Pan-cancer analyses of clinical prognosis, immune infiltration, and immunotherapy efficacy for TRPV family using multi-omics data

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A B S T R A C T

Background: Transient receptor potential cation channel subfamily V (TRPV) play an essential in cancer initiation, progression, and treatment. TRPV expression alteration are shown relate to multiple cancers prognosis and treatment of cancers but are less-studied in pan-cancer. In this study, we characterize the clinical prediction value of TRPV at pan-cancer level.

Methods: Several databases were used to examine the transcript expression difference in tumor vs. normal tissue, copy-number variant (CNV) and single nucleotide polymorphisms (SNP) mutation of each TRPV members in pan-cancer, including The Cancer Genome Atlas (TCGA) and cBio-Portal. We performed K-M survival curve and univariate Cox regression analyses to identify survival and prognosis value of TRPV. CellMiner were selected to explore drug sensitivity. We also analyzed association between tumor mutation burden (TMB), microsatellite instability (MSI), tumor immune microenvironment and TRPV family genes expression. Moreover, we investigated the relationship between TRPVs expression and effectiveness of immunotherapy in multiple co-horts, including one melanoma (GSE78220), one renal cell carcinoma (GSE67501), and three bladder cancer cohorts (GSE111636, IMvigor210, GSE176307 and our own sequencing dataset (TRUCE-01)), and further analyzed the changes of TRPVs expression before and after treatment (tislelizumab combined with nab-paclitaxel) of bladder cancer. Next, we made a special effort to investigate and study biological functions of TRPV in bladder cancer using gene set enrichment analysis (GSEA), and conducted immune infiltration analysis with TRPVs family genes expression, copy number or somatic mutations of bladder cancer by TIMER 2.0. Finally, real-time PCR and protein expression validation of TRPVs within 10 paired cancer and para-carcinoma tissue samples, were also performed in bladder cancer.

Results: Only TRPV2 expression was lower in most cancer types among TRPV family genes. All TRPVs were correlated with survival changes. Amplification was the significant gene alternation in all TRPVs. Next, analysis between TRPVs and clinical traits showed that TRPVs were related to

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pathologic stage, TNM stage and first course treatment outcome. Moreover, TRPV expression was highly correlated with MSI and TMB. Immunotherapy is a research hotspot at present, our result showed the significant association between TRPVs expression and immune infiltration indicated that TRPV expression alternation could be used to guide prognosis. In addition, we also discovered that the expression level of TRPV1/2/3/4/6 was positively or negatively correlated with objective responses to anti-PD-1/PD-L1 across multiple immunotherapy cohort. Further analysis of drug sensitivity showed the value to treatment. Based on the above analysis, we next focused on TRPV family in bladder cancer. The result demonstrated TRPV also played an important role in bladder cancer. Finally, qPCR assay verified our analysis in bladder cancer.

Conclusion: Our study firstly revealed expression and genome alternation of TRPV in pan-cancer. TRPV could be used to predict prognosis or instructing treatment of human cancers, especially bladder cancer.

1. Introduction

Cancer is a public safety and health problem which seriously endanger human health. Worldwide, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred in 2020. Approximately 19.3 million new cancer cases and 10 million died of cancer [1]. Gene alternation play an important role in cancer initiation and development. With the constant refinement of TCGA etc. database [2], the analysis to gene alternation and expression can be used to the research of cancer occurrence mechanism, diagnosis, treatment and so on [3].

Transient receptor potential cation channel subfamily V (TRPV), is one of seven channel superfamily subtypes. TRP has clear Ca2+ selectivity [4]. TRPV can be activated by many physical and chemical factors include temperature, pH, vanillin to play a part in sensory, thermoregulatory, cell signaling, osmotic pressure regulation and other functions. TRPV subfamily has 6 members, divided into two subgroups (TRPV1-TRPV4 and TRPV5/TRPV6) according to sequence homology, functional similarities, and Ca2+ selectivity [5].

Since TRPV has significant role in organism, TRPV alternation relate to many kinds of diseases, include prostate cancer, lung cancer etc. cancers [6,7]; cardiac hypertrophy [8]; irritable bowel syndrome; pain-related diseases (diabetic neuropathy, Migraine, acute dental pain and chronic pulpitis); chronic lung diseases etc. involved a wide range of organs and tissues [9].

It has been previous showed TRPV expression alternates in cancers. TRPV is vital to tumor tumorigenesis due to its role of cell cycle regulation. TRPV4 expression increased in breast cancer cells, enhances angiogenesis and vascular remodeling by promotes endothelial growth in turn leads to tumor growth [10]. TRPV6 expression increased in breast cancer, colon cancer, thyroid cancer and several types of other cancers, leads to the entering of free calcium into cell increased through TRPV6 channel [11]. In addition, TRPV1 expression in bone cancer is significantly increased; Hence, TRPV is possible to be a drug target to cancer treatment. For example, specific blocking TRPV can be used for intractable pain in cancer treatment [7,9].

However, at present, all the studies of TRPV correlate with cancer are confined to specific cancer, there is a lack of correlation between TRPV and pan-cancer. Therefore, based on TCGA, cBioPortal, CellMiner, GSEA and other databases, we analyzed expression and gene alternation of TRPV in pan-cancer; survival curve were plotted to identify the relationship between TRPV expression and prognosis. Next, we explored changes of biological functions due to TRPV expression alternation from multiple perspectives. Our research determined that TRPV expression related to prognosis of many cancers. Changes in TRPV expression were associated with multiple clinical traits, and had an impact on anti-tumor immunity through affecting immune cell infiltration, MSI and TMB. Furthermore, the effects of TRPV to chemotherapy and immunotherapy were also showed in our study. Finally, the same analysis was performed based on bladder cancer and added enrichment analysis to reveal the correlation between TRPV and related signaling pathways.

2. Methods and materials

2.1. Data gathering

We obtained transcriptome data, mutation data and clinicopathological data of 33 kinds of cancer from cBioPortal and TCGA database. Both normal and tumor issues were included. Compound activity data was acquired from CellMiner database. The UCSC Xena database was used to get the transcription expression data, associated clinical data, survival data, mutation data, immunophenotype data, and stem index evaluation data of 33 different cancer types in TCGA. The microarray expression data of three GEO immunotherapy datasets, GSE111636 (n = 11), GSE78220 (n = 28), and GSE67501 (n = 11) were all quantile-normalized, and the genes were annotated in their respective microarray platform files GPL17586, GPL11154, and GPL14951. The IMvigor210 mRNA-sequencing dataset, a cohort of 348 MIBC patients treated with Atezolizumab (PD-L1 inhibitor), was also used for validation of TRPVs family genes as immunotherapy efficacy markers.

Furthermore, in our ongoing single-arm phase II clinical study of tislelizumab combined with low-dose nab-paclitaxel (TRUCE-01, NCT04730219) for muscle-invasive urothelial bladder carcinoma, the mRNA transcriptome sequencing data and clinical information of 29 cases, which included baseline 15 cancer tissues and 15 paired cancer tissues after immunotherapy, were acquired to explored the changes of TRPVs expression, and the statistical significance of differential expression was evaluated using the paired Wilcoxon test.

According to Response Evaluation Criteria in Solid Tumors (RECIST) criteria, responders were defined as patients who had complete or partial responses (CR/PR) after immunotherapy; non-responders were those who had stable disease or progressive disease (SD/PD). Data processing and image production were based on R packets.

2.2. TRPVs expression in pan-cancer

TRPV expression value of 43 cancers was established using median. Then, we selected 18 cancers and compared TRPV expression between tumor and normal tissues. Limited by the databases, only the cancer which sample size was larger than 5 was picked. Meanwhile, we compared the expression level of every member gene in TRPV using heatmaps. Correlation between two parameters was assessed by Pearson correlation coefficient (r).

2.3. TRPVs mutations analysis

Mutation data was based on CellMiner database, include 2683 samples. We calculated the mutation level of every TRPV member in 33 cancers and compared the difference between different mutation types. Data were presented as median, and Kruskal-Wallis (K–W) test was used for testing.

2.4. Survival analysis

Establish Kaplan-Meier (K-M) curve to show the correlation between the survival of patients and TRPV. Then, univariate Cox regression models were created to evaluate Hazard Ratio (HR). TRPV expression regarding Overall Survival (OS), Disease-Specific Survival (DSS), Progression-free Survival (PFS) and Disease-free Survival were displayed by K-M curve. We also analyzed the impact of TRPV mutations on OS. All kinds of mutations were divided into Mutation group and Non-mutation group, we considered Statistical significance when $P < 0.05$.

2.5. TRPVs expression and clinical traits in pan-cancer

To explore relationship between TRPV expression and tumor pathological stages, we selected 17 types of tumor tissue. TRPV expression levels of these tumor tissues at different pathological stages were calculated separately and then using K–W test to test. Moreover, complete Response (CR) and Partial Response (PR) were divided into treatment-effective groups, Progression Development (PD) and Stable Disease (SD) were divided into Treatment ineffective group. Next, we analyzed correlation of TRPV expression and first course treatment outcome in selected types of cancers.

2.6. Correlation between TRPVs expression and immune cell infiltration

According to the study of Veśteinn Thorsson et al. [12]. divided pan-cancer tumor tissue into 6 immune subtypes, then separately calculated correlation between TRPV and 6 immune subtypes. We calculated the immune score of CD8$^+$ T cell, NK cell and NKT cell based on multiple databases. Afterward, we evaluated the association of each TRPV member gene with potential immunological cells. The using database included TIMER, EPIC, MCPCOUNTER, CIBERSORT, CIBERSORT-ASS, QUANTISEQ and XCELL. We based on ESTIMATE algorithm scored the immune microenvironment of pan-cancer tumor tissues; then assessed its relationship with TRPVs.

2.7. TRPVs expression and tumor stemness scores

Based on TRPV expression data calculated RNA stemness score (RNAss), then analyzed correlation between TRPV expression and RNAss. Pearson correlation coefficients (r value) were represented to analyze the magnitude of correlation. A positive r value indicated positive correlation, and negative r value indicated negative correlation. High score meant more stem cell content and lower grade of tumor differentiation.

2.8. TRPVs expression in pan-cancer and TMB, MSI and immune checkpoint related genes

Data acquired from TCGA database was used to analyze correlation between TRPV expression and TMB and MSI. Pearson correlation coefficients (r value) was used to describe the correlation magnitude. The Result was shown by bubble plot. Performed co-expression analysis between the expression of TRPV in pan-cancer and PD-1 and CTLA4-related genes, includes whether there was a statistical difference, and the correlations were positive or negative. The results were presented in heatmaps.

2.9. Drug screening based on TRPVs expression

Through analyzing the correlation of TRPVs expression and drug sensitivity from CellMiner database, it could guide selection of drugs that were effective or ineffective when TRPVs expression alternated. The treatment efficacy was computed using z-score value.

2.10. TRPVs expression and clinical efficacy of immune checkpoint inhibitors

We picked IMvigor210, GSE111636, GSE78220, GSE67501, GSE176307 and our TRUCE-01 to analyze the correlation between TRPV expression in bladder cancer, renal cell carcinoma, melanoma and the clinical efficacy of immune checkpoint inhibitors. The results were divided into Respond and Non-respond, statistically difference was set at $p < 0.05$.

2.11. TRPVs evidence in the specific bladder cancer cohort

In bladder cancer, the methods of correlation analysis between TRPV expression and tumor microenvironment (stem cell index, TMB, MSI) was described above, and the results were uniformly demonstrated by univariate Cox analysis. In addition, we selected age (>65 or not), gender (male or female), pathological stage, Non-papillary or papillary cancer and TNM stage eight clinical traits to analyze their correlation with TRPV expression. We based on TIMER database, analyzed correlation between TRPV expression and immune cell content in bladder cancer. We used Box plots to show the results, and statistical significance was evaluated using wilcox. Then differential analysis of correlation between immune cell content and TRPV gene alteration (Mutation or Non-mutation) in bladder was performed and visualized. Furthermore, we studied the biological functions of TRPV in bladder cancer by Gene Set Enrichment Analysis (GSEA), data was acquired from KEGG database. Through computing normalized enrichment score divided the samples into high expression group and low expression group, the 20 most relevant pathways were shown.

2.12. Quantitative real-time reversetranscription PCR (qRT-PCR)

The tissues were obtained from 10 patients by transurethral resection whose tissue, including cancer and adjacent normal tissues, were collected and quickly frozen from patients. Total RNA was isolated from cell using E.Z.N.ATM Hp total RNA Kit (OMEGA). The cDNA for pRT-PCR was synthesized from total RNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Rockford, IL, USA). Quantitative real-time PCR was performed to test the expression using TOROGreen qPCR Master Mix (Toroivd). Our primer sequence used in PCR are shown in Table 1.

3. Results

3.1. Differential co-expression analysis and prognostic significance of TRPV genes in pan-cancer

A detailed flow chart of this study is provided in Fig. 1. First, to explore the expression of TRPV, we surveyed TRPV gene subfamily expression in pan-cancer and the member gene expression in 33 cancers. Data based on TCGA database. Results are shown in Fig. 2A, in pan-cancer, TRPV2 had the highest expression level followed by TRPV4. The remaining TRPV1, 3, 5, 6 were expressed at low level; The expression of TRPV in specific cancer is ranked LAML, SKCM, TGCT, LUBC, LAML, PRAD from TRPV1 to TRPV6 (Supplementary Fig. S1).

To determine weather cancer related to TRPV expression alteration, we selected 18 cancer tissues to compare the gene expression between normal and cancer tissues. Then sorted the expression form small to large, the result can be seen in Supplementary Fig. S2. We found that expression of TRPV decreased or increased in pan-cancer by comparing tumor group and normal group in different species of cancers, and some tumor tissues have several TRPV expression altered. Furthermore, the co-expression relationship could be observed in Fig. 2C, including TRPV1 and TRPV5 (correlation coefficient (Cor) = 0.27), TRPV5 and TRPV6 (Cor = 0.25), TRPV2 and TRPV6 (Cor = −0.21).

Meanwhile, we analyzed the pairwise correlation among TRPVs. The results were illustrated in Figs. 2B and 7 genes showed a positive correlation, with 6 being negative. Only one gene had no correlation to other genes. Among all TRPVs, TRPV1 had most significantly positively correlation with TRPV5($r = 0.27$), well TRPV2 and TRPV6 were the most significantly negatively ($r = −0.21$).

Next, to explore the effect of TRPV expression alteration to prognosis, we analyzed the relationship between overall survival (OS), progression-free survival (PFS), disease-free survival (DFS), disease specific Survival (DSS) and TRPVs by using K-M curves (Supplementary Fig. S3). The K-M curve indicated that expression of TRPVs was related to OS, DFS, PFS and DSS in many kinds of cancers (Supplementary Table S1; Additional data1). Then, univariate cox proportional hazard model was used to assess the risk factors for mortality (Fig. 2D-G). Among results reported with statistical significance, TRPV2 and TRPV3 had the most significant difference

Table 1

The primers used for real-time PCR are designed and synthesized by Sango Biotech (Shanghai, China).

| Gene Name	Primer Type	Primer Sequence	Product Length
TRPV2	Forward primer	5'-TCAGGTTGGAGACATTAGATGGA-3'	155
	Reverse primer	5'-TCGGTAGTTGAGGTTGACTCTT-3'	
TRPV4	Forward primer	5'-CTACGGCACCTATCGTCACC-3'	215
	Reverse primer	5'-TTAGGCGTTTCTTGTGGGTCA-3'	
TRPV6	Forward primer	5'-AGGACCAATAACCGCACGAG-3'	158
	Reverse primer	5'-ATGTCTGGAACCTCTACCAGC-3'	
GAPDH	Forward primer	5'-CGGAGTCAACGGATTTGGTC-3'	180
	Reverse primer	5'-TTCCCGTTCTCAGCCTTGAC-3'	
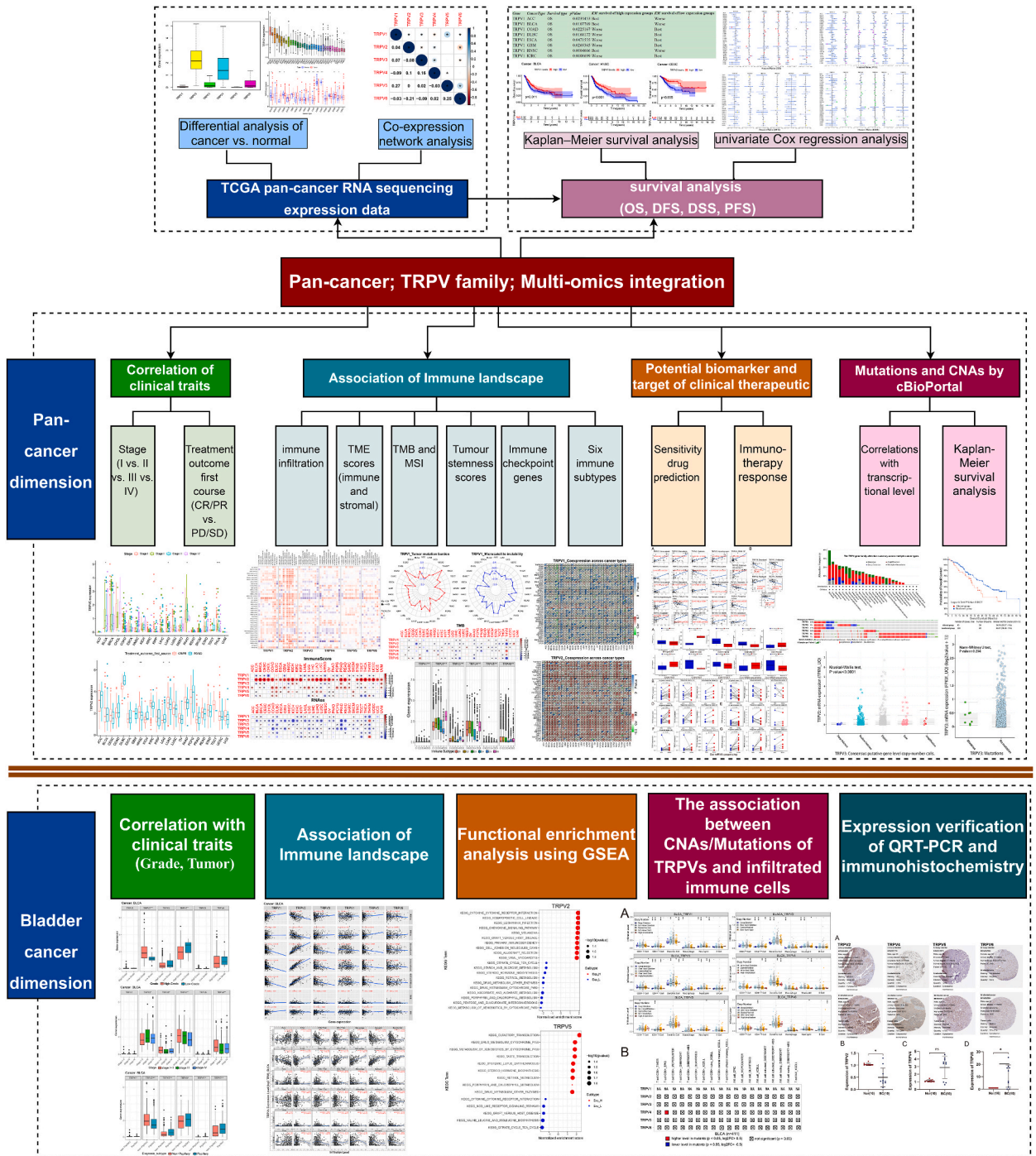
Fig. 1. The analysis flow diagram of the study on TRPV family genes role in human cancers.

(Supplementary Fig. S3, Only the most significant results were shown). In COAD etc. cancers, TRPV upregulation related to worse OS, but in ACC etc. 55 cancers relate to higher OS. The univariate Cox proportional hazard model analyses results showed that high expression of TRPV was associated with poor OS in GBM etc. 21 cancers but was a protective factor in HNSC etc. 11 cancers. DSS was the most significantly affected by TRPV expression alternated, with DFS was least affected. Meanwhile, we found that TRPV2 and TPRV4 had the most notable effect on survival, had more influence on OS, PFS, DFS and DSS. High expression of TRPV3 and TRPV6 were risk factors to all kinds of cancers (Fig. 2D-G).
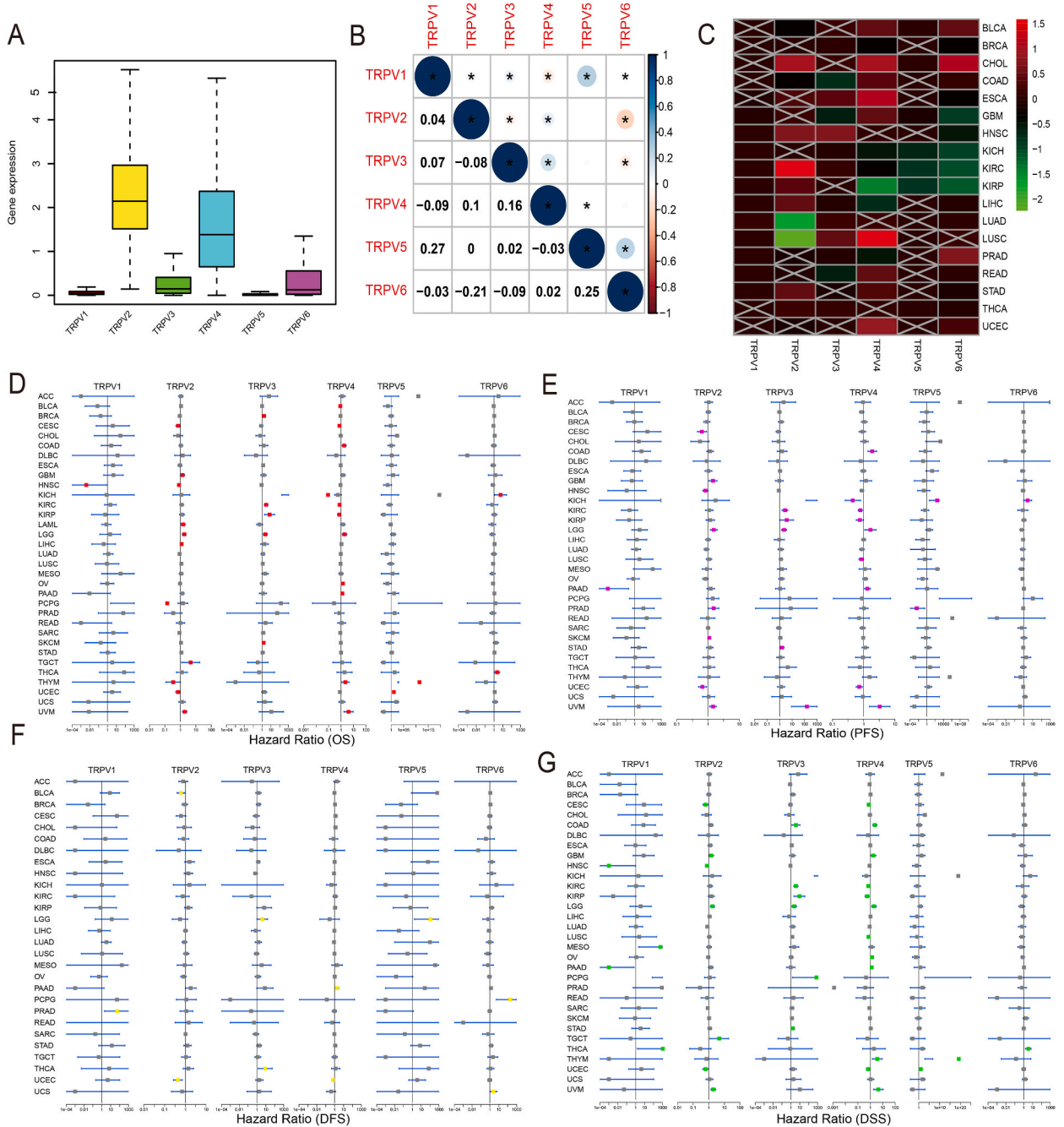
Fig. 2. TRPVs gene family expression in pan-cancer and the univariate Cox regression was calculated to analyze the relationship between TRPVs expression and clinic prognosis in pan-cancer which Results was shown using forest plots. **(A)** Boxplot of TRPVs expression based on TCGA database. **(B)** Correlation graph of each member of TRPVs. **(C)** Heat map of TRPVs expression between cancer tissues and noncancerous normal tissues. **(D)** OS, overall survival, **(E)** PFS, Progression-free Survival, **(F)** DFS, disease-free survival, **(G)** DSS, disease-specific survival.

3.2. Genomic alteration of TRPV family genes in pan-cancer

In this pan-cancer investigation, genomic changes in TRPV family members were not uncommon. Fig. 3 demonstrates the alteration of TRPV in pan-cancer. We investigated 2683 pan-cancer cancerous tissues from the cBioPortal database and found 13.01% had TRPV altered, include mutation, amplification, deep deletion and multiple alterations. Copy number variation (CNV or copy-number alterations, CNA) was the main alteration which was evidently higher to the frequency of mutation (2.35%). While amplification (8.54%) was most common in CNA, while deep deletion (1.49%) and multiple alteration (0.63%) were of low frequency (Fig. 3A).
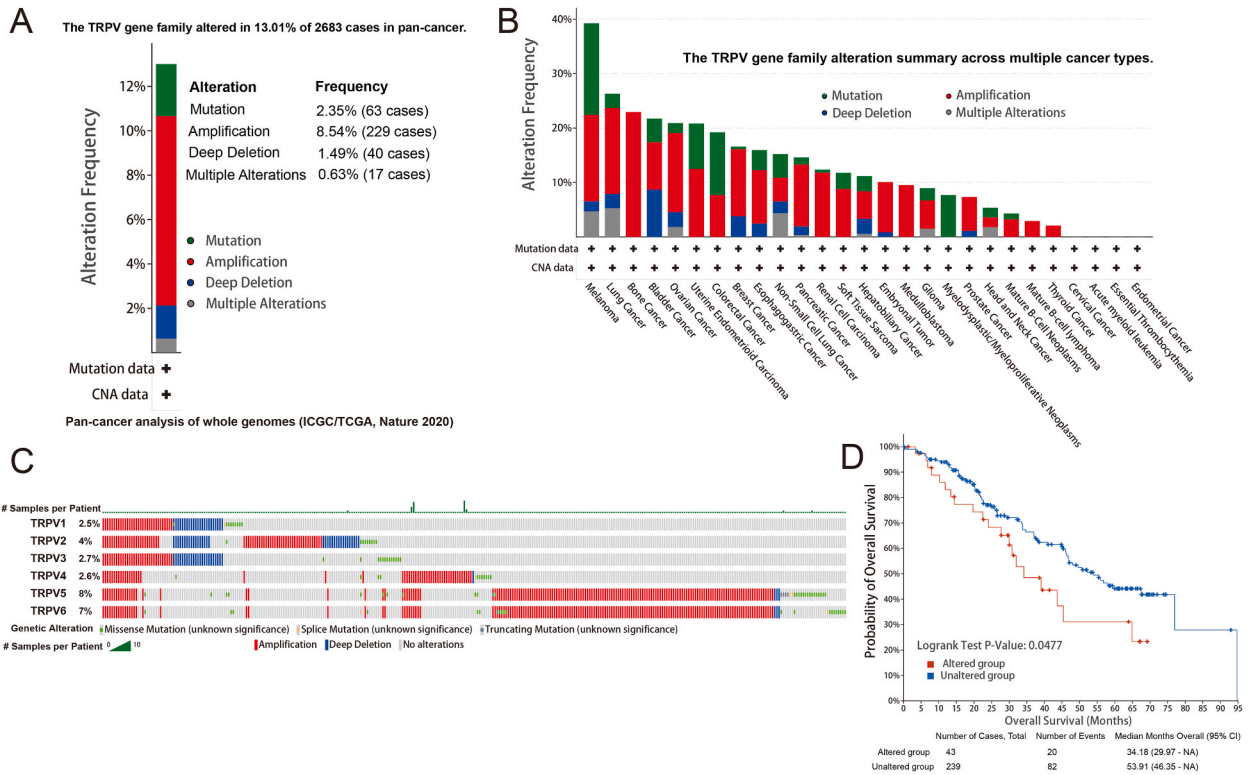
Fig. 3. Gene alternation of TRPVs in pan-cancer was defined by cBioPortal database. **(A)** The overall changes of TRPVs in pan-cancer included mutation, amplification, deep deletion, and multiple alternations. **(B)** TRPV alternation in 27 kinds of cancers. **(C)** The alternation frequency of TRPVs in pan-cancer. **(D)** Kaplan–Meier curve was used to compare overall survival with and without TRPV alternations.

TRPVs had different degrees of alteration, but amplification was predominated in pan-cancer. In Lung cancer, Bone cancer, Ovarian cancer etc. cancers, the predominant alteration is amplification; In melanoma, colorectal cancer, myelodysplastic/myeloproliferative neoplasms etc. Cancers, the predominant alteration is mutation; In Non-Small Cell Lung Cancer, the frequency of multiple alterations, amplification, mutation were almost coincide which were higher to multiple alteration; Head and Neck Cancer had a same frequency of multiple alteration, amplification and mutation; Bladder Cancer showed a lower frequency of mutation compared with multiple alteration and amplification (Fig. 3B). TRPV5 and TRPV6 had the greatest frequency of amplification and almost occur simultaneously (Fig. 3C). TRPV2 had a higher frequency of deep deletion compared with other TRPVs. Patients with TRPVs alteration had significantly shorter median survival compared with patients did not have TRPVs alteration (Fig. 3D).

3.3. The impact of TRPVs genomic variation on gene expression

To explore how TRPV CNA and Single Nucleotide Polymorphism (SNP) act on expression, we analyzed CNA and SNP in each number of TRPV (Fig. 4A-F). CNA includes deep deletion, shallow deletion, amplification and gain, we observed no significantly statistically difference between CNA and expression (Fig. 4G-L). However, statistical difference was detected between SNP and expression. When SNP occurred, TRPV1, TRPV2, TPRV3 expression were downregulated; but amplification and gain could increase the expression of some TRPVs.

3.4. Correlation between TRPV expression and clinical traits acorss pan-cancer

We next examined the relationship between TRPV expression and clinical traits. Cancer stage and first course treatment outcome were selected to evaluate the relationship. We found TRPV3 increased with the increase of tumor stage in KICH and KIRP; and, TRPV3, TRPV4, TRPV6 related to tumor stage in BLCA. TRPV2, TRPV6 expression changed with tumor stage altered in THCA (Fig. 5A; Supplementary Fig. S4D). In addition, BRCA and SKCM had the highest expression of TRPVs in stage4 (Fig. 5B; Supplementary Fig. S4B).

On the other hand, we analyzed correlation between first course treatment outcome and TRPV expression (Fig. 5C and D; Supplementary Fig. S4E-H). Only few cancers had TRPVs expression change in the SD + PD group than in the CR + PR group after first course treatment. Compared with CR + PR group, TRPV1 expression increased in LGG; TRPV2 expression decreased in HNSC and LUAD and its expression increased in DLBC and LGG; The expression of TRPV3 decreased in ESCA but increased in STAD; however in
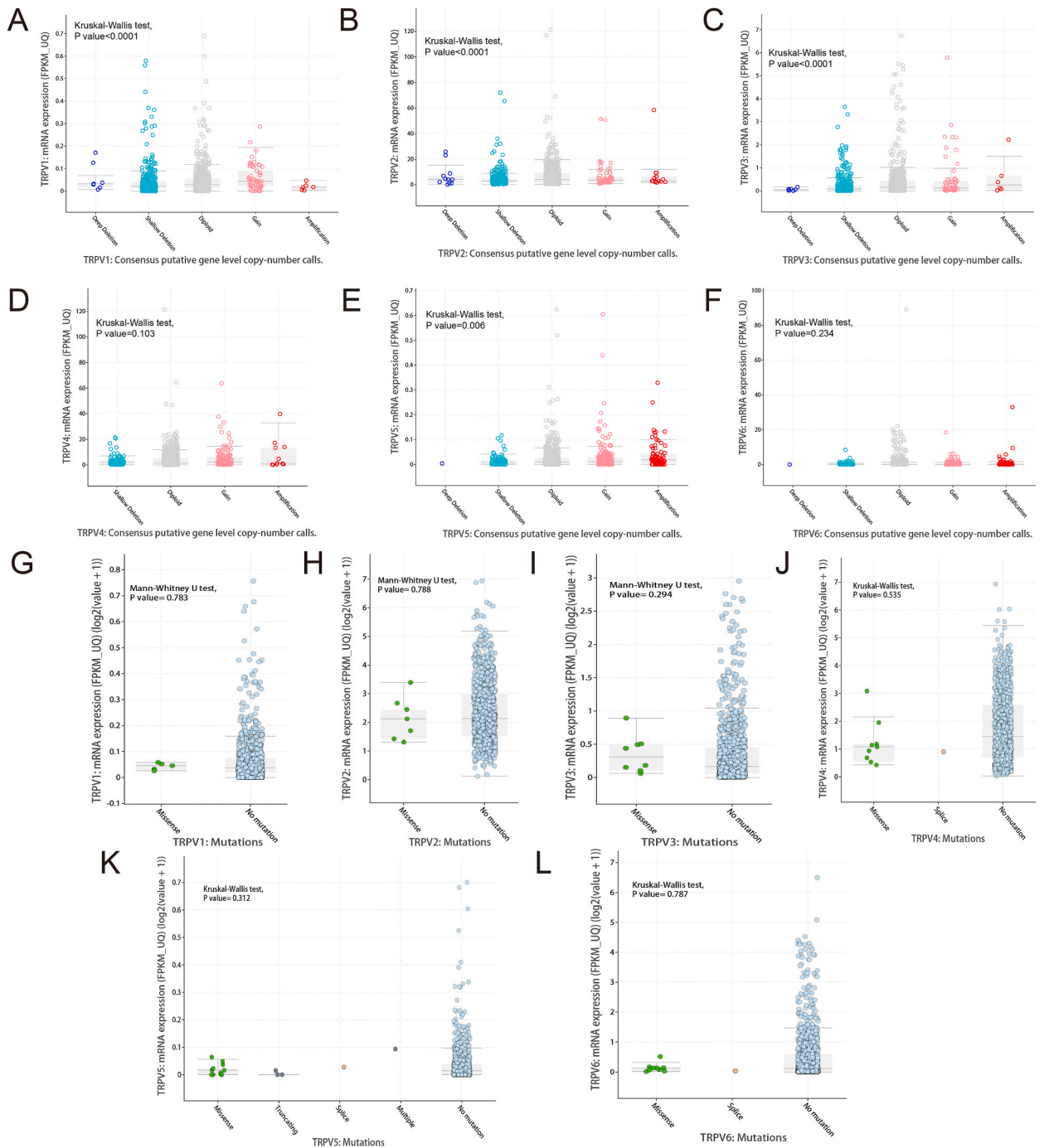
Fig. 4. TRPVs expression related to Copy number alternations were analyzed based on ICGC/TCGA pan-cancer datasets (2683 samples) from cBioPortal **(A–F)**. Similarly, associations of TRPVs expression with SNVs were also analyzed **(G–L)**.

HNSC and LUSC, the expression of TRPV4 decreased in the SD + PD group. Interestingly, TRPV5 expression was significantly decreased in COAD, KICH and PCPG, as well as increased in PRAD in the SD + PD group but these cancers expressed TRPV5 all at a low level (Supplementary Fig. S4G). Finally, TRPV6 expression decreased in LUAD, BLCA and PRAD (Supplementary Fig. S4H).

3.5. Correlation between TRPV expression and tumor microenvironment in pan-cancer

In according with the study of Veśteinn Thorsson et al., immune subtype was assigned to C1–C6. TRPV expression in each immune
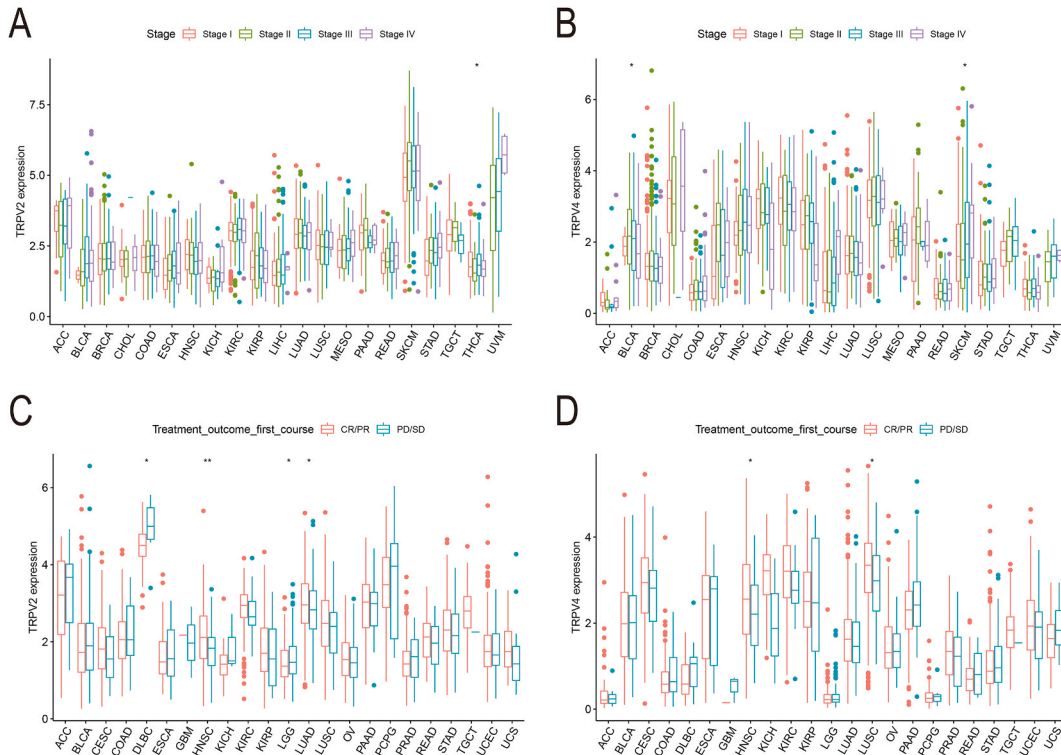
Fig. 5. Correlation analysis of TRPV2 **(A)** and TRPV4 **(B)** expression with pathological staging with different cancers were performed, respectively. Similarly, association of TRPV2 **(C)** and TRPV4 **(D)** expression with response status after initial treatment of patients acorss various cancer types.

subtype was shown in Fig. 6A. Upon analysis we found in immune subtypes include C1 (wound healing), C2 (IFN-γ dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet) and C6 (TGF-β dominant), TRPV expression features displayed significant difference. TRPV2 expression related to C1–C6 had the same trend to TRPV4. Meanwhile, the expression of TRPV2 and TRPV4 were at a high level in 6 kinds of immune subtypes compared with the other TRPVs, which were markedly expressed at a low level.

Role of TRPV in pan-cancer immune microenvironment have not been reported, we based on ESTIMATE algorithm, used TIMER database to analyze stromal score and immune score of TRPV in pan-cancer (Fig. 6B and C). The analysis revealed high immune score significantly positively related to TRPV2 expression increased, but weakly negatively related to TRPV1 expression decreased in almost all kinds of cancers. And notably, the stromal score result had a similar trend to immune score in nearly all tumor types.

Furthermore, we analyzed the correlation between TRPV expression and immune cell infiltration, include CD8$^+$ T cells, Natural killer (NK) cells and NKT cells. Results are available in Fig. 6E, we found there was a distinct positive correlation between TRPV2 expression and several immune cells infiltration while TRPV1 up regulated was positively but weakly related to immune cell infiltration, indicted TRPV1 and TRPV2 increased had a better prognosis; TRPV3 and TRPV4 had a negative correlation to NK cell content in almost all species of cancers, indicted TRPV3 and TRPV4 increased were unfavorable to anti-tumor immunity. For special cancer types, HPV (+) HNSC and UVM etc. was closely related to TRPV expression.

3.6. Association between TRPV genes and RNAss

Increasing evidence suggests that stemness markers of tumor cell expression grow is highly associated to drug resistance, cancer recurrence, tumor proliferation. Therefore, we evaluated the correlation of RNA stemness score and TRPV expression. The results were illustrated in Fig. 6D, in 33 types of cancers, most cancers TRPV expression negatively corrected to RNAss, with COAD, PAAD etc. significantly. Only THYM RNAss was associated with all TRPVs and had a strong positively statistical difference to TRPV2 and TRPV6. Meanwhile, TPRV2 affected RNAss more significantly compared with other TRPVs.

3.7. Correlation between TRPV and immunological indicators (i.e., TMB, MSI and immune checkpoint)

Due to MSI and TMB have important connection with the sensitivity of immune checkpoint inhibitors, we analyzed the correlation between TRPV expression and levels of TMB and MSI. Results are shown in Figs. 7A and 6B and Supplementary Figs. S5 and 25 of 33 cancers TRPV expression related to MSI with TRPV1 the most significantly which was associated with 12 cancers. MSI in COAD, LUSC and TGCT varied most significantly with TRPV. In relationship between TRPV and TMB, most cancers related negatively with TRPV,
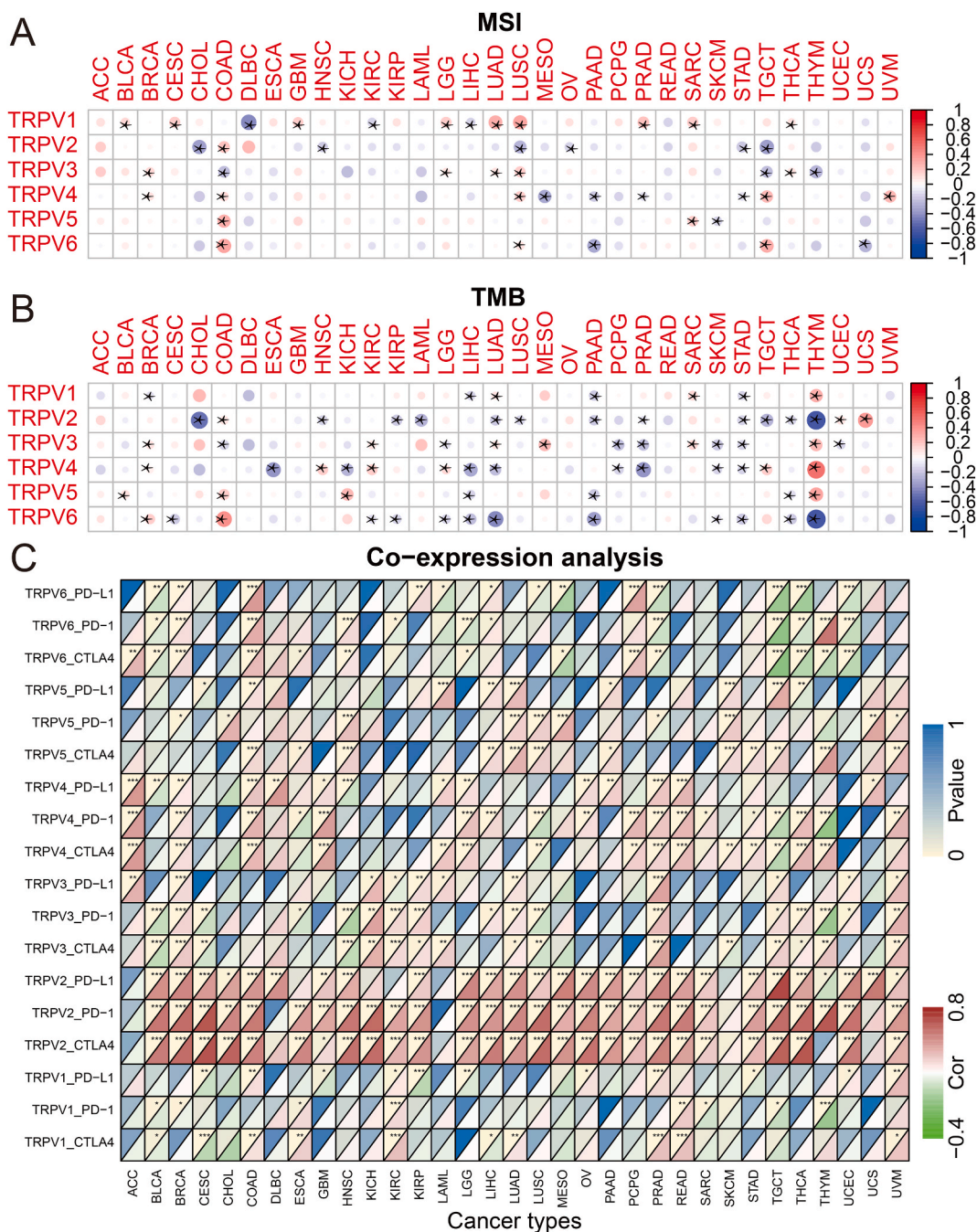
Fig. 6. (A) The relationship of expression of TRPVs and immune subtypes based on TCGA database. C1 (wound healing), C2 (IFN-g dominant), C3 (inflammatory), C4 (lymphocyte depleted) and C6 (TGF-b dominant). **(B–D)** Correlation between TRPVs expression and immune score, stromal score or RNAss score. **(E)** Several algorithms were performed to analyze the potential correlation of TRPVs expression level and the infiltration level of CD8$^+$ T cell, NK cell and T cell NK.

THYM had the most significant relationship to TRPV among these cancers.

The expression of immune checkpoint is essential to immunotherapy. We selected PD-1, PD-L1 and CTLA-4 as our study subjects, which are the top hit immune checkpoint. Then explored the relationship between TRPV and the immune checkpoints. The results are shown in Fig. 7C and Supplementary Fig. S6, most of the 33 cancers had positive relationship between TRPV2 expression and immune checkpoints; Each cancer had at least one TRPV related to immune checkpoint, and most were positive. The most significant difference between TRPV and immune checkpoint was presented in PRAD.

Fig. 7. TCGA databased was used to display the relationship between TMB, MSI, immune checkpoints' expression, and TRPVs expression in different cancer types. **(A)** Correlation between TRPVs expression and MSI. **(B)** Correlation between TRPVs expression and TMB. **(C)** Correlation between TRPVs expression and known immune checkpoints' mRNA expression in 33 cancers.

3.8. Analysis of TRPV and drug sensitivity

Because of drug resistance in anti-tumor therapy, we based on CellMiner database selected TRPV as potential targets for drug screening (Fig. 8, Supplementary Table S2). The result exhibited that TRPV2 expression increased was most disadvantaged for drug screening, which was positively related to IC50 of Vemurafenib, Dabrefenib, Bafetinb, Hypothemycin, Selumetinb, Cobimetinb and ABT-199, indicated that TRPV2 expression level increased with treatment effect decreased; TRPV1 expression was negatively related to IC50 of Depsipeptide, Dolastatin 10 and Eribulin mesylate; Drug sensitivity to SR16157, and Fulvestrant was negatively related to TRPV3 according to IC50; TRPV4 expression was positively associated with IC50 of Vemurafenib, Dabrafenib, and Hypothemycin was
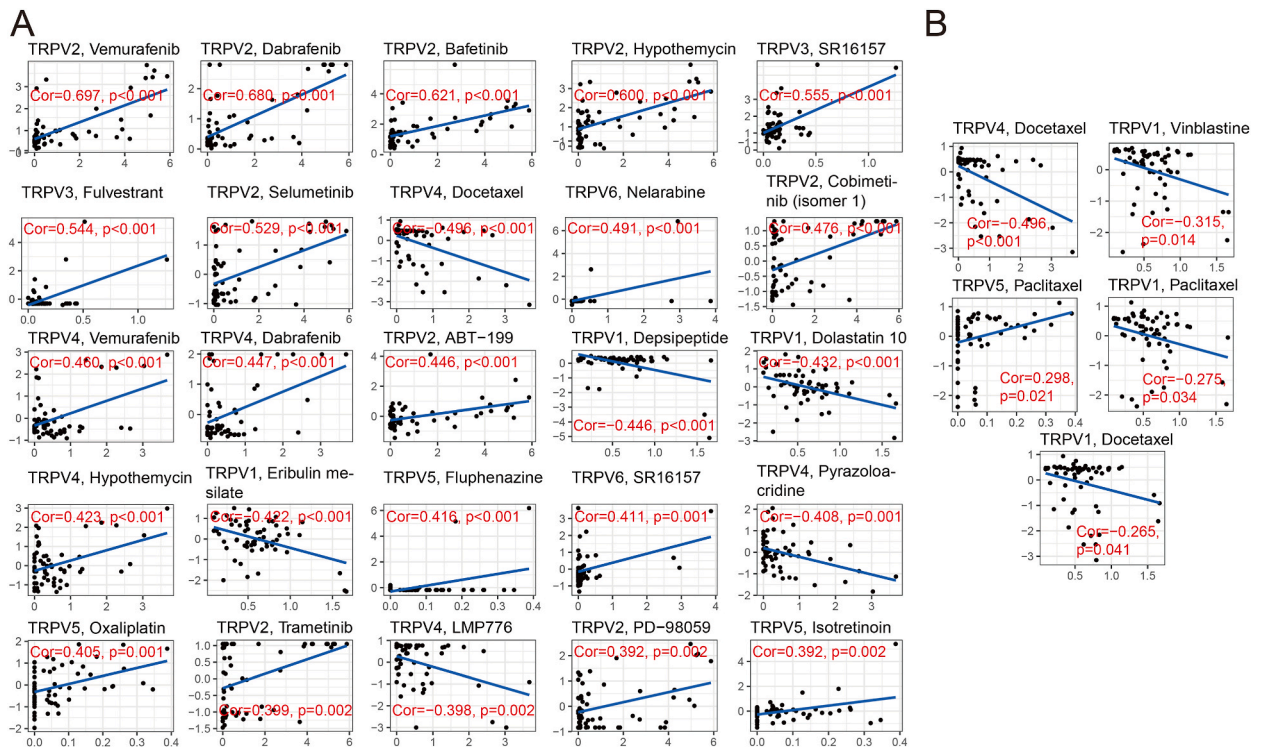
Fig. 8. Drug response of TRPVs expression alternation in TCGA cancers. **(A)** Top 25 drugs significantly associated with TRPV family genes (P < 0.01) were obtained. **(B)** The significant correlation between commonly used chemotherapeutic drugs for cancer and the expression of TRPV genes (P < 0.05).

negatively correlated with IC50 of Docetaxel, Pyrazoloacridine; Likewise, IC50 was used to evaluate drug sensitivity of TRPV5 and TRPV6, the result showed that IC50 of Fluphenazine was positively correlated to TRPV5, and IC50 of Nelarabine and SR16157 was negatively correlated to TRPV6.

3.9. Effect of TRPV genes expression on the clinical effects of immunotherapy via integrating several datasets

Immunotherapy is essential to cancer treatments; thus, it is of great significant to assess how gene expression correlated to immunotherapy effects. We selected three tumor tissues, analyzed the correlation between TRPV expression in bladder cancer, melanoma and renal cell carcinoma and immunotherapy effects based on GEO database, iMvigor210 cohort and our mRNA sequencing results. The analysis results shown in Fig. 9A determined that only TRPV4 expression decreased in melanoma showed significant difference to respond to immunotherapy. In addition, p value of TRPV4 low expression in bladder cancer and respond to immuno-therapy was close to 0.05, had borderline significance. But regrettably, TRPV2, which was strongly related to tumor microenvironment. Furthermore, the results of our sequencing showed that TRPV2 expression after treatment was decreased and TRPV6 was increased. TRPV3 aslo showed the trend of expression decreasing which p = 0.055. Unfortunately, our mRNA sequencing TRUCE01 results of response to tislelizumab combined with nab-paclitaxel therapy did not have significant statistical significance, the reason for this may due to the sample size.

Apart from this, we also investigated the potential mechanism behind the expression of TRPV genes and immunotherapy response. Our sequencing TRUCE01 results found that the expression level of TRPV3/TRPV6, and TRPV1 respectively in bladder cancer cases with response or non-response to tislelizumab combined with nab-paclitaxel therapy significantly decreased after treatment (p = 0.049); however the expression level of TRPV2 increased after treatment in responsive cases (Fig. 9B–G). Interestingly, these results indicated that the molecular mechanism underlying these responses was significantly associated with the expression of TRPV-familiy genes. This is also the part that we will continue to studyin depth in the follow-up work.

3.10. Serial supplementary analysis of TRPV family genes in bladder cancer

We identified the correlation between TRPV expression and gene alternation, immune microenvironment and clinical character-istics above. We found that TRPV strongly related to clinical traits and prognosis in most cancers. Meanwhile, significant difference of TRPV2 and survival, immune score and stemness index in pan-cancer was determined. Therefore, we want to further investigate the correlation of TRPV and bladder cancer, includes expression, gene alternation and immune-related characteristics.
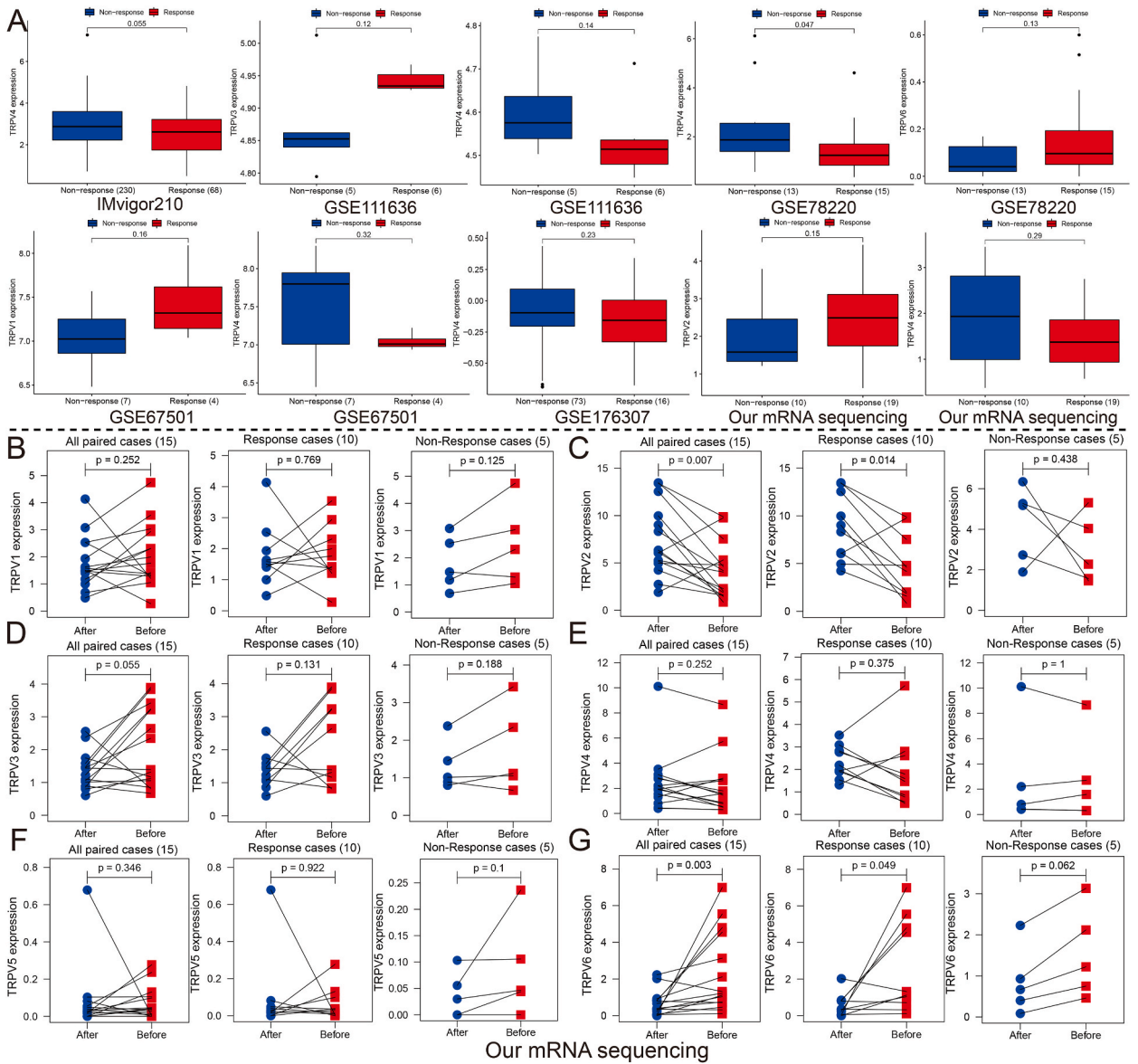
Fig. 9. Expression level of TRPV family genes in different immunotherapy response groups in immunotherapy cohort. **(A)** Differential expression analysis was performed based on IMvigor210, GSE111636, GSE78220, GSE67501, GSE176307 and our mRNA sequencing. **(B–G)** The differences in TRPVs expression before and after immunotherapy were compared by paired wilcox test in our TRUCE-01 data.

3.11. The biological function of TRPV in bladder cancer

To begin with, we based on GSEA database explored biological function of TRPV in bladder cancer (Supplementary Fig. S7, Supplementary Table S3). substance metabolic, drug metabolic and some signaling pathways are positively enriched in high level expression of TRPV1 and TRPV3, probably related to drug treatment of cancer. TRPV2, in addition to associating with positive regulation of cytokine–cytokine receptor interaction and cell-adhesion molecule, correlated with multiple diseases. The diseases mainly encompassed immune-related diseases, such as graft-versus-host disease and primary immunodeficiency; melanoma; infectious disease. These results showed TRPV2 probably closely related to immune-related treatments. Similar to TRPV2, TRPV4 and TRPV5 also positive regulate substance metabolic and especially drug metabolic. Diseases like systemic lupus erythematosus enriched in TRPV4 or TRPV5 high expression. Notably, TRPV6 positively regulated NK cell mediated cytotoxicity, Toll-like receptor signaling pathway and SLE and other autoimmune diseases; indicated that TRPV6 closely interlinked to body immune status.

3.12. Correlation analyses of TRPV with immune subtypes and clinical traits, tumor stemness, immunological characteristics in bladder cancer

We separately analyzed the correlation between TRPV expression and immunotyping and clinical traits in bladder cancer. The result showed in Supplementary Fig. S8 revealed that TRPV2 achieved statistically difference in immune subtypes and multiple clinical traits. C2 immune subtype was related to TRPV2 high expression, while C6 was associated with low level of TRPV2 expression. As for clinical traits, TRPV2 expression increased more often with high grade, high stage, high tumor grade (T) and more likely been non-papillary carcinoma. TRPV4 expression related to grade, stage and tumor grade (T, N), indicated that it could predict tumor growth. The correlation between TRPV6 and immune subtypes was opposite to TRPV2, low expression level of TRPV6 is more probable to appear in C1, C3 and C6. Tumor diagnosis subtype was the most closely related to TRPV among clinical traits, correlation between all TRPVs were observed.

The result shown in Fig. 10 depicted how TRPV related to RNAss, DNAss, immune score, stromal score, ESTIMATE score, MSI and TMB. TRPV1 expression positive associated with RNAss, with TRPV2 and TRPV6 negatively; relationship between TRPV2 expression and DNAss was negative association, while TRPV5 and TRPV6 were positive. The analysis to immune score, stromal score and ESTIMATE score revealed that only TRPV3 negatively related to the three scores. Further analysis of TMB and MSI determined that only TRPV1 positively correlated with MSI; TRPV5 was confirmed moderately correlation with TMB.
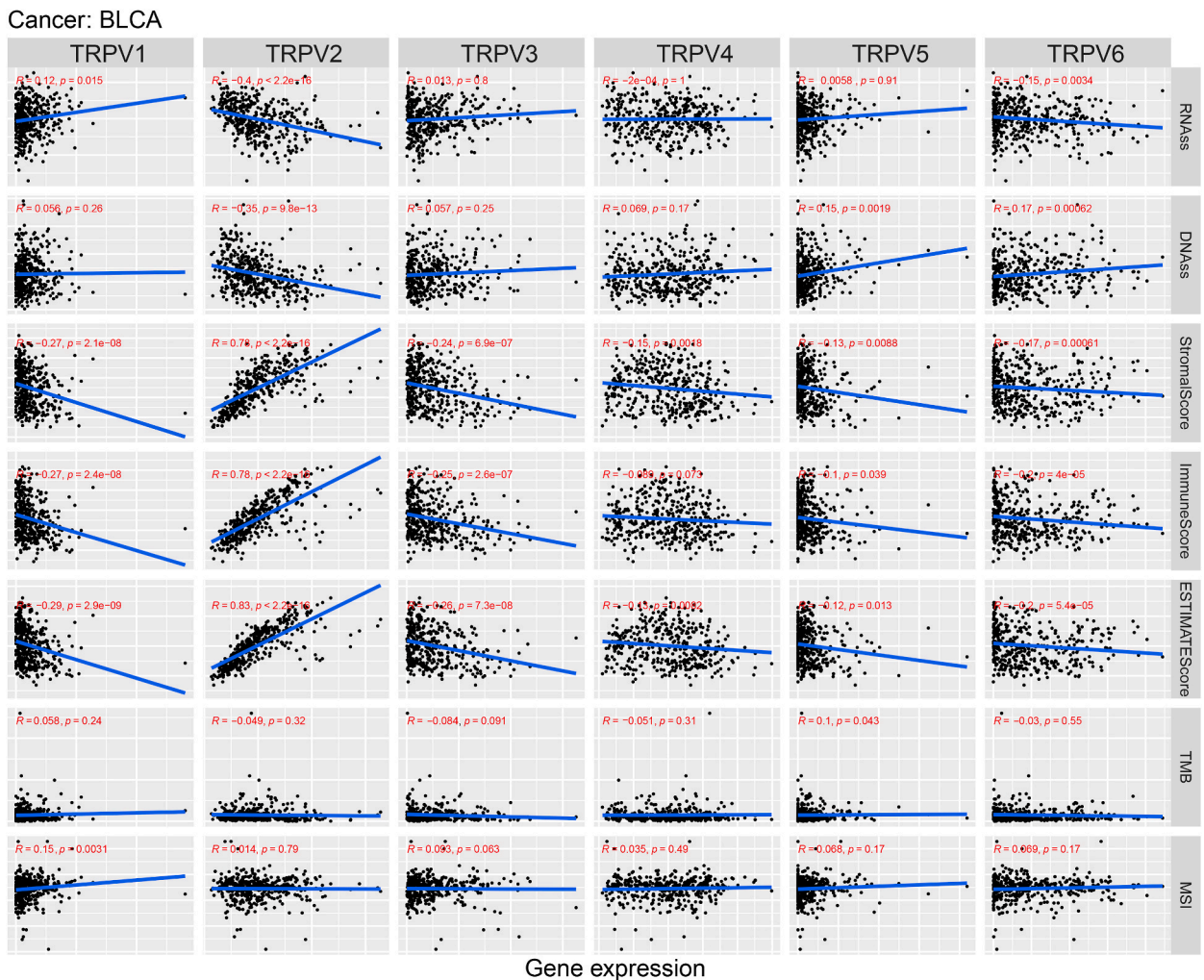

Fig. 10. Correlation between the TRPVs family genes expression and RNAss, DNAss, StromaScore, ImmuneScore, ESTIMATEScore, TMB and MSI based on TCGA database. RNAss, stemness scores in BLCA; DNAss, stemness scores based on DNA-methylation; TMB, tumor mutation burden; MSI, microsatellite instability.

3.13. Correlation between TRPV genes expression (or genomic variations) and immune microenvironment in bladder cancer

Tumor immune microenvironment had an important influence on immune treatment. Hence, we according to TIMER database analyzed TRPV expression and bladder cancer immune microenvironment. The results are presented in Fig. 11, except no significant difference was observed between TRPV4 and immune microenvironment, other TRPVs all showed effects to immune microenvironment. Among TRPVs TRPV2 had the most significant influence, which strongly correlated with B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophage and dendritic cells; indicated that the immune infiltration of high level TRPV2 expression was more visible, favored immune treatment. Except for TRPV2, other TRPVs denoted more likely a negative correlation with immune cell infiltration, suggested that was unfavorable to immune treatment.

In order to further explore the reason of TRPV expression alternation and changes of immune microenvironment, we analyzed the correlation between CNA in TRPV and immune microenvironment. The results can be seen in Fig. 12A. All CNA of TRPVs would cause the decrease of $CD4^+$ T cells content, included gene amplification and deletion. The amplification and deletion of TRPV1, TRPV2, TRPV3, TRPV4 caused dendritic cells content decreased. TRPV5 and TRPV6 CNA had same effects, which deletion reduced $CD4^+$ T cells content and high amplification decreased B cells content.

Mutation is another essential of gene expression alteration. Next, we explored the effects of TRPV mutation to immune cell content, the results are shown in Fig. 12B. We firstly analyzed the correlation between TRPV mutation and immune cell content above. The algorithm based on EPIC revealed that only TRPV mutation in bladder could affect $CD8^+$ T cells content. In turn every number of TRPV
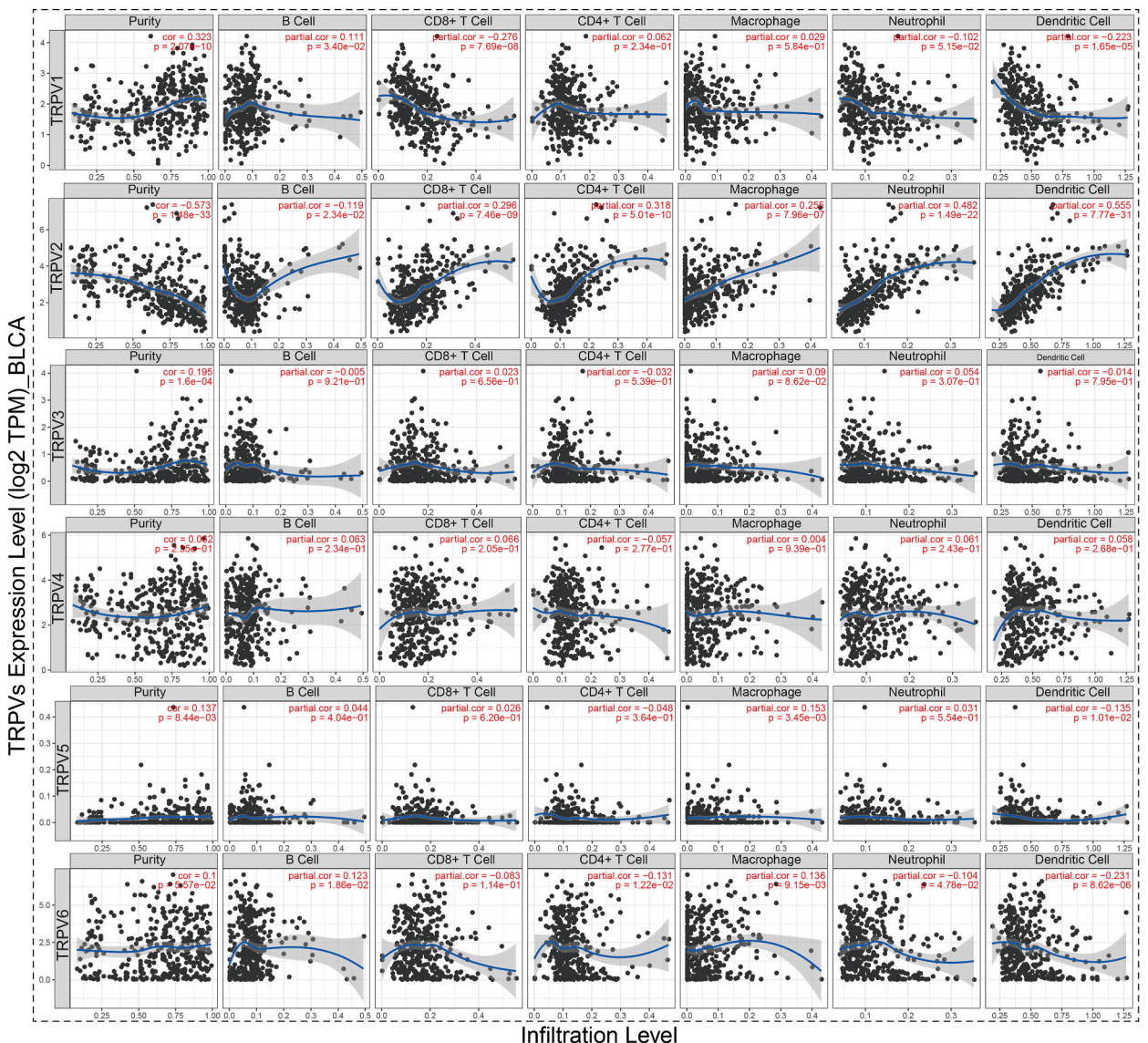

Fig. 11. Correlation between B cell, CD4+T cell, CD8+T cell, macrophage, neutrophil, dendritic cell and TRPVs expression using TIMER database.
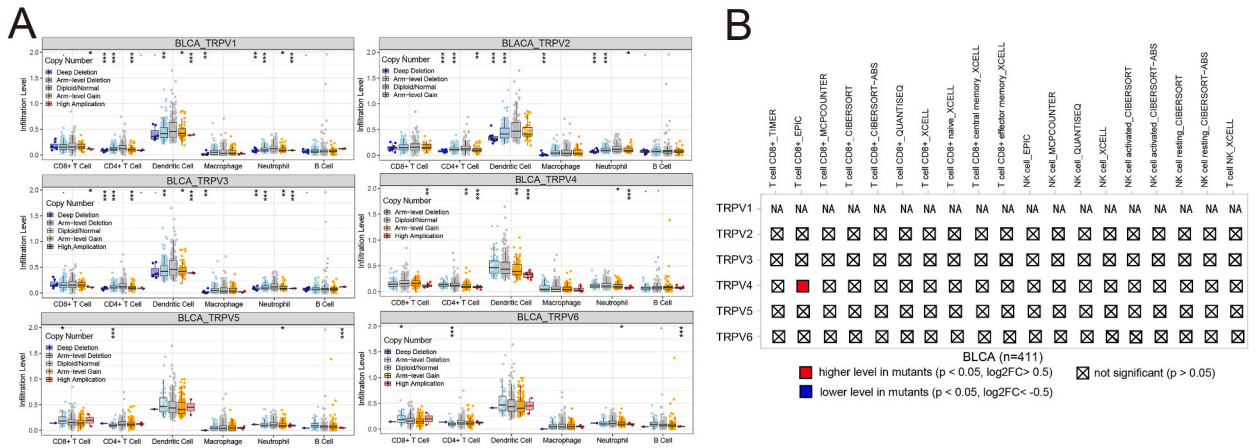
Fig. 12. The association between copy number variations **(A)** or mutations **(B)** of TRPV genes and immune infiltration in BLCA.
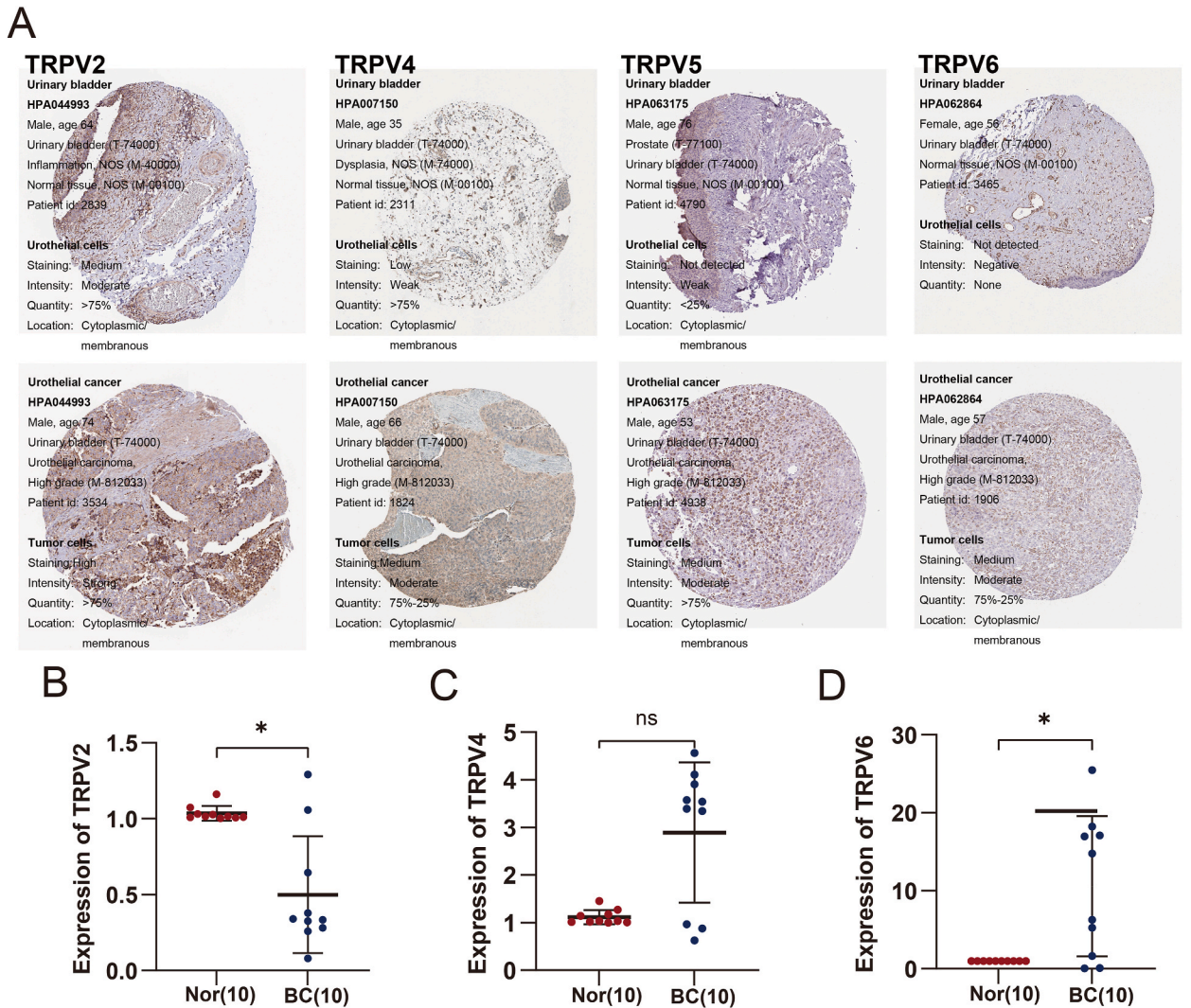

Fig. 13. Verification of TRPVs expression in protein and mRNA level. **(A)** The sample displayed TRPV2, TRPV4, TRPV5, and TRPV6 expression in protein level based on HPA database. **(B–D)** The expression levels of TRPV2, TRPV4 and TRPV6 was determined by real-time PCR.

was analyzed and we found that only TRPV4 mutation caused CD8$^+$ T cells content increased in bladder cancer.

3.14. Verification of TRPV expression in bladder cancer

Through investigating HPA database we found that the expression of TRPV2, TRPV4, TRPV5 and TRPV6 was consistent with our analysis in bladder cancer, the result is shown in Fig. 13A. Next, we selected 10 pairs of tumor and normal tissues to verify, each pair of tissues was taken from the same patient. The results can be seen in Fig. 13B-D. Among them, TRPV5 was excluded because of the low expression that could not be detected. The expression of TRPV2 is decreased in cancer tissues, and the expression of TRPV6 is increased in tumor tissues, which was squared with our previous analysis. However, the expression level of TRPV4 in tumor and adjacent tumor tissues was not statistically significant in our validation, which might be due to the small sample size.

4. Discussion

TRPV subfamily has six members, which structure and function are similar. Existing studies revealed that TRPV has an impact on occurrence, treatment and prognosis in part cancers. However, present study of the correlation between TRPV and pan-cancer still has many gaps. For this reason, we based on TCGA and other databases, analyzed the effects that TRPV exert on pan-cancer. Via analyzing the association between survival, immune microenvironment and prognosis, we found that TRPV significantly correlated with cancer and could be used to guide cancer immunotherapy or judge the prognosis.

First, we analyzed TRPV expression in pan-cancer, found that TRPV2 and TRPV4 expression were strongly increased compared with other TRPVs; SKCM and LUSC expressed TRPV2 and TRPV4 at the highest level. But, due to the difference of cancers, only part of TRPV expression were significant different compared normal tissue with tumor. Most of tumor had a high level of TRPV with part cancers expression decreased, such as TRPV2 expression decreased in LUSC and LUAD. When exploring the reason of TRPV expression alteration, we found that amplification, swallow deletion and gain could affect TRPV expression, but mutation had no effect.

Further survival analysis revealed that patient of cancer with TRPV expression changed had worse OS compared with patient did not have TRPV expression alteration, especially in TRPV2 and TRPV4. Previous study had suggested that TRPV4 was upregulated in hepatocellular carcinoma and colon cancer [13,14] and TRPV2 inhibited proliferating of glioblastoma stem-like cells [15]. Besides, TRPV was also associated with prognosis of prostate cancer and breast adenocarcinoma [16,17]. The above results demonstrated that changes of TRPV expression could be considered as prediction of prognosis. In addition, 10 cancers which TRPV expressed at a high level remised after first course treatment; 6 cancers TRPV low expression remised after first course treatment. The results were similar to PFS that we analyzed.

TRPV expression had significant correlation with immune subtypes. TRPV2 and TRPV4 expressed highest in C6 immune subtype. The trend of effect that TRPV2 and TRPV4 to immune subtypes was consistent. C6 immune subtype had the highest level of TRPV expression, reflected the highest characteristic feature of TGF-β and a higher infiltration of lymphocytes. C6 immune subtype had better prognosis [18]. TME features could serve as markers for predicting tumor cell responses to immunotherapy [19]. TRPV2 was significant correlated with immune score and stromal score, indicated that there were more non-tumor components in TMB when expression increased. Thus, better immune response related to TRPV2 high expression and tumor probably had a more significant effect on immunotherapy, meaning the tumor was 'hot' tumor [20]. PD-1, PD-L1 and CTLA4 are the most widely used immune checkpoint [21]. Our results demonstrated that TRPV2 showed a significant positive correlation with PD-1, PD-L1, and CTLA4 expression in almost all cancers, exceptions were made only in ACC, LAML, and SKCM. It shows that high expression of TRPV2 is more likely to benefit from immunotherapy. Next, the analysis of relationship between TRPV and drug sensitivity showed that TRPV2 was also specific for drug screening, and its elevated expression was associated with resistance to some chemotherapy drugs. Results of Simona Laurino et al. showed TRPV2 overexpression promoted cisplatin-induced apoptosis resistance, confirmed our point [22].

MSI and TMB are two promising predictive biomarkers of immunotherapeutic effect [23]. We therefore analyzed whether TRPV correlated with MSI and TMB. The results illustrated that TRPV had various degrees of correlation between MSI in 25 cancers and TMB in 27 cancers, suggesting that TRPV expression may affect cancer response to immunotherapy. Furthermore, the relevance between TRPV and TMB was more pronounced and mostly negative, revealing that patients with low TRPV expression and high TMB were more prone to benefit from immunotherapy. The study of Xuehui Jiang et al. also confirmed the application value of TRPV in immunotherapy [24].

To fill the gap of TRPV in bladder cancer, we focused on analyzing the role of TRPV expression in bladder cancer. Firstly, we performed an enrichment analysis of bladder cancer and found that multiple pathways were enriched in bladder cancer. TRPV2 was mostly positive enriched in immune-associated pathways. Then, the analysis of the relationship between clinical traits and TRPV demonstrated that high expression of TRPV4 was associated with low stage, papillary carcinoma and low T stage, suggesting that TRPV4 had certain value in clinical diagnosis and treatment of bladder cancer. TPPV increase with the progression of cancer stage [25]. Thirdly, the analysis of RNAss and DNAss showed that TRPV2 had a clear negative correlation between the two, and its high expression was not favorable for the proliferation and metastasis of bladder cancer, H. Mizuno et al. showed the similar result [26]. Fourth, the trend of TRPV expression on immune score, stromal score and ESTIMATE score showed consistency, and only TRPV2 expression was positively correlated with immune score, stromal score and ESTIMATE score; Indicated that TRPV2 could be used as an independent factor of determining the degree of tumor malignancy, and elevated TRPV2 expression indicated that the tumor was more suitable for immunotherapy. Tiffany M Link et al. identified that lack of TRPV2 leaded to the impairing of zymosan-, immunoglobulin G (IgG)- and complement-mediated particle binding and phagocytosis in macrophages [27]. Fifth, there is also a significant correlation between TRPV in bladder cancer immune infiltration and tumor microenvironment, suggesting that it can predict the effect of

immunotherapy. Studies have shown that TRPV affects immune infiltration and prognosis of renal cell carcinoma [28]. In addition, we also analyzed TRPV mutation and copy number variants in bladder cancer and found that various types of copy number variants were associated with decreased immune cell content. Interestingly, only TRPV4 mutation affected CD8$^+$ T cell content. After analysis, we verified the expression level of TRPV in bladder cancer, and the verification results were highly consistent with our analysis, further proving the accuracy and clinical significance of our results.

Our analysis shows that TRPV2 had a significant trend in transcription, genetic changes, and the association with immune infiltration and immune cells. Transcriptional alterations in TRPV2 may result from amplification, shallow deletion, or gain. The immune-related analysis of TRPV2 showed that high expression of TRPV2 was correlated with better therapeutic effect and prognosis from multi-angle such as immunotyping, ESTIMATE score, and immune checkpoint co-expression, showed that TRPV was important to clinical application. Takahiro Yamada et al. confirmed that active TRPV2 channel could lead to apoptosis of T24 cells [29]. Besides, TRPV4 also had certain significance for pan-cancer, and played an obvious role in observing the level of immune infiltration and guiding immunotherapy. Further analysis in bladder cancer also confirmed the importance of TRPV2. We added enrichment analysis to show that TRPV2 had a close association with immunity. In addition, the results of Xiaoyi Mo et al. showed that TRPV2 was associated with apoptosis by PTEN, suggesting that TRPV2 was related to multiple pathways [30]. But increased TRPV2 expression was more likely to be C2 immune subtype in bladder cancer, different to pan-cancer and reflecting higher level of INF-γ [18].

In this article, our analysis involved multi-omics and performed a systematic analysis of the expression and alteration of TRPV family in pan-cancer, which had not been analyzed in previous studies. Moreover, we further analyzed the correlation between TRPV and clinical traits, tumor microenvironment, immune cell content, etc. for clinical guidance. Finally, we did a detailed analysis of TRPV in bladder cancer and confirmed the role of TRPV in bladder cancer.

However, our study still had limitations. First, we only analyzed the relationship between TRPV expression and gene alteration of cancer based on the database, without the support of relevant experimental results; Second, for some analyses, there probably had bias because of the lack of relevant data and small sample size. Third, the molecular mechanisms of TRPV's role in pan-carcinoma remain to be studied. In summary, TRPV is a gene that plays an important role in cancer, and its expression affects tumor microenvironment, immune infiltration and other aspects. TRPV is also a promising gene family in clinical application, but the study of its molecular mechanisms still has a long way to go.

5. Conclusion

In conclusion, our study demonstrated that TRPV family genes, from a systems-wide perspective of multi-omics and multi-datasets, was highly related to survival and prognosis. Moreover, the correlation between TRPV and immune infiltration, immunotherapy response displayed all that the expression of TRPV could be used to guide treatment. Furthermore, analysis of TRPV in bladder cancer also revealed the crucial role of TRPV. However, further experiment still needed to verify in the future.

Author contributions

Hl. H. and C. S. designed this study; C. S. and Y. S. wrote the manuscript; Z. Z., Y. Z., and Sb. Y. screened the database and collected the data; K.L., Yd. L and C. S. performed the bioinformatic analysis; Hl. H. and C. S. revised the manuscript; C. F., Z. L., Zl. W., Zj. W., Sw H, Hy. C, Zl. L., J. G., and P. L. provided critical comments; All authors contributed to the article and approved the submitted version.

Funding

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics approval and consent to participate

This research has been conducted in accordance with the Declaration of Helsinki and has been approved by the ethics committee of the 2nd Affiliated Hospital of Tianjin Medical University Ethics code KY2021K003 with proper written documentation of informed consent.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16897.

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