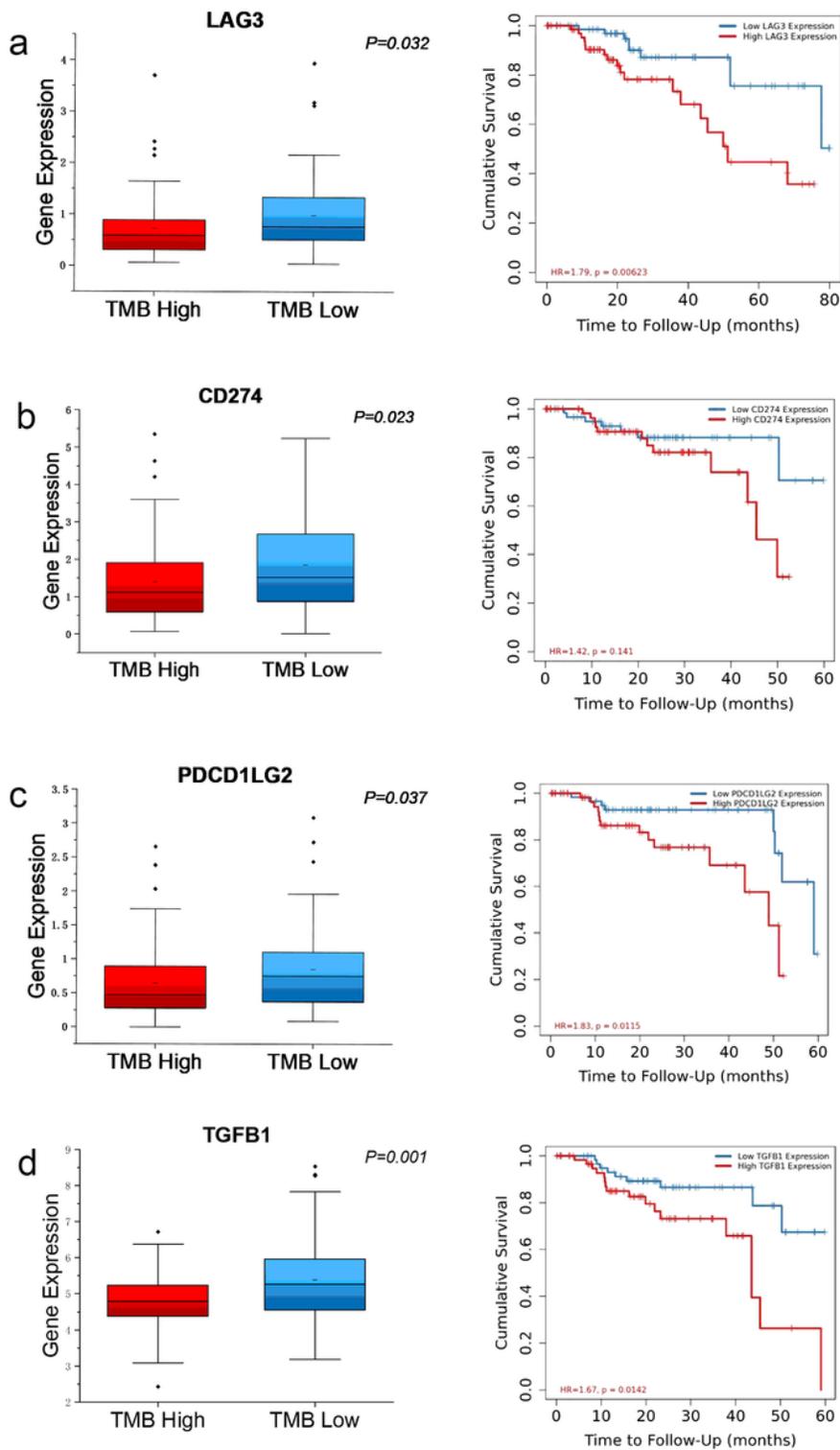


Figure 4

Association between TMB and immune checkpoint gene expression and the association between immune checkpoint genes expression and OS in PRCC. LAG3 (a), CD274 (b), PDCD1LG2 (c), TGFB1 (d). TMB tumor mutational burden; PRCC papillary renal cell carcinoma.

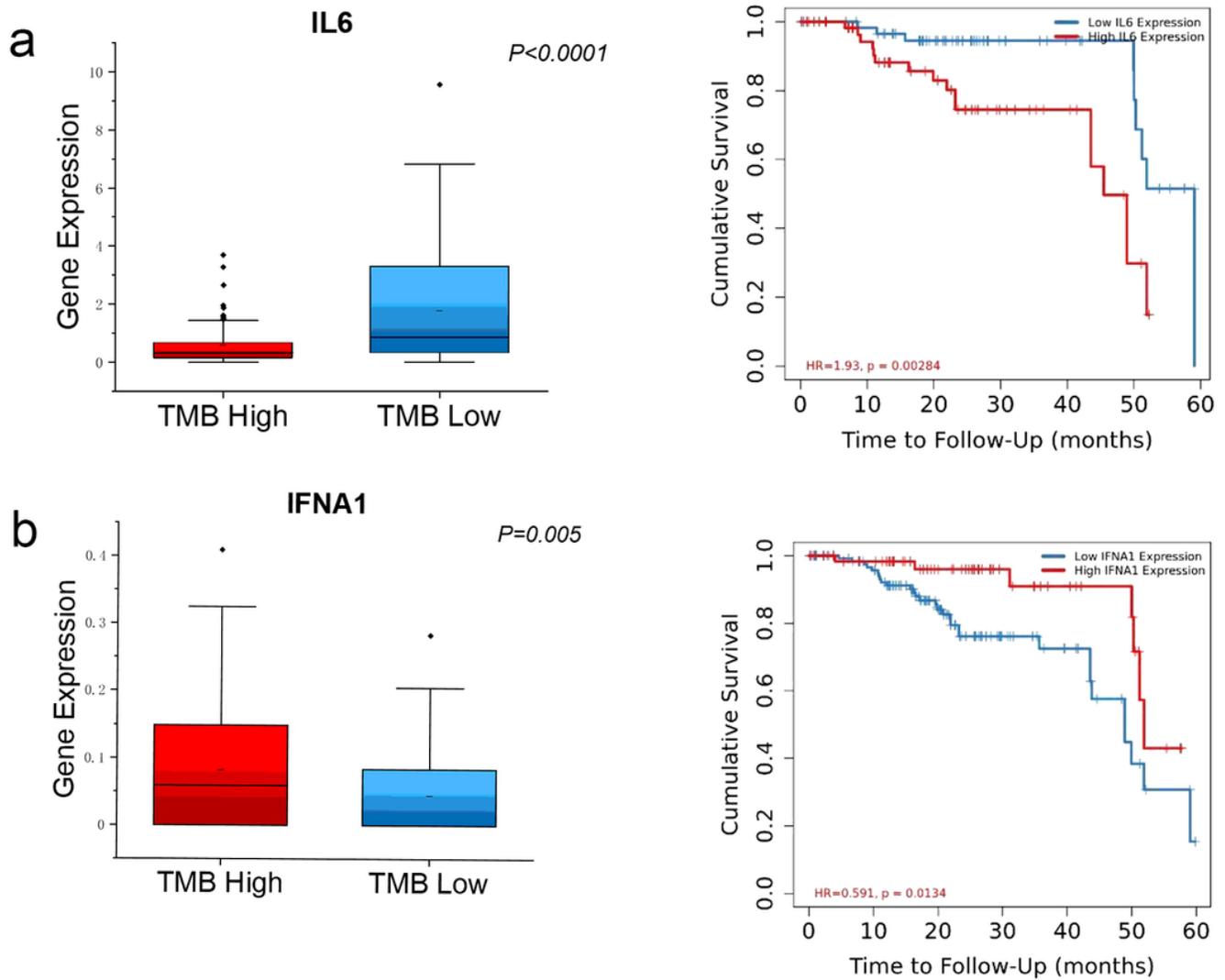


Figure 5

Association between TMB level and cytokine-related gene expression and association between cytokine-related genes expression and OS in PRCC. IL6 (a), IFNA (b). TMB tumor mutational burden; PRCC papillary renal cell carcinoma.