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Research article

A combined analysis of bulk and single-cell sequencing data reveals metabolic enzyme, pyruvate dehydrogenase E1 subunit beta (PDHB), as a prediction biomarker for the tumor immune response and immunotherapy

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ABSTRACT

Pyruvate dehydrogenase E1 subunit beta (PDHB) is located in mitochondria and catalyzes the conversion of glucose-derived acetyl-CoA. The detailed roles of PDHB in human cancers is unclear. Here, through comprehensive bioinformatics analysis, we found that PDHB was aberrantly expressed in multiple human cancers and is associated with patients' clinical stage. The abnormal expression of PDHB was related to the prognostic values of cancers, such as kidney renal clear cell carcinoma (KIRC) and kidney renal papillary cell carcinoma (KIRP). The Wanderer database with clinical data from Cancer Genome Atlas (TCGA) showed a significant correlation between PDHB expression and the pathologic stage of KIRP patients. We also evaluated the mutation profiles of PDHB in pan-cancer, and showed its roles on the patients' prognosis. At last, from several immunity algorithms, we demonstrated that the expression of PDHB was correlated with the infiltration of various immune cells in pan-cancer. Moreover, the aberrant PDHB had effects on the response to immune checkpoint inhibitors in cancer patients, such as anti-PD-1. Taken together, our study demonstrated the prognostic values of PDHB in pan-cancers. PDHB may be a potential molecular marker to predicting the immune response in cancer patients.

1. Introduction

In recent years, several high-throughput sequencing technologies have been developed to analyze the cancer genomics datasets. Exploring the novel cancer-associated biomarkers by performing pan-cancer analysis in cancer genomics databases has become promising research issues [1,2].

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Pyruvate dehydrogenase E1 subunit beta (PDHB) is a major subunit of pyruvate dehydrogenase (PDH) [3]. PDH can catalyze the decarboxylation of pyruvate and participating in the glycolytic pathway, thereby regulating cellular energy metabolism [4]. As an important subunit of PDH, PDHB may favorably alter the reprogramming of tumor metabolism [5]. Upon prolonged hypoxic condition, Eguchi et al. found the downregulated PDHB and increased mitophagy in cancer cells [6]. PDHB could be an oncogenic factor by tuning glycolytic metabolism, contributing to the malignant behaviors of breast cancer cells [5]. However, the derailed roles and underlying mechanisms of PDHB in pan-cancer has not been elucidated.

Here, we performed a pan-cancer analysis to explore the profiles of PDHB in human cancers. In addition to the expression profiles of PDHB, we also analyzed its prognostic values, mutation status, and underlying biological functions. This comprehensive analysis of PDHB in pan-cancer could provide a basis for the clarification of PDHB in the pathogenesis of cancers.

2. Materials and methods

2.1. Gene expression analysis based on pan-cancer database

The bioinformatics network resources, summarized in Table S1, were used to evaluate the detailed roles of PDHB in pan-cancer.

The analysis of PDHB expression between normal tissues and tumor tissues was completed by tumor immune estimation resource, version 2 (TIMER2.0) [7]. We also integrated the normal samples from GTEx with tumor sample from TCGA [8] to confirm PDHB expression in several tumors. UALCAN has also been used to analysis PDHB expression in pan-cancer [9]. At the same time, we downloaded and analyzed immunochemistry (IHC) images of PDHB in normal and tumor tissues from the Human Protein Atlas (HPA) [10]. We obtained the transcriptome microarray GSE781 [11] to further analyze PDHB expression in KIRC samples.

2.2. The effects of PDHB on patients' survival prognosis

GEPIA2 and Kaplan-Meier plotter were used to analyze the prognostic values of PDHB in cancer patients [12,13], including overall survival (OS), disease-free survival (DFS), First progression (FP) and Post-progression survival (PPS). The relationship between PDHB expression and survival status of patients with different cancers was analyzed by Log-rank test. The effects of PDHB expression on the clinical features of KIRC and KIRP patients were analyzed using Wanderer database [14]**.** UCSC Xena (http://xena.ucsc.edu) [15] has a variety of visualization and analytical capabilities to confirm the prognostic values of PDHB in patients with KIRC and KIRP.

2.3. Genetic alteration of PDHB in pan-cancer

We used the cBioPortal tool [16] to analyze the genetic changes of PDHB in pan-cancer. Gene alteration and mutation site of PDHB could be obtained from the "Oncoprint", "Cancer Types Summary" and "Mutations" modules in cBioPortal. At the same time, we also compared cancer survival data in all TCGA databases based on the genetic alteration of PDHB. The survival data, including OS, PFS and DFS, could be obtained from the "Comparison/Survival" module.

2.4. The effects of PDHB on immune response

We used "Diff Exp" module in TIMER2.0 database to analyze the relationship between PDHB expression and infiltration of various immune cells in pan-cancer tissues, including B cells, NK cells, macrophages, DC cells, $CD8^+$ T cells, monocytes and neutrophils. The TIMER, TIDE, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, MCPCOUNTER and EPIC algorithms are used for the estimation of their relationships. In addition, tumor immunotherapy gene expression resource (TIGER) [17] was used to analyze the effects of PDHB on the immunotherapeutic response.

2.5. Single cell sequencing analysis

CancerSEA [18], a single-cell sequencing database, can be used to provide different functional states of cancer cells at the single-cell level. Based on the expression profiles of PDHB, we re-analyzed single-cell sequencing data from CancerSEA to evaluate the tumor functional states. At the same time, t-SNE maps were obtained from CancerSEA directly to display the distribution of tumor cells at single-cell levels.

2.6. The functions enrichment analysis of PDHB

We constructed a protein-protein interaction network (PPI) using STRING [19]. We obtained the top 100 PDHB-related genes in the GEPIA2 database, and performed the Pearson analysis about the correlation between PDHB and the top six related genes by the "Gene-Corr" panel in TIMER2.0. Correlation coefficients (R) and p-values were calculated and displayed in the corresponding graph panels. To gain a more comprehensive understanding of the biological functions of PDHB, we visualized functional enrichment using the GSEA package in Xiantao Xueshu (https://www.xiantao.love/products).

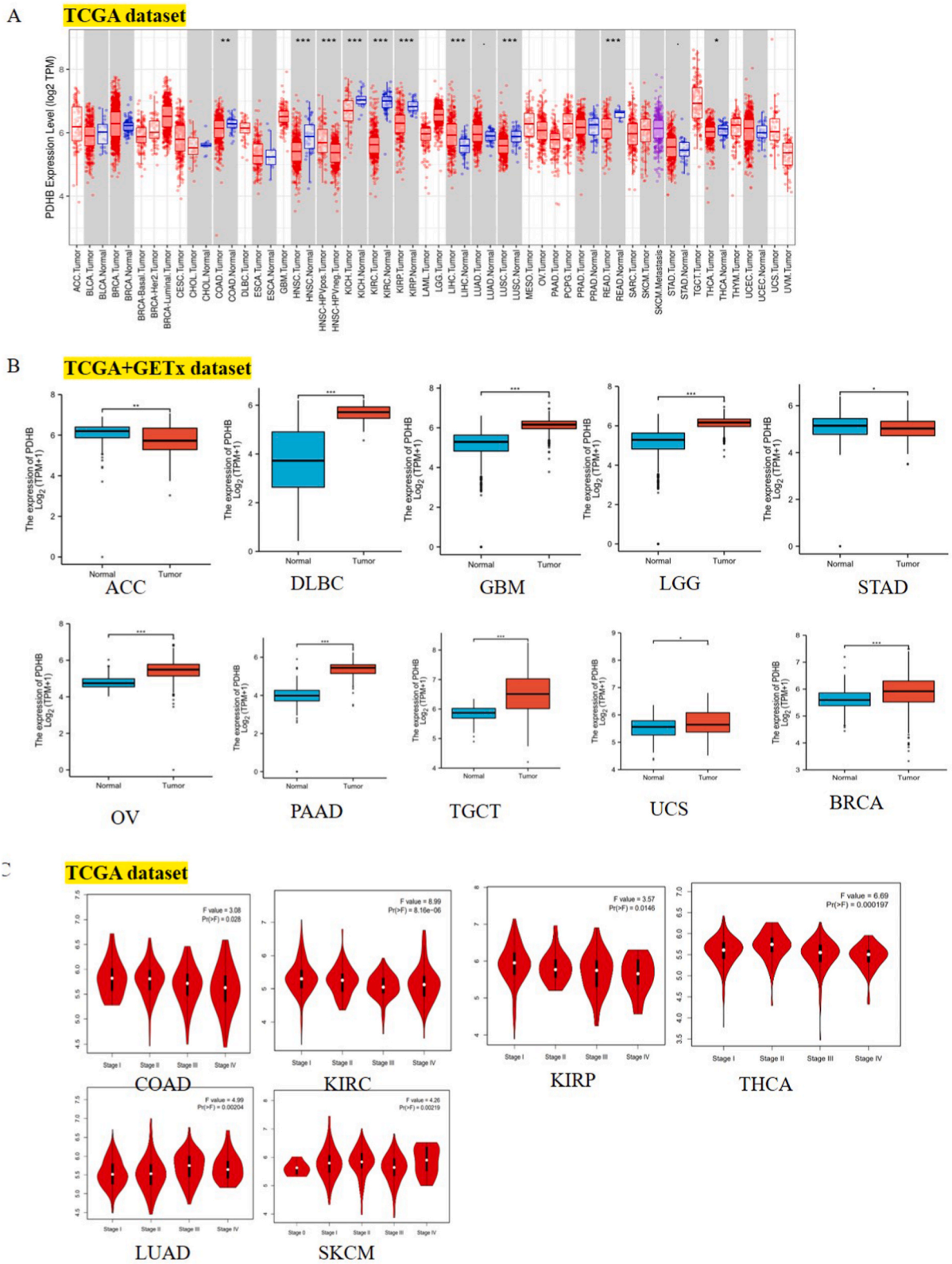

Fig. 1. Expression levels of PDHB in pan-cancer. (A) PDHB expression levels in different cancer types in the TIMER2.0 database. (B) Differential PDHB expression levels between TCGA cancer and GTEx normal tissues. (C) Expression levels of PDHB in major pathological stages of cancer. *$p <$ 0.05, **$p < 0.01$, and ***$p < 0.001$.

2.7. Statistical analysis

Wilcoxon rank sum test and Spearman rank test were used to examine the different expression and correlations between two groups, respectively. Survival outcomes were analyzed by Kaplan-Meier survival curves using the log-rank test. A Cox proportional hazards regression model was used to calculate the hazard ratio (HR). Meanwhile, ANOVA and K independent sample tests in the statistical software package SPSS 12.0 (IBM Analytics) were used to analyze the relationship between PDHB expression and clinic pathological features. p-value <0.05 was considered statistically significant.

3. Results

3.1. The expression profiles of PDHB in pan-cancer

Firstly, we explored PDHB expression in tumor and normal tissues using TIMER2.0. As shown in Fig. 1A, PDHB was lowly expressed in most cancers, such as COAD (Colon adenocarcinoma), HNSC (Head and Neck squamous cell carcinoma), KICH (Kidney Chromophobe), KIRC (Kidney renal clear cell carcinoma), KIRP (Kidney renal papillary cell carcinoma), LUSC (Lung squamous cell carcinoma), READ (Rectum adenocarcinoma) and THCA (Thyroid carcinoma). However, PDHB was highly expressed in LIHC (Liver hepatocellular carcinoma). Since several tumor types have no or very little normal control tissue, we combined TCGA and GETx databases to found that PDHB was highly expressed in Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (DLBC), Glioblastoma multiforme (GBM), Brain Lower Grade Glioma (LGG), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Testicular Germ Cell Tumors (TGCT), Uterine Carcinosarcoma (UCS), and Breast invasive carcinoma (BRCA). On the contrary, PDHB was lowly expressed in Adrenocortical carcinoma (ACC) and Stomach adenocarcinoma (STAD) (Fig. 1B). In addition, the pan-cancer analysis using UALCAN was used to further support the aberrantly expressed of PDHB in human cancers (Fig. S1). We also used GEPIA2 to analyze the relationship between PDHB expression and tumor pathological stage. The results showed that PDHB was negatively correlated with the pathological stage of COAD, KIRC, KIRP and THCA, and positively correlated with the pathological stage of LUAD and SKCM (Fig. 1C). However, in other tumor tissues, PDHB was not significantly correlated with the pathological stage (Fig. S2). Next,

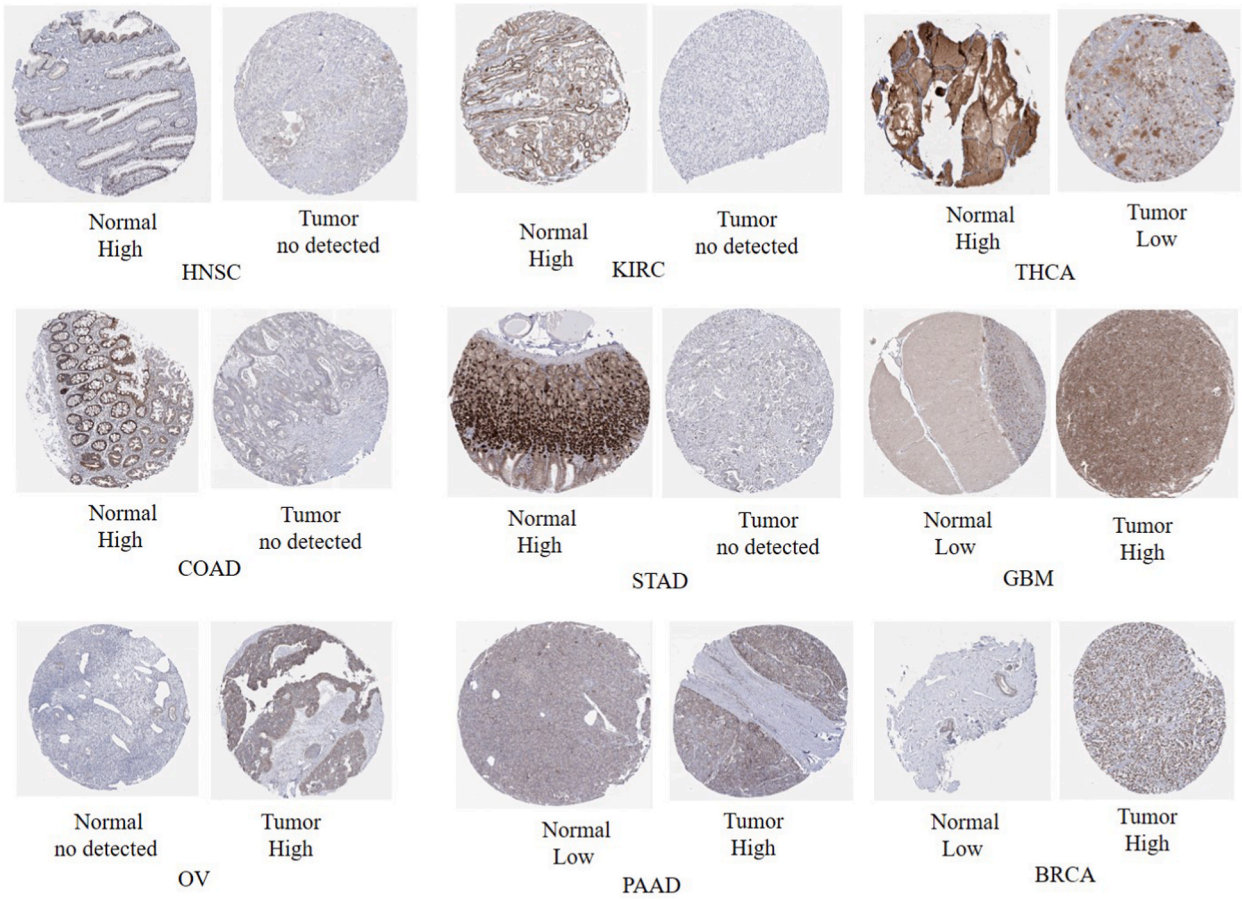

Fig. 2. Comparison of PDHB gene expression between immunohistochemistry in normal and tumor tissues.

we screened out a microarray dataset (GSE781) from the GEO database, and the results showed that the expression level of PDHB in KIRC was lower than that in normal kidney tissue (Fig. S3).

To gain a better clarification of PDHB expression at the protein level, we also analyzed the IHC results provided by the HPA database. The results showed that PDHB was moderately or highly expressed in GBM, OV, PAAD and BRCA, while lowly or negatively expressed in HNSC, KIRC, THCA, COAD and STAD (Fig. 2). Collectively, these findings suggested the downregulated PDHB at the transcription and protein levels in KIRC and THCA.

3.2. Prognostic values of PDHB in pan-cancer

We explored the association between PDHB expression and survival prognostic values in multiple cancers. The data from GEPIA2 indicated that aberrantly repressed PDHB mainly affected the survival prognosis in kidney cancer patients. The lower expression level of PDHB, the worse OS and DFS in patients with KIRC (Fig. 3A and B). Moreover, patients with low level expression of PDHB displayed unfavorable OS in patients with KIRP (Fig. 3A). Similarly, UCSC Xena showed that the KIRC and KIRP patients with high levels of PDHB displayed favorable OS (Fig. S4). In addition, the expression of PDHB was statistically significant with the pathological stage of KIRP patients ($P = 0.019$) (Table S2). Unfortunately, PDHB expression was not correlated with clinical features of KIRC patients (Table S3). Thus, the above-mentioned results demonstrated the downregulated PDHB showed poor prognosis in kidney cancer patients.

Next, we analyzed the survival data from Kaplan-Meier plotter. We found that highly expressed PDHB was positively correlated with OS, FP and PPS in patients with lung cancer and gastric cancer. And highly expressed PDHB was positively correlated with PPS, DMFS and RFS in patients with breast cancer. However, highly expressed PDHB was negatively correlated with OS, FP and PPS in ovarian cancer (Figure S5).

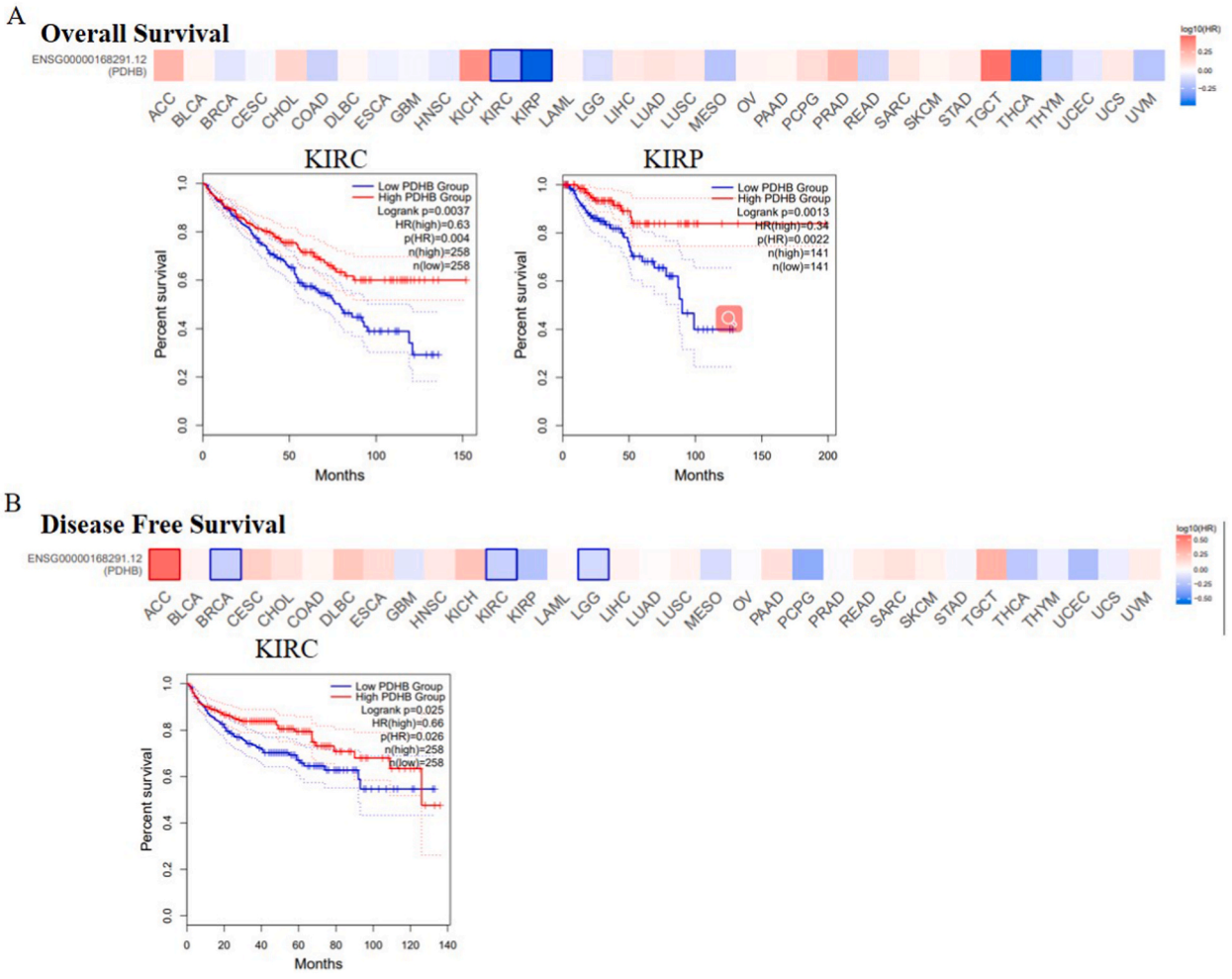

Fig. 3. Prognostic values of PDHB expression in pan-cancer. (A–B) Relationship between PDHB expression and overall survival and disease free survival in different cancers from GEPIA database.

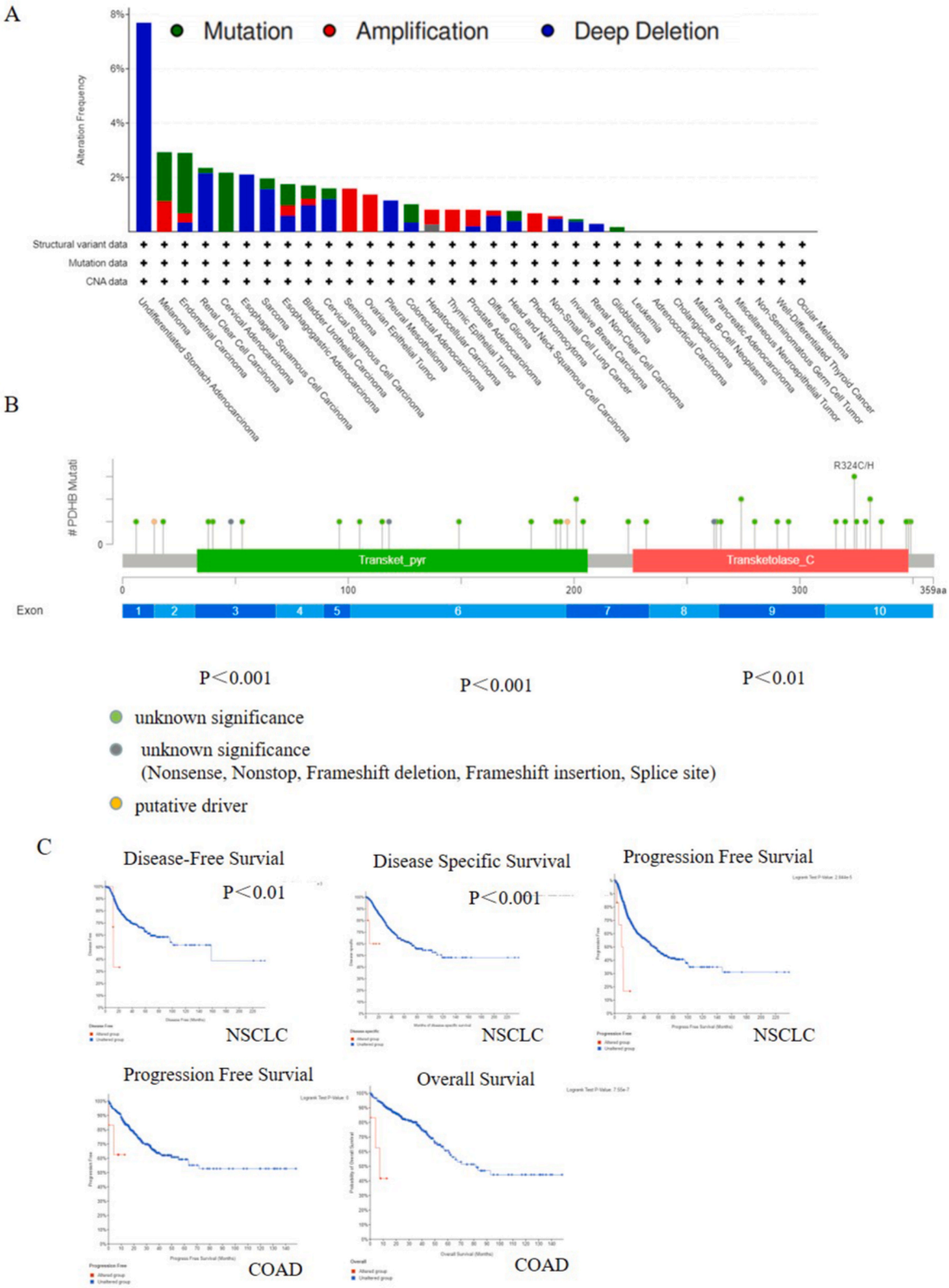

Fig. 4. Analysis of genetic alterations in PDHB in TCGA tumors by the cBioportal tools. (A) Summary of mutations in PDHB in the TCGA pan-cancer dataset. (B) The main mutation sites of PDHB genome alterations. (C) The mutation status of PDHB on the prognosis of patients with NSCLC and COAD.

3.3. Genetic alteration of PDHB in pan-cancer

Genetic alterations play an important role in the development of human malignant tumors [20]. In this case, we analyzed the genetic variation of PDHB in malignant tumors by cBioPortal database. Mutations and deep deletions were the main types of genetic

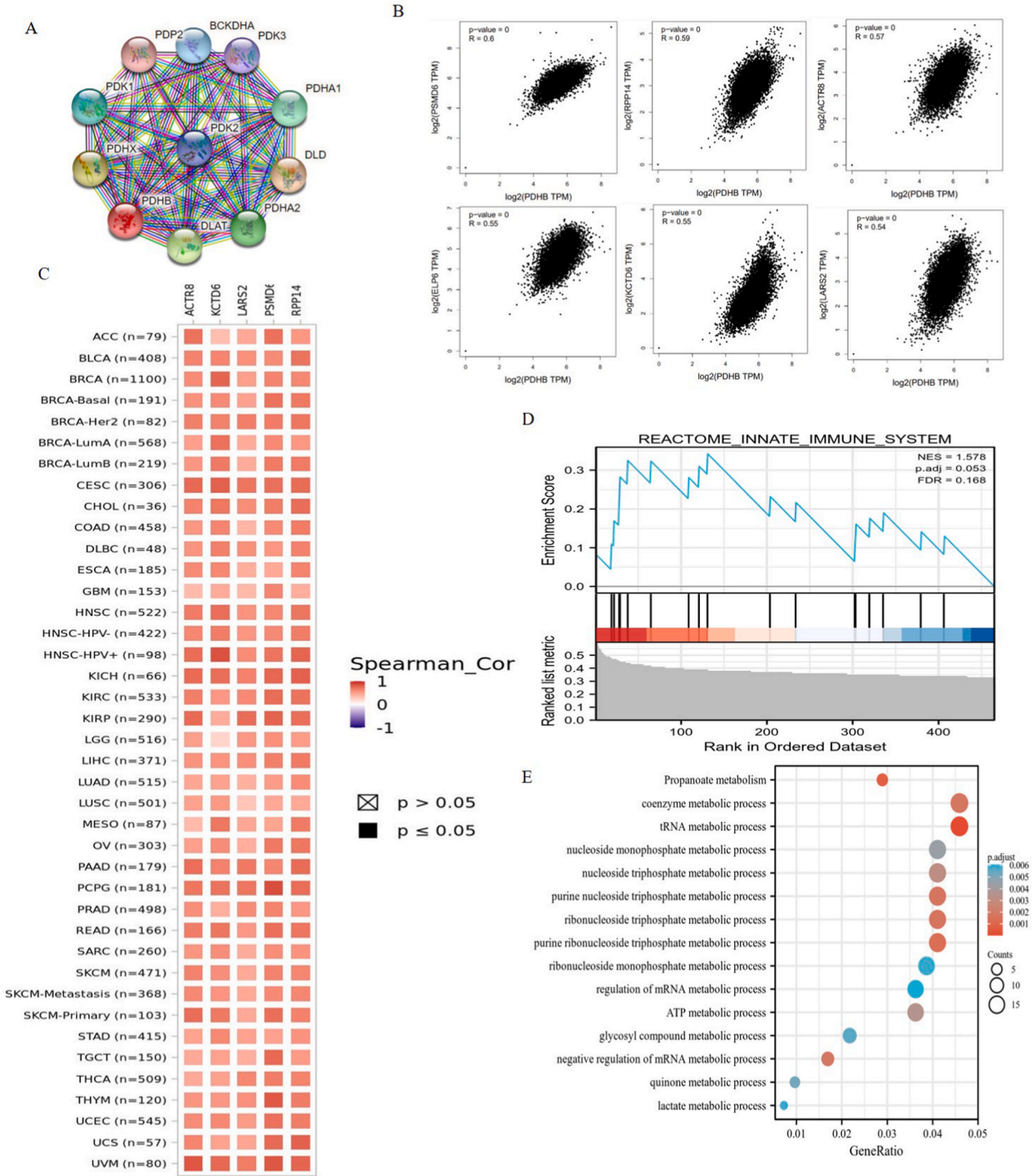

Fig. 5. Co-expression network and enrichment pathway analysis of PDHB. (A) PDHB interaction network from STRING website. (B) Expression correlation between PDHB-related genes (PSMD6, RPP14, ACTR8, ELP6, KCTD6 and LARS2) and PDHB in GEPIA2. (C) The heatmap indicated the positive expression correlation between PDHB-related genes (PSMD6, RPP14, ACTR8, ELP6, KCTD6 and LARS2) and PDHB in pan-cancer. (D) GO/KEGG functional enrichment analysis of PDHB-interacted molecules. (E) GSEA pathway analysis of PDHB-interacted molecules.

alterations in PDHB genome. The highest frequency (>5%) of PDHB changes in undifferentiated STAD was "deep deletion". Endometrial carcinoma and cervical adenocarcinoma had the highest rates (about 2%) with "mutation" as the predominant types (Fig. 4A). Subsequently, we further evaluated the mutation sites and prognostic values of PDHB genetic alteration. The mutation of PDHB, R324 C/H, in exon 10 could be the most frequent point mutation (Fig. 4B). We also studied the roles of PDHB genetic alteration on the clinical survival prognosis of patients. The NSCL patients with PDHB mutations displayed favorable DFS, DSS and PFS. In COAD, the patients with PDHB mutations had good PFS and OS (Fig. 4C).

3.4. Enrichment pathway analysis and co-expression network of PDHB

The molecular mechanism of PDHB in tumorigenesis is currently unclear. To better understand the underlying mechanisms of PDHB, we performed functional enrichment analysis of PDHB-associated genes in pan-cancers. Firstly, we predicted the functional interacting molecules of PDHB through the STRING website. The results showed that PDHB could bind to various molecules, such as PDK2, PDK1, BCKDHA, PDHA and PDHC (Fig. 5A). We then obtained the top 100 genes associated with PDHB by the GEPIA2. The expression of PDHB was positively correlated with PSMD6, RPP14, ACTR8, ELP6, KCTD6 and LARS2 (Fig. 5B). At the same time, the heatmap data showed that in most cancers, PDHB had a strong positive correlation with the five genes mentioned above (Fig. 5C). Next, we perform the functional enrichment analysis to evaluate the biological functions of PDHB in pan-cancers. As shown in Fig. 5D, GSEA functional enrichment indicated the promising roles of PDHB in the regulation of innate immune system. More importantly, GO/KEGG functional enrichment analysis showed that PDHB-associated molecules mainly participated in several metabolic processes, including coenzyme metabolic process, tRNA metabolic process, ribonucleoside triphosphate metabolic process, etc (Fig. 5E). These pathways suggested that aberrant PDHB could play an important role in tumor pathogenesis through regulating the metabolic and immune signaling.

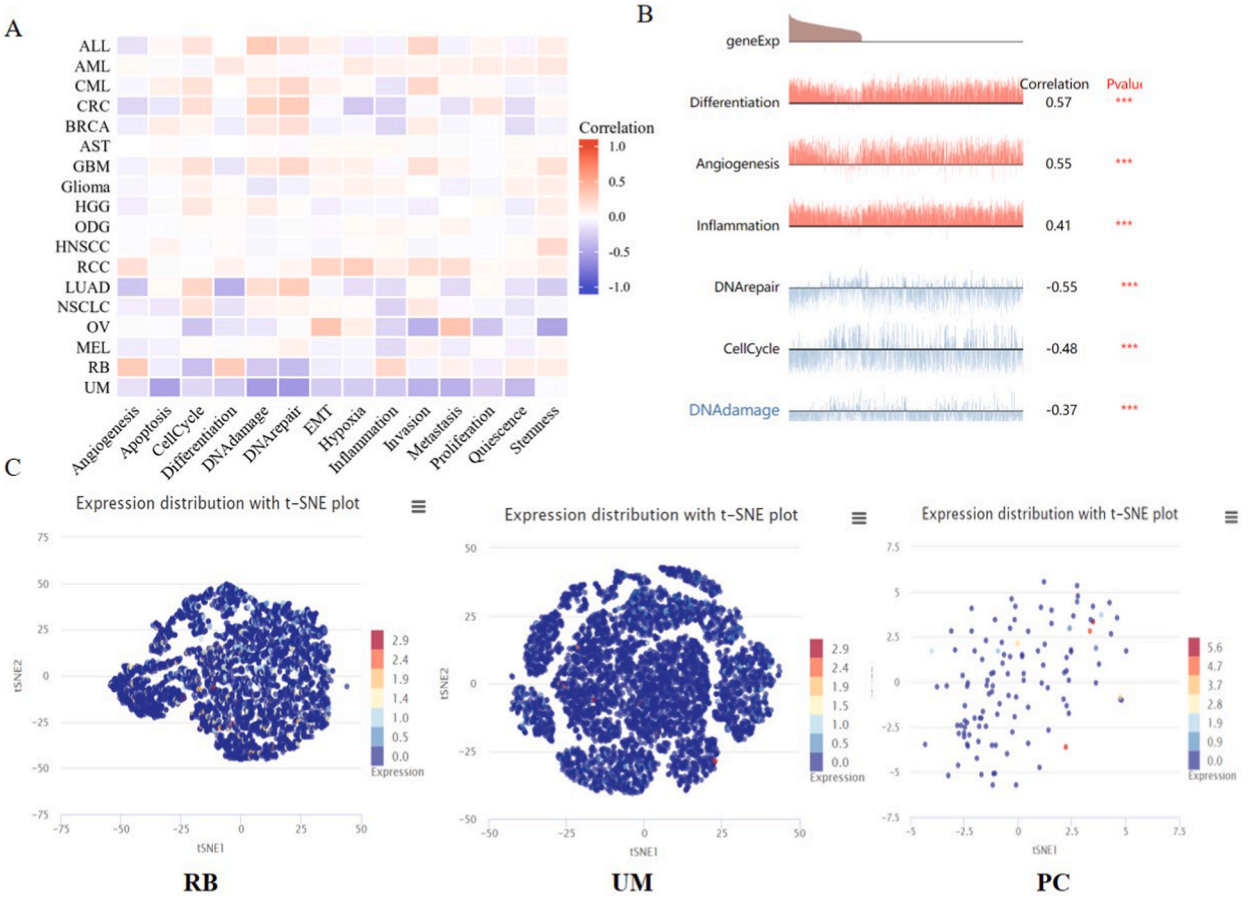

Fig. 6. Expression patterns of PDHB at single-cell level and their correlation with tumor functional status. (A) Correlation between PDHB expression and functional status of PDHB in different tumors in CancerSEA. (B) Correlation between PDHB expression and different functional states in RB. (C) T-NSE plots showed the single-cell expression distribution of PDHB in RB, UM, and PC samples, respectively.

3.5. Expression patterns of PDHB at single cells and its functional status

Single-cell transcriptome sequencing is a key technique for the analysis of cancer cells, immune cells, endothelial cells and stromal cells [21,22]. By using the CancerSEA database, we analyzed the relationship between PDHB expression and its functional status in different cancer single cells. For example, in most cancers, the expression of PDHB displayed negatively correlated with the inflammation (Fig. 6A). Fig. 6B showed that PDHB in RB was negatively correlated with DNA repair response and cell cycle, while positively correlated with differentiation, angiogenesis and inflammation. In addition, the single cell distribution of PDHB expression profiles in RB, UM and PC were displayed using T-SNE plots (Fig. 6C). Taken together, PDHB might play a key role in the development and progression of human cancers though regulating several pathways, especially like inflammatory response.

3.6. The roles of PDHB on the immune response in pan-cancer

Immune infiltration plays a crucial role in the tumor progression and treatment [23]. To investigate the roles of PDHB in the regulation of immune micro-environment, we performed a pan-cancer analysis of the correlation between PDHB expression and immune cell infiltration using the TIMER2.0 database. We applied various algorithms, including TIMER, CIBERSORT, CIBERSORT-ABS, TIDE, XCELL, MCPCOUNTER, QUANTISEQ and EPIC, to explore the correlation between different immune cells and PDHB expression. The results showed that PDHB expression was positively correlated with the estimated infiltration value of DC cells in PAAD. In addition, PDHB was positively correlated with the estimated infiltration value of macrophages in HNSC-HPV (−), PAAD,

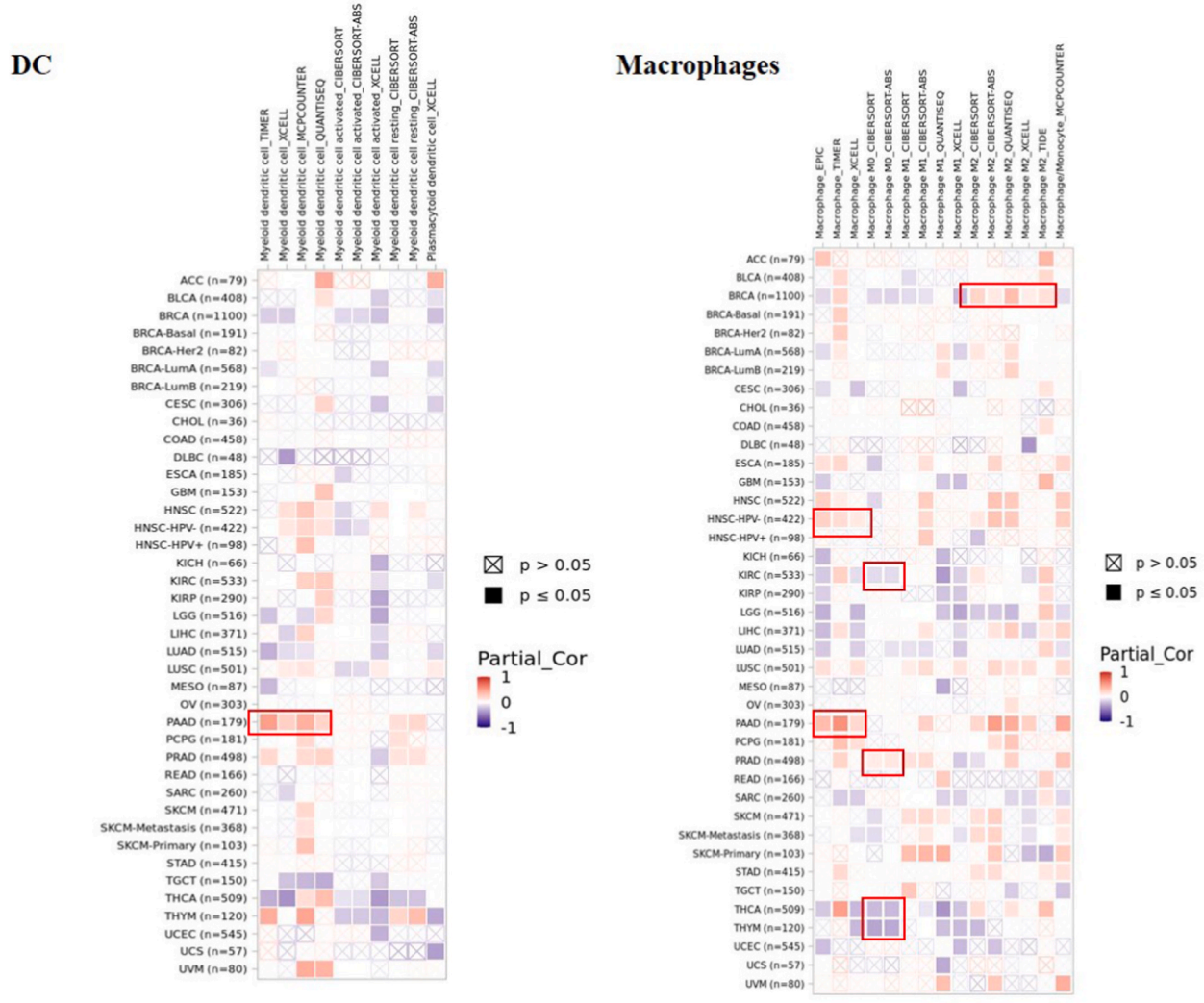

Fig. 7. The roles of PDHB expression level in the tumor infiltration of DC cells and macrophages. Several algorithms, such as TIMER, CIBERSORT, CIBERSORT-ABS, TIDE, XCELL, MCPCOUNTER, QUANTISEQ and EPIC, were used to analyze the relation between the infiltration of DC cells and macrophages and PDHB expression.

PRAD and BRCA. However, PDHB was negatively correlated with the infiltration of macrophages in KIRC, THCA and THYM (Fig. 7). In addition, aberrantly expressed PDHB did not affect the infiltration of other immune cells, such as $CD8^+$ T cells, B cells, NK cells, monocytes and neutrophils (Fig. S6).

3.7. The regulation of PDHB on immunotherapy response

TIGER platform was used to analyze the effect of PDHB on the immunotherapy response in pan-cancer patients. Fig. 8A shows that

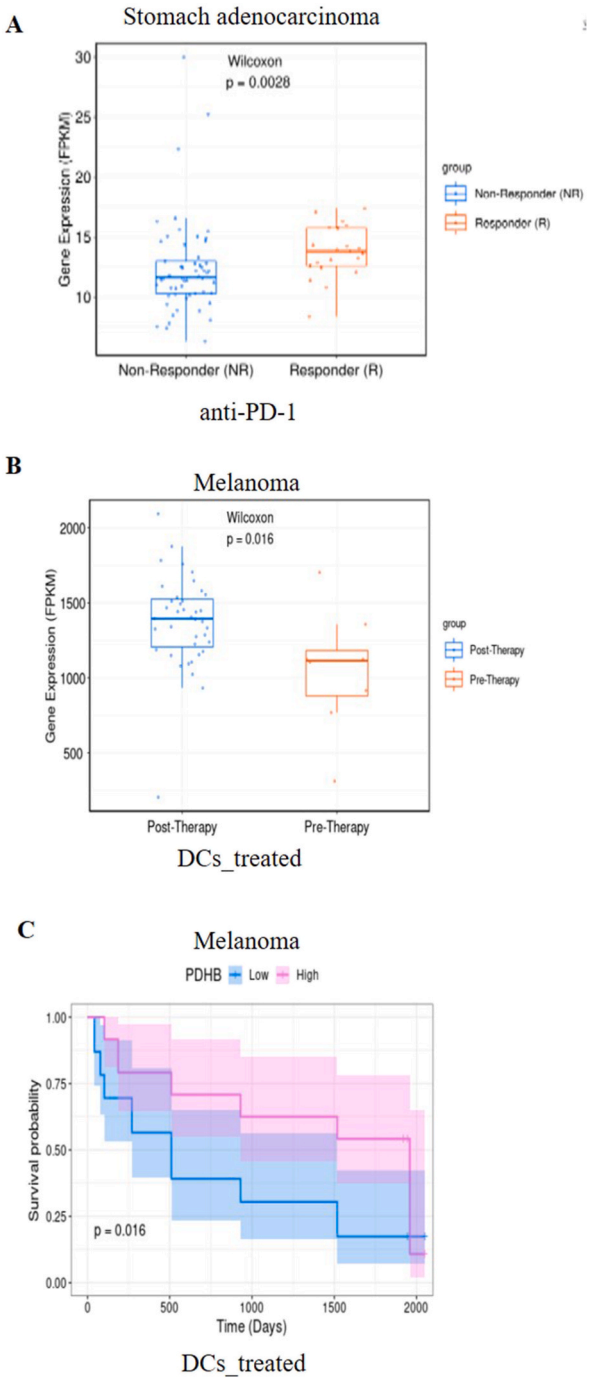

Fig. 8. Effect of PDHB on immunotherapy response in pan-cancer patients. (A) PDHB levels in anti-PD-1 response patients. (B) PDHB levels in patients with DCs immunotherapy. (C) Upon DCs immunotherapy, the riles of PDHB on the patients' survival rate.

PDHB expression was significantly increased in anti-PD-1 response STAD patients. Meanwhile, PDHB expression was significantly up-regulated in melanoma patients with treatment with DCs (Fig. 8B). After DCs treatment, the patients with high PDHB expression displayed higher survival rates than those with low PDHB expression (Fig. 8C). All these results demonstrated the clinical effects of abnormal PDHB in determining patients' response to the immunotherapy in cancer patients.

4. Discussion

Recently, Tsvetkov et al. discover a new form of cell death, named copper-dependent death [24]. Copper-dependent death occurs through direct binding of copper to fatty acylated components of the tricarboxylic acid (TCA) cycle, resulting in toxic protein stress and ultimately cell death [25]. Changes in intracellular copper levels influence cancer initiation and progression [26]. PDHB, a mitochondrial enzyme that links the Krebs cycle and glycolysis, plays an important role in the copper-dependent death signaling pathway [24]. It has been experimentally proven that the activity of PDHB could accelerate the growth of tumor cells [27]. Overexpression of PDHB inhibits the migration and growth of NPC cells by inhibiting RasV12-driven ERK signaling [28]. Wang et al. experimentally found that MEG3 could induce ER stress by upregulating PDHB, leading to the inhibition of cell proliferation and invasion in colorectal cancer cells [29]. However, the potential mechanisms of PDHB in cancers have not been clarified in details.

Therefore, we focused on the underlying roles of PDHB in human pan-cancer. We analyzed PDHB expression in 34 cancers from several databases such as TCGA. The expression profiles of PDHB at transcription and protein levels were different in different types of cancers, indicating that PDHB may have different mechanisms and functions in cancers. In addition, the GEPIA2 database results showed that kidney cancer patients with low PDHB expression level displayed unfavorable survival prognosis. Kaplan-Meier plotter showed that high PDHB expression was positively correlated with patients' prognosis in lung cancer, gastric cancer and breast cancer, while high PDHB expression was negatively correlated with patients' prognosis in ovarian cancer. These results suggested that PDHB might serve as a novel prognostic biomarker for cancer patients.

Nowadays, single-cell sequencing can provide a great opportunity to discover the functional status of candidate genes in individual cancer cells [30,31]. Kim et al. delineated the evolution features of chemo-resistant phenotype in the triple-negative breast cancer (TNBC) patients by single-cell sequencing [32]. Zhang et al. used single-cell sequencing to obtain 11,138 T cells from colon cancer patients, and systematically studied the tissue distribution, clonal expansion, migration, and developmental transition or differentiation of T cells [33]. In our study, we explored the PDHB expression and functional status at the single-cell level through the CancerSEA database. The results showed that PDHB in ALL and CRC was positively correlated with DNA damage and repair. PDHB in RB is positively associated with angiogenesis and inflammation. These results suggested that PDHB might play a key role in the development and progression of human cancers though regulating several pathways, especially like inflammatory response.

Immune responses have been shown to play an important role in cancer progression and treatment [34]. Experimental results by Zhang et al. showed that the phosphorylation of pyruvate dehydrogenase E1 subunit complex could promote tumor immune escape [35]. We analyzed the relationship between PDHB and different tumor-infiltrating immune cells in pan-cancer tissues by TIMER2.0. The results indicate that PDHB was negatively correlated with the infiltration of macrophages in KIRC, THCA and THYM. In addition, the results of TIGER platform showed that the expression of PDHB was significantly increased in STAD patients with anti-PD-1 response. After DC treatment, the survival rate of patients with high PDHB expression was also higher than that of patients with low PDHB expression. All these results suggested the important functional roles of PDHB in the immune response of cancer patients.

In conclusion, we used comprehensive bioinformatics analysis to investigate the expression profiles, prognostic values and genetic alterations of PDHB and its roles in immune infiltration in pan-cancer. The expression of PDHB at the single cell level and its functional signaling pathways were also discussed. PDHB may be potential biomarkers for prognosis and immunotherapy response in pan-cancer. These findings might help to elucidate the underlying roles of PDHB in carcinogenesis from various perspectives.

Author contribution statement

Yan Y: Conceived and designed the experiments; Yang K and Zhang F: Performed the experiments; Liu Y and Liang Q: Analyzed and interpreted the data. Zhang F: Contributed reagents, materials, analysis tools or data. Wu G and Xu Z: Wrote the paper. All authors approved final version of manuscript.

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Data availability statement

Data associated with this study has been deposited at https://www.jianguoyun.com/p/De9pxtcQtuCECxjPr98EIAA.

Additional information

Supplementary content related to this article has been published online at [URL].

Declaration of interest's statement

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13456.

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