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# A pan-cancer analysis of oncogenic protein tyrosine phosphatase subfamily PTP4As

Mingyang Yu, Chunxu Lin, Min Wei *

*Center for Drug Research and Development, Guangdong Pharmaceutical University, Guangzhou, 510006, China*

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ABSTRACT

*Objective:* The objective of this study was to conduct a comprehensive pan-cancer analysis of Protein tyrosine phosphatase 4A (PTP4As), specifically PTP4A1, PTP4A2, and PTP4A3, to investigate their aberrant expression, genomic alterations, prognostic values, and molecular functions. The aim was to evaluate the roles of PTP4As in cancer development and progression, as previous research has primarily focused on PTP4A3 and yielded inconsistent results regarding their expression in cancers.

*Methods:* A meticulous and extensive analysis of PTP4As was performed across diverse cancer types. mRNA expression levels of PTP4A isoforms were examined, and correlations between protein expression and mRNA expression were investigated. Genomic alterations affecting PTP4As, such as amplification, were analyzed. Survival analysis was conducted to assess the prognostic values of PTP4As in different cancers. Additionally, pathway enrichment analysis was performed to identify signaling pathways and biological processes associated with PTP4A2 and PTP4A3.

*Results:* The analysis revealed that PTP4A3 exhibited the most prevalent up-regulation at the mRNA level among the PTP4A isoforms. PTP4A2 mRNA expression in cancer generally displayed an up-regulated trend. However, inconsistent results were observed for PTP4A1 expression, even within the same cancer type but across different datasets, indicating the need for further investigation. The correlation between PTP4As protein expression and mRNA expression was found to be weak, indicating the complexity of their regulatory mechanisms. Genomic analysis showed that amplification was the major type of alteration affecting PTP4As, although it did not always translate into higher expression. Survival analysis revealed that high PTP4As expression was typically associated with unfavorable prognoses in several cancers, although exceptions existed. Pathway enrichment analysis unveiled novel signaling pathways and biological processes potentially influenced by PTP4As.

*Conclusion:* The pan-cancer analysis of PTP4As provided insights into their aberrant expression, genomic alterations, prognostic values, and molecular functions. PTP4A3 exhibited the most prevalent up-regulation, while PTP4A2 showed a general up-regulated trend. Inconsistent results were observed for PTP4A1 expression, warranting further investigation. These findings contribute to our understanding of the molecular mechanisms through which PTP4As may contribute to cancer pathogenesis.

## 1. Introduction

Protein tyrosine phosphatases (PTPs), in coordination with kinases, elaborately regulate the phosphorylation of proteins to control the intensity and duration of signaling transduction in cells. Aberrant activity of PTPs have been demonstrated to correlate with diseases initiation and progress, such as cancers and diabetes. But unlike kinases, which have been intensively studied as drug targets and consequently kinase inhibitors have achieved great success in cancer treatment, PTPs are understudied proteins and there are no PTPs-targeting drugs approved as cancer therapeutics in clinic so far.[1,2]

Protein tyrosine phosphatases (PTP4As) is one of the most oncogenic PTPs subfamilies. PTP4As, also known as PRLs (phosphatase of regenerating liver) , including PTP4A1, PTP4A2 and PTP4A3, can act on both tyrosine and serine/threonine residues in a protein to remove phosphate modification. The oncogenic role of PTP4As have been demonstrated in varying cancers and PTP4A3 is the best studied one and proposed to be a biomarker of tumor progression and metastasis. PTP4A3 expression was elevated in tumor tissues, compared with healthy tissues, or in advanced versus early stage tumors, spanning colon,[3,4] breast,[5,6] gastric,[7] ovarian,[8]

* Corresponding author.
*E-mail address:* min.wei@gdpu.edu.cn (M. Wei).

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liver,[9] prostate,[10] lung,[11] AML (acute myeloid leukemia)[12,13] and B-ALL (B-cell acute lymphoblastic leukemia).[14] Its role in T-ALL progression was also demonstrated by using genetic and chemical approaches.[15] Importantly, high PTP4A3 expression was often correlated with poor prognosis in patients with colon,[16] breast,[5,6,17] gastric,[18,19] liver,[9] lung[11], ovarian cancers[20] and AML.[12]

Similarly, both PTP4A1 and PTP4A2 dysregulation also correlate with prognosis in cancer patients. Specifically, elevated PTP4A1 expression was detected in non-small cell lung,[21] liver[22,23] cancer and associated with poor prognosis in non-small cell lung[21] and liver cancer.[23] PTP4A2 overexpression was also observed in breast,[24] nasopharyngeal[25] and liver cancer.[26] Interestingly, PTP4A2 overexpression was associated with poor prognosis in nasopharyngeal cancer[25] while showed favorable prognosis for breast cancer.[24] Notably, the direction of its dysregulation varies among different cancer types. For example, decreased PTP4A1 expression was observed in ovarian, breast, and lung cancers and PTP4A2 was significantly down-regulated in kidney carcinomas compared to normal tissue.[26]

Most of the previous studies about PTP4As are limited to small sample size and different experimental techniques were used, making it difficult for comparison between independent studies. In addition, PTP4A1 and PTP4A2 were poorly characterized compared with PTP4A3. In order to have an overview of pan-cancer dysregulation of PTP4As in large patient cohorts, we harness the large cancer patient datasets in multiple public database to perform a detailed and thorough pan-cancer analysis, including: (1) compared PTP4As mRNA and protein expression between cancer and normal tissues; (2) investigated the correlation between PTP4As genomic alteration, mRNA and protein expression; (3) explored the prognostic value of PTP4As among cancers; (4) examined the potential molecular function of PTP4As.

## 2. Materials and methods

### 2.1. Datasets and platforms used for analysis

Differential gene expression analysis was done using GEIPA2 (http://gepia2.cancer-pku.cn).[27] The TCGA pan-cancer data contains 33 cancer types but we only use the 30 datasets with at least 10 samples for each group, which are ACC (Adrenocortical carcinoma), BLCA (Bladder Urothelial Carcinoma), BRCA (Breast invasive carcinoma) , COAD (Colon adenocarcinoma), ESCA (esophageal carcinoma), GBM (Glioblastoma multiforme), HNSC (Head and Neck squamous cell carcinoma), 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), OV (Ovarian serous cystadenocarcinoma), PRAD (Prostate adenocarcinoma), READ (Rectum adenocarcinoma), SKCM (Skin Cutaneous Melanoma), STAD (Stomach adenocarcinoma), TGCT (Testicular Germ Cell Tumors), THCA (Thyroid carcinoma), THYM (Thymoma), UCEC (Uterine Corpus Endometrial Carcinoma), UCS (Uterine Carcinosarcoma). In total, 9184 primary tumor samples, 697 adjacent normal samples from TCGA and 4829 normal tissue samples from GTEx were used for this study.

Oncomine (https://www.oncomine.org) database was used to further validate the significant aberrant PTP4As expression, focusing the ones showing the similar or opposite trend to GEPIA2 and the findings which are not consistent with previous publications.[28] We only include the cancer types which were supported by at least two analyses with the same up or down-regulation trend.

Clinical Proteomic Tumor Analysis Consortium (CPTAC) data sets in UALCAN (http://ualcan.path.uab.edu/analysis.html),[29] including breast, ovarian, colon, KIRC, UCEC and LUAD cancers were used for differential protein expression analysis of PTP4As. UALCAN was also used for overall survival analysis of PTP4As based on mRNA seq data in TCGA data sets.

The cBioPortal (http://www.cbioportal.org/),[30] an open-source platform for analyzing and visualizing multidimensional cancer genomics and clinical data, was used for genomic alteration analysis of PTP4As. TCGA datasets, including 10 967 samples in 32 cancer types, were used for analysis.

TCGA, Oncomine, CTPAC and cBioportal data belong to public databases. The patients included in these databases have obtained ethical approval, ensuring adherence to ethical standards. Users are granted free access to download the relevant data for research purposes and publish related articles. As our study relies on open-source data, there are no ethical concerns or conflicts of interest associated with our research.

### 2.2. Differential gene expression analysis using GEPIA, ONCOMINE and UALCAN

For differential mRNA expression analysis of PTP4As, TCGA tumor samples were compared to adjacent normal samples also from TCGA. In GEPIA2, all mRNA expression data were presented as log2 (TPM + 1) for plotting. One-way ANOVA was used for differential analysis with $p$ value $< 0.01$ and fold change threshold equal to 2. Oncomine was used to validate PTP4As expression with the parameter as: $p$ value $< 0.01$, fold change $>2$, and gene ranking in the top 10%. Proteomic data available in a subset of tumors in Clinical Proteomic Tumor Analysis Consortium (CPTAC) was analyzed between tumor samples and normal samples. Normalization using Log2 spectral count ratio values was performed within each sample profile, then normalized across samples. $T$-test was used for differential analysis with $p$ value $< 0.01$.

### 2.3. Survival analysis using ULCAN

ULCAN was used to determine prognostic significance of PTP4As across TCGA data sets. Cancer patient samples were grouped into high group and low/medium group with TPM values in the upper quartile and lower quartile, respectively. The correlation between PTP4As expression level and patient overall survival (OS) was depicted by plotting a Kaplan-Meier survival curve. Log ranks $p$ value and hazard ratio (HR) with 95% confidence intervals were calculated and $p$ value $< 0.01$ was set as the threshold for significant difference.

### 2.4. Genomic alteration analysis using cBioportal

Genomic alterations of PTP4As, including gene fusion, amplification, deletions and mutations, were analyzed by using cBioPortal in TCGA datasets. A summary of genetic alterations of PTP4As was presented under OncoPrint tab. The Cancer Type Summary tab displayed alteration frequency of individual PTP4A across 32 cancer types. The tab Plot visualized the association between mRNA expression level of PTP4As and genetic alterations in each cancer study.

## 3. Results

### 3.1. Pan-cancer analysis of PTP4A1 expression

To comprehensively explore PTP4As expression in cancer, we compared the mRNA expression of PTP4As in TCGA cancer datasets with normal tissues across 30 different cancer types. PTP4A1 was significantly up-regulated in 6 cancer types (Fig. 1A), including GBM, LGG, LUAD, PAAD, ESCA, and STAD while down-regulated in KICH and TGCT (Fig. 1D). No significant differential PTP4A1 expression was found in other cancer types compared to that in normal tissues, including liver, ovarian and breast cancers, while the three cancer types showed aberrant PTP4A1 expression previously.[22,23,26]

In order to validate these findings in other independent studies, we next examined PTP4A1 expression using ONCOMINE, where the numbers represent the datasets showing statistically significant over-expression (red) or down-expression (blue) of PTP4As mRNA in tumor

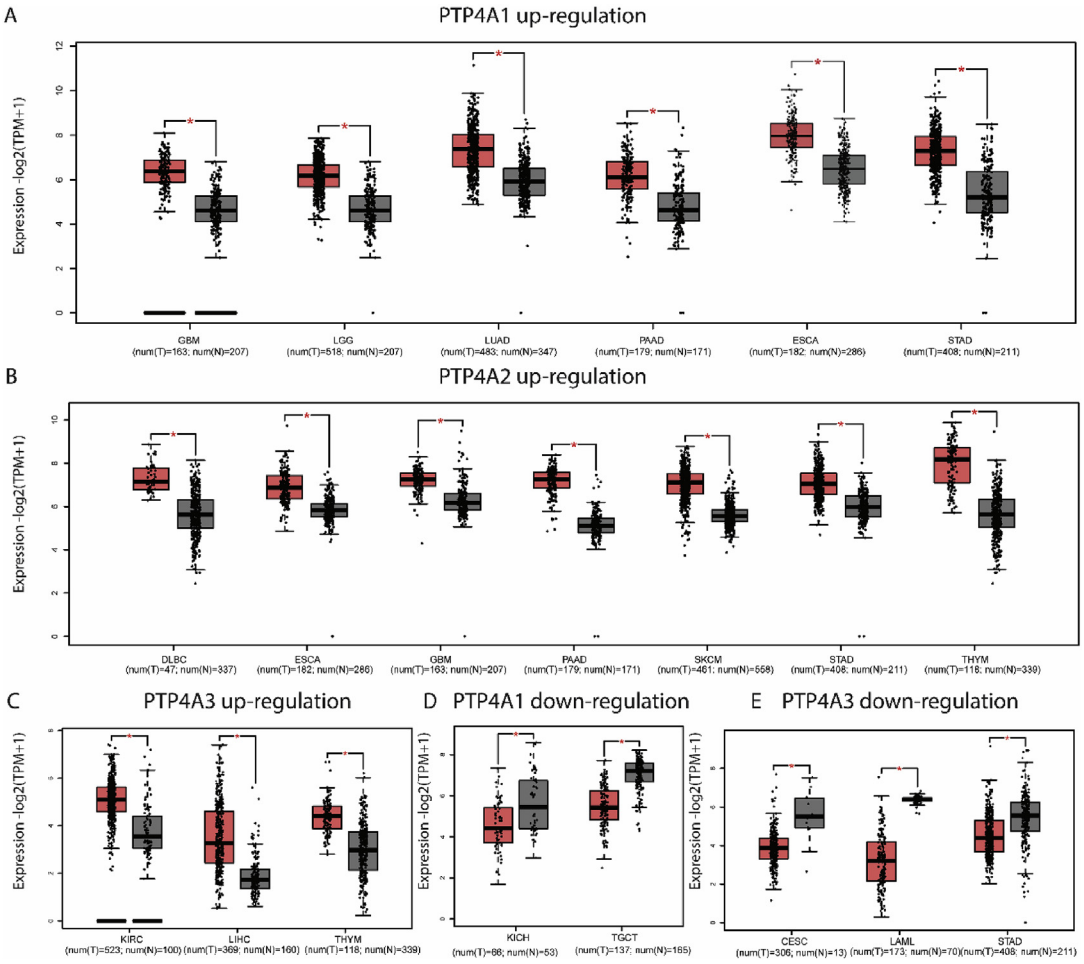



**Fig. 1.** Aberrant PTP4As expression in tumors.
PTP4As mRNA expression across TCGA cancers versus normal tissues compiled in GEPIA2. A. PTP4A1 up-regulation; B. PTP4A2 up-regulation; C. PTP4A3 up-regulation; D. PTP4A1 down-regulation; E. PTP4A3 down-regulation. *$P < 0.01$.

samples *vs.* normal tissues (Fig. 2). Surprisingly, PTP4A1 is frequently down-regulated in ONCOMINE datasets. For example, its down-regulation was consistently demonstrated in colorectal and lung cancer, opposite to the TCGA data analysis (Fig. 2).

As there is inconsistency about PTP4A1 dysregulation in liver, ovarian and breast cancers between TCGA datasets and previous studies in literature, we examined PTP4A1 expression in these 3 cancer types in Oncomine in detail. No significant dysregulation of PTP4A1 expression was found in the 5 liver cancer studies. Only 1 out of 7 ovarian cancer datasets showed PTP4A1 down-regulation compared to normal tissues, 1 out of 13 breast cancer studies showed PTP4A1 up-regulation and also 1 out of 13 breast cancer studies showed PTP4A1 down-regulation.

The new aberrant PTP4A1 expressions identified by Oncomine analysis include bladder cancer where PTP4A1 was significantly down-regulated. On the contrary PTP4A1 was up-regulated in sarcoma and lymphoma (Table 1).

Taken together, our analysis reveals an absence of consistent trends in PTP4A1 mRNA expression, even within the same cancer type. While we observed a general up-regulation of PTP4A1 in the TCGA datasets, contrasting findings of extensive down-regulation were observed in the ONCOMINE datasets. These discordant results highlight the need for further research and validation to clarify the role of PTP4A1 in cancer and determine the factors contributing to the observed discrepancies across different datasets.

### 3.2. *Pan-cancer analysis of PTP4A2 expression*

The TCGA pan-cancer analysis revealed that PTP4A2 was significantly up-regulated across DLBC, ESCA, GBM, PAAD, SKCM, STAD and THYM (Fig. 1B) compared with that in normal tissues. Contrarily to the previous reports, no aberrant expression of PTP4A2 was found in breast and liver cancers. Oncomine analysis validated PTP4A2 up-regulation in ESCA and SKCM (Fig. 2 and Table 2). No differential PTP4A2 expression was discovered in PAAD, STAD and GBM. There is some inconsistency about PTP4A2 expression in DLBC. Specifically, 3 analyses showed PTP4A2 up-regulation while 2 analyses showed PTP4A2 down-regulation (Table 2). However, the sample size of these analyses is relatively small, so further studies are needed to determine PTP4A2 expression in DLBC.

As PTP4A2 up-regulation was reported in breast and liver cancer in literature but not revealed in GEPIA2 analysis, we examined its expression in Oncomine liver and breast cancer data sets in detail. Among 5 independent studies, only one study showed PTP4A2 down-regulation (Mas liver, $n = 19$ for normal tissue, $n = 38$ for cancer tissue) and the largest data set, Roessler Liver 2, which compared 220 normal Liver samples with 225 hepatocellular carcinoma cases did not identify significant difference about PTP4A2 expression. Similar pattern of PTP4A2 expression was observed in breast cancer. PTP4A2 up-regulation was revealed both in situ and invasive breast cancer in 4 analyses and PTP4A2 down-regulation was observed in other types of breast cancer (Table 2). However, in the largest breast cancer data set, Curtis Breast studies (2136

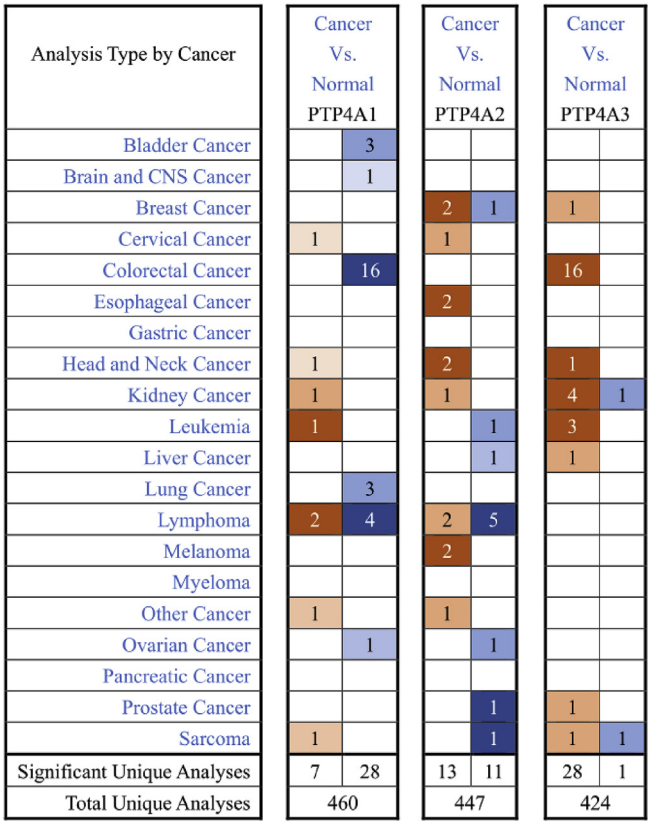



**Fig. 2.** Validation of aberrant PTP4As mRNA expression in ONCOMINE.
The numbers represent the analysis meeting the threshold: $p$ value $< 0.000\ 1$, Foldchange $>2$, gene rank in top 10%. The color of the cells reflects the direction of the PTP4As regulation with red for up-regulation and blue for down-regulation. The intensity of the color indicates gene rank percentile in the analysis.

samples) which contains normal breast ($n = 144$), invasive ductal and invasive Lobular breast carcinoma ($n = 90$), invasive ductal breast carcinoma ($n = 1556$), invasive lobular breast carcinoma ($n = 148$), medullary breast carcinoma ($n = 32$), mucinous breast carcinoma ($n = 46$), tubular breast carcinoma ($n = 67$), the foldchange of PTP4A2 mRNA are very mild compared to that in normal breast tissues.

Though there are new cancer types demonstrating aberrant PTP4A2 expression in Oncomine analysis including lung cancer (down-regulation), kidney (up-regulation) and head/neck cancer (up-regulation), larger scale of analysis was required to further validate these findings due to the limited sample size used.

In summary, our analysis indicates that PTP4A2 exhibits a general up-regulation across various cancers, particularly in esophageal, melanoma, and lymphoma. However, it is important to note that there is often inconsistency between different studies, even within the same type of cancer. These discrepancies underscore the complexity and heterogeneity of PTP4A2 regulation in different cancer contexts. Further investigations are warranted to elucidate the underlying mechanisms responsible for these variations and to validate the potential role of PTP4A2 as a biomarker or therapeutic target in specific cancer types.

### 3.3. Pan-cancer analysis of PTP4A3 expression

While TCGA pan-cancer data revealed up-regulation of PTP4A3 only in KIRC, LIHC, THYM (Fig. 1C) and down-regulation in CESC, LAML and STAD (Fig. 1E). Oncomine analysis exhibited its up-regulation across most cancer types (Fig. 2 and Table 3). In particular, significant PTP4A3 up-regulation was found in colorectal, kidney, B-cell acute lymphoblastic leukemia and prostate cancer and down-regulation of PTP4A3 was identified in sarcoma though with limited sample size. Although PTP4A3 up-regulation was also revealed in 2 independent breast cancer studies in Oncomine (Table 3), the sample size of the studies is really small. The analysis of the largest breast cancer data set, Curtis Breast studies (2136 samples), showed trend of PTP4A3 up-regulation in certain types of breast cancers, but none of them meets the threshold with foldchange no less than 2.

Although LIHC showed PTP4A3 up-regulation in GEPIA2 analysis, only one out of 10 analyses (Chen Liver) exhibited significant PTP4A3 up-regulation in liver cancer *vs* normal tissues. The largest liver cancer data set in Oncomine, Roessler Liver 2, demonstrated a trend of PTP4A3 up-regulating but with the foldchange <2. In addition, no PTP4A3 dysregulation was found in CESC, LAML and STAD in Oncomine, which was not consistent with GEPIA2 analysis.

While PTP4A3 up-regulation was reported in lung cancer, AML and ovarian cancers in previous publications, no aberrant PTP4A3 expression was found in lung and ovarian cancer and AML in Oncomine data sets (Fig. 2).

In summary, our analysis reveals that PTP4A3 displays widespread and significant up-regulation across various cancer types, with notable emphasis on colorectal, kidney, prostate cancers, and B-cell acute lymphoblastic leukemia (B-ALL). These findings suggest that PTP4A3 may play a crucial role in the pathogenesis of these cancers.

### 3.4. Proteomic data analysis revealed discrepant PTP4As protein expression in cancers

The differential gene analysis in GEPIA2 and Oncomine was performed at mRNA level, which is based on the assumption that mRNA abundance can reflect the expression and function of the corresponding proteins. However, recent large scale proteomic and transcriptomic profiling experiments clearly demonstrated that the correlation between mRNA and protein is not very strong with a squared Pearson correlation coefficient of ~0.4.[31] As protein is the final product of gene expression that connects genotype to phenotype, it is important to examine PTP4As expression at protein level.

We then compared differential PTP4As expression at mRNA or protein level using the PTP4As proteomic data available in a subset of tumors in CPTAC, including breast, colon, KIRC, UCEC and LUAD cancers. Similar to mRNA expression, PTP4A1 protein is significantly down-regulated in colon cancer and KIRC (Fig. 3A). However, dysregulation of PTP4A1 protein expression in BRCA, UCEC and LUAD is detected

**Table 1**
New aberrant PTP4A1 expression identified in ONCOMINE.

| Cancer type | Dataset | Tumor (cases) | Normal tissue(cases) | Foldchange | $p$ value ($T$-test) |
|---|---|---|---|---|---|
| Lymphoma | Choi | Acute Adult T-Cell Leukemia/Lymphoma (22) | $CD4^+$ T-Lymphocyte (6) | 2.17 | 3.90E-5 |
| | Choi | Chronic Adult T-Cell Leukemia/Lymphoma (19) | $CD4^+$ T-Lymphocyte (6) | 2.31 | 4.81E-4 |
| | Basso | Primary Effusion Lymphoma (9) | B-Lymphocyte (10) | 2.12 | 8.84E-5 |
| Sarcoma | Barretina | Leiomyosarcoma (26) | Adipose Tissue (9) | 2.28 | 5.73E-8 |
| | Detwiller | Leiomyosarcoma (6) | Normal Tissue (15) | 2.53 | 0.002 |
| Bladder | Sanchez-Carbayo | Superficial Bladder Cancer (28) | Bladder (48) | −3.48 | 9.88E-19 |
| | Sanchez-Carbayo | Infiltrating Bladder Urothelial Carcinoma (81) | Bladder (48) | −2.23 | 5.35E-14 |
| | Lee | Superficial Bladder Cancer (126) | Bladder Mucosa (68) | −2.06 | 1.40E-12 |

**Table 2**
Aberrant PTP4A2 expression in ONCOMINE.

| Cancer type | Dataset | Tumor (cases) | Normal tissue (cases) | Foldchange | *p* value (*T*-test) |
|---|---|---|---|---|---|
| Lung | Wachi | Squamous Cell Lung Carcinoma (5) | Lung (5) | −2.143 | 1.59E-4 |
| Esophageal Cancer | Garber | Squamous Cell Lung Carcinoma (13) | Lung (6) | −2.397 | 1.00E-3 |
| | Wang | Esophageal Adenocarcinoma (9) | Esophagus (24) | 3.366 | 3.51E-7 |
| | Wang | Barrett's Esophagus (19) | Esophagus (24) | 2.524 | 4.17E-6 |
| | Kimchi | Esophageal Adenocarcinoma (8) | Esophagus (24) | 3.924 | 2.93E-4 |
| Kidney | Jones | Renal Pelvis Urothelial Carcinoma (8) | Kidney (23) | 2.703 | 1.16E-10 |
| | Yusenko | Renal Wilms Tumor (4) | Kidney (23) | 2.158 | 4.00E-3 |
| Melanoma | Talantov | Benign Melanocytic Skin Nevus (18) | Skin (7) | 2.672 | 2.95E-8 |
| | Talantov | Cutaneous Melanoma (45) | Skin (7) | 4.025 | 1.74E-11 |
| Head and Neck | Pyeon | Oropharyngeal Carcinoma (6) | Tonsil (4) | 2.186 | 5.16E-7 |
| | Pyeon | Floor of the Mouth Carcinoma (5) | Oral Cavity (9) | 3.016 | 3.53E-5 |
| | Pyeon | Oral Cavity Carcinoma (4) | Oral Cavity (9) | 2.129 | 6.00E-3 |
| Breast | Radvanyi | Invasive Ductal Breast Carcinoma (25) | Breast (5) | 2.967 | 5.39E-4 |
| | Ma | Invasive Ductal Breast Carcinoma (9) | Breast (14) | 2.449 | 5.05E-5 |
| | Ma | Ductal Breast Carcinoma in Situ (9) | Breast (14) | 2.431 | 1.7E-4 |
| | TCGA | Mixed Lobular and Ductal Breast Carcinoma (7) | Breast (61) | 2.206 | 8.55E-5 |
| | TCGA | Mucinous Breast Carcinoma (4) | Breast (61) | −2.842 | 5.76E-4 |
| | Finak | Invasive Breast Carcinoma (53) | Breast (6) | −9.714 | 1.87E-22 |
| Lymphoma | Compagno | Germinal Center B-Cell-Like Diffuse Large B-Cell Lymphoma (9) | B-Lymphocyte (15) | −2.544 | 8.81E-7 |
| | Compagno | Diffuse Large B-Cell Lymphoma (44) Germinal Center B-Cell-Like | B-Lymphocyte (15) | −2.015 | 1.11E-11 |
| | Alizadeh | Diffuse Large B-Cell Lymphoma (34) | B-Lymphocyte (21) | 4.249 | 6.51E-8 |
| Lymphoma | Rosenwald | Diffuse Large B-Cell Lymphoma (38) | B-Lymphocyte (8) | 3.108 | 4.00E-3 |
| | Alizadeh | Follicular Lymphoma (9) | B-Lymphocyte (21) | 3.082 | 6.53E-5 |
| | Rosenwald | Follicular Lymphoma (7) | B-Lymphocyte (8) | 2.801 | 2.00E-3 |
| | Basso | Centroblastic Lymphoma (28) | B-Lymphocyte (15) | −2.387 | 7.17E-16 |
| | Eckerle | Primary Cutaneous Anaplastic Large Cell Lymphoma (7) | T-Lymphocyte and Natural Killer Cell (41) | −2.028 | 9.88E-19 |
| | Eckerle | Classical Hodgkin's Lymphoma (4) | T-Lymphocyte (31) | −2.380 | 4.00E-3 |
| | Brune | Hodgkin's Lymphoma (12) | B-Lymphocyte (10) | −2.275 | 2.20E-6 |

**Table 3**
Aberrant PTP4A3 expression in ONCOMINE.

| Cancer type | Dataset | Tumor (cases) | Normal tissue (cases) | Foldchange | *p* value (*T*-test) |
|---|---|---|---|---|---|
| Breast | Turashvili | Invasive Ductal Breast Carcinoma (5) | Ductal Breast Cell (10) | 2.441 | 2.00E-3 |
| | Richardson 2 | Ductal Breast Carcinoma (40) | Breast (7) | 2.288 | 2.81E-6 |
| Colorectal | Skrzypczak 2 | Colon Carcinoma (5) | Colon (10) | 7.712 | 6.64E-12 |
| | Skrzypczak 2 | Colon Carcinoma Epithelia (5) | Colon (10) | 9.765 | 2.91E-11 |
| | Skrzypczak 2 | Colon Adenoma Epithelia (5) | Colon (10) | 10.291 | 2.61E-7 |
| | Skrzypczak 2 | Colon Adenoma (5) | Colon (10) | 4.932 | 2.29E-6 |
| | Sabates-Bellver | Colorectal Carcinoma (36) | Colorectal Tissue (24) | 10.632 | 1.34E-18 |
| | Sabates-Bellver | Colorectal Adenocarcinoma (45) | Colorectal Tissue (24) | 2.786 | 2.74E-13 |
| | Skrzypczak | colorectal Carcinoma (36) | Colorectal Tissue (24) | 2.732 | 3.62E-13 |
| | Skrzypczak | Colorectal Adenocarcinoma (45) | Colorectal Tissue (24) | 2.786 | 5.74E-13 |
| | TCGA | Colon Adenocarcinoma (101) | Colon (19) | 2.832 | 1.02E-25 |
| | TCGA | Rectal Adenocarcinoma (60) | Colon (19), Rectum (3) | 2.904 | 2.18E-19 |
| | TCGA | Cecum Adenocarcinoma (22) | Colon (19), Rectum (3) | 2.354 | 1.56E-8 |
| | Gaedcke | Rectal Adenocarcinoma (65) | Rectum (65) | 4.472 | 6.55E-32 |
| | Kaiser | Cecum Adenocarcinoma (17) | Colon (5) | 2.708 | 3.83E-7 |
| | Kaiser | Colon Mucinous Adenocarcinoma (13) | Colon (5) | 2.418 | 2.45E-6 |
| | Kaiser | Rectosigmoid Adenocarcinoma (10) | Colon (5) | 2.600 | 1.69E-5 |
| | Kaiser | Rectal Adenocarcinoma (8) | Colon (5) | 2.455 | 1.45E-4 |
| | Hong | Colorectal Carcinoma (70) | Colon (12) | 3.209 | 7.51E-11 |
| Kidney | Yesenko | Renal Oncocytoma (4) | Kidney (5) | 3.035 | 1.63E-5 |
| | Yesenko | Clear Cell Renal Cell Carcinoma (26) | Kidney (5) | 2.784 | 4.90E-7 |
| | Yesenko | Chromophobe Renal Cell Carcinoma (4) | Kidney (5) | 2.645 | 7.94E-4 |
| | Beroukhim | Non-Hereditary Clear Cell Renal Cell Carcinoma (27) | Renal (11) | 2.433 | 1.58E-9 |
| | Beroukhim | Hereditary Clear Cell Renal CellCarcinoma (32) | Renal (11) | 2.064 | 3.80E-7 |
| Luekemia | Maia | B-Cell Acute Lymphoblastic Leukemia (20) | Immature B-Lymphocyte (2) Pre-B Lymphocyte (5) | 4.243 | 2.45E-11 |
| | Haferlach | B-Cell Childhood Acute Lymphoblastic Leukemia (359) | Peripheral Blood Mononuclear Cell (74) | 2.609 | 2.18E-66 |
| | Haferlach | B-Cell Acute Lymphoblastic Leukemia (147) | Peripheral Blood Mononuclear Cell (74) | 2.933 | 2.72E-38 |
| Prostate | Arredouani | Prostate Carcinoma (13) | Prostate Gland (8) | 2.665 | 1.18E-4 |
| | Vanaja | Prostate Adenocarcinoma (32) | Prostate Gland (8) | 2.071 | 3.47E-5 |
| Sarcoma | Wallace | Prostate Adenocarcinoma (69) | Prostate Gland (20) | 2.309 | 9.41E-4 |
| | Cutcliffe | | Fetal Kidney (3) | | 6.20E-6 |
| | Cutcliffe | Clear Cell Sarcoma of the Kidney (14) | Fetal Kidney (3) | −5.282 | 6.20E-6 |
| | Detwiller | Synovial Sarcoma (4) | Normal Tissue (15) | −2.638 | 3.00E-3 |

despite the fact that no significant change of PTP4A1 mRNA level in cancer samples compared to that in adjacent normal samples (Fig. 3B).

CPTAC analysis suggested down-regulation of PTP4A2 protein in KIRC compared to that in adjacent normal tissues, consistent with mRNA

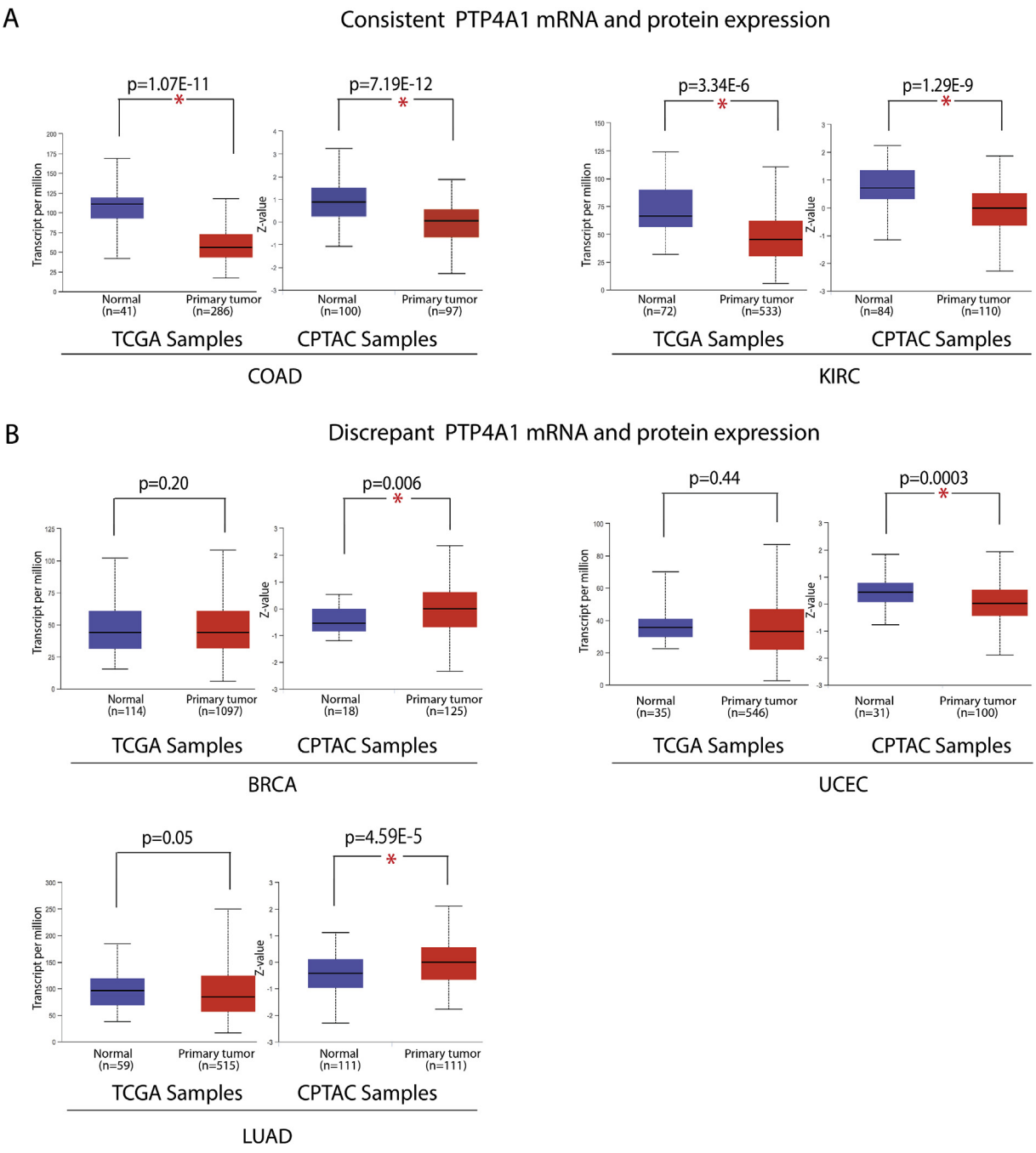



**Fig. 3.** Comparison of differential PTP4A1 expression at mRNA and protein level in cancers.
CPTAC data sets in UALCAN were used for differential protein expression analysis of PTP4A1, then compared with the differential mRNA expression of PTP4A1 in the corresponding TCGA datasets. A. Consistent protein and mRNA differential expression of PTP4A1 in COAD and KIRC. B. Discrepant protein and mRNA differential expression of PTP4A1 in BRCA, UCEC and LUAD.

alteration (Fig. 4A). However, the discrepancy between PTP4A2 mRNA and protein dysregulation was found in BRCA, COAD, UCEC and LUAD (Fig. 4B).

Particularly, there is no significant difference of PTP4A2 protein expression in BRCA and LUAD *vs*. normal tissues even though PTP4A2 mRNA is up-regulated in cancer samples. PTP4A2 protein showed a pronounced higher lever in cancer samples *vs.* normal tissues although PTP4A2 mRNA level is comparable in COAD. A bigger variance was observed in UCEC, where PTP4A2 mRNA was up-regulated while its protein expression was down-regulated in cancers *vs*. normal tissues.

Both mRNA and protein expression of PTP4A3 were found up-regulated in BRAC and KIRC cancers (Fig. 5A). However, PTP4A3 mRNA dysregulation is not translated into aberrant protein expression in COAD, UCEC and LUAD (Fig. 5B). Unexpectedly, PTP4A3 protein expression in COAD and LUAD is similar to that in normal tissues, notwithstanding the strong evidence of PTP4A3 mRNA up-regulation. In UCEC, the uncoupled mRNA and protein expression of PTP4A3 is toward another direction, where unchanged PTP4A3 mRNA in UCEC contrasts with decreased PTP4A3 protein level compared to that in normal tissues.

Collectively, our analysis of PTP4As expression at both the mRNA and protein levels highlights a lack of consistent correlation between mRNA expression and protein expression. These findings suggest the presence of additional regulatory mechanisms governing PTP4As protein expression beyond transcriptional regulation alone. Consequently, exploring the

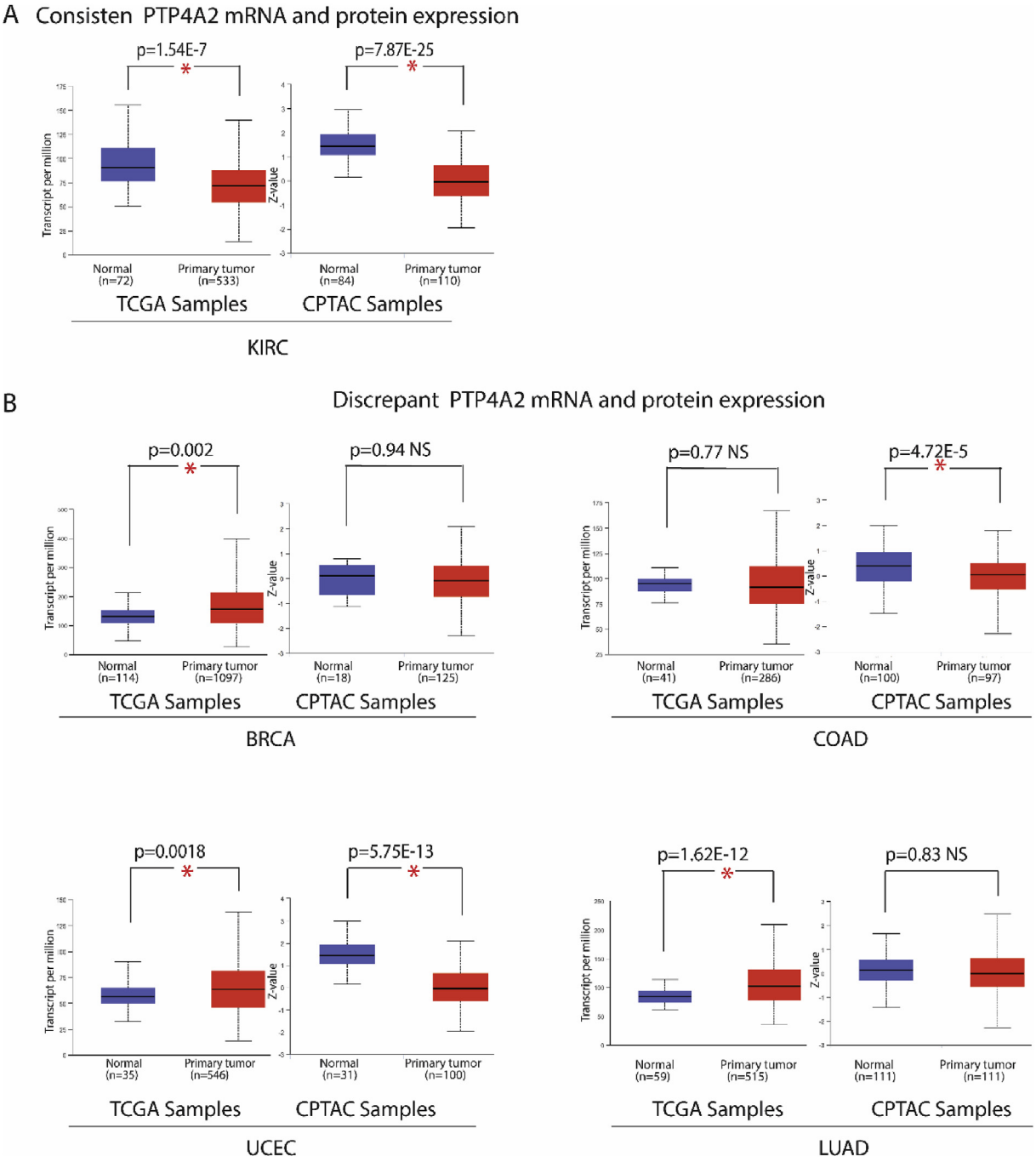



**Fig. 4.** Comparison of differential PTP4A2 expression at mRNA and protein level in cancers.
CPTAC data sets in UALCAN were used for differential protein expression analysis of PTP4A2, then compared with the differential mRNA expression of PTP4A2 in the corresponding TCGA datasets. A. Consistent protein and mRNA differential expression of PTP4A 2 in KIRC; B. Discrepant protein and mRNA differential expression of PTP4A2 in BRCA, COAD, UCEC and LUAD.

differential protein expression of PTP4As would provide a more robust approach to investigate their association with tumorigenesis and cancer progression. By examining protein expression, we can gain deeper insights into the functional relevance of PTP4As and their potential as biomarkers or therapeutic targets in cancer research.

### 3.5. Prognostic value of PTP4As in cancer

The Kaplan-Meier curve and log rank test analyses revealed that high PTP4As expression generally is an unfavorable marker for cancers. Specifically, high expression of PTP4A1 correlated with worse prognosis of CESC, ESCA, KIRC and PAAD but better prognosis of KIRC (Fig. 6A). High PTP4A2 expression suggested decreased survival of KIRC, LIHC, LUSC and SARC patients while increased survival of BCLA and THYM patients (Fig. 6B and D). High PTP4A3 expression associated with lower survival rate in KIRC, KIRP, LUSC, READ, TGCT and UCEC cancers (Fig. 6C) while higher survival rate in LUAD patients (Fig. 6E).

### 3.6. Genomic alteration of PTP4As in cancer

The cBioPortal was used to evaluate genetic alterations of PTP4As in TCGA cancers. The analysis revealed that the major contributor for

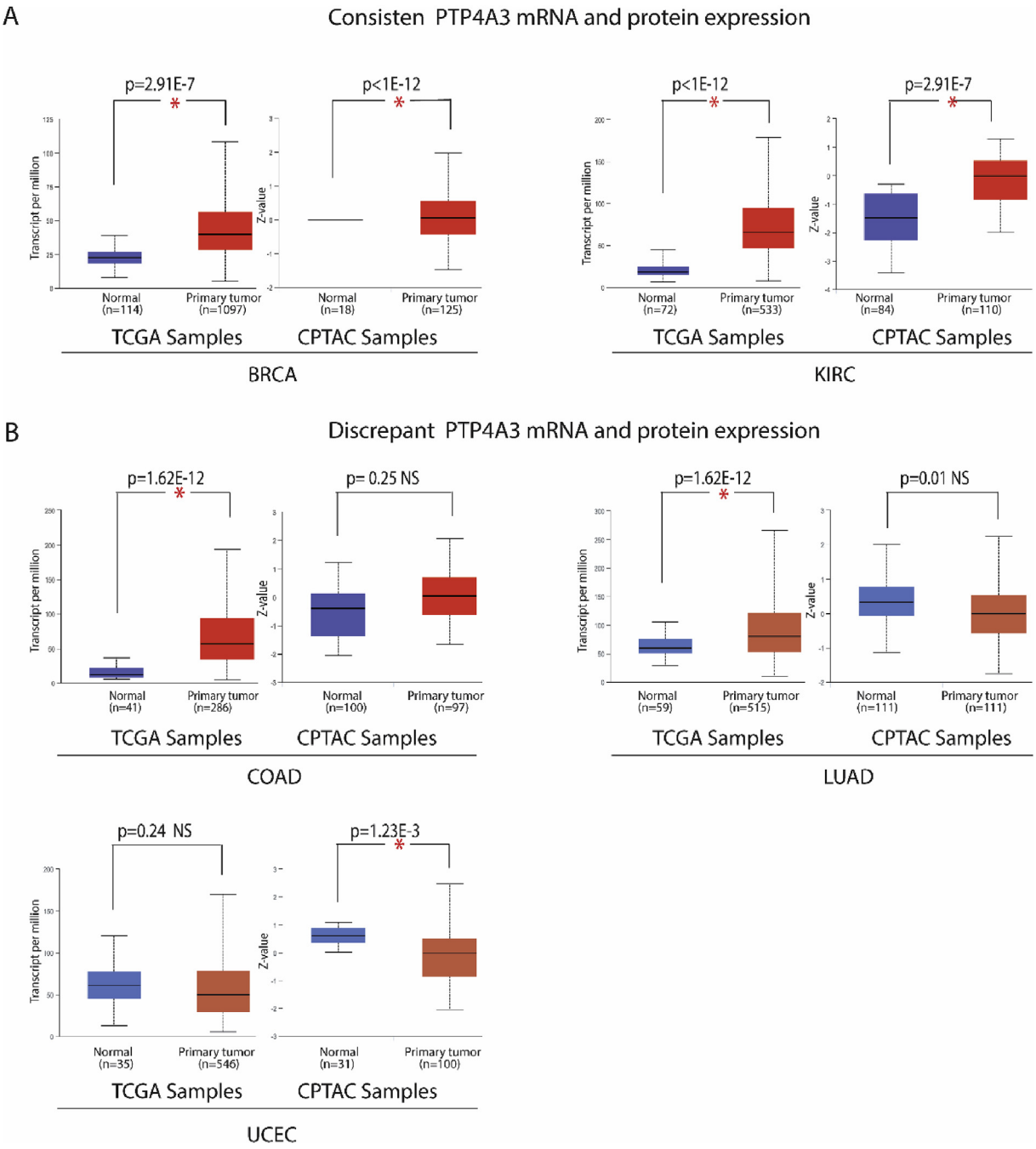



**Fig. 5.** Comparison of differential PTP4A3 expression at mRNA and protein level in cancers.
CPTAC data sets in UALCAN were used for differential protein expression analysis of PTP4A3, then compared with the differential mRNA expression of PTP4A3 in the corresponding TCGA datasets. A. Consistent protein and mRNA differential expression of PTP4A3 in BRCA and KIRC; B. Discrepant protein and mRNA differential expression of PTP4A3 in COAD, UCEC and LUAD.

PTP4A3 genetic alteration is amplification while for PTP4A1 and PTP4A2 deep deletion is also prevalent besides amplification in some cancer types (Fig. 7A–C). Notably, there is high inter-tumor heterogeneity of PTP4As genetic alteration frequency. Among 32 types of cancers examined, 8 types of cancers do not display any PTP4A1 genetic alterations, 10 types of cancers show no PTP4A2 genetic alterations and 4 types of cancers contain no PTP4A3 genetic alterations. High PTP4As genetic alteration revealed by the top 5 cancer types are: lung squamous, stomach, melanoma, liver and esophagus cancer for PTP4A1 (Fig. 7A); ovarian, PCPG, esophagus, uterine cancer and sarcoma for PTP4A2 (Fig. 7B); ovarian, uterine, esophagus, breast and liver cancer for PTP4A3 (Fig. 7C). In ovarian cancer, PTP4A3 genetic alteration rate is as high as 26%.

In order to explore how PTP4As genetic alteration contributes to their expression, we next plotted mRNA expression of PTP4As *vs.* genetic alteration across different cancer types (Fig. 7D–F). Not surprisingly, higher percentage of amplification of PTP4As are translated into higher mRNA expression, reflected by clustering of the red dots on the top part of the scatter plot within the same cancer type. Contrarily, the blue dots, representing samples with PTP4As deletion, clustered on the bottom of the plot within the same cancer type (Fig. 7D–F). In addition, the average PTP4A2 mRNA level in PCPG which showed the highest deep deletion

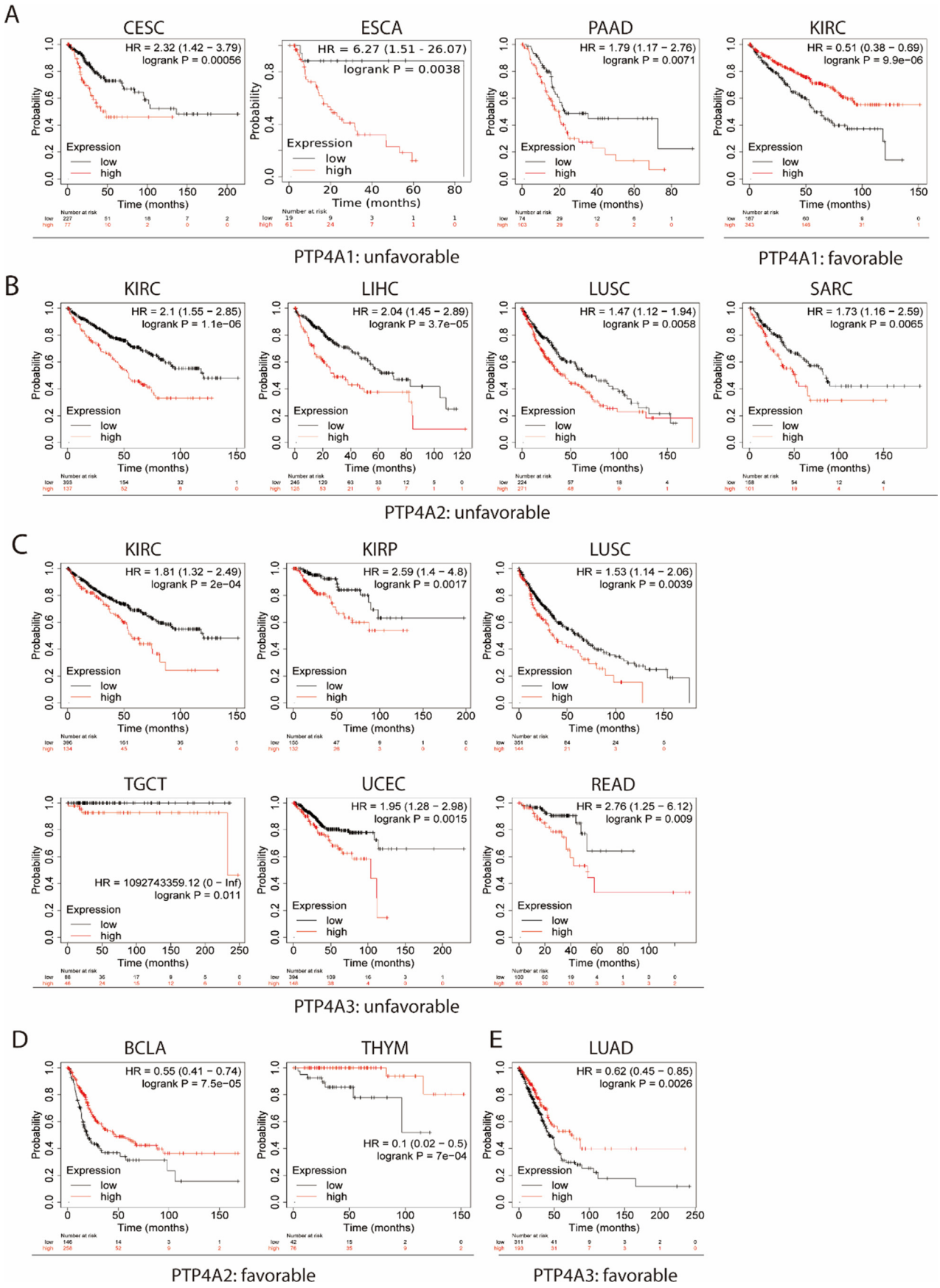



**Fig. 6.** Prognostic value of PTP4As in cancers.
The lower quartile and upper quartile of PTP4As expressing tumors samples were used for determining their prognostic values: A. PTP4A1; B. and D. PTP4A2; C. and E. PTP4A3. $p$ value $< 0.01$ was considered significant.

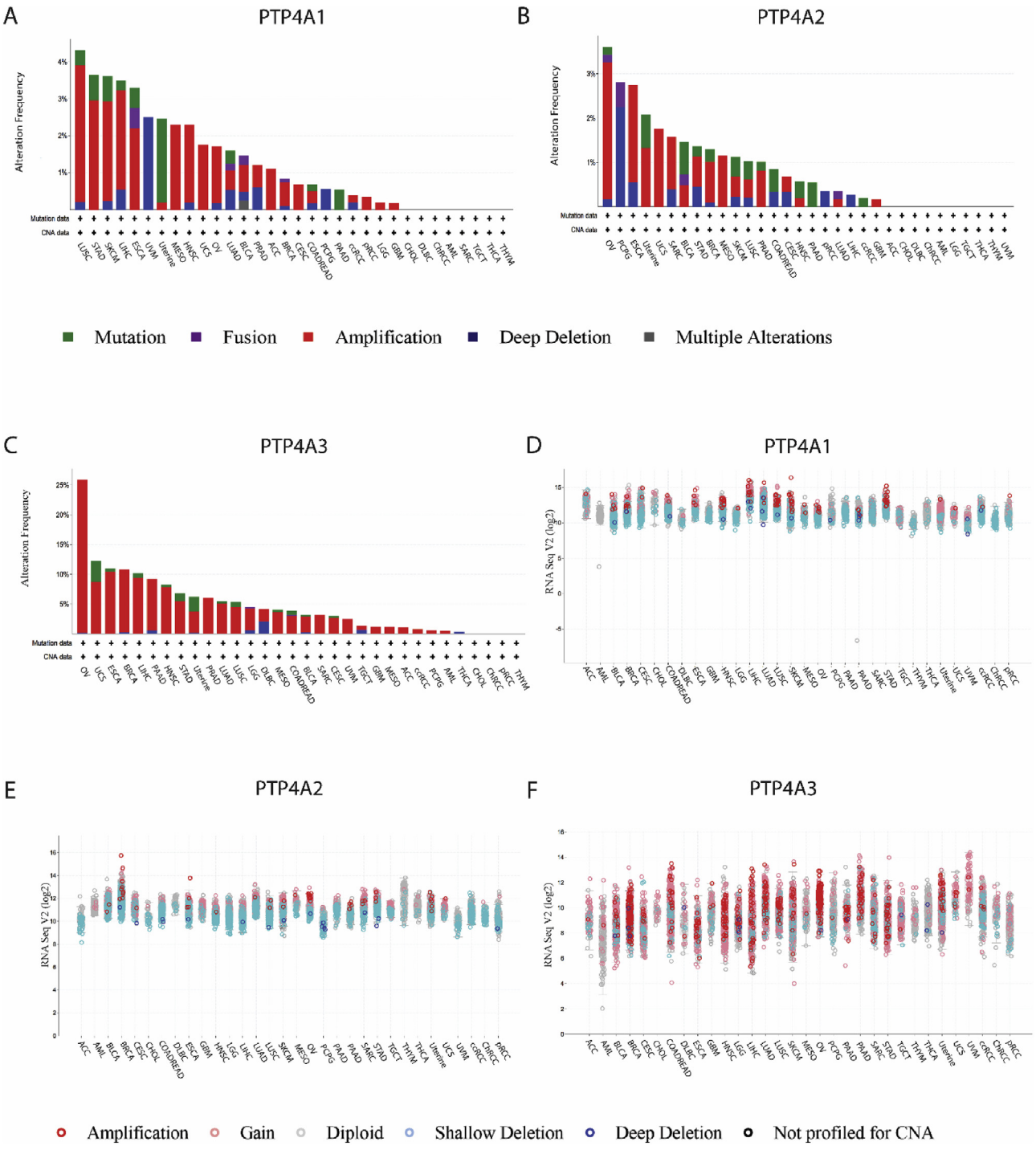



**Fig. 7.** Genetic alterations of PTP4As in cancers.
Genetic alterations of PTP4As were analyzed using cBioportal. The pan-cancer genetic alteration frequency of PTP4As. The profiling of different genetic alterations of PRLs across various cancer types (A-C). The relevance between genetic alterations and mRNA expression suggested that genetic alterations were largely correlated to the mRNA expression (D-F).

rate, is much lower compared with that in ovarian cancer, which demonstrated the highest amplification rate for PTP4A2 (Fig. 7E). Noticeably, there are also high rate of shallow deletions (normally heterozygous deletion) and gains (a few additional copies) of PTP4As but it seems those alterations do not affect mRNA expression.

However, PTP4As amplification or deletion is not always translated into mRNA expression. For example, melanoma exhibited the highest PTP4A3 mRNA expression level despite its amplification rate (2.5%) is much lower compared with that in ovarian cancer (25.68%) and breast cancer (10.52%) (Fig. 7C and F).

### 3.7. Pathways related to PTP4As expression in cancers

In order to understand the molecular mechanism related to expression of PTP4A2 and PTP4A3, the two PTP4As consistently up-regulated in cancers, we next examined the correlated genes to their expression.

For PTP4A2, we focused on melanoma, where PTP4A2 was consistently up-regulated. The top 200 genes positively associated with PTP4A2 mRNA expression level in melanoma was obtained from cBioportal (TCGA pan cancer atlas data set) and analyzed by PANTHER. As shown in Fig. 8A, the top significant pathways enriched includes T-cell or B-cell activation, Fas signaling and apoptosis signaling pathway and the genes over-represented in these pathways included CASP10, CFLAR, BIRC3, CDC42, PIK3CG, RPPRC, ITPR1, LCP2, CD80 and MAP3K2 (Fig. 8B).

Similar analysis was performed for examination of PTP4A3 correlated genes in KIRC. The top 200 genes were analyzed by PANTHER and major enriched pathways (Fig. 8D) included integrin signaling, Notch signaling and axon guidance mediated by netrin. The genes over-represented in these pathways contained DLL4, Notch3, VASP, UNC5B, NFATC4 and several collagen family proteins (Fig. 8C).

## 4. Discussion

PTP4As have been largely known for their oncogenic property. PTP4A1 was firstly identified in 1991 as one of the immediately up-regulated early genes in regenerating liver and also expressed in the course of liver regeneration. Sequence homology analysis identified a PTP signature motif in PTP4A1 *in vitro* analysis demonstrated its phosphatase activity toward an artificial substrate DiFMUP (6,8-Diflfluoro-4-Methylumbelliferyl Phosphate), establishing that it belongs to a new subfamily of PTPs, and therefore named as phosphatases of regenerating liver.[32] Later, PTP4A2 and PTP4A3 were determined as members of the PTP4As family based on a sequence homology search.[33] PTP4As share high degree of amino acid sequence similarity.

The first key study suggesting the oncogenic role of PTP4As was published in 2001 by Saha′s group when they examined the differential gene expression in colorectal human cancer samples from different stages *vs*. normal tissues, namely metastatic, primary, benign colorectal tumors and normal colorectal epithelium samples, PTP4A3 was identified constantly up-regulated in all the metastatic samples.[3] Subsequently extensive efforts have been put into characterizing the roles of PTP4As in cancers, including identifying their aberrant expression in cancer cell lines and human cancer samples *vs.* normal counterparts, determining their contribution to cancer initiation and progression by ectopic expression or knockdown of PTP4As, examining prognostic value of PTP4As and molecular pathways involved in tumor progression. As a result, the interest to explore PTP4As as new therapeutic cancer targets has been rising in the last two decades, leading to the development of several PTP4As inhibitors or antibodies.[34]

Due to the high cost and risks in drug discovery, it would be beneficial to confirm the roles of PTP4As in larger collection of cancer datasets before investing more resources and efforts to develop drugs targeting PTP4As. Therefore we harness the datasets deposited in multiple public cancer databases, including TCGA, Oncomine and cBioportal to perform a pan-cancer analysis of PTP4As. We found that PTP4A3 is extensively up-regulated across a variety of cancer types at mRNA level and shows relatively higher genetic alterations in cancers compared to PTP4A1 or PTP4A2. Although up-regulation of PTP4A2 was observed across cancer types, there was also frequent inconsistent reports even in same type of subtypes of cancers. The general trend of PTP4A1 mRNA expression is controversial in most cancer types and need further studies and/or analysis of current data. Our previous study in T-cell acute lymphoblastic leukemia (T-ALL) determined that both PTP4A3 and PTP4A2 mRNA were significantly up-regulated while PTP4A1 is down-regulated in T-ALL samples compared with healthy bone marrow.[15] Therefor it needs more evidence to relate aberrant PTP4A1 expression to tumor progression.

Nonetheless, the caveat here is that the mRNA expression of PTP4As is not well correlated with the protein expression when we compared proteomic data in CPTAC with RNA seq data in TCGA. Specifically, the high mRNA level of PTP4As is not always translated into high protein expression. On the other hand, aberrant protein expression of PTP4As were detected despite of the similar mRNA level. Since protein is the real workhorse that drives phenotype of biological systems, it is not solid enough to draw the conclusion based on aberrant mRNA expression of PTP4As. Currently, there are still very limited proteomic data in CPTAC, 57 studies focusing on several major cancer types and PTP4As protein data was missing in many of the studies. Further characterization of PTP4As protein expression in broader variety of cancers are needed to better understand their roles in cancer.

Genomic analysis revealed extremely low frequency of genetic alteration of PTP4A1 and PTP4A2 in cancer, with overall percentage of 1.4% and 1%, respectively. The top 5 cancer types exhibiting genomic alterations range within 3%–5% and 1%–4% for PTP4A1 and PTP4A2, respectively. Considering the more extensive abnormal expression of PTP4A1 and PTP4A2 at mRNA and protein level, it is likely the regulation of their expression occurs not only at the genomic level, but also at transcription and translational level. For PTP4A3, genetic alteration concurs at a much higher percentage, 6% for pan-cancer and 10–26% for top 5 cancer types and the major alteration type is amplification. However, high PTP4A3 amplification does not translate into up-regulated mRNA or protein expression, suggesting again the multiple level regulation of PTP4A3 expression.

Due to the oncogenic role of PTP4A3, molecular mechanism of PTP4A3 has been extensively studied. Integrin $\beta$1 and keratin 8, critical players in cell adhesion and migration regulation are reported PTP4A3 substrates in colon cancer cell models.[35,36] Other interacting protein of PTP4A3 includes integrin $\alpha$1,[37] stathmin,[38] CDH22,[38] ezrin,[39] elongation factor 2,[40] CNNM.[41] Most of the PTP4A3 substrates and interacting proteins are crucial regulator of cell adhesion and motility and the downstream signaling pathway affected includes p53, PTEN/PI3K/Akt, Src/ERK1/2, Rho family GTPases related pathways.[34]

However, most of these studies were performed in cell line models, the clinical relevance of these findings needs to be validated. Our current analysis of PTP4A3 correlated genes in large KIRC patient samples demonstrated the most significantly enriched pathway is integrin signaling, which is consistent with the finding in cell line models. A notable finding was that Notch signaling and netrin mediated axon guidance pathway were also enriched in this analysis. The Notch signaling is frequently activated in cancer and plays critical role in cancer progression, including cell proliferation, differentiation and survival,[42] but no previous studies have related PTP4A3 to Notch activation.

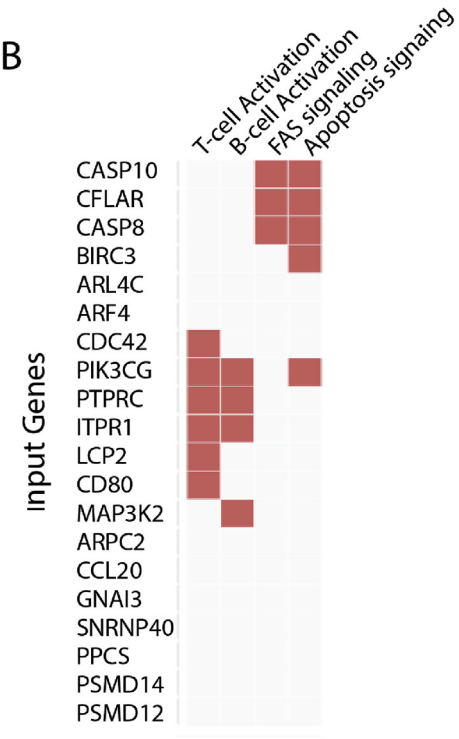
Netrins are a family of laminin-like proteins that were originally known as neuronal guidance molecules to regulate axonal growth and cell migration during development.[43] Later rising evidence suggest that they also critical cancer regulator. For example, netrin-1, the best well-characterized netrins, has been established as a key regulator to enhance cell invasion, migration, angiogenesis in various cancer models, including non-small cell lung cancer,[44] glioblastoma,[45] prostate.[46] However, there is no previous reports about the interaction between PTP4As and netrins or netrin pathway related proteins. So, the suggested interactions between PTP4A3 and Notch signaling or netrin in the current study would be an interesting direction for further study.

How PTP4A2 contributes to cancer progression is less well-defined compared with PTP4A3 and there are very limited studies providing deep insights about the signaling pathways affected by PTP4A2. PTP4A2 knockout in mice impaired its angiogenesis, which was likely via regulation of VEGF-A, DLL-4/NOTCH-1 signaling pathway.[47] In lung cancer, PTP4A2 is demonstrated to promote tumor cell migration and invasion via ERK signaling.[48] It is also reported that PTP4A2 could promote oncogenesis through a phosphatase independent mode, namely through the interaction CNNM3, the magnesium transporter, to regulate intracellular magnesium concentration.[49] Our current analysis implied that PTP4A2 might be involved in T-cell/B-cell activation signaling, Fas signaling and apoptosis signaling. Further mechanistic studies are required to elucidate the exact role of PTP4A2 in cancers.

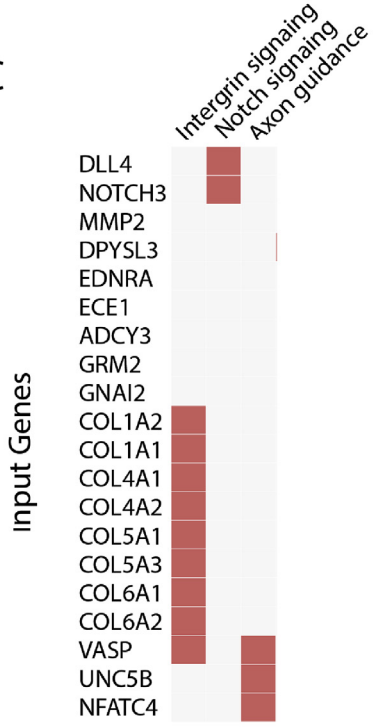
A

| Index | Term | p-value | q-value |
|---|---|---|---|
| 1 | T cell activation Homo sapiens P00053 | 0.000090 | 0.004977 |
| 2 | B cell activation Homo sapiens P00010 | 0.002534 | 0.049759 |
| 3 | FAS signaling pathway Homo sapiens P00020 | 0.003603 | 0.049759 |
| 4 | Apoptosis signaling pathway Homo sapiens P00006 | 0.003619 | 0.049759 |
| 5 | Cell cycle Homo sapiens P00013 | 0.010888 | 0.107451 |
| 6 | Inflammation mediated by chemokine and cytokine signaling pathway Homo sapiens P00031 | 0.011722 | 0.107451 |
| 7 | Axon guidance mediated by netrin Homo sapiens P00009 | 0.036031 | 0.247714 |
| 8 | Huntington disease Homo sapiens P00029 | 0.036031 | 0.247714 |
| 9 | mRNA splicing Homo sapiens P00058 | 0.049015 | 0.292634 |
| 10 | N-acetylgluosamine metabolism Homo Sapiens p02756 | 0.049015 | 0.292634 |

B




C




D

| Index | Term | p-value | q-value |
|---|---|---|---|
| 1 | Integrin signalling pathway Homo sapiens P00034 | 5.00E-07 | 0.000015 |
| 2 | Notch signaling pathway Homo sapiens P00045 | 0.000548882 | 0.008233225 |
| 3 | Axon guidance mediated by netrin Homo sapiens P00009 | 0.003277995 | 0.032779948 |
| 4 | Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway Homo sapiens P00026 | 0.009051064 | 0.067882979 |
| 5 | Alzheimer disease-presenilin pathway Homo sapiens P00004 | 0.017483341 | 0.104900046 |
| 6 | Metabotropic glutamate receptor group II pathway Homo sapiens P00040 | 0.036030988 | 0.168679316 |
| 7 | Endothelin signaling pathway Homo sapiens P00019 | 0.039358507 | 0.168679316 |

(caption on next page)

**Fig. 8.** PANTHER pathway enrichment analyses of co-expressed genes implies distinct roles of PTP4A3 and PTP4A2 in cancer.
The top 200 genes in melanoma positively associated with PTP4A2 mRNA expression (Spearman′s correlation ≥0.405, $p$ value ≤ 9.11E-16) was obtained from cBioportal and analyzed by PANTHER on Enrichr. The top 10 significant pathways are visualized using Appyters and displayed in (A) and genes overrepresented in the pathways with $q$ value < 0.05 was presented in (B). The top 200 genes in KIRC positively associated with PTP4A3 mRNA expression (Spearman′s correlation ≥0.521, $p$ value ≤ 7.0E-37) was obtained from cBioportal and analyzed by PANTHER on Enrichr. The 7 pathways with $p$ value < 0.05 are visualized using Appyters and displayed in (D) and genes overrepresented in the pathways with $q$ value < 0.05 was presented in (C). The $q$ value is an adjusted $p$ value calculated using the Benjamini-Hochberg method for correction for multiple hypotheses testing.

## Author contribution statement

YU Mingyang: Investigation, Visualization and Data curation; LIN Chunxu: Investigation, Visualization; WEI Min: Conceptualization, Writing- Reviewing and Editing.

## Conflict of interest

All authors have no conflict of interest to declare.

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## Abbreviations

| | |
|---|---|
| PTP | protein tyrosine phosphatases |
| PTP4A | protein tyrosine phosphatase 4A |
| PRL | phosphatase of regenerating liver |
| AML | acute myeloid leukemia |
| ALL | acute lymphoblastic leukemia |
| ACC | adrenocortical carcinoma |
| BLCA | bladder urothelial carcinoma |
| BRCA | breast invasive carcinoma |
| COAD | colon adenocarcinoma |
| ESCA | esophageal carcinoma |
| GBM | glioblastoma multiforme |
| HNSC | head and Neck squamous cell carcinoma |
| 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 |
| OV | ovarian serous cystadenocarcinoma |
| PRAD | prostate adenocarcinoma |
| READ | rectum adenocarcinoma |
| SKCM | skin cutaneous melanoma |
| STAD | stomach adenocarcinoma |
| TGCT | testicular germ cell tumors |
| THCA | thyroid carcinoma |
| THYM | thymoma |
| UCEC | uterine corpus endometrial carcinoma |
| UCS | uterine carcinosarcoma |

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