The Pan-Cancer Landscape of Crosstalk Between TRP Family and Tumour Microenvironment Relevant to Prognosis and Immunotherapy Response

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Background: Transient Receptor Potential (TRP) channel is a kind of channel protein widely distributed in peripheral and central nervous system. They can be regulated by natural aromatic substances and serve as a therapeutic target for many diseases. However, the role and function of the TRP family genes in tumours remain unclear.

Methods: Gene alterations (mutation, copy number, methylation), expression, clinical features, and prognostic value of the TRP family genes were evaluated in pan-cancer using data from The Cancer Genome Atlas and Genotype-Tissue Expression databases. TRP score was calculated by the ssGSEA function of the R package “GSVA”. The association of TRP score and the tumour microenvironment (TME), especially the tumour immune microenvironment (TIME), along with immunotherapy response were explored in-depth.

Results: TRP family genes were involved in tumour progression and highly associated with poor prognosis in a variety of cancers. TRP score was positively associated with malignant pathways in pan-cancer, such as IL6–JAK–STAT3 signalling, interferon-gamma response, and inflammatory response. All pathways were closely associated with TIME. Elevated TRP score also correlated with multiple immune-related characteristics of the TIME in pan-cancer. Moreover, the TRP score was a predictive biomarker for immune checkpoint inhibitor (ICI) treatments in patients with tumours.

Conclusions: TRP family genes play a key role in pan-cancer and are closely associated with TME. Patients with high TRP scores have excellent immune-activated TIME and immunotherapy sensitivity. Therefore, the TRP score could be a potential biomarker for patients with tumours treated with ICI.

Keywords: TRP family, pan-cancer, prognosis, tumour microenvironment, immunotherapy response
INTRODUCTION

Ion channels mediate many reactions in cell physiology and are often dysregulated in various diseases, especially in most types of tumours (1–3). Most of these channels are expressed on the cell surface and are responsible for turning intracellular signals on and off, which enable them to easily access drug targets (4). However, as an important ion channel, the involvement of transient receptor potential (TRP) family genes in tumours and their therapeutic use as targets remain unclear.

Recent research indicated the TRP channel family as potential biomarkers and/or drug targets in tumour treatment (5). For instance, TRP channels, such as TRPC1, TRPC5/6, TRPM4, TRPM7/8, TRPV1/2, TRPV4, and TRPV6 are closely associated with progression and act as novel therapeutic targets in breast invasive carcinoma (6). TRPV4 is also a proven immunomodulatory related prognostic biomarker in ovarian cancer and colorectal cancer (7, 8). In addition, TRPM8 is a potential therapeutic target in prostate adenocarcinoma (9), colon adenocarcinoma (10), breast invasive carcinoma (11), and bladder urothelial carcinoma (12).

In our study, we performed a pan-cancer systematic analysis of TRP family genes in 33 tumour types, including gene alteration (mutation, copy number, methylation), expression, clinical features, and prognostic value of the TRP family. The tumour microenvironment (TME), especially the tumour immune microenvironment (TIME), plays an important role in the development of tumours (13). TIME constructed by immune cells is essential for tumour progression and cancer treatment, including chemo-, radio-, and especially immunotherapy (14–18). Thus, we calculated a TRP score and explored its association with TIME and immunotherapy response to indicate immunotherapy efficacy.

MATERIALS AND METHODS

Data Collection

The expression profiles and clinical information of The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) were downloaded from the UCSC Xena (https://xenabrowser.net/datapages/) database. UCSC Xena is a cancer genomics data analysis platform that supports the visualization and analysis of a variety of omics data of cancer samples (18). The platform has built-in public data sets, including data from large cancer research projects such as TCGA and ICGC. The detailed sample sizes of tumour and normal tissues in the TCGA and GTEx databases (including tumour type, sample size of tumour tissues from TCGA, sample size of normal tissues from TCGA, and sample size of normal tissues from GTEx) are provided in Supplementary Table 1.

The immune cell infiltration data of the TCGA database were downloaded from the Immune Cell Abundance Identifier (ImmuCellAI) (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) and TIMER2 databases (http://timer.cistrome.org/). The immunotherapy datasets GSE135222 and GSE91061 were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov).

Online Analysis

The gene alterations of the TRP family, including mutation, copy number, methylation, were conducted using the Gene Set Cancer Analysis (GSCA) database (http://bioinfo.life.hust.edu.cn/GSCA/#/). GSCA is an integrated database for genomic and immunogenomic gene set cancer analysis.

TRP Score Analysis

The single-sample gene set enrichment analysis (ssGSEA) function of R v4.1.1 package ‘GSVA’ was used to calculate the TRP score of each patient in the TCGA cohort.

Prognostic Analysis of TRP Score

Univariate Cox regression (uniCox) analyses were performed to explore the effect of TRP score on the survival of patients, including overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) indicators, in pan-cancer using the R packages ‘survminer’ and ‘survival.’

Gene Enrichment Analysis

To explore the biological functions of the TRP family and its role in various cancers, gene set variation analysis (GSVA) enrichment analysis was performed using the R package ‘GSVA’ to evaluate the correlation between TRP score and 50 HALLMARK pathways based on the MSigDB database (http://software.broadinstitute.org/gsea/msigdb/index.jsp).

Tumour Microenvironment Analysis

The R package ‘ESTIMATE’ was used to calculate the stromal score, immune score, and tumour purity score of each patient in the TCGA cohort. The association between the TRP score and these scores was analysed. The TME-related pathways were obtained, and pathway scores were calculated according to a previous publication (19). We further analysed the correlation between TRP score and immune cell infiltration, immunomodulatory genes, MHC genes, and chemokine/chemokine receptors at the pan-cancer level. The visualization of all heatmaps in this step was conducted using the ‘ggplot2’ R package.

Statistical Analysis

All data used in this article are presented as the mean ± standard deviation (SD). Differences between various groups were analysed and compared using the Student’s t-test. Statistical analysis was performed using R v4.1.1 (https://www.r-project.org/). The R packages ‘ggplot2’ and ‘ggpubr’ were used for the statistics of all histograms. Pearson correlation coefficient was used in all correlation analyses. P < 0.05 was considered statistically significant and indicated as follow: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

RESULTS

mRNA Level and Prognostic Value of TRP Family

We first explored the differential expression of 28 TRP family genes based on the TCGA and GTEx databases in 33 tumour
types. The sample size for the cancer types is listed in Supplementary Table 1. As shown in Figure 1A, 28 TRP family genes were commonly differentially expressed in 33 tumour types. We further assessed the correlation between TRP family genes based on TCGA pan-cancer data (Figure 1B). Additionally, we performed a uniCox analysis of each gene in the 33 tumours (Figure 2A). The results indicated that most TRP family genes were risk factors in tumours. We calculated the risk scores (i.e., the number of tumours in which genes are risk factors minus the number of tumours in which genes are protective factors) and found that TRPM4 was the most significant risk factor (Figure 2B). High expression of TRPM4 predicted worse overall survival in lower-grade glioma (LGG), uveal melanoma (UVN), kidney renal clear cell carcinoma (KIRC), pancreatic adenocarcinoma (PAAD), mesothelioma (MESO), and thyroid carcinoma (THCA).

Gene Alterations of TRP Family

We further explored the gene alterations of the TRP family and their association with the mRNA levels. We comprehensively described the genetic alteration numbers of TRP family genes, including mutation, fusion, amplification, homozygous deletion, and multiple alterations, in the TCGA cohort (Figure 3). We described the variant classification, variant type, single-nucleotide variant (SNV) class, variants per sample, variant classification summary, and top10 mutated genes (Figure 4A). For the SNV percentage, the total deleterious mutation percentage (i.e., the number of samples with at least one deleterious mutation site/the number of samples with SNV mutation data) showed the highest deleterious mutation frequency of TRPM6 in skin cutaneous melanoma (SKCM) (Figure 4B). In addition, TRPM6 also had the highest mutation frequency (15%) among TRP family genes in pan-cancer (Figure 4C).

Next, we described the methylation information of TRP family genes in pan-cancer. Results indicated that the methylation levels of TRP family genes were general negatively correlated with mRNA levels (Figure 5A). Subsequently, we assessed the methylation status of the TRP family genes and found that the methylation levels of TRP family genes differed between cancer types (Figure 5B). We further analysed the copy number variants (CNVs) of TRP family genes. The proportion of different types of CNV (including heterozygous amplification, homozygous deletion, homozygous amplification, and homozygous deletion) of each gene in pan-cancer was shown (Figure 6A). The CNV of TRPC4AP was positively correlated with its mRNA level in 27 of 33 tumour types (Figure 6B). The CNV of homozygous or heterozygous amplification was positively correlated with mRNA level, and the CNV of homozygous or heterozygous deletion was negatively correlated with mRNA level (Figures 6C, D).

The Estimation, Differential Distribution, and Survival Analysis of TRP Score

We conducted ssGSEA to estimate the TRP score across 33 tumour types in the TCGA cohort. The TRP score was the highest in SKCM and lowest in LGG (Figure 7A). In addition, the TRP score was higher in tumour tissues compared to the adjacent normal tissues in head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), KIRC, prostate adenocarcinoma (PRAD), and THCA (Figures 7B–F), while lower in colon adenocarcinoma (COAD), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), and lung squamous cell carcinoma (LUSC) (Figures 7G–J). As for the results of uniCox analysis: (1) For OS, TRP score was a risk factor in LGG, UVM, thymoma (THYM), and KIRC, whereas a protective factor in bladder urothelial carcinoma (BLCA) (Figure 8A); (2) For DSS, TRP score was a risk factor in LGG, UVM, and THYM, while a protective factor in BLCA and kidney renal papillary cell carcinoma (KIRP) (Figure 8B); (3) For DFI, TRP score was a risk factor in breast invasive carcinoma (BRCA), whereas a protective factor in LIHC, LUSC, and stomach adenocarcinoma (STAD) (Figure 8C); (4) For PFI, TRP score was a risk factor in LGG, UVM, glioblastoma multiforme (GBM), and THYM, while a protective factor in adrenocortical carcinoma (ACC), LIHC, and BLCA (Figure 8D).

GSVA of TRP Score

To analyse the potential pathways affected by the TRP score, we performed a GSVA based on 50 HALLMARK pathways. The association between the TRP score and GSVA scores in pan-cancer is shown in Figure 9. We observed that the TRP score was positively associated with many malignant pathways in pan-cancer, such as IL6–JAK–STAT3 signalling, interferon-gamma response, and inflammatory response. All these pathways were also closely associated with TIME.

Relationship Between TRP Score and the TME

We further explored the association between TRP score, stromal, and immune scores in pan-cancer (Figure 10A). Results revealed that TRP score was positively associated with an immune score, stromal score, and ESTIMATE score. We further obtained and calculated TME-related pathways according to the published paper, including immune-related pathways, stromal-related pathways, and DNA repair-related pathways. The results indicated that the TRP score was closely associated with immune-related pathways, including immune checkpoint, CD8 T effector, and antigen processing machinery, in pan-cancer (Figure 10B).

Immune Infiltrating Analysis

The above results showed that the TRP score was highly correlated with the immune score. Therefore, we further explored the correlation of TRP score with immune cells in TME. Based on the ImmuCellAI database, we found that at the pan-cancer level, the TRP score is highly correlated with most immune cells, indicating an immune-activated TME (Figure 11A). The correlation of immune cell infiltration based on the TIMER2 database also illustrated that the TRP score had a significant positive correlation with immune-activated TME in pan-cancer (Figure 11B).
In addition, we observed that the TRP score was highly correlated with immune-activating genes (Figure 12A), chemokines (Figure 12B), chemokine receptors (Figure 12C), and MHC genes (Figure 12D) in most tumour types. The above results all indicated that patients with high TRP scores have elevated immune cell infiltration, which may have a positive impact on immunotherapy.

The Association Between TRP Score and Immunotherapy Response

It was reported that patients with a high tumour mutation burden (TMB) or microsatellite instability (MSI) may be sensitive to immune checkpoint inhibitor (ICI) treatment (20–22). We found that the TRP score was associated with TMB in four cancer types and MSI in seven cancer types. TRP score was
FIGURE 2 | The prognostic value of TRP family genes. (A) Heatmap of the uniCox results of TRP family genes in each tumour type. (B) The risk score of each TRP family gene in pan-cancer.

FIGURE 3 | The genetic alteration numbers of TRP family genes. The genetic alteration numbers of TRP family genes in each tumour type. The number represents the occurrence of mutations, fusions, amplifications, homozygous deletions, or multiple alterations per sample.
positively correlated with the TMB value in BRCA (Figure 13A) and MSI value in BRCA, HNSC, and uterine corpus endometrial carcinoma (UCEC) (Figure 13B). Based on these results, we suspected that patients with high TRP scores are sensitive to immunotherapy. To verify this hypothesis, we collected immunotherapy data and calculated the TRP score. Through Kaplan-Meier analysis, we found that patients undergoing ICI treatment with high TRP scores had better OS or progression-free
survival (PFS). The percentage of responsive patients was higher in the high TRP score group (Figures 1A–F).

**DISCUSSION**

Cancer is a multifactorial disease whose progression depends on the dysregulation of multiple pathways involved in cellular metabolism, immune evasion, avoidance of cell death, inflammation, migration, and invasion (23). Ion channels, as major regulators of cellular function, are implicated in a variety of physiological and pathophysiological relevant mechanisms (23). However, the relevance of ion channels as potential anti-tumour mechanisms is not well studied.

TRP channels are ion channels (Ca²⁺ permeable) that act as cellular sensors and are involved in the detection of temperature (heat and cold), chemical, and mechanical stimuli (24). In pathological conditions, such as cancer, dysregulation of signalling pathways can modulate the levels of certain TRP channels, thereby altering cellular sensitivity and response selection to the extracellular environment (25, 26). In this study, we explored the differential expression and prognostic value of 28 TRP family genes in 33 tumour types based on the TCGA and GTex databases. We found that TRP family genes are extensively involved in tumour progression and serve as risk factors for most tumours—especially TRPM2, TRPM4, and TRPM8 leading to poor prognosis of patients with cancer. To further explore the potential oncogenic mechanisms of TRP family genes, we comprehensively described the number of genetic alterations in TRP family genes, including mutations, fusions, amplifications, homozygous deletions, and methylations, and found that TRPM6 through deleterious mutations and TRPC4AP through increased CNVs may promote tumour progression. Among these TRP family genes, it has been shown previously that TRPV2 in brain tumours (2), TRPV6 in PRAD (27), and TRPC6 in STAD (28) promote tumour progression—which validates our analysis. In addition, several reports have explored their oncogenic mechanisms. For instance, TRPM4 regulates the proliferation, migration, and invasion of prostate cancer cells by altering Ca²⁺ signalling and thus upregulates β-linked protein oncogene signalling along with its nuclear localisation (29). TRPM8-mediated Ca²⁺ influx promotes glioma progression by activating Ca²⁺ activated K⁺ ion channels with large conductance (30). TRPM2 mediates cancer cell migration via Ca²⁺ and Zn²⁺ (31). We also found that mutations in TRPM6 can lead to ion channel dysfunction causing primary hypomagnesaemia and secondary hypocalcaemia (32). Therefore, we hypothesize that TRP channel molecules in tumour cells promote tumour progression by regulating Ca²⁺ signalling.

Tumour development and metastasis are inextricably linked to the structure and function of the TME (33, 34). Specifically, a TME in which immune effector cells inhibit tumour growth can be used to assess the response of tumour cells to immunotherapy and has been effectively applied to influence clinical outcomes.

![Image](image_url)
FIGURE 7 | The differential distribution of TRP score. (A) The TRP score distribution in 33 tumour types in TCGA cohort. (B–J) The differential distribution of TRP score in paired tumour and adjacent normal tissues. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. 

Wu et al. TRP Family Pan-Cancer Landscape

Frontiers in Immunology | www.frontiersin.org April 2022 | Volume 13 | Article 837665
FIGURE 8 | The survival analysis of TRP score. (A–D) Forest plots of the Cox analysis results of TRP score in pan-cancer. (A) Overall survival, (B) disease-specific survival, (C) disease-free interval, and (D) progression-free interval.
We further established a TRP score based on the ssGSEA method in pan-cancer. TRP scores were higher in tumour tissue than adjacent normal tissue in HNSC, KICH, KIRC, PRAD, and THCA, while lower in COAD, LIHC, LUAD, and LUSC. In addition, high TRP scores can predict the poor prognosis of many cancers, including that of LGG, UVM, THYM, KIRC, BRCA, and GBM in accordance with previous results. Through in-depth analyses, we observed that the TRP

| FIGURE 9 | GSVA of TRP score. Heatmap of the correlation between TRP score and 50 HALLMARK pathways in pan-cancer. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. |
FIGURE 10 | TME analysis of TRP score. (A) Heatmap of the correlation between TRP score and immune score, stromal score, ESTIMATE score, and tumour purity score in pan-cancer. (B) Heatmap of the correlation between TRP score and TME-related pathways. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

FIGURE 11 | Immune infiltration analysis. (A, B) Correlation between TRP score and immune cell infiltration based on (A) ImmuCellAI or (B) TIMER2 database. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
FIGURE 12 | Correlation between TRP score and immune-related genes. (A–D). Correlation of TRP score with (A) immune-activating genes, (B) chemokines, (C) chemokine receptors, or (D) MHC genes in pan-cancer. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

FIGURE 13 | Correlation between TRP score and TMB and MSI values. (A, B) Radar plot of the correlation between TRP score and (A) TMB or (B) MSI. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
FIGURE 14 | The association between TRP score and immunotherapy response. (A) The Kaplan-Meier PFS analysis of TRP score in GSE135222 cohort. (B, D, F) The percentage of responsive and progressive patients in high- and low-TRP score groups in (B) GSE135222, (D) GSE91061, and (F) PMID32472114 cohorts. (C, E) The Kaplan-Meier OS analysis of TRP score in (C) GSE91061 and (E) PMID32472114 cohorts. CR, complete response; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.
score was positively associated with many malignant pathways in pan-cancer, such as IL6-JAK–STAT3 signalling, interferon-gamma response, and inflammatory response—all of which were closely associated with TIME. Therefore, we further explored the correlation of the TRP score with immune cells in the TME. We found that at the pan-cancer level, the TRP score is highly correlated with most immune cells, indicating an immune-activated TME. Moreover, we observed that the TRP score was highly correlated with immune-activating genes, chemokines, chemokine receptors, and MHC genes in most tumour types. The above results all indicated that patients with high TRP scores are rich in immune cell infiltration, which may have a positive impact on immunotherapy. TMB and MSI are critical biological markers of ICI response capable of predicting immunotherapy response across tumour types (20–22). Previous studies have shown that patients with high TMB/MSI had improved response rates and showed better immunotherapy treatment outcomes (37). Considering the genetic alterations of the TRP family genes in pan-cancer, we performed TMB and MSI analyses of TRP scores and collected additional immunotherapy data. Similar to the results of genetic alterations of TRP family genes in pan-cancer, our findings show that TRP scores correlate with TMB in four cancer types and MSI in seven cancer types and that patients with high TRP scores in a variety of cancers, including BRCA, are sensitive to immunotherapy. In addition, we found that patients with high TRP scores treated with ICIs had better OS or PFS in a comprehensive validation analysis of multiple immunotherapy datasets. Therefore, our results confirm that elevated TRP scores are highly correlated with pan-cancer TIME and that TRP score was a potential biomarker for ICI treatment efficacy in patients with tumours.

In conclusion, our study demonstrated that TRP family genes are risk factors for tumours and play crucial roles in tumorigenesis and progression. Elevated TRP scores were highly associated with multiple immune-related features of TIME in pan-cancer. In addition, the TRP score is a potential biomarker for treatment efficacy in patients with tumours receiving ICI therapy. The results of this study provide insight into the potential anti-tumour mechanisms of ion channels and provide a novel and effective immune anti-tumour strategy for tumour immunology research.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

GW conceived and designed the study. GW, MH and XY wrote the manuscript and participated in data analysis. WW and JZ participated in discussion and language editing. KR and XC reviewed the manuscript. QX provided all the funding for this study. All authors contributed to the article and approved the submitted version.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.837665/full#supplementary-material
## GLOSSARY

| Abbreviation | Description |
|--------------|-------------|
| ACC          | adrenocortical carcinoma |
| BLCA         | bladder urothelial carcinoma |
| BRCA         | breast invasive carcinoma |
| CNVs         | copy number variants |
| COAD         | colon adenocarcinoma |
| DFI          | disease-free interval |
| DSS          | disease-specific survival |
| GBM          | glioblastoma multiforme |
| GEO          | Gene Expression Omnibus |
| GSCA         | Gene Set Cancer Analysis |
| GSVA         | gene set variation analysis |
| GTEx         | Genotype-Tissue Expression |
| HNSC         | head and neck squamous cell carcinoma |
| ICI          | immune checkpoint inhibitor |
| ImmuCellAI   | Immune Cell Abundance Identifier |
| KICH         | kidney chromophobe |
| KIRC         | kidney renal clear cell carcinoma |
| KIRP         | kidney renal papillary cell carcinoma |
| LGG          | lower-grade glioma |
| LIHC         | liver hepatocellular carcinoma |
| LUAD         | lung adenocarcinoma |
| LUSC         | lung squamous cell carcinoma |
| MESO         | mesothelioma |
| MSI          | microsatellite instability |
| OS           | overall survival |
| PAAD         | pancreatic adenocarcinoma |
| PFI          | progression-free interval |
| PFS          | progression-free survival |
| PRAD         | prostate adenocarcinoma |
| SD           | standard deviation |
| SKCM         | skin cutaneous melanoma |
| SNV          | single-nucleotide variant |
| ssGSEA       | single-sample gene set enrichment analysis |
| STAD         | stomach adenocarcinoma |
| TCGA         | The Cancer Genome Atlas |
| THCA         | thyroid carcinoma |
| THYM         | thymoma |
| TIME         | tumour immune microenvironment |
| TMB          | tumour mutation burden |
| TME          | tumour microenvironment |
| TRP          | transient receptor potential |
| UCEC         | uterine corpus endometrial carcinoma |
| uniCox       | Univariate Cox regression |
| UVM          | uveal melanoma |