A Pan-cancer Analysis of the Prognostic and Immunological Role of PPP1R14A

JiaJie Lu
Guangzhou Medical University Second Affiliated Hospital https://orcid.org/0000-0002-3866-8773
Rihong Huang
Guangzhou Medical University Second Affiliated Hospital
Haojian Wang
Guangzhou Medical University Second Affiliated Hospital
Yuecheng Peng
Guangzhou Medical University Second Affiliated Hospital
Yongyang Fan
Guangzhou Medical University Second Affiliated Hospital
Zejia Feng
Guangzhou Medical University Second Affiliated Hospital
Zhaorong Zeng
Guangzhou Medical University Second Affiliated Hospital
Yunxiang Ji
Guangzhou Medical University Second Affiliated Hospital
Yezhong Wang
Guangzhou Medical University Second Affiliated Hospital
Zhaotao Wang (wangzhaotao@gzhmu.edu.cn)
The Second Affiliated Hospital of Guangzhou Medical University https://orcid.org/0000-0001-5049-8626

Primary research

Keywords: PPP1R14A, pan-cancer, prognosis, diagnosis, genetic alternation, immune, phosphor-ylation

Posted Date: September 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-882685/v1

Abstract

Background

Despite emerging evidence revealed the remarkable roles of Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A (PPP1R14A) in cancer tumorigenesis and progression, no pan-cancer analysis is available. Our research, for the first time, comprehensively investigated the potential carcinogenic mechanism of PPP1R14A across 33 tumors using bioinformatic techniques.

Methods

TCGA datasets and the CPTAC datasets embedded in UALCAN were first applied to study the differential expression of PPP1R14A in various cancer types at the transcription and protein levels, respectively. Besides, we also conducted relevant prognostic survival analysis and used the GEPIA2 database to explore the association between PPP1R14A expression and pathological stages. In addition, cBioPortal and UALCAN databases were employed to analyze the genetic alterations and post-transcriptional phosphorylation of PPP1R14A. Then based on TCGA, we analyzed the relationship between PPP1R14A and immune infiltration, the correlation with tumor mutational burden (TMB), microsatellite instability (MSI) and immune checkpoint molecules (ICMs), and whether it is expected to be a predictive indicator in cancer patients, which was achieved by receiver operating characteristic (ROC) curve. Finally, STRING, GEPIE2 and TIMER2.0 databased were used to explore the potential mechanism of PPP1R14A in cancer and find molecules that have potential close interactions with PPP1R14A.

Results

PPP1R14A is down-expressed in major malignancies and there is a significant correlation between the PPP1R14A expression and the prognosis of patients. Pan-cancer survival analysis indicated that the high expression of PPP1R14A in most cases was associated with poor overall survival (OS), disease-specific survival (DSS), and progress-free interval (PFI) across patients with various malignant tumors, containing adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA). The results of ROC analysis subsequently exhibited that the molecule has a high reference significance in diagnosing a variety of cancers. Besides, we detected that the frequency of PPP1R14A genetic changes including genetic mutations and copy number alterations (CNAs) in uterine carcinosarcoma reached 16.07%, and these alterations brought misfortune to the survival and prognosis of cancer patients. In addition, the methylation within the promoter region of PPP1R14A DNA was enhanced in a majority of cancers. Downregulated phosphorylation levels of phosphorylation sites including S26, T38, etc. in most cases took place in several tumors, such as breast cancer, colon cancer, etc. PPP1R14A remarkably correlated with the levels of infiltrating cells and immune checkpoint genes.

Conclusions

Our research summarized and analyzed the carcinogenic effect of PPP1R14A in different tumors comprehensively and provided a theoretical basis for promising therapeutic and immunotherapy

strategies.

Introduction

Cancer is a persistent public health challenge facing the world at present. According to estimates from global cancer statistics in 2019, cancer becoming the main obstacle to the growth of population life expectancy in the 21st century is the first or second leading cause of death before the age of 70 years in 112 of 183 countries and ranks third or fourth in a further 23 countries[1]. Although the early diagnosis and innovative methods of reducing mortality have been made great efforts in various cancers, the incidence rate of malignant cancer has increased, especially embodying in the fact that there will be about 1.2 million new cancer cases and 400000 cancer deaths in 2020 compared with 2018, which is a major problem of human health[2-5]. Due to the complexity of tumorigenesis, pan-cancer analysis of the same gene has attracted more and more attention, which not only helps us to find its common phenotypic characteristics but also helps us to deeply understand the internal regulation mechanism of key molecules and the relationship between potential clinical prognosis[6].

Belonging to the protein phosphatase 1 (PP1) inhibitor family, Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A (PPP1R14A), often known by the alias, 17 kDa PKC-Potentiated Inhibitory Protein of PP1 (CPI-17), has more than 1000 times inhibitory activity during phosphorylation, resulting in a molecular switch to regulate the phosphorylation state of PPP1CA substrate and smooth muscle contraction. Previous studies have shown that PPP1R14A is associated with a pivotal role in the occurrence and development of tumors, including sporadic vestibular glioma, human melanoma, schwannoma, etc.[7-9]. In addition, Jin et al. demonstrated that the downregulation of CPI-17 induces merlin dephosphorylation, inhibits Ras activation, and abolishes the tumorigenic transformation phenotype[10]. However, most studies on the function of PPP1R14A in cancers were limited to specific types of cancer. Therefore, deeply examining the regulatory functions and molecular mechanisms of PPP1R14A in pan-cancer is particularly important to provide new insights into relevant carcinogenic mechanisms as well as directions and strategies for the clinical treatment of cancer

Our research, for the first time, utilized a series of online datasets and databases based on TCGA to perform a pan-cancer expression analysis of PPP1R14A, the identified key molecule, and included a series of relevant studies, including PPP1R14A differential expression, clinical survival prognosis, genetic alteration, promoter DNA methylation, protein phosphorylation, immune infiltration landscape, and putative signaling pathway, etc. to explore the underlying mechanism in the tumorigenesis and tumor suppression across different cancer species. We strongly believe that the analysis of PPP1R14A provides new insights into the carcinogenic role of PPP1R14A across multiple malignancies and further deepens our understanding of the individual management for cancer precision therapy.

Methods And Materials

PPP1R14A expression pattern in human pan-cancer

The dysregulation of the PPP1R14A expression between various types of cancer and normal tissues was investigated by combining the data, which had been uniformly processed by the Toil process in UCSC Xena (https://xenabrowser.net/datapages/) [11, 12], for normal tissues from the GTEx and The Cancer Genome Atlas (TCGA) databases and visualized by R ggplot2 (version: 3.3.3) package. Besides, To compare a gene's expression level between tumor and matched normal tissues across all TCGA cancer types, the "Gene_DE" Module of TIMER2.0, a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types based on TCGA cohorts[13], was applied to carry out this analysis.

The UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html) [14], an interactive web resource for analyzing cancer OMICS data, allowed us to conduct protein expression analysis of the (Clinical proteomic tumor analysis consortium) CPTAC datasets. Herein, we explored the expression levels of the total protein or phosphoprotein (with phosphorylation at the S26, T38, S101, S103, S107, and S109 sites) of PPP1R14A (NP_001230876.1) between primary tumor and normal tissues, respectively, by entering "PPP1R14A". PPP1R14A promoter methylation levels between different cancers and corresponding adjacent tissues were evaluated whereafter. The significance of differences was evaluated using Student's t-test, and $p < 0.05$ was considered statistically significant. And the available datasets of six tumors were selected, namely, breast cancer, ovarian cancer, colon cancer, KIRC, UCEC, and LUAD.

Additionally, we obtained violin plots of the PPP1R14A expression in different pathological stages (stage I, stage II, stage III, and stage IV) of all TCGA tumors via the "Pathological Stage Plot" module of GEPIA2 (http://gepia2.cancer-pku.cn/#index) [15]. The log2 [TPM (Transcripts per million) +1] transformed expression data were applied for the violin as well as corresponding box plots.

Prognostic analysis.

The connection between the PPP1R14A expression and the prognosis of patients, including overall survival (OS), disease-specific survival (DSS) and progression-free interval (PFI) in 33 types of cancers were examined using Kaplan-Meier curves [16]. The hazard ratios (HRs) and 95% confidence intervals were calculated using univariate COX regression survival analysis. R survival (version: 3.2-10) and survminer (version: 0.4.9) packages were employed for statistical analysis and visualization.

Genetic alteration analysis

After logging into the cBioPortal web (https://www.cbioportal.org/) [17, 18], we chose the cases in "TCGA Pan-Cancer Atlas Studies", with both mutations and copy number alterations (CNAs) meanwhile, in the "Quick select" section and entered "PPP1R14A" for queries of the genetic alterations and characteristics of PPP1R14A. The results of the alteration frequency, mutations, and CNAs status across all TCGA tumors were observed in the "OncoPrint" and "Cancer Types Summary" modules. Moreover, the "Comparison/Survival" module was employed to obtain the data and diagrams for Kaplan-Meier plots with log-rank analysis on the overall, disease-free, disease-specific and progression-free survival differences for the TCGA cancer cases with or without PPP1R14A genetic alterations.

Receiver operating characteristic (ROC) analysis

To better assess the performance of a diagnostic test over the range of possible values of a predictor variable, ROC curves, the most useful tool in the early stages of evaluation of a new diagnostic test, as well as the area under ROC curves (AUC) were employed through using R pROC (version: 1.17.0.1) and ggplot2 (version: 3.3.3) packages[19, 20]. And the display would take place only when AUC surpassed 0.9.

Pan-cancer analysis of the correlation of PPP1R14A expression with immune cells infiltration.

The data of 33 types of cancer and normal tissues in TCGA were downloaded from the Genomic Data Commons (GDC) data portal website. For reliable immune score evaluation, Immuneeconv, an R software package that integrates the two latest algorithms, TIMER and xCell [13, 21-29]. The horizontal axis represents different tumor tissues, the vertical axis represents diverse types of immune lymphocytes, different colors represent correlation coefficients, and the stronger the correlation, the darker the color. The significance of the two groups of samples passed the Wilcox test.

Analysis of the relationship of TRIM family expression between TMB/MSI with ICMs

The mRNA-seq data, comprised of tumor mutational burden (TMB) and microsatellite instability (MSI) scores, were obtained from TCGA [30, 31]. Correlation analyses between the TRIM family expression and TMB/MSI, immune checkpoint molecules (ICMs) were performed using Spearman's method. R ggstatsplot and pheatmap packages were applied to analyses and visualization. P value <0.05 was the significance threshold in this study.

PPP1R14A-related gene enrichment analysis

Using the query of a single protein name ("PPP1R14A") and organism ("Homo sapiens"), we first searched in the STRING website (https://string-db.org/)[32] and set the following main parameters: Network type ("physical network"), the minimum required interaction score ["custom value (0.235)"], active interaction sources ("Textmining, Experiments, and Databases ") and max number of interactors to show ("no more than 50 interactors" in 1st shell) subsequently. Finally, the available experimentally and putatively determined PPP1R14A-interacting proteins were obtained. Meanwhile, the "Similar Gene Detection" module of GEPIA2 was applied to obtain the top 100 PPP1R14A-correlated targeting genes based on the datasets of all TCGA tumors and normal tissues. To compare the PPP1R14A-correlated and interacted genes, an intersection analysis showed by the Venn diagram was conducted. Moreover, we used the "Gene_Corr" module of TIMER2.0 to supply the heatmap data of the overlapped genes, which contains the partial correlation (cor) and P value in the purity-adjusted Spearman's rank correlation test. Then R clusterProfiler (version: 3.14.3) and org.Hs.eg.db packages[33] were used to carry out and visualize Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. To control false-positive discovery rates, modified P values and Benjamini/Hochberg algorithm were introduced. $P < 0.05$ and false discovery rate (FDR) < 0.05 were considered statistically significant.

Results

Expression landscape of PPP1R14A across cancers

According to the results from the TIMER2.0 database, PPP1R14A exhibited consistent mRNA expression in 23 types of human common cancer. The PPP1R14A expression was significantly lower in cancer versus adjacent normal tissues in the BLCA, BRCA, CESC, COAD, GBM, KICH, KIRP, LUAD, LUSC, PCPG, PRAD, READ, STAD, UCEC and higher in CHOL, HNSC, LICH datasets (Fig 1A). We also compared the PPP1R14A expression using the data directly from the TCGA and GTEx. The downregulated PPP1R14A mRNA expression was observed in tumor tissues versus normal tissues in the ACC, BLCA, BRCA, CESC, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LUAD, LUSC, OV, PCPG, PRAD, READ, SKCM, STAD, TGCT, THCA, USEC, UCS datasets, and the upregulated PPP1R14A expression profile was detected in CHOL, DLBC, HNSC, PAAD, THYM datasets (Fig 1B). Further comparison of the PPP1R14A protein expression according to the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database demonstrated that the PPP1R14A protein expression was significantly decreased in advanced tumor tissues versus normal tissues in breast cancer, KIRC, colon cancer, LUAD, ovarian cancer, and UCEC (Fig 1C).

Pan-cancer overview of the correlation between PPP1R14A expression and clinicopathology

To investigate the association between the PPP1R14A expression and clinicopathological features in multiple cancers, we assessed the PPP1R14A expression in cancer patients of stages I, II, III, and IV. The results from the TCGA database revealed that the expression of PPP1R14A was significantly altered in BLCA, COAD, KIRC, KIRP, LUSC, STAD, and TGCT (Fig 2).

Prognostic significance of PPP1R14A across cancers

We evaluated the relationship between PPP1R14A and the prognosis of patients across cancers. According to this study results, PPP1R14A expression significantly acted as a risk factor for OS in ACC ($p=0.001$), BLCA ($p=0.007$), CESC ($p=0.002$), gliomas ($p < 0.0001$), KIRP ($p=0.003$), LUSC ($p=0.013$), MESO ($p=0.047$), READ ($p=0.013$), SKCM ($p=0.014$), STAD ($p=0.024$), THCA ($p=0.045$) and protective factor in HNSC ($p=0.031$), LAML ($p=0.029$), LIHC ($p=0.027$), LUAD ($p=0.039$) (Fig 3). Then we found the correlation of PPP1R14A expression with DSS of patients, which indicated that high expression of PPP1R14A unfavorably impacted DSS in ACC ($p=0.001$), BLCA ($p=0.014$), CESC ($p<0.0001$), COAD ($p=0.002$), ESCA($p=0.018$), gliomas ($p<0.001$), KIRP ($p=0.001$), READ ($p=0.01$)，SKCM ($p=0.032$)， STAD ($p=0.004$) and friendly influenced in LIHC ($p=0.045$) (Fig 4). Besides, we analyzed the relationship between PPP1R14A expression and the PFI of patients across cancers. Elevated PPP1R14A expression remarkably indicated poor outcomes for the PFI of patients in ACC ($p<0.001$), BRCA ($p=0.023$), CESC ($p<0.001$), COAD ($p=0.007$), gliomas ($p<0.001$), KIRP ($p=0.002$), LUSC ($p=0.037$), OV ($p=0.018$), READ ($p=0.025$), SKCM ($p=0.02$), STAD ($p=0.007$),TGCT ($p=0.011$), and UCS ($p=0.041$) and signed a friendly prognosis in LIHC ($p=0.037$), THCA ($p=0.027$) (Fig 5). To sum up, the above results show that PPP1R14A expression is related to the prognosis of patients significantly.

Genetic alteration and methylation status of PPP1R14A

We investigated the pan-cancer alterations of PPP1R14A using the cBioPortal (TCGA, Pan-Cancer Atlas) database. The results demonstrated that the alteration frequency of PPP1R14A was 2.3% across 32 various cancers and took the lead with 16.07% in samples of uterine carcinosarcoma (Fig 6A-B). Among the different types of genetic alterations, amplification was the most common type. We also examined the potential relationship between genetic alterations in PPP1R14A and the prognosis of patients with different types of cancer. As shown in Fig 6C, tumor patients with genetic alterations in PPP1R14A had worse OS, DFS, DSS, and PFS than patients without alterations. We also investigated the promoter DNA methylation of PPP1R14A using the UALCAN database. A significant increase in the methylation levels within promoter regions of PPP1R14A was observed in BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PCPG, READ, THCA tissues compared to normal tissues (Fig 7). While PPP1R14A promoter methylation levels in SARC and UCEC decreased.

Phosphorylation profile of PPP1R14A across multiple tumors

We examined alterations in PPP1R14A phosphorylation levels between primary tumor tissues and normal tissues (Fig 8). The CPTAC database includes six types of cancer, namely, breast cancer, KIRC, colon cancer, LUAD, ovarian cancer, and UCEC. Lower levels of S26 phosphorylation of PPP1R14A were observed in breast cancer, colon cancer, LUAD, ovarian cancer, and UCEC samples compared to normal samples. In contrast, S26 phosphorylation of PPP1R14A was increased in KIRC. T38 phosphorylation of PPP1R14A was decreased in colon cancer tissues compared to normal tissues. S101 phosphorylation of PPP1R14A was remarkably decreased in LUAD and USEC compared to normal adjacent tissues. In addition, S103 phosphorylation of PPP1R14A was significantly decreased in breast cancer and colon cancer compared to normal adjacent tissues. Moreover, S109 phosphorylation of PPP1R14A was remarkably decreased in ovarian cancer and colon cancer compared to normal adjacent tissues. Decreased S103 and S109 phosphorylation as well as S107 and S109 phosphorylation in PPP1R14A were observed in colon cancer, respectively. S101, S107, and S307 phosphorylation of PPP1R14A were significantly increased in USEC. These findings suggest that the phosphorylation of the S26, T38, S101, S103, S107, and S109 residues of PPP1R14A plays a crucial role in oncogenesis.

ROC analysis

To better comprehend the efficiencies of PPP1R14A prognostic prediction, we evaluated ROC curve analysis (Fig 9). Results demonstrated that BLCA(AUC = 0.923), BRCA(AUC = 0.982), CESC(AUC = 0.956), CHOL(AUC = 0.910), COAD(AUC = 0.954), ESCA(AUC = 0.937), KICH(AUC = 0.945), KIRP(AUC = 0.901), LAML(AUC = 1.000), LUAD(AUC = 0.982), LUSC(AUC = 0.987), OV(AUC = 0.905), READ(AUC = 0.953), TGCT(AUC = 0.969), THYM(AUC = 0.917), UCEC(AUC = 0.951) and UCS(AUC = 0.987).

Immune cell infiltration landscape

Because of the distinct relationship between PPP1R14A and the immune response, we performed a pan-cancer analysis of the association between PPP1R14A expression and the immune infiltration levels based on the TIMER and XCELL algorithms. As shown in Fig 10A, the expression of PPP1R14A was significantly associated with the abundance of infiltrating immune cells: B cells in 14 types of cancer, CD4 + T cells in 18 types of cancer, CD8+ T cells in 11 types of cancer, macrophages in 17 types of cancer, neutrophils in 10 types of cancer, and DCs in 13 types of cancer. We further used the xCell online tool to examine the relationship between PPP1R14A expression and the infiltration of different types of immune cell subtypes. Among 38 subtypes of immune cells, we found that the PPP1R14A expression negatively correlated with these subtypes in SARC, TGCT etc., positively and significantly associating with ESCA, KIRP etc. In addition, hematopoietic stem cells, endothelial cells, etc. were positively and T CD4+ memory cells, mast cells, etc. were negatively associated with the PPP1R14A expression in these different cancers (Fig 10B).

Pan-cancer analysis of the association lies in PPP1R14A with ICMs and TMB/MSI

To closely estimate the relationship between the PPP1R14A expression and the tumor environment across carcinomas, we further investigated the relationships between the PPP1R14A expression and ICMs. Notably, we observed that the expression of PPP1R14A negatively correlated with most ICMs in SARC and TGCT (Fig 11). In contrast, the expression of PPP1R14A positively correlated with most ICMs in ESCA, KICH, KIRP, PRAD, and UVM (Fig 11). TMB and MSI are two emerging biomarkers associated with the immunotherapy response. The relationship between the PPP1R14A expression and TMB was investigated. The expression level of PPP1R14A remarkably, in addition to the phenomenon of significant positive correlation in OV, negatively correlated with TMB in several tumors, including BLCA, BRCA, COAD, KIRP, LIHC, Lung carcinomas, PRAD, SARC, SKCM and STAD (Fig12A and C). The correlation of the PPP1R14A expression with MSI was also investigated in 33 types of cancer, and it indicated that BRCA, HNSC, THCA exhibited positive correlations, while COAD, ESCA, Renal carcinomas, SARC, STAD exhibited negative correlations (Fig 12A and B).

PPP1R14A-related gene enrichment analysis

To better understand the interplay among the PPP1R14A-related genes, STRING database screening and PPI network with 119 edges and 51 nodes construction were performed, and visualization was carried out using Cytoscape (Fig 13A). Furthermore, the GEPIA2 approach was performed and we obtained the top 100 PPP1R14A-correlated genes and constructed a Venn diagram with molecules that have a putative physical binding relationship with PPP1R14A, and it is found that there are two molecules, including TAGLN and PPP1R12B in the overlapping area (Fig 13B). The corresponding heatmap exhibits the relationship between PPP1R14A and the two intersecting molecules in the various cancer types (Fig 13C).

Consistent with PPI network analysis, functional enrichment clustering of these genes showed a strong association with calcium-mediated signaling, regulation of phosphatase activity, integrin-mediated signaling pathway, positive regulation of transforming growth factor beta receptor signaling pathway, adenylate cyclase-inhibiting dopamine receptor signaling pathway, regulation of protein

autophosphorylation, angiotensin-activated signaling pathway, regulation of cAMP-mediated signaling, positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway in GO as well as cGMP-PKG signaling pathway, cAMP signaling pathway, Apelin signaling pathway, Calcium signaling pathway, Ras signaling pathway, Neurotrophin signaling pathway, Rap1 signaling pathway, Glioma in KEGG (Fig 13D-E).

Discussion

PPP1R14A, a widely expressed serine-threonine phosphatase regulating many cellular processes such as actin contraction, glycogen metabolism, cell cycle, protein synthesis and neuronal signal transduction, etc., is an inhibitor of protein phosphatase1(PP1)[34, 35]. In recent years, emerging publications have reported functional associations between PPP1R14A and clinical diseases, especially tumors[36, 37]. However, this part of the research is limited to schwannoma, mesothelioma, and some malignancies of the gastrointestinal tract, making it difficult for us today to have a qualitative and quantitative understanding of the role of the molecule in other tumors and the deeper functions of the above-mentioned tumors. Hence, whether PPP1R14A plays a role in the pathogenesis of different tumors through multifarious molecular mechanisms remains unclear. Therefore, to provide researchers with macro and relatively rough understanding about molecular characteristics of PPP1R14A gene expression, survival prognosis, somatic mutations, and CNAs, DNA methylation, protein phosphorylation profile, etc. in various malignant tumors, we performed comprehensive exploration of bioinformatics of PPP1R1AA genes in different tumors.

We evaluated the expression of this molecule at the mRNA and protein levels in various malignant tumors. Due to TIMER2.0, a database based on TCGA, exists many tumor samples lacking a control group. In conjunction with GTEx, we launched a further study. The final result surprisingly showed that the expression of PPP1R14A was maladjusted in most tumors. It is worth noting that after combining more normal GTEx samples, the significantly high expression of PPP1R14A in LIHC disappeared. Whether this is a deviation caused by the normal samples of the analysis, or a true objective law, awaits further exploration by researchers. In UALCAN, a database based on the CPTAC, PPP1R14A showed a remarkably low expression in all 6 types of adult cancers given, which is consistent with the analysis results of the transcription level.

And through the analysis of the prognostic survival of PPP1R14A in various cancers, we are interested to find a seemingly strange phenomenon, which seems extremely inconsistent with our previous general cognition[38]. For example, if we focus on Fig 3-5, we can observe that among BLCA, COAD and KIRP patients, the PPP1R14A high expression group showed a worse prognosis. But as shown in Fig 1, PPP1R14A in cancers of these three categories all showed lower expression levels, clearly telling us that this is a "tumor suppressor gene". However, it exhibited a role in promoting cancer progression in patients with these three types of tumors, which was embodied in the manifestation that the up-regulation of PPP1R14A promotes the malignant process of tumors, thereby makes such patients suffer from a lower survival probability. And this, more surprisingly, is just a microcosm. Looking at Fig 3-5, it is not difficult to

find that in most cases, the role of PPP1R14A in the comparison of tumor and normal samples is inconsistent with the role shown in the tumor cohort, indicating that the role of PPP1R14A in the two processes of initiation and progression of cancers is opposite in most cases. As shown in Fig 2, we can easily observe the results further confirming our viewpoints that as cancer progresses, the expression level of PPP1R14A gradually and steadily increases in BLCA, COAD and KIRP. And the counter-example is in KIRC. The overall expression trend of PPP1R14A in this tumor is very ambiguous, which may mean that it does not have the effect of promoting the progress of KIRC to some extent. Sure enough, the survival analysis of OS, DSS and PFI were not significant.

Taken together, PPP1R14A may be a potentially unique molecule, and it is likely to play completely different roles in the origin and progression of most cancers, which is different from our general understanding. At the same time, the ROC results explained that PPP1R14A, whose AUC values surpass 0.9 in multiple malignant tumors such as BLCA, COAD and KIRP, reached the outstanding level in the diagnostic test evaluation. Due to space limitations, many results with AUC greater than 0.7 were not displayed.

Since the genetic mutations and CNAs of somatic cells have been revealed to be closely related to the occurrence and development of tumors, they have attracted the attention and involvement of many researchers around the world, and we are no exception. The results of our experiments showed that the PPP1R14A is genetically altered in tumors. Although the frequency of genetic alterations is not as high as expected, it is enough to have a significant friendly influence on the prognosis of OS, DFS, DSS and PFS.

Generally suppressing the gene expression by changing chromatin structure, DNA stability, and DNA conformation, DNA methylation is a major form of epigenetic modification of DNA that regulates the gene expression without altering the sequence of DNA[39-41]. The link between DNA methylation and cancer was gradually discovered in recent decades. A large number of studies have shown that certain key genes, including PPP1R14A that has been experimentally demonstrated by Peng et al. as the expression of PPP1R14A in gastric cancer cell lines, are regulated by promoter region methylation[42].

In addition, PPP1R14A was also evaluated for changes in the degree of methylation and the possibility of becoming tumor markers in other cancers, such as GBM, HNSC, CRC, etc.[43-46], and it showed that PPP1R14A promoter methylation status was expected to become a prognostic and promising predictive biomarker.

Besides, our analysis results more broadly explained that the PPP1R14A promoter methylation level has been significantly altered in BLCA, COAD, KIRP and other cancers, clarifying that it is expected to become a novel prognostic marker and potential therapeutic target. Hypermethylation within promoter regions often leads to the silencing or inactivation of tumor suppressor genes in cancerous cells [39-41]. Our results revealed that the methylation level of PPP1R14A is generally significant in most common cancers, whose scene is largely consistent with the down-regulation of the expression levels of PPP1R14A we observe in Fig 1. However, we also noted many seemingly contradictory thought-provoking points. For example, in CHOL, HNSC and other malignant tumor samples, the methylation expression profile of the

high promoter region of PPP1R14A is related to the high expression level of transcription. However, things in nature are often not that simple. Smith et al. gave us an overview of promoter DNA hypermethylation that can guide gene expression activation, although this seems a bit abnormal[47]. Nevertheless, this is just an immature conclusion. Hence, the relationship between DNA methylation and PPP1R14A expression warrants more in-depth study.

Emerging experiments have revealed that post-transcriptional phosphorylation modification of genes often leads to great changes in the function of the original protein molecules, which is inseparable from the origin and progression of malignant tumors[48, 49]. Researches have reported that Rho-kinase (ROCK) participating in this phosphorylation process to a certain extent, CPI-17 Thr(38) phosphorylation can selectively inhibit myosin light chain phosphatase (MLCP), and then mediate the contraction process of smooth muscle[35, 50]. Besides, studies have clarified that ROCK inhibitors showed a promising outlook on K-Ras-induced glioma, lung cancers and hematological malignancies[51, 52]. These seemed to guide us that the phosphorylation process of PPP1R14A mediated by ROCK may be somewhat related to the occurrence and development of tumors. Even so, almost no research has come to a conclusion about the mode of action of phosphorylated PPP1R14A protein factor in many cancers until today. Based on this, we analyzed it with UALCAN. The feedback results showed that in most cases, PPP1R14A tends to have low phosphorylation levels in tumors, suggesting that PPP1R14A phosphorylation may play a crucial role in tumorigenesis.

With the in-depth exploration of the tumor immune microenvironment by scholars, one hidden immune checkpoint molecule after another is gradually emerging, which means that immunotherapy is also developing. The advent of immunotherapy has transformed the clinical oncology landscape in recent years, with significant improvements in long-term survival in some patients. But despite this, the clinical application of immune checkpoint blockade in glioma patients remains challenging due to the “cold phenotype” of glioma and multiple factors inducing resistance[53]. Therefore, more suitable marker molecules warrant urgently to be discovered.

Among investigated biomarkers in immune checkpoint targeted therapy till now, TMB and MSI have recently emerged as a potential predictor of response to immunotherapy in various tumor types[54, 55].

Our results demonstrated that the expression pattern of PPP1R14A in cancers represented by ESCA, KIRP, and PCPG has a positive relationship with either ICMs or the infiltration of tumor immune lymphocytes indicating that although the higher level of immune cell infiltration in solid tumors in these patients seeming to be a good thing, it seems to be “braked” by ICMs, and immunotherapy may be the key to releasing the brake pedal. Looking at the relevant results in reverse has to guide significance for clinical treatment as well. From this, we can find that the expression of PPP1R14A has a negative relationship with ICMs and immune cell infiltration levels in malignant tumors such as SARC or TCGT, etc. This may to some extent indicate the application of immunotherapy to the patients of these cancers with high PPP1R14A expression levels is not very wise, if such a situation exists. However, the heartbreaking thing is that it is not over yet. Because the next results reflected that PPP1R14A is negatively correlated with

TMB/MSI in many tumors, which means that neoantigens, the target of immune cells, would be less in these cancer patients with high PPP1R14A, and immune checkpoint targeted therapy may be deflated as a result. However, things are not so bad. We perceived that the degree of such correlation is not as high as we imagined. Coupled with the aid of clinical screening methods, immunotherapy can still be expected here. And it was worth noting that the results of TMB and MSI related to SARC warned us that immunotherapy may not be really suitable for SARC patients.

To have a deeper understanding of the molecular properties of PPP1R14A, we explored it and the molecules potentially related to it. The results of GO and KEGG indicated that these molecules are significantly enriched in many core pathways that lead to the initiation and progression of various malignant tumors, including calcium-mediated signaling, integrin-mediated signaling pathway, positive regulation of transforming growth factor beta receptor signaling pathway, cAMP signaling pathway, Apelin signaling pathway, Ras signaling pathway, Neurotrophin signaling pathway, Rap1 signaling pathway and so forth[56-64]. It is worth mentioning that Jin et al. have profoundly explained the role of PPP1R14A in tumorigenic transformation, which is basically achieved by inhibits merlin activation, promotes Ras activation, being consistent to a certain extent with our enrichment results[10]. Meanwhile, through the screening of the Venn diagram, we found two overlapping molecules, TAGLN and PPP1R12B with potential interaction with PPP1R14A. As shown in Fig 13C, PPP1R14A and TAGLN have a high and extensive synchronization pace in a variety of malignant tumors, especially renal carcinomas, implying that the two molecules are likely to work together to tumorigenesis. However, more deeply rooted explorations merit being carried out by scholars.

Taken together, to some extent helping to understand the role of PPP1R14A in tumorigenesis from the perspective of clinical tumor samples, our first pan-cancer analysis of PPP1R14A showed that PPP1R14A expression was statistically correlated with clinical prognosis, DNA methylation, protein phosphorylation, immune cell infiltration, ICMs/TMB/MSI, etc. in multiple tumors.

Conclusions

Our research, for the first time, found that PPP1R14A has expression imbalance in most of the 33 tumors collected by TCGA, which is closely related to the prognosis of corresponding patients. The results of ROC reflected that the molecule has great promise as a diagnostic factor in major tumors. At the same time, we also conducted a multi-omics, multi-angle, and multi-functional analysis of PPP1R14A's genetic changes, including mutations, CNAs, methylation, and post-transcriptional phosphorylation modifications. What's more, our results on immune analyses explained the unique immunological landscape of PPP1R14A. However, this experiment only stays at the stage of rudimentary theoretical verification, and the actual more objective and in-depth mechanism needs to be added by researchers. Future prospective and experimental studies on PPP1R14A expression and immune cell infiltration in different cancer populations may provide more insights for the development of tumor mechanisms and treatment strategies for PPP1R14A to improve the efficacy of immunotherapy.

Abbreviations

ACC: Adrenocortical carcinoma; AUC: Area under ROC curves; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; CNAs: Copy number alterations; COAD: Colon adenocarcinoma; CPI-17: 17 kDa PKC-Potentiated Inhibitory Protein of PP1; CPTAC: Clinical Proteomic Tumor Analysis Consortium database; DLBC: B-cell lymphoma; DSS: Disease-specific survival; ESCA: Esophageal carcinoma; FDR: False discovery rate; GBM: Glioblastoma multiforme; GO: Gene Ontology; HNSC: Head and neck squamous cell carcinoma; ICMs: Immune checkpoint molecules; KEGG: Kyoto Encyclopedia of Genes and Genomes; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; MLCP: Myosin light chain phosphatase; MSI: Microsatellite instability; OS: Overall survival; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PFI: Progress-free interval; PPP1R14A: Protein Phosphatase 1 Regulatory Inhibitor Subunit 14A; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; ROC: Receiver operating characteristic; ROCK: Rho-kinase; SARC: Sarcoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; TMB: Tumor mutational burden; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma;

Declarations

Acknowledgments

We acknowledge TCGA and various databases used in this research for providing us with such meaningful platforms and datasets, making this work can be carried out.

Author Contributions

Conceptualization, Z.W., Y.W. and Y.J.; Supervision, Z.W.; methodology, investigation, visualization J.L.; All authors have contributed to drafting the manuscript and read and agreed to the published version of the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (81901117), Natural Science Foundation of Guangdong Province (2019A1515010926) and College Students' science and technology innovation project of Guangzhou Medical University (2020A024) and Health and Technology Project of Guangzhou (20211A010062).

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the UCSC Xena repository, (https://tcga.xenahubs.net). Data used included the Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/), the Genotype-Tissue Expression projects (GTEx, https://commonfund.nih.gov/GTEx). And some analyses and visualization of this study are derived from public databases, as follows. Gene Expression Profiling Interactive analysis 2 (GEPIA2) database (http://gepia2.cancer-pku.cn/#index), TIMER2.0 database (http://timer.comp-genomics.org/). STRING database (https://string-db.org), Cytoscape software (http://www.cytoscape).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of Interest

The authors report no conflicts of interest in this work.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries**. *CA Cancer J Clin* 2021, **71**(3):209-249.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries**. *CA Cancer J Clin* 2018, **68**(6):394-424.

3. **Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries**. *CA: a cancer journal for clinicians* 2020, **70**(4):313.

4. Gao Q, Xie H, Zhan H, Li J, Liu Y, Huang W: **Prognostic Values of Long Noncoding RNA GAS5 in Various Carcinomas: An Updated Systematic Review and Meta-Analysis**. *Front Physiol* 2017, **8**:814.

5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F: **Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012**. *Int J Cancer* 2015, **136**(5):E359-E386.

6. Kang Y, Zhang Y, Sun YJFig: **Comprehensive Analysis of the Expression Characteristics of the Enhancer of the Zeste Homolog 2 Gene in Pan-Cancer**. 2021, **12**:658241.

7. Xu J, Zhang Y, Shi Y, Yin D, Dai P, Zhao W, Zhang TJO, neurotology : official publication of the American Otological Society ANSEAoO, Neurotology: **CPI-17 Overexpression and Its Correlation With the NF2 Mutation Spectrum in Sporadic Vestibular Schwannomas**. 2020, **41**(1):e94-e102.

8. Riecken L, Zoch A, Wiehl U, Reichert S, Scholl I, Cui Y, Ziemer M, Anderegg U, Hagel C, Morrison HJO: **CPI-17 drives oncogenic Ras signaling in human melanomas via Ezrin-Radixin-Moesin family proteins**. 2016, **7**(48):78242-78254.

9. Hagel C, Dornblut C, Schulz A, Wiehl U, Friedrich RE, Huckhagel T, Mautner VF, Morrison H: **The putative oncogene CPI-17 is up-regulated in schwannoma**. *Neuropathology and applied neurobiology* 2016, **42**(7):664-668.

10. Jin H, Sperka T, Herrlich P, Morrison H: **Tumorigenic transformation by CPI-17 through inhibition of a merlin phosphatase**. *Nature* 2006, **442**(7102):576-579.

11. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN *et al*: **Visualizing and interpreting cancer genomics data via the Xena platform**. *Nature biotechnology* 2020, **38**(6):675-678.

12. Vivian J, Rao AA, Nothaft FA, Ketchum C, Armstrong J, Novak A, Pfeil J, Narkizian J, Deran AD, Musselman-Brown A *et al*: **Toil enables reproducible, open source, big biomedical data analyses**. *Nature biotechnology* 2017, **35**(4):314-316.

13. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XJNar: **TIMER2.0 for analysis of tumor-infiltrating immune cells**. 2020, **48**:W509-W514.

14. Chandrashekar D, Bashel B, Balasubramanya S, Creighton C, Ponce-Rodriguez I, Chakravarthi B, Varambally SJN: **UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses**. 2017, **19**(8):649-658.

15. Tang Z, Kang B, Li C, Chen T, Zhang ZJNar: **GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis**. 2019, **47**:W556-W560.

16. Liu J, Lichtenberg T, Hoadley KA, Poisson LM, Lazar AJ, Cherniack AD, Kovatich AJ, Benz CC, Levine DA, Lee AV *et al*: **An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics**. *Cell* 2018, **173**(2).

17. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E *et al*: **Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal**. *Sci Signal* 2013, **6**(269):pl1.

18. Cerami E, Gao J, Dogrusoz U, Gross B, Sumer S, Aksoy B, Jacobsen A, Byrne C, Heuer M, Larsson E *et al*: **The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data**. 2012, **2**(5):401-404.

19. Mandrekar JN: **Receiver operating characteristic curve in diagnostic test assessment**. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2010, **5**(9):1315-1316.

20. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, Müller M: **pROC: an open-source package for R and S+ to analyze and compare ROC curves**. *BMC Bioinformatics* 2011, **12**:77.

21. Aran D, Hu Z, Butte AJGb: **xCell: digitally portraying the tissue cellular heterogeneity landscape**. 2017, **18**(1):220.

22. Zeng D, Li M, Zhou R, Zhang J, Sun H, Shi M, Bin J, Liao Y, Rao J, Liao WJCir: **Tumor Microenvironment Characterization in Gastric Cancer Identifies Prognostic and Immunotherapeutically Relevant Gene Signatures**. 2019, **7**(5):737-750.

23. Wang J, Sun J, Liu L, Flies D, Nie X, Toki M, Zhang J, Song C, Zarr M, Zhou X *et al*: **Siglec-15 as an immune suppressor and potential target for normalization cancer immunotherapy**. 2019, **25**(4):656-666.

24. Frost F, Cherukuri P, Milanovich S, Boerkoel CJJoc, medicine m: **Pan-cancer RNA-seq data stratifies tumours by some hallmarks of cancer**. 2020, **24**(1):418-430.

25. Izzi V, Davis M, Naba AJC: **Pan-Cancer Analysis of the Genomic Alterations and Mutations of the Matrisome**. 2020, **12**(8).

26. Zhang Q, Huang R, Hu H, Yu L, Tang Q, Tao Y, Liu Z, Li J, Wang GJi: **Integrative Analysis of Hypoxia-Associated Signature in Pan-Cancer**. 2020, **23**(9):101460.

27. Sturm G, Finotello F, Petitprez F, Zhang J, Baumbach J, Fridman W, List M, Aneichyk TJB: **Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology**. 2019, **35**(14):i436-i445.

28. Li B, Severson E, Pignon J, Zhao H, Li T, Novak J, Jiang P, Shen H, Aster J, Rodig S *et al*: **Comprehensive analyses of tumor immunity: implications for cancer immunotherapy**. 2016, **17**(1):174.

29. Sturm G, Finotello F, List M: **Immunedeconv: An R Package for Unified Access to Computational Methods for Estimating Immune Cell Fractions from Bulk RNA-Sequencing Data**. *Methods Mol Biol* 2020, **2120**:223-232.

30. Bonneville R, Krook MA, Kautto EA, Miya J, Wing MR, Chen H-Z, Reeser JW, Yu L, Roychowdhury S: **Landscape of Microsatellite Instability Across 39 Cancer Types**. *JCO Precis Oncol* 2017, **2017**.

31. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang T-H, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA *et al*: **The Immune Landscape of Cancer**. *Immunity* 2018, **48**(4).

32. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P *et al*: **STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets**. *Nucleic Acids Res* 2019, **47**(D1):D607-D613.

33. Yu G, Wang L, Han Y, He QJOajoib: **clusterProfiler: an R package for comparing biological themes among gene clusters**. 2012, **16**(5):284-287.

34. Virshup DM, Shenolikar S: **From promiscuity to precision: protein phosphatases get a makeover**. *Mol Cell* 2009, **33**(5):537-545.

35. **Regulation of Cellular Protein Phosphatase-1 (PP1) by Phosphorylation of the CPI-17 Family, C-kinase-activated**

PP1 Inhibitors.

36. Riecken LB, Zoch A, Wiehl U, Reichert S, Scholl I, Cui Y, Ziemer M, Anderegg U, Hagel C, Morrison H: **CPI-17 drives oncogenic Ras signaling in human melanomas via Ezrin-Radixin-Moesin family proteins**. *Oncotarget* 2016, **7**(48):78242-78254.

37. Xu J, Zhang Y, Shi Y, Yin D, Dai P, Zhao W, Zhang T: **CPI-17 Overexpression and Its Correlation With the NF2 Mutation Spectrum in Sporadic Vestibular Schwannomas**. *Otology & neurotology : official publication of the American Otological Society, American Neurotology Society [and] European Academy of Otology and Neurotology* 2020, **41**(1):e94-e102.

38. Lu J, Peng Y, Huang R, Feng Z, Fan Y, Wang H, Zeng Z, Ji Y, Wang Y, Wang Z: **Elevated TYROBP expression predicts poor prognosis and high tumor immune infiltration in patients with low-grade glioma**. *BMC cancer* 2021, **21**(1):723.

39. Esteller M: **Epigenetic gene silencing in cancer: the DNA hypermethylome**. *Hum Mol Genet* 2007, **16 Spec No 1**:R50-59.

40. Toh TB, Lim JJ, Chow EK-H: **Epigenetics in cancer stem cells**. *Molecular cancer* 2017, **16**(1):29.

41. Wang M, Ngo V, Wang W: **Deciphering the genetic code of DNA methylation**. *Briefings in bioinformatics* 2021.

42. Peng Y, Wu Q, Wang L, Wang H, Yin F: **A DNA methylation signature to improve survival prediction of gastric cancer**. *Clin Epigenetics* 2020, **12**(1):15.

43. Li D, Guo J, Wang S, Zhu L, Shen Z: **Identification of novel methylated targets in colorectal cancer by microarray analysis and construction of co-expression network**. *Oncology letters* 2017, **14**(3):2643-2648.

44. Butler M, Pongor L, Su Y-T, Xi L, Raffeld M, Quezado M, Trepel J, Aldape K, Pommier Y, Wu J: **MGMT Status as a Clinical Biomarker in Glioblastoma**. *Trends in cancer* 2020, **6**(5):380-391.

45. Bouras E, Karakioulaki M, Bougioukas KI, Aivaliotis M, Tzimagiorgis G, Chourdakis M: **Gene promoter methylation and cancer: An umbrella review**. *Gene* 2019, **710**:333-340.

46. Kanazawa T, Misawa K, Shinmura K, Misawa Y, Kusaka G, Maruta M, Sasaki T, Watanabe Y, Carey TE: **Promoter methylation of galanin receptors as epigenetic biomarkers for head and neck squamous cell carcinomas**. *Expert Rev Mol Diagn* 2019, **19**(2):137-148.

47. Smith J, Sen S, Weeks RJ, Eccles MR, Chatterjee A: **Promoter DNA Hypermethylation and Paradoxical Gene Activation**. *Trends in cancer* 2020, **6**(5):392-406.

48. Lin WH, Chang YW, Hong MX, Hsu TC, Lee KC, Lin C, Lee JL: **STAT3 phosphorylation at Ser727 and Tyr705 differentially regulates the EMT-MET switch and cancer metastasis**. *Oncogene* 2021, **40**(4):791-805.

49. Im JY, Kim DM, Park H, Kang MJ, Kim DY, Chang KY, Kim BK, Won M: **VGLL1 phosphorylation and activation promotes gastric cancer malignancy via TGF-β/ERK/RSK2 signaling**. *Biochimica et biophysica acta Molecular cell research* 2021, **1868**(1):118892.

50. Satoh K, Fukumoto Y, Shimokawa H: **Rho-kinase: important new therapeutic target in cardiovascular diseases**. *Am J Physiol Heart Circ Physiol* 2011, **301**(2):H287-296.

51. Nakabayashi H, Shimizu K: **HA1077, a Rho kinase inhibitor, suppresses glioma-induced angiogenesis by targeting the Rho-ROCK and the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) signal pathways**. *Cancer science* 2011, **102**(2):393-399.

52. Rath N, Olson MJEr: **Rho-associated kinases in tumorigenesis: re-considering ROCK inhibition for cancer therapy**. 2012, **13**(10):900-908.

53. Qi Y, Liu B, Sun Q, Xiong X, Chen Q: **Immune Checkpoint Targeted Therapy in Glioma: Status and Hopes**. *Frontiers in immunology* 2020, **11**:578877.

54. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS *et al*: **Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer**. *Science (New York, NY)* 2015, **348**(6230):124-128.

55. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS *et al*: **Genetic basis for clinical response to CTLA-4 blockade in melanoma**. *The New England journal of medicine* 2014, **371**(23):2189-2199.

56. Kadio B, Yaya S, Basak A, Djè K, Gomes J, Mesenge C: **Calcium role in human carcinogenesis: a comprehensive analysis and critical review of literature**. *Cancer metastasis reviews* 2016, **35**(3):391-411.

57. Cooper J, Giancotti FG: **Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance**. *Cancer Cell* 2019, **35**(3):347-367.

58. Shah S, Brock EJ, Ji K, Mattingly RR: **Ras and Rap1: A tale of two GTPases**. *Seminars in cancer biology* 2019, **54**:29-39.

59. Perdomo-Pantoja A, Mejía-Pérez SI, Gómez-Flores-Ramos L, Lara-Velazquez M, Orillac C, Gómez-Amador JL, Wegman-Ostrosky T: **Renin angiotensin system and its role in biomarkers and treatment in gliomas**. *J Neurooncol* 2018, **138**(1):1-15.

60. Masoumi J, Jafarzadeh A, Khorramdelazad H, Abbasloui M, Abdolalizadeh J, Jamali N: **Role of Apelin/APJ axis in cancer development and progression**. *Advances in medical sciences* 2020, **65**(1):202-213.

61. Alshehri MM, Robbins SM, Senger DL: **The Role of Neurotrophin Signaling in Gliomagenesis: A Focus on the p75 Neurotrophin Receptor (p75(NTR)/CD271)**. *Vitamins and hormones* 2017, **104**:367-404.

62. Griffin N, Faulkner S, Jobling P, Hondermarck H: **Targeting neurotrophin signaling in cancer: The renaissance**. *Pharmacological research* 2018, **135**:12-17.

63. Hashemzehi M, Beheshti F, Hassanian SM, Ferns GA, Khazaei M, Avan A: **Therapeutic potential of renin angiotensin system inhibitors in cancer cells metastasis**. *Pathology, research and practice* 2020, **216**(7):153010.

64. Batlle E, Massagué J: **Transforming Growth Factor-β Signaling in Immunity and Cancer**. *Immunity* 2019, **50**(4):924-940.

Figures

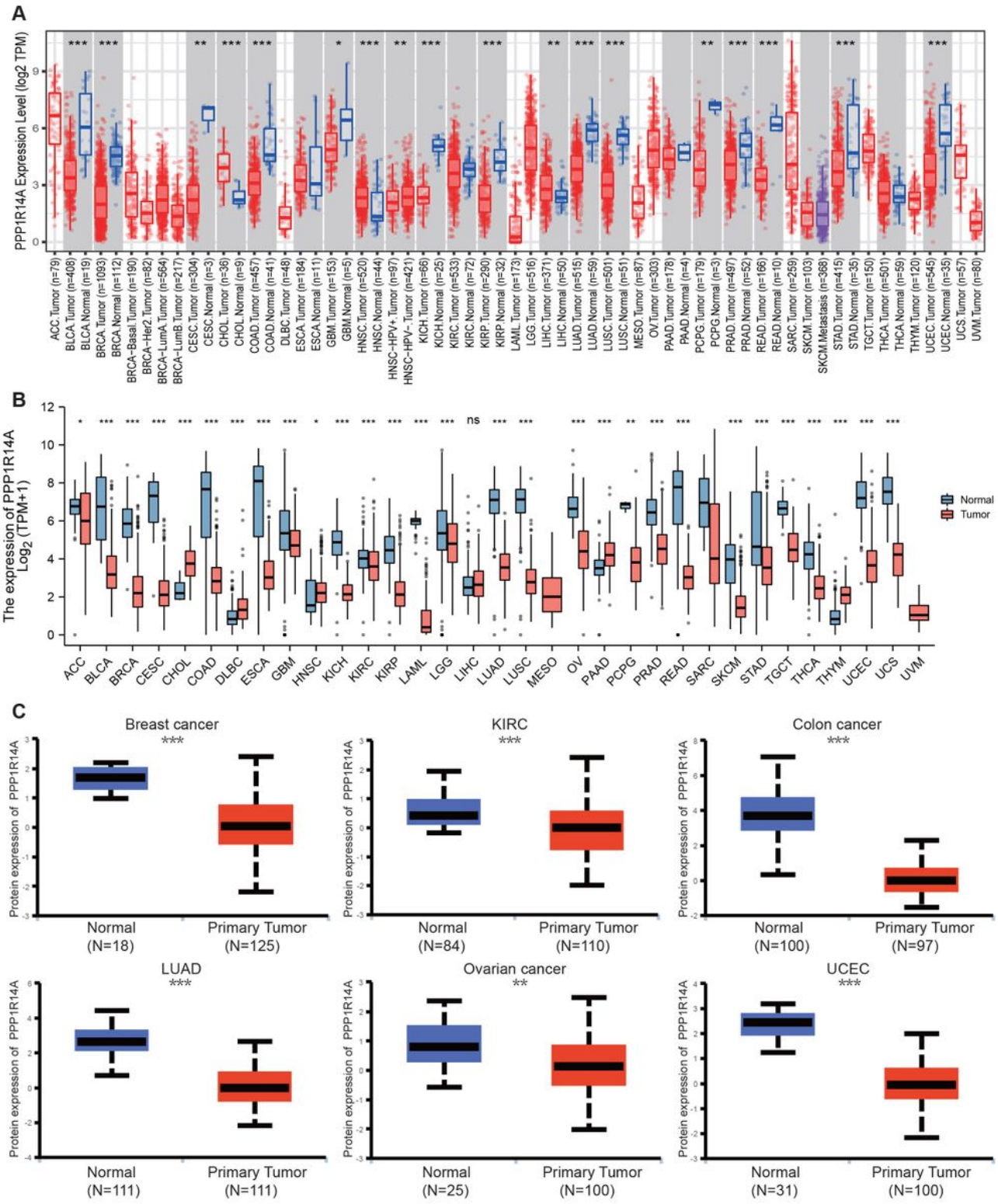

Figure 1

Aberrant mRNA expression of PPP1R14A in pan-cancer. The results from the TIMER2.0 database indicated that the PPP1R14A expression was remarkably dysregulated in 18 cancer types. The red and blue boxes represent tumor tissues and normal tissues, respectively (A). The expression level of PPP1R14A in different cancer types from TCGA and GTEx (B). The PPP1R14A protein expression level in

normal tissues and primary tissues of breast cancer, KIRC, colon cancer, LUAD, ovarian cancer and UCEC was examined using the CPTAC datasets (C). ns, p≥0.05; *, p< 0.05; **, p<0.01; ***, p<0.001.

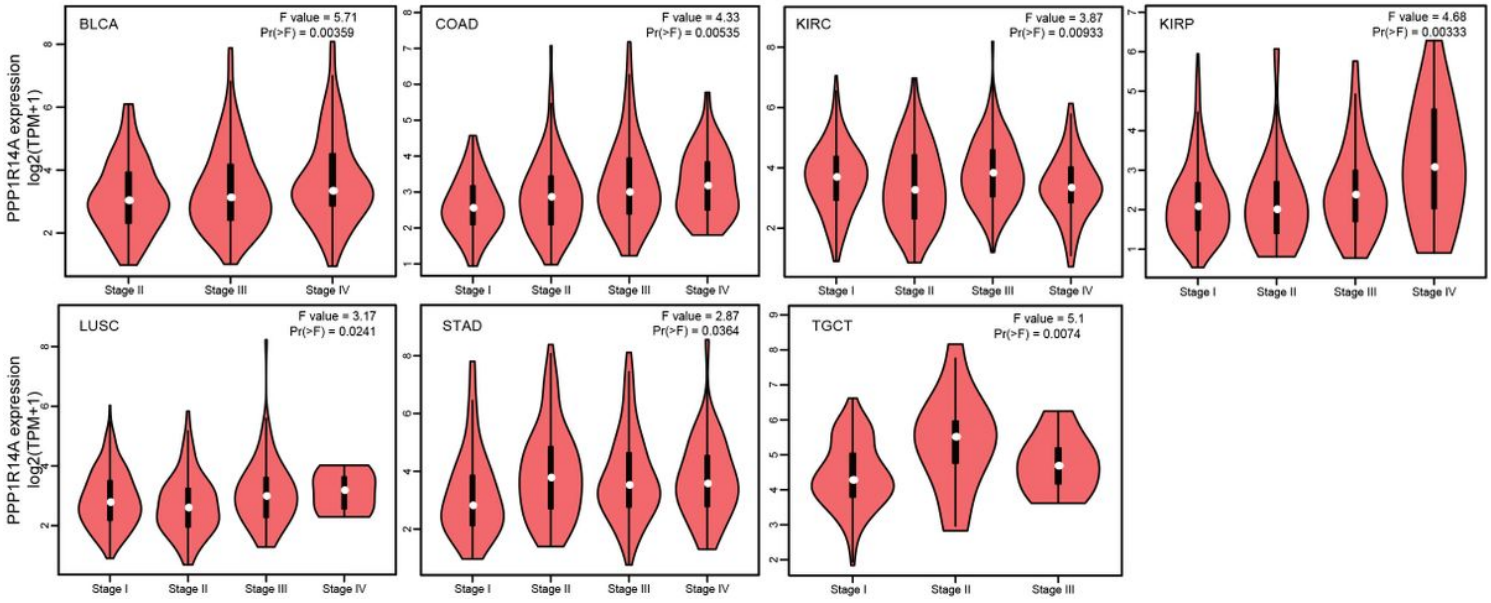

Figure 2

Correlations between the PPP1R14A expression and the main pathological stages, including stage I, stage II, stage III, and stage IV of BLCA, COAD, KIRC, KIRP, LUSC, STAD and TGCT.

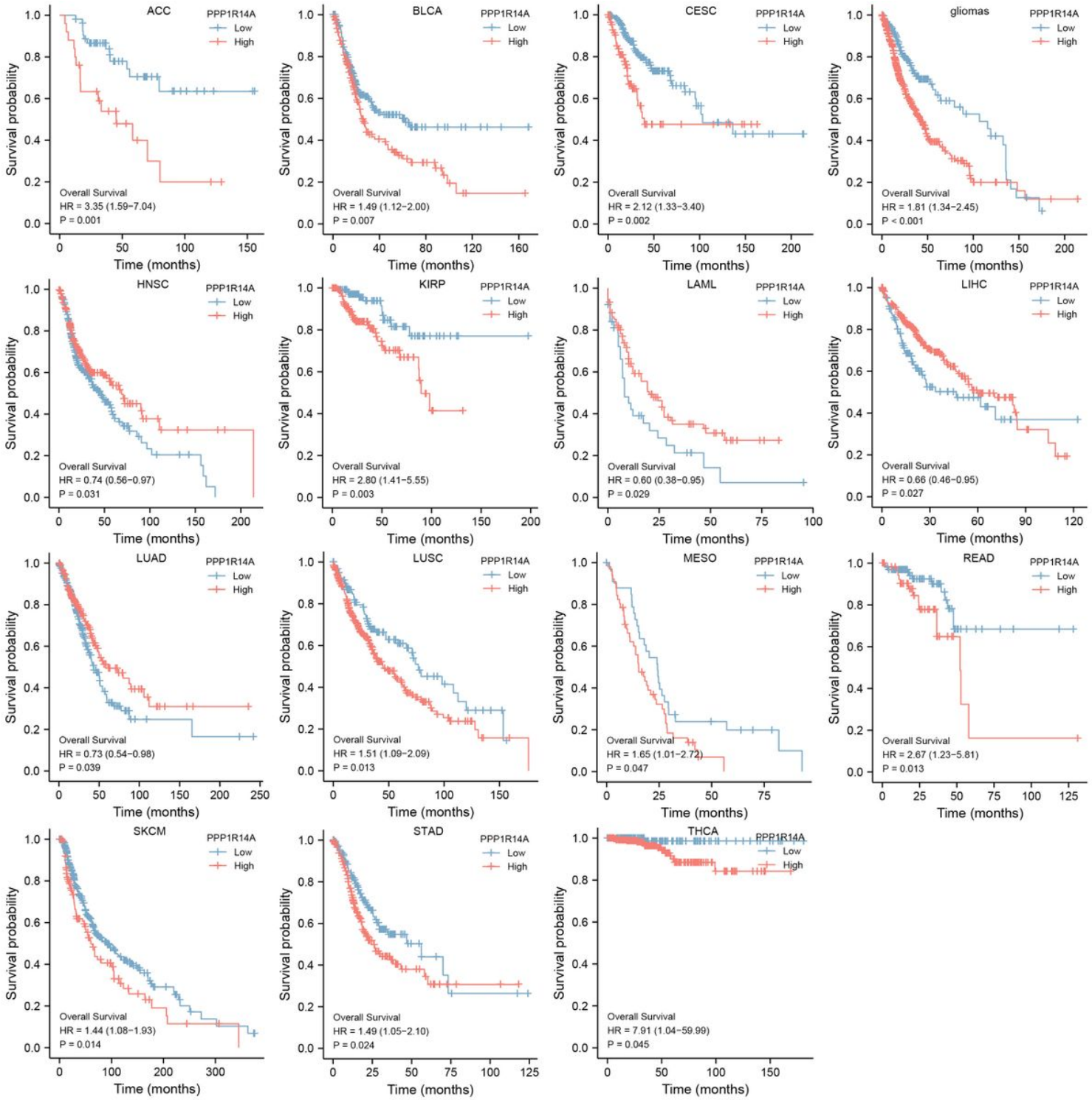

Figure 3

Association between the PPP1R14A expression and the OS of cancer patients. Kaplan-Meier survival curves for patients stratified by the different expressions of PPP1R14A in ACC, BLCA, CESC, gliomas, HNSC, LAML, LIHC, LUAD, LUSC, MESO, READ, SKCM, STAD, and THCA.

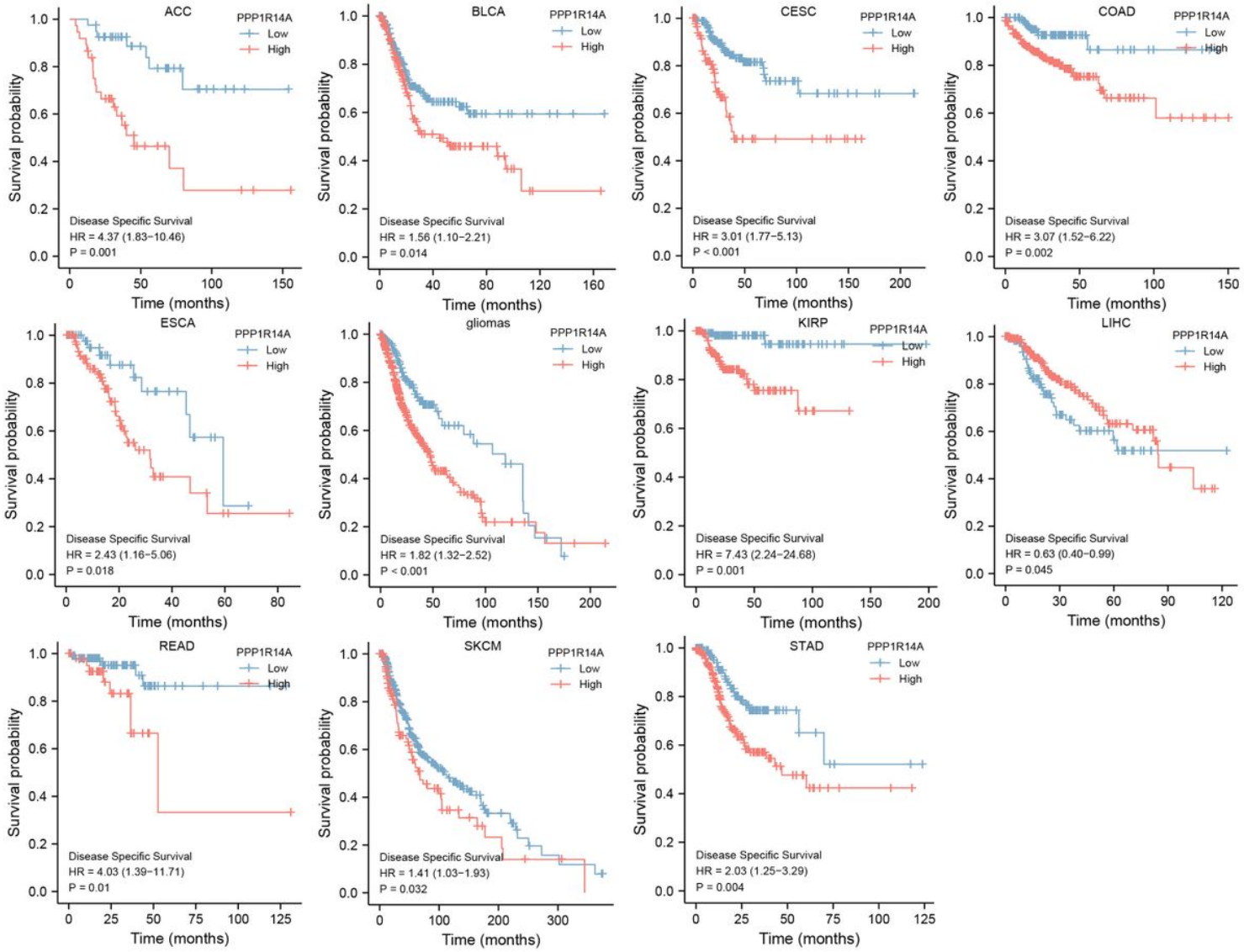

Figure 4

Association between the PPP1R14A expression and the DSS of cancer patients. Kaplan-Meier survival curves for patients stratified by the different expressions of PPP1R14A in ACC, BLCA, CESC, COAD, ESCA, gliomas, KIRP, LIHC, READ, SKCM, and STAD.

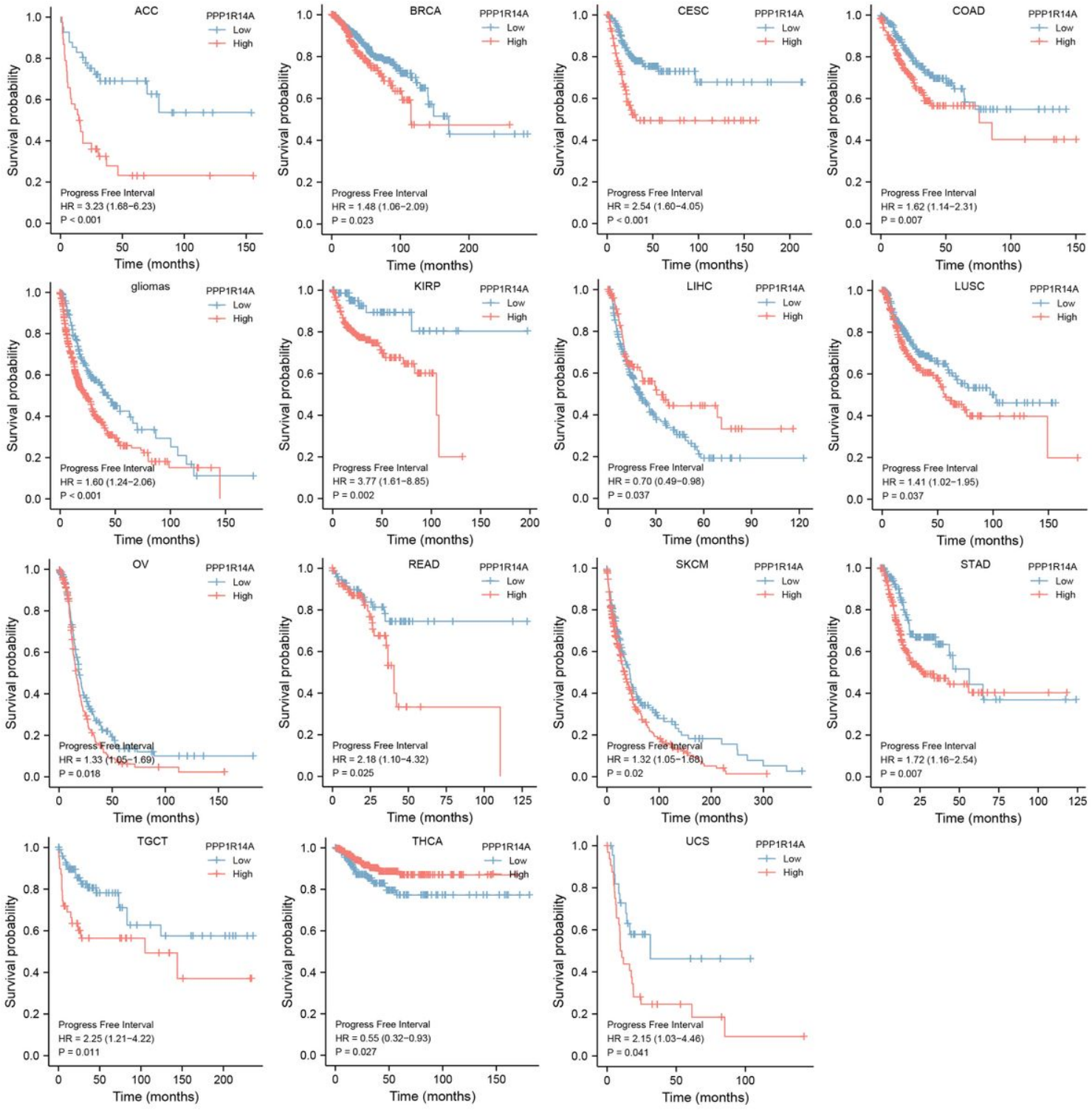

Figure 5

Association between the PPP1R14A expression and the PFI of cancer patients. Kaplan-Meier survival curves for patients stratified by the different expressions of PPP1R14A in ACC, BRCA, CESC, COAD, gliomas, KIRP, LIHC, LUSC, OV, READ, SKCM, STAD, TGCT, THCA and UCS.

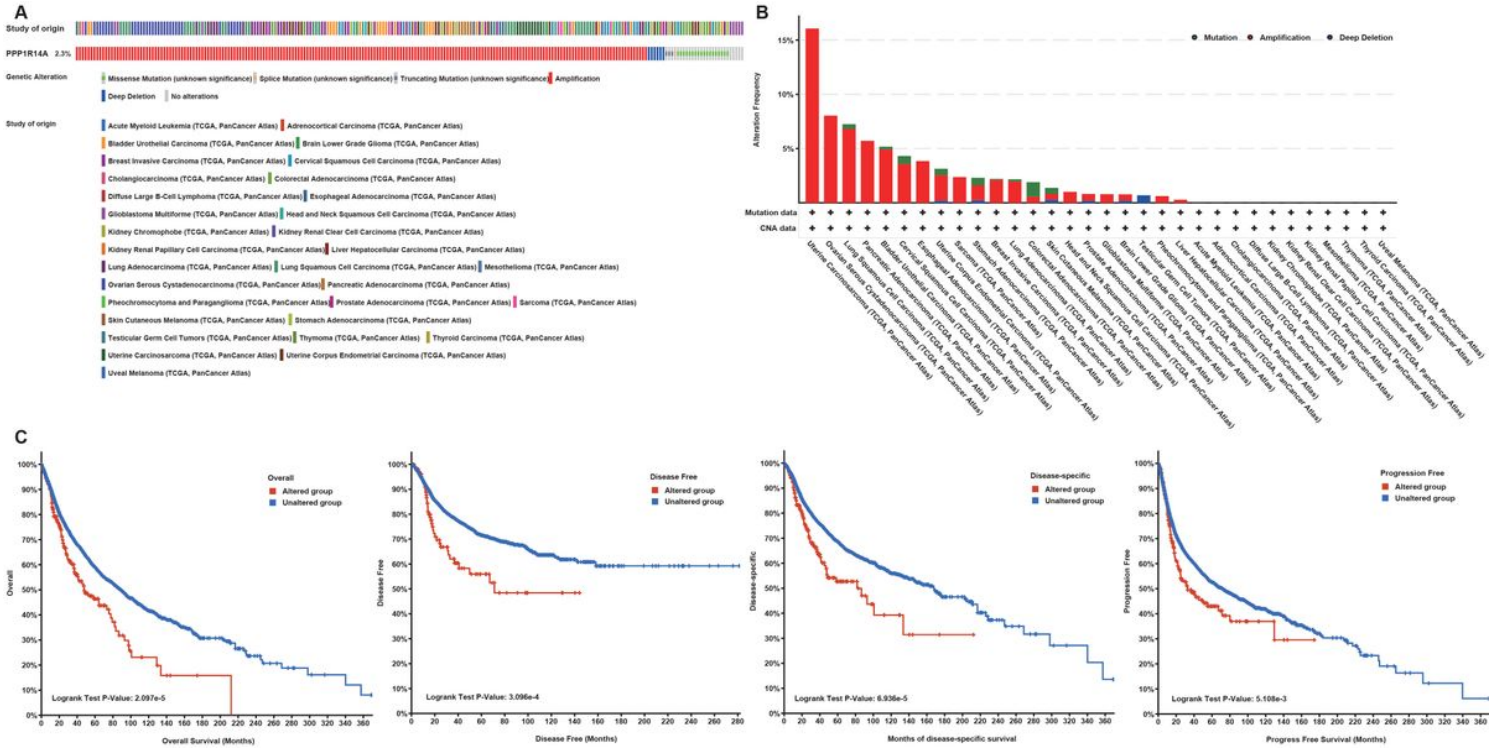

Figure 6

Summary of genetic alterations of PPP1R14A in different tumors (A). The alteration frequency with different types of copy number variations and mutations were examined (B). The effect of PPP1R14A alteration status on overall, disease-free, disease-specific and progression-free survival of cancer patients was investigated (C).

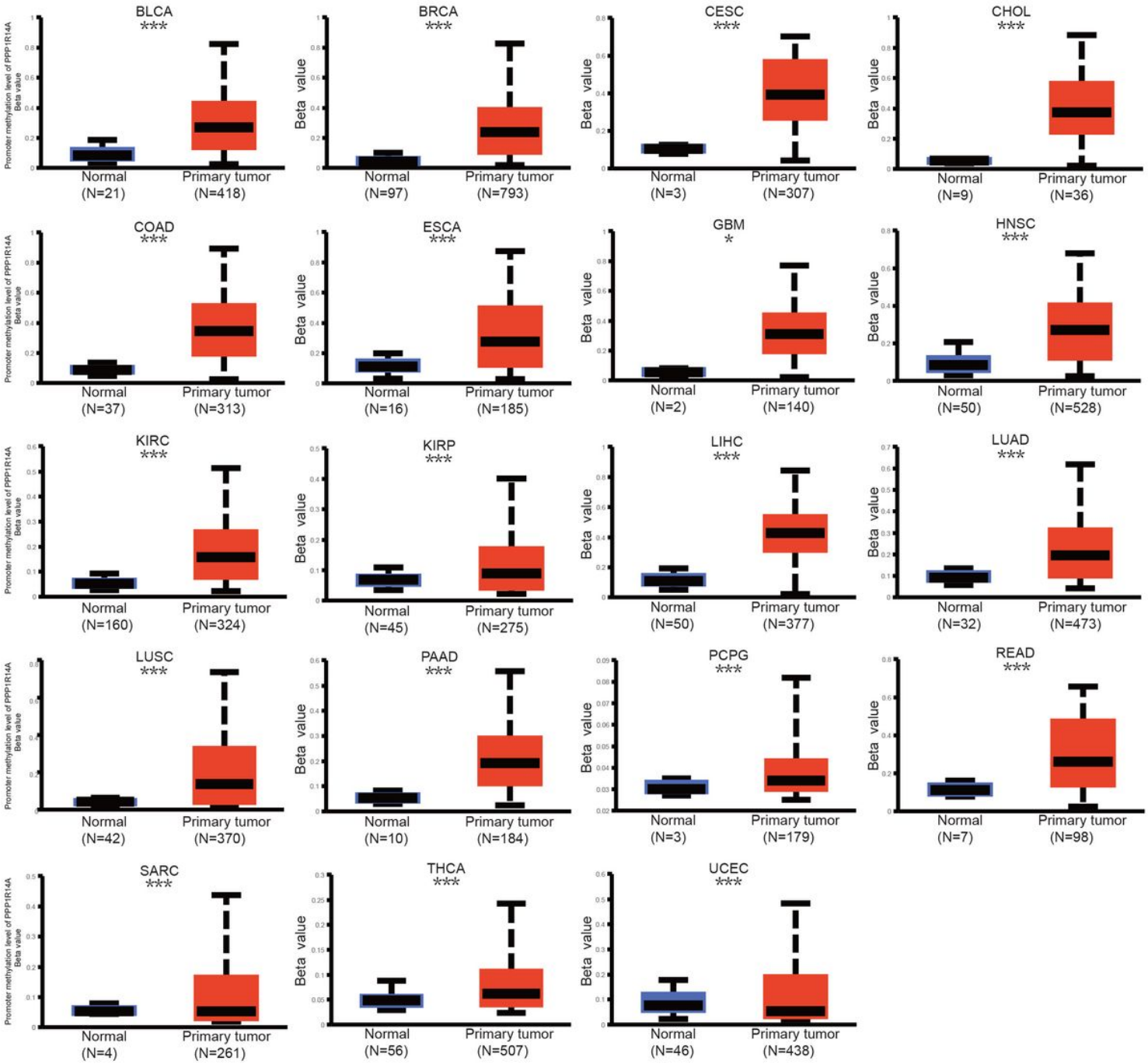

Figure 7

Promoter methylation level of PPP1R14A in pan-cancer. The results were obtained from the UALCAN database. ∗,p < 0.05; ∗∗,p < 0.01; ∗∗∗; p < 0.001.

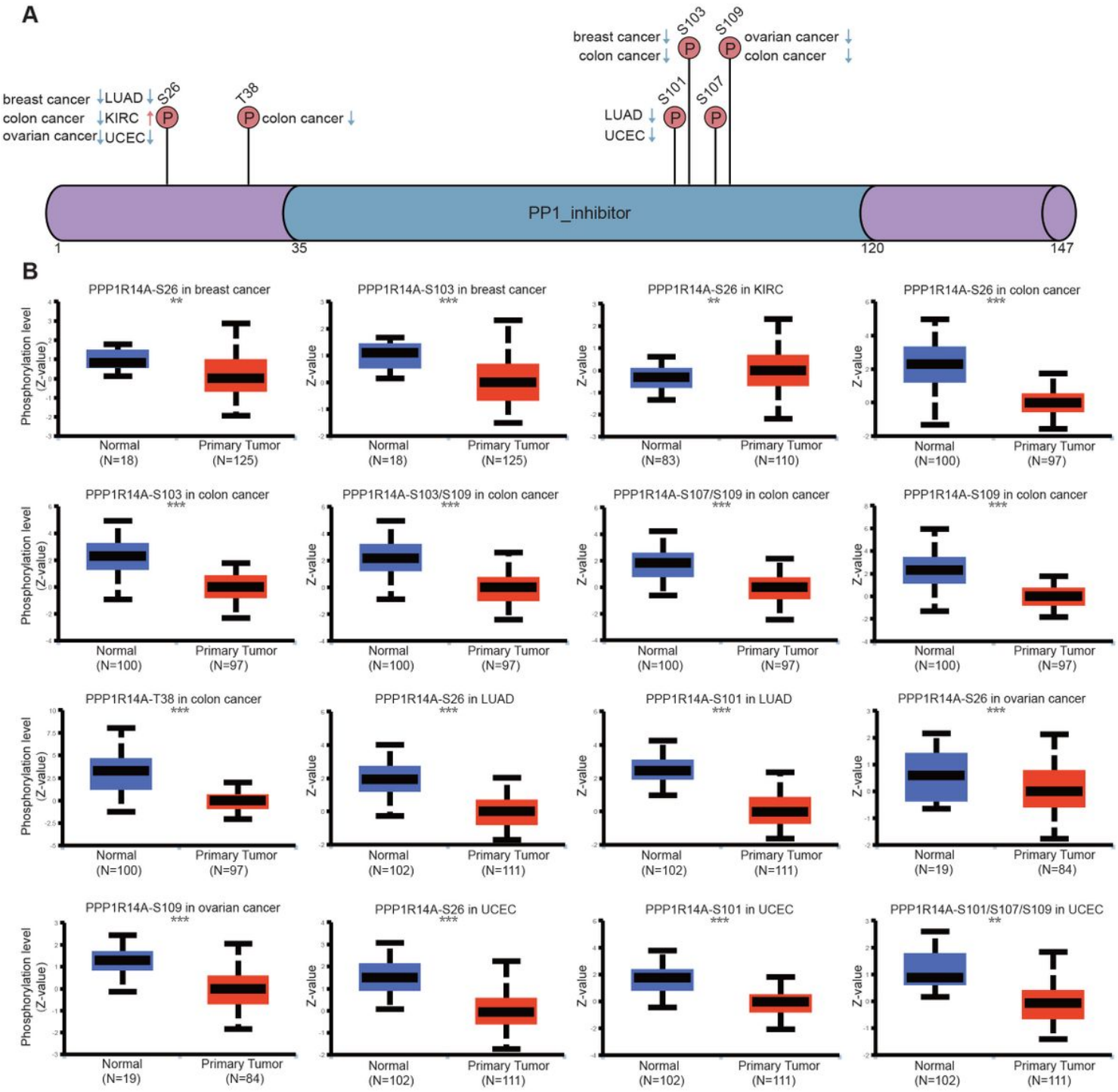

Figure 8

Phosphorylation of PPP1R14A in several selected cancers according to the CPTAC database. The schematic diagram and phosphorylation sites of the PPP1R14A protein are shown (A). The phosphorylation of PPP1R14A at S26, S101, S101/S107/S109, S103, S103/S109 S107/S109, S109 and T38 was analyzed in breast cancer, clear cell RCC, colon cancer, LUAD, ovarian cancer, and UCEC (B). ∗∗,$p < 0.01$; ∗∗∗, $p < 0.001$.

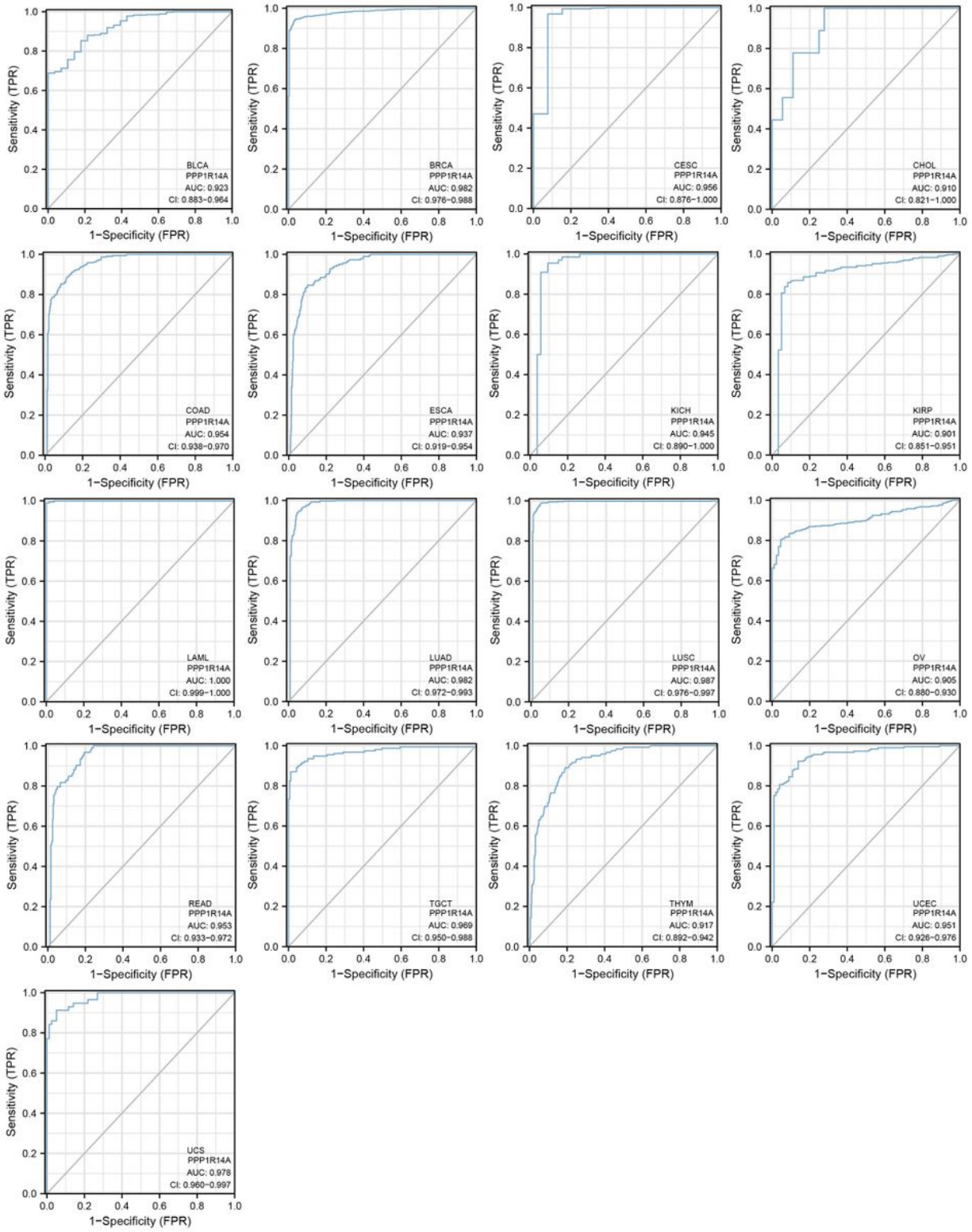

Figure 9

ROC curves for PPP1R14A across BLCA, BRCA, CESC, CHOL, COAD, ESCA, KICH, KIRP, LAML, LUAD, LUSC, OV, READ, TGCT, THYM, UCEC and UCS.

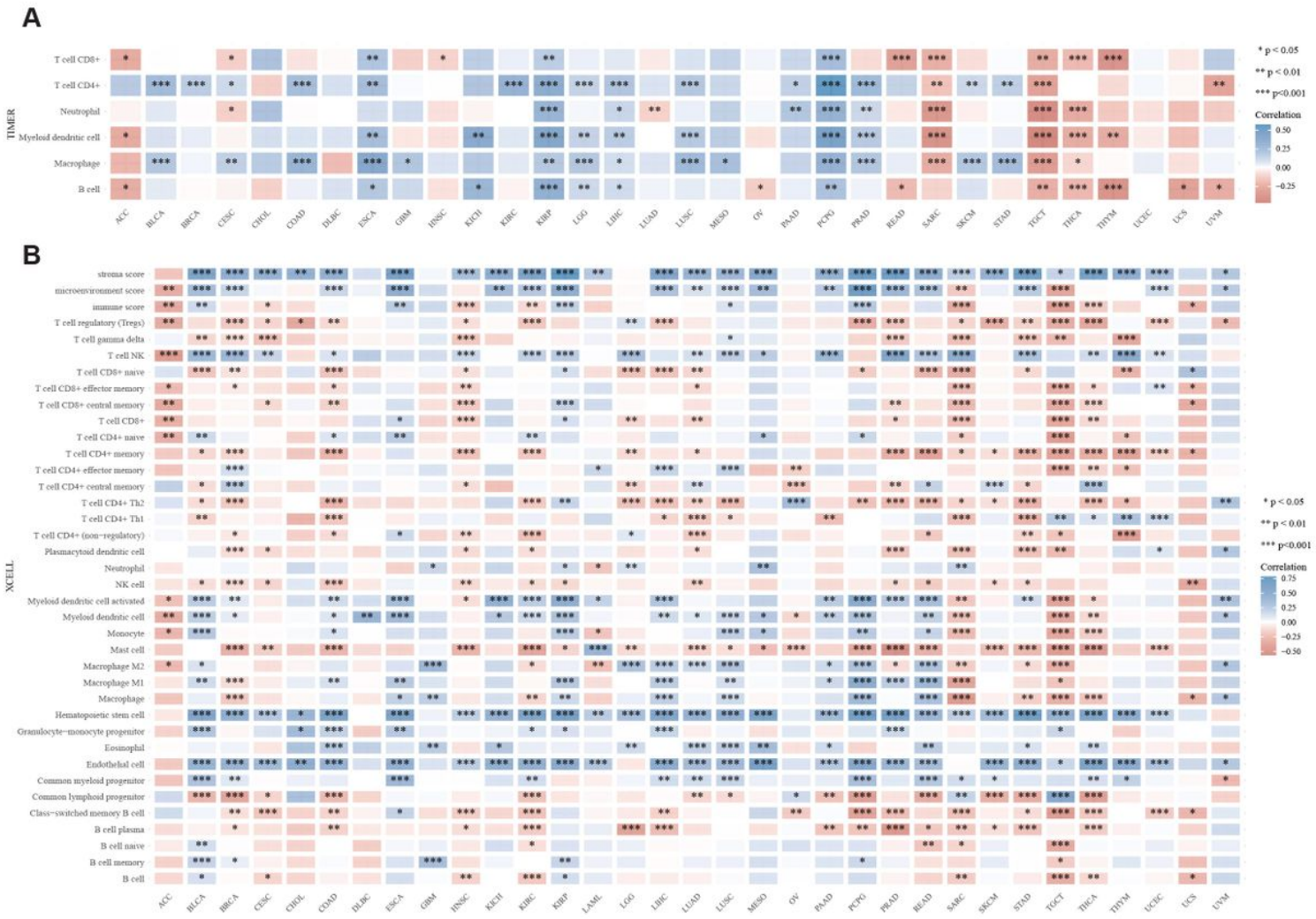

Figure 10

The PPP1R14A expression correlated with immune infiltration. The PPP1R14A expression significantly correlated with the infiltration levels of various immune cells in different tumors based on TIMER (A) and XCELL (B) algorithms.

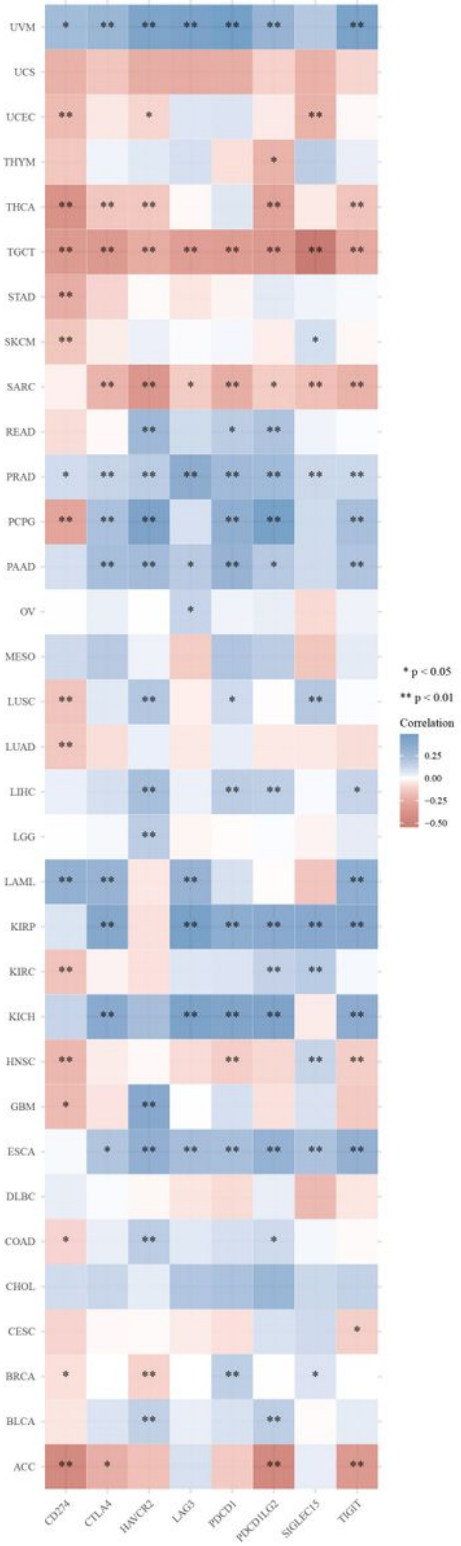

Figure 11

Correlation analyses of the PPP1R14A expression with immune checkpoint genes in pan-cancer.

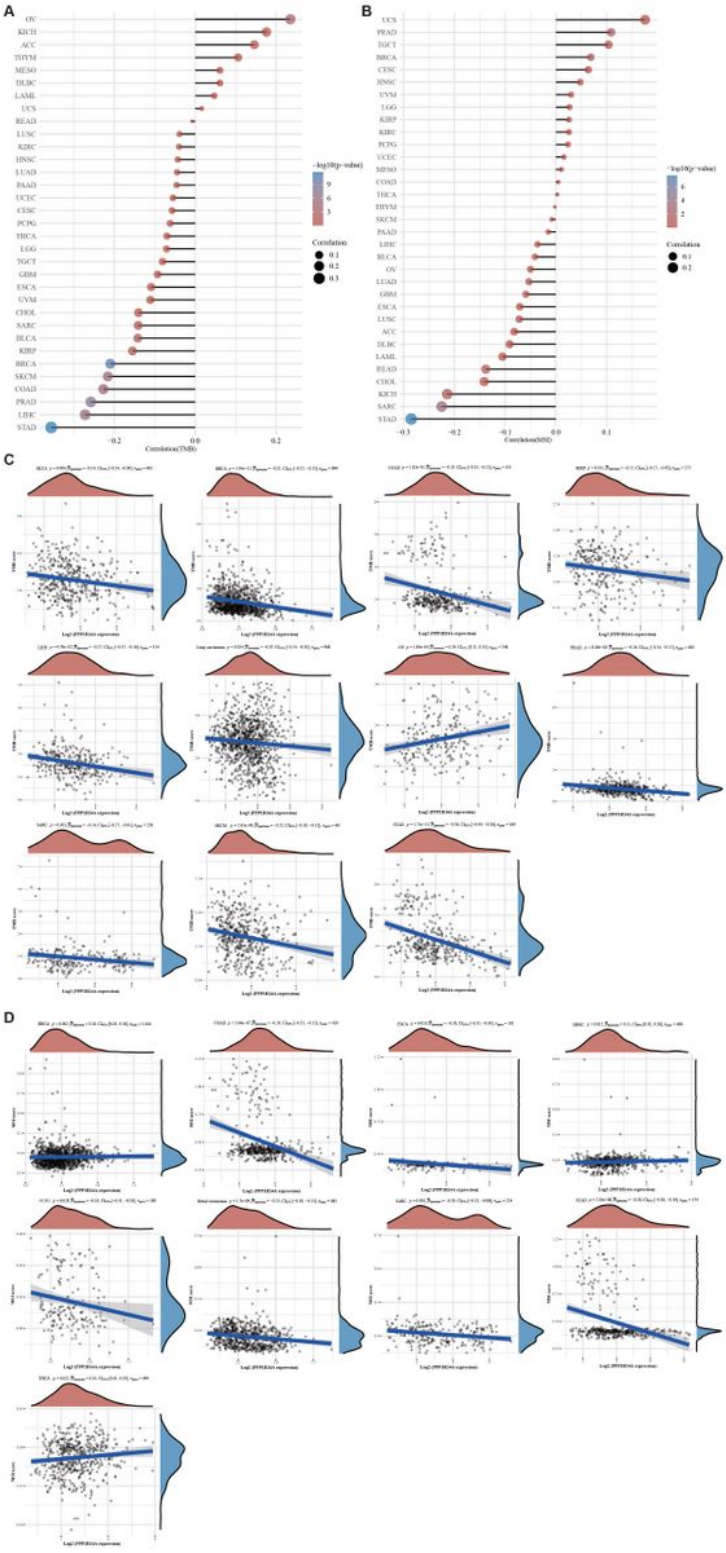

Figure 12

Correlation between the PPP1R14A gene expression and TMB and MSI in pan-cancer. A stick chart shows the relationship between the PPP1R14A gene expression and TMB in diverse tumors. The red curve represents the correlation coefficient, and the blue value represents the range (A). A stick chart shows the association between the PPP1R14A gene expression and MSI in diverse tumors (B). Relationship between the PPP1R14A gene expression and TMB (C) or MSI (D) in pan-cancer.

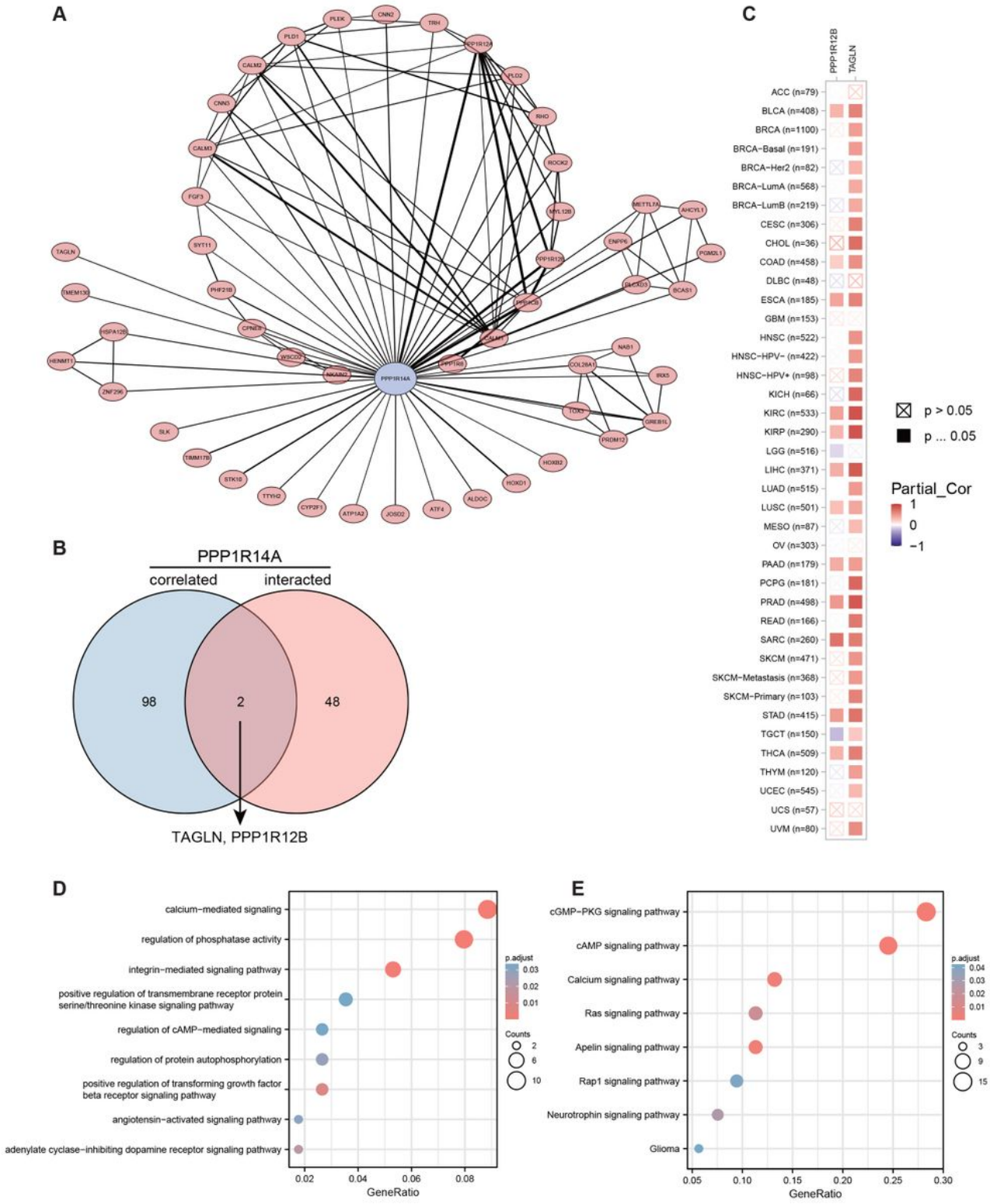

Figure 13

PPP1R14A-related gene enrichment analysis. A PPI network displays the available experimentally determined PPP1R14A-binding proteins. PPP1R14A is marked by blue color, line thickness represents combined score (A). Using the GEPIA2 approach, we also obtained the top 100 PPP1R14A-correlated genes and constructed a Venn diagram with molecules that have a physical binding relationship with PPP1R14A, and it is found that there are two molecules, including TAGLN and PPP1R12B in the

overlapping area (B). The corresponding heatmap exhibits the relationship between PPP1R14A and the two intersecting molecules in the detailed cancer types (C). Based on the PPP1R14A-binding and related genes, GO (D) and KEGG (E) pathway analysis was performed and displayed through bubble charts.