ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
In press.

Pan-cancer Analysis Predicts *PDCD4* as a Potential Diagnostic, Prognostic and Immune Infiltration-related Biomarker

Haili Jiang[1,2], Anni Xie[3], Ting Wang[2], Wenjing Shi[4], Die Hu[3], Rui Sheng[3], Chaozheng Gao[3], and Tian Xie[1,5]

[1] Nanjing University of Chinese Medicine, Nanjing, China
[2] Department of Oncology Integrated Traditional Chinese and Western Medicine, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China
[3] Anhui University of Chinese Medicine, Hefei, Anhui, China
[4] Anhui Medical University, Hefei, Anhui, China
[5] School of Pharmacy, Hangzhou Normal University, Hangzhou, China

Received: 6 October 2024; Received in revised form: 6 November 2024; Accepted: 22 November 2024

ABSTRACT

Programmed cell death protein 4 (*PDCD4*) is an oncogene involved in the cell cycle and apoptosis, enhancing drug sensitivity in tumor cells and inhibiting tumor development. However, the relationship between *PDCD4* and tumor immune microenvironment remains unclear.

The Cancer Genome Atlas (TCGA) database was used to collect *PDCD4* data and somatic mutation data for 33 cancer types. Gene Expression Profiling Interactive Analysis (GEPIA) database was used to obtain the distribution map of *PDCD4* gene in human tissues and the prognostic expression heat map of cancer. Human Protein Atlas (HPA) database was used to explore the expression differences of *PDCD4* RNA in different cell lines, and *PDCD4* expression and clinical data were obtained from Gene Expression Omnibus (GEO) database.

We found significant differences in the expression of *PDCD4* in different cancers and associated with patient prognosis. *PDCD4* is closely related to the tumor microenvironment, sensitive to immunomodulators, and involved in immune regulation. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that *PDCD4* plays a crucial role in tumor metastasis, affecting the survival and prognosis of tumor patients. The differential expression of *PDCD4* in various tumor tissues and its involvement in immunomodulatory mechanisms suggest its potential as a biomarker for prognosis and immunotherapy.

PDCD4 was closely related to tumor immune microenvironment and immune efficacy indexes. It is suggested that it may be used as a biomarker to predict immune efficacy.

Keywords: Immunity; Biomarker; Pan-cancer; *PDCD4*; Prognosis

Corresponding Author: Tian Xie, MD, PhD;
Nanjing University of Chinese Medicine, Nanjing, China.
Tel: (+86 0571) 2886 8269, Fax: (+86 0571) 2886 8269, Email: tianxie@hznu.edu.cn

*The first and second authors contributed equally to this study

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Programmed cell death protein 4 (PDCD4) is a groundbreaking discovery in oncology and a protein that inhibits tumor progression by blocking the translation process, which was first discovered by Japanese scientist Yoshinori Suzuki and his team while studying apoptosis in 1996[1]. All that set the stage for extensive exploration into this novel programmed cell death-associated protein.

Since the advent of *PDCD4* research, substantial advancements have been made with *PDCD4* proving instrumental in diverse biological phenomena such as cell proliferation, transformation, migration, and programmed cell death itself.[2,3] As such, *PDCD4* has emerged as a central regulator in multiple biological pathways, interacting with essential cellular components like the eukaryotic initiation factor eIF4A and transcription factor EB (TFEB), modulating their functionality.[4,5] Given its critical role in numerous physiological processes, disruptions in *PDCD4*'s normal functioning can contribute significantly to disease pathogenesis. Indeed, empirical evidence has established that *PDCD4* was a potent tumor suppressor, where its expression levels bear a telling correlation with the size and differentiation state of solid tumors.[6]

In more recent years, researchers have delved deeper into understanding the intricate interplay between *PDCD4* and various signaling pathways.[7-10] A case in point is the strategic manipulation of the *PDCD4*-ATG5 axis, which can effectively suppress autophagy in gastric epithelial cells, possibly reverting precancerous changes and introducing innovative treatment paradigms for gastric cancer.[11] Moreover, *PDCD4* exhibits considerable promise in combinational therapies; in treating glioblastoma multiforme (GBM), even though postsurgical adjuvant radiotherapy is standard care, *PDCD4*'s downregulation has been shown to confer radioresistance upon tumor cells,[11] suggesting that targeting *PDCD4* could be a game-changer in GBM therapy, offering fresh prospects for patients.

Despite the wealth of literature addressing *PDCD4*'s involvement in cancer onset and progression, there remains a set of critical knowledge gaps. These include elucidating the precise ways in which *PDCD4* influences the differentiation status of cancer cells, deciphering the molecular mechanisms governing *PDCD4* expression, and exploring whether the tumor microenvironment affects *PDCD4*'s role in tumor cell differentiation. To bridge these gaps and provide a comprehensive overview of *PDCD4*'s roles across various malignancies, this study embarks on a meticulous examination of numerous databases, transcending conventional single-cancer research models. By adopting a broader and more holistic analytical framework, we aim to reveal commonalities and disparities in *PDCD4*'s actions across different cancer types. Ultimately, the results of this study bolster *PDCD4*'s standing as both a potential disease biomarker and therapeutic target, underpinning the advancement of precision medicine with robust scientific grounding.

MATERIALS AND METHODS

Data Collection

PDCD4 data (including genomic and clinicopathologic information) for the cancer types involved in this study were obtained from The Cancer Genome Atlas (TCGA) database (http://cancergenome.nih.gov/) (Table 1). Somatic mutation data were also accessed from TCGA. *PDCD4* gene distribution maps and cancer prognosis thermograms were generated using the Gene Expression Profiling Interactive Analysis (GEPIA) database, which is a web server for cancer and normal gene expression profiling and interactive analyses (http://gepia2.cancer-pku.cn/). Human Proteome Atlas (HPA) (https://www.proteinatlas.org/) is a comprehensive database built upon various omics technologies. By leveraging HPA resources, we were able to investigate the distribution of *PDCD4* within cells, as well as its function and characterization in different cellular environments. Additionally, expression and clinical data for renal cell carcinoma, lung adenocarcinoma, and metastatic melanoma cohorts were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). The *PDCD4* gene was brought into the above website for data analysis.

Clinical Parameters and Survival Prognosis Analysis Strategy

The differential expression and activity of *PDCD4* in tumor and normal tissues were analyzed using the limma package of R software (version 4.1.0), with associated boxplots constructed via the `ggpubr` package. The correlation between *PDCD4* mRNA expression and

Table 1. The types of human cancers employed in our research.

Abbreviation	Full name
ACC	Adrenocortical carcinoma
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/rectum adenocarcinoma/esophageal carcinoma
DLBC	Lymphoid neoplasm diffuse large B-cell lymphoma
ESCA	Esophageal carcinoma
FPPP	FFPE (formalin fixation and paraffin embedding) pilot phase II
GBM	Glioblastoma multiforme
GBMLGG	Glioma
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney Chromophobe
KIPAN	Pan-kidney cohort (KICH+KIRC+KIRP)
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
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
STAD	Stomach adenocarcinoma
SKCM	Skin cutaneous melanoma
STES	Stomach and esophageal carcinoma
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma
UVM	Uveal melanoma
OS	Osteosarcoma
ALL	Acute lymphoblastic leukemia
NB	Neuroblastoma
WT	High-risk Wilm’s tumor

clinical parameters such as tumor stage, age, and gender were also examined. *PDCD4*'s value in cancer diagnosis and prognosis was evaluated by processing data and conducting receiver operating characteristics curve (ROC) tests with the `pROC` package, with the `ggplot2` package utilized for visual presentation. Survival analysis involved the use of `survminer` and survival R packages to execute one-way Cox regression analysis for overall survival (OS), progression-free survival (PFS), disease-specific survival (DSS), and disease-free survival (DFS). The *p* values and 95% confidence intervals for the hazard ratios were calculated for each cancer type and depicted in a forest plot generated by the R software package.

Effect of Gene Mutation in Pan-cancer

The mutation frequency of the *PDCD4* gene in pan-cancer tissues was analyzed via the cBioPortal online platform (https://www.cbioportal.org/), and the corresponding data visualization graphs were produced. The expression relationship data of *PDCD4* and 44 RNA modification genes in each sample were acquired from the University of California Santa Cruz (UCSC) database (https://genome.ucsc.edu/). Methylation analysis of *PDCD4* was conducted using UALCAN (https://ualcan.path.uab.edu/index.html) and MethSurv (https://biit.cs.ut.ee/methsurv/), resulting in the generation of corresponding visualization graphs.

Related Tumor Microenvironment Component Analysis Methods

ESTIMATE, a technique for estimating the number of infiltrating immune and stromal cells in tumor tissue, was employed in this study. Using the `ESTIMATE` and `limma` packages in R software, the immune and stromal scores corresponding to the number of immune and stromal cells in each tumor tissue case were calculated. Furthermore, the `CIBERSORT` algorithm in R software was utilized to estimate immune cell infiltration in each case. Correlation plots were drawn using the `ggplot2`, `ggpubr`, and `ggExtra` packages in R. The Tumor-Immune System Interaction Database (TISIDB, http://cis.hku.hk/TISIDB/) was used to investigate the potential relationship between *PDCD4* expression and 3 types of immunomodulators (immunosuppressants, immune agonists, and major histocompatibility complex [MHC] molecules).

Gene set Enrichment Analysis

The Gene Ontology (GO) gene set `c5.go.v7.4` and the Kyoto Encyclopedia of Genes and Genomes (KEGG) gene set `c2.cp.kegg.v7.4` were downloaded from the Gene Set Enrichment Analysis website (GSEA, https://www.gsea-msigdb.org/gsea/index.jsp). Samples were then divided into high- and low-expression groups according to the median *PDCD4* expression. Enrichment analysis was conducted on these samples using the `limma`, `enrichment plot`, `clusterProfiler`, and `org.Hs.eg.db` R packages.

Gene Heterogeneity and Immunotherapy Response Analysis Strategy

Data on MATH, HRD, and NEO associated with *PDCD4* were sourced from the UCSC database. MATH, HRD, and NEO are utilized to evaluate genetic heterogeneity, the risk of inherited tumors, and the generation of tumor neoantigens. Lollipop plots of these data were drawn using the `maftools` package in R language. The Tumor mutation burden (TMB) and the microsatellite instability (MSI) are key predictors of immune checkpoint inhibitor (ICI) response in solid tumors and are closely related to tumor prognosis. This study used the R package `fmsb` to explore the relationship between *PDCD4* expression, TMB, and MSI, and generated radar plots to visualize the findings. The correlation between *PDCD4* expression and immunotherapy efficacy was further confirmed in 3 independent immunotherapy cohorts, categorizing partial and complete remission cases as responders and stable and progressive cases as non-responders. Lastly, the Wilcoxon test was employed to examine the differences in *PDCD4* expression between the 2 groups.

Statistical Analysis

Statistical analysis was conducted using R software and its accompanying package. *PDCD4* expression between groups was compared using the Wilcoxon test, while its correlation was analyzed via the Spearman correlation test. Survival curves were drawn employing the Kaplan-Meier method, and the relationship between *PDCD4* expression and survival was examined through Cox proportional regression to obtain the risk ratio. A two-tailed p value<0.05 was deemed significant.

RESULTS

Expression of *PDCD4* in Normal Tissues and Its Localization

PDCD4 is widely expressed in human tissues and widely distributed in cytoplasm and nucleus. Among the tissues analyzed in the GETx and HPA datasets, pancreatic tissue had the highest level of *PDCD4* expression (Figure 1).

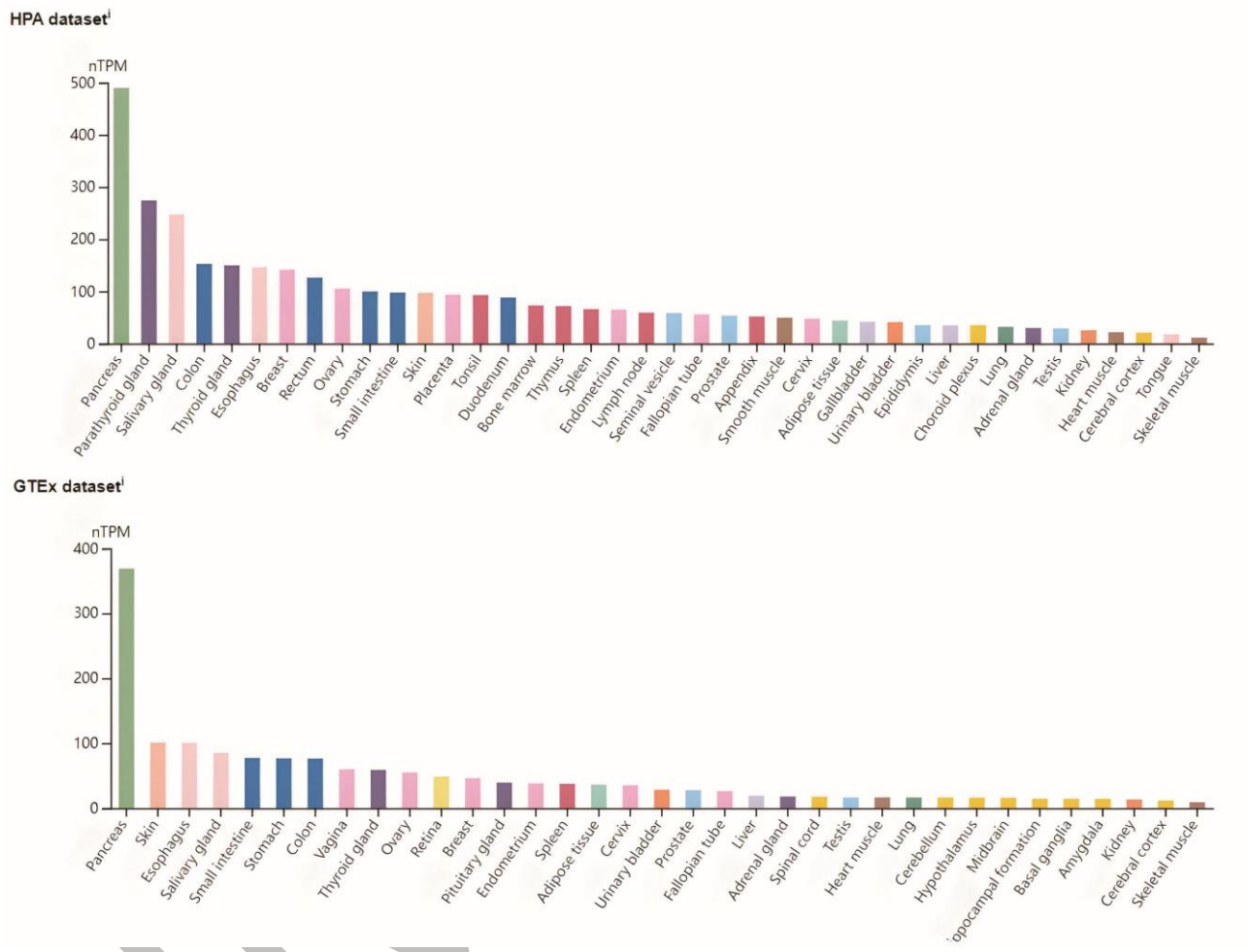


Figure 1. Expression of programmed cell death protein 4 (*PDCD4*) in tissues: comparison of RNA expression levels of *PDCD4* in different tissues.

PDCD4 Expression Levels in Pan-cancer Tissues

Using the TGCT database, the analysis revealed that *PDCD4* expression was highest in thyroid carcinoma (THCA) and lowest in GBM among the 33 tumor tissues examined (Figure 2A). Furthermore, significant variations in *PDCD4* expression levels were observed across the 16 comparison cohorts. Specifically, the downregulation of PDCD4 expression was observed in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), and uterine corpus endometrial carcinoma (UCEC). Conversely, upregulation was detected in cholangiocarcinoma (CHOL), liver hepatocellular carcinoma (LIHC), and pheochromocytoma and paraganglioma (PCPG) tissues compared to their respective normal counterparts (Figure 2B). Comparable results were obtained using the TIMER2.0 database (Figure 2C). Subsequently, we delved into the relationship between *PDCD4* expression levels and clinical parameters among cancer patients. Notably, *PDCD4* expression was higher in the non-elderly (<65 years) population in adrenocortical carcinoma (ACC), BLCA, kidney renal clear cell carcinoma

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

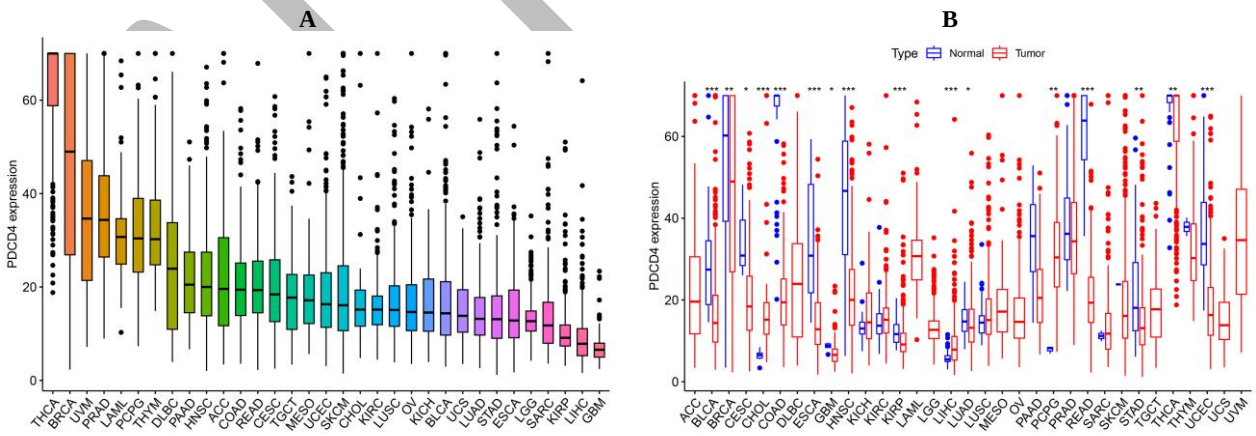
(KIRC), ovarian serous cystadenocarcinoma (OV), sarcoma (SARC), and THCA, but higher expression was observed in the elderly (>65 years) group in uterine carcinosarcoma (UCS) (Figure 2D). Male patients with lung adenocarcinoma (LUAD) had higher *PDCD4* expression levels compared to female patients. Conversely, notably elevated *PDCD4* expression was observed among female patients with BRCA, kidney renal papillary cell carcinoma (*KIRP*), and LIHC (Figure 2E). A strong correlation was observed between *PDCD4* expression and the clinical stage of ACC, BRCA, KIRP, and testicular germ cell tumors (TGCT) (Figure 2F). From this, we concluded that *PDCD4* expression was more prominent in BRCA, which also showed differential results in within-group cohort comparisons. Further, we observed that the expression of *PDCD4* in BRCA also differed significantly in gender and stage.

Analysis of the Diagnostic and Survival Prognostic Value of *PDCD4* in Pan-cancer Tissues

In this study, we analyzed the diagnostic efficacy of *PDCD4*, a pan-cancer biomarker, using ROC curves. Specifically, *PDCD4* exhibited a high diagnostic value with AUC>0.9 and $p<0.05$ in READ, PCPG, COAD, CHOL, and UCEC cancer tissues. Furthermore, *PDCD4* exhibited a diagnostic value degree of AUC>0.8 and $p<0.05$ in CESC, ESCA, and HNSC (Figure 3A).

The correlation between *PDCD4* expression and OS, PFS, DFS, and DSS in tumor patients was analyzed using the Cox risk-proportional regression model and presented in the form of a forest plot (Figure 3B). High *PDCD4* expression predicted poorer OS in KIRP, LIHC, LUAD, and THYM (HR>1, $p<0.05$), but protective effects in SARC. Elevated *PDCD4* expression was associated with improved PFS in BRCA, KIRC, LGG, PRAD, and SARC. DSS analysis showed higher *PDCD4* expression predicted poorer prognosis in KIRP and PCPG, but better DSS in BRCA, KIRC, LGG, THCA, and UVM. High *PDCD4* shortened DFS in BLCA and KICH patients, but prolonged DFS in BRCA, LUAD, and PRAD patients.

Subsequently, a survival analysis of the *PDCD4* gene across multiple cancer types was conducted and visually represented using Kaplan-Meier curves (Figure 3C). Elevated *PDCD4* expression predicted a favorable prognosis for KIRC, LAML, LUAD, MESO, SKCM, and UVM patients. For BRCA, KIRC, LGG, and SARC, higher *PDCD4* expression indicated a positive prognosis for PFS. It favorably influenced DFS in BRCA, LUAD, and PRAD, as well as DSS in BRCA, KIRC, and UVM, aligning with Cox risk prediction. An intriguing observation was made: LGG patients with high *PDCD4* expression had higher survival rates until 8 years, but survival rates favored those with low *PDCD4* expression beyond this threshold.



Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

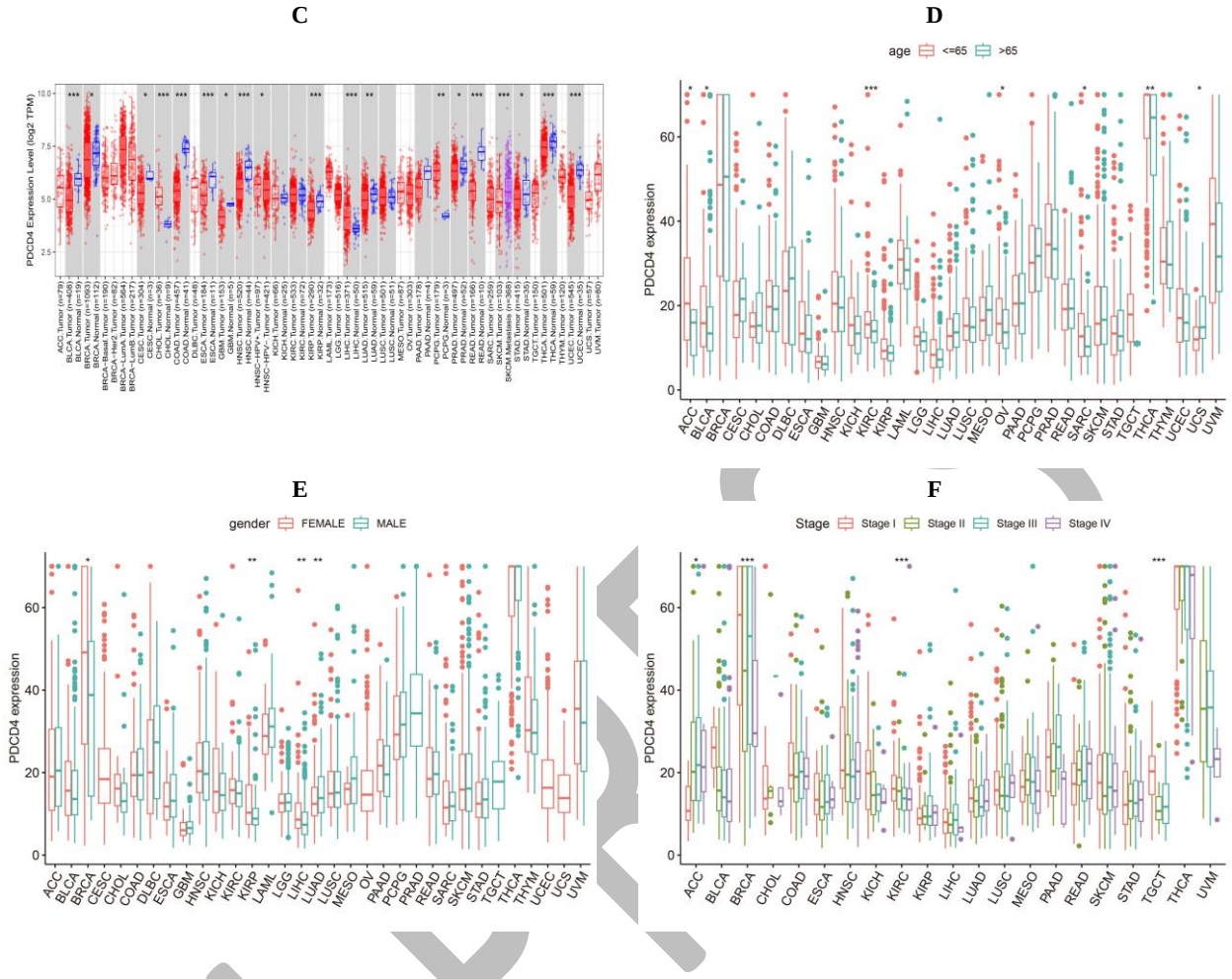
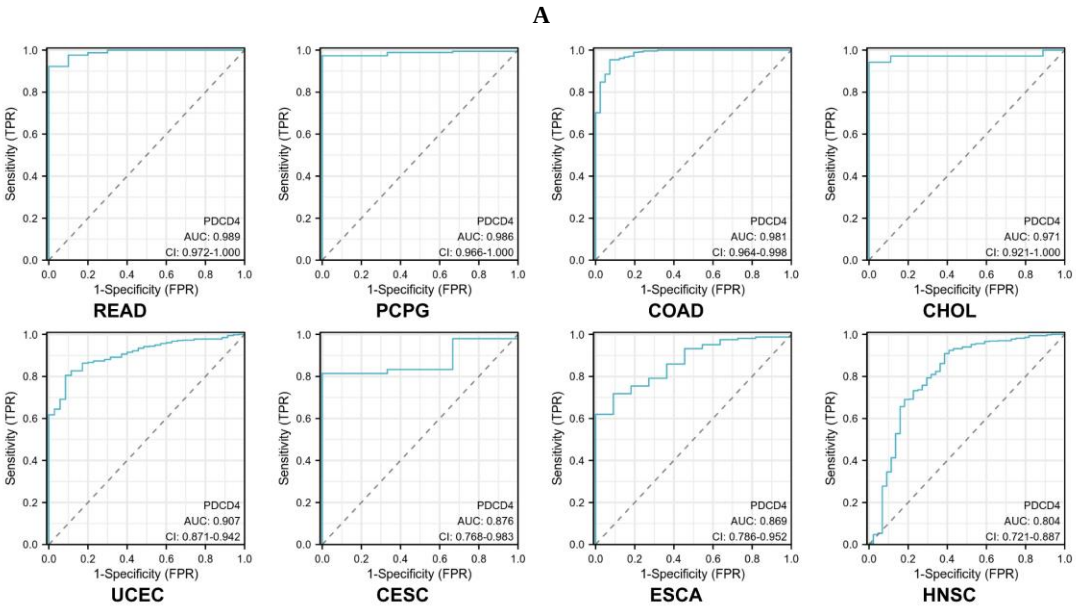
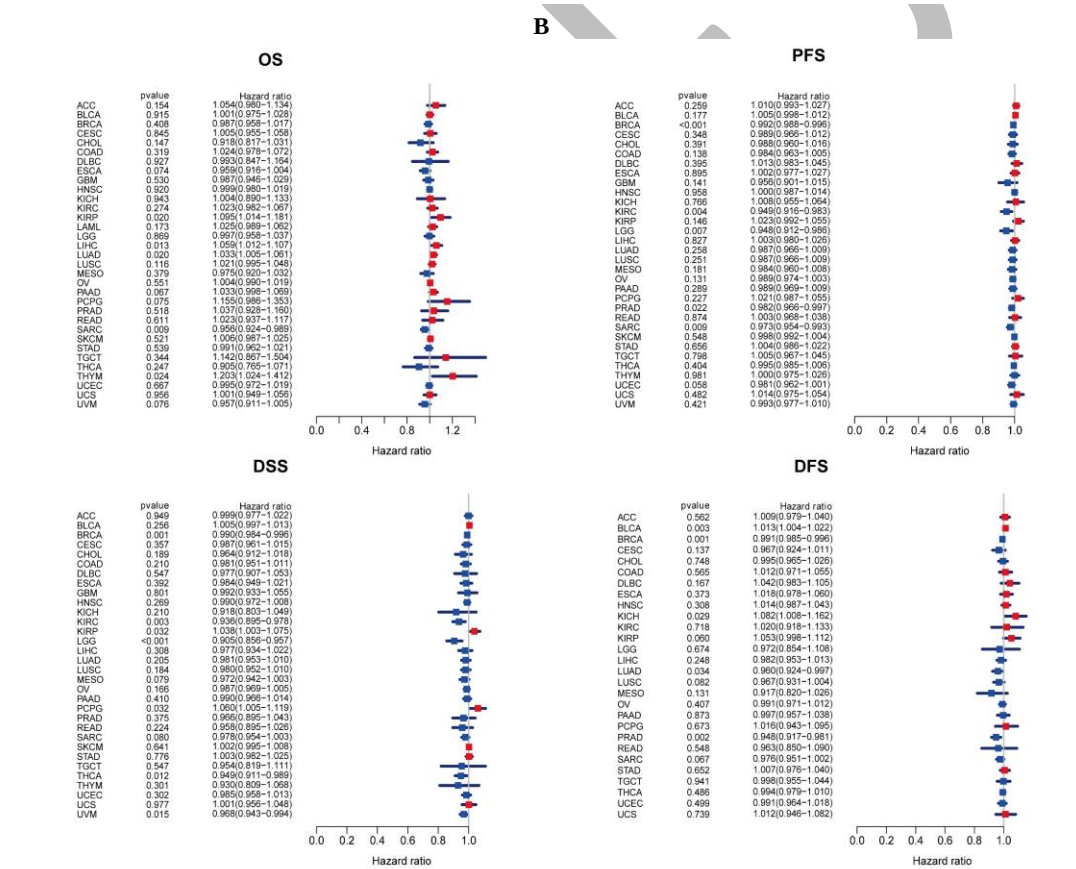


Figure 2. Expression pattern and correlation analysis of programmed cell death protein 4 (*PDCD4*) in pan-cancer: A. Expression differences of *PDCD4* in 33 different tumor types. B. Differences in the expression of *PDCD4* between 33 types of cancers and normal tissues in the TCGA database. C. Expression differences of *PDCD4* between different types of cancers and normal tissues in the TIMER2.0 database. D. Effect of age difference on gene expression of *PDCD4* in 33 types of cancers. E. Effect of gender differences on gene expression of *PDCD4* in 33 cancers. F. Effect of clinical stage on gene expression of *PDCD4* in 33 cancers. Red boxes represent tumor tissues and blue boxes represent normal tissues. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)


A
Sensitivity (TPR)
1-Specificity (FPR)
PDCD4
READ
AUC: 0.989
CI: 0.972-1.000
PCPG
AUC: 0.986
CI: 0.966-1.000
COAD
AUC: 0.981
CI: 0.964-0.998
CHOL
AUC: 0.971
CI: 0.921-1.000
UCEC
AUC: 0.907
CI: 0.871-0.942
CESC
AUC: 0.876
CI: 0.768-0.983
ESCA
AUC: 0.869
CI: 0.786-0.952
HNSC
AUC: 0.804
CI: 0.721-0.887


B
OS
PFS
DSS
DFS
Hazard ratio

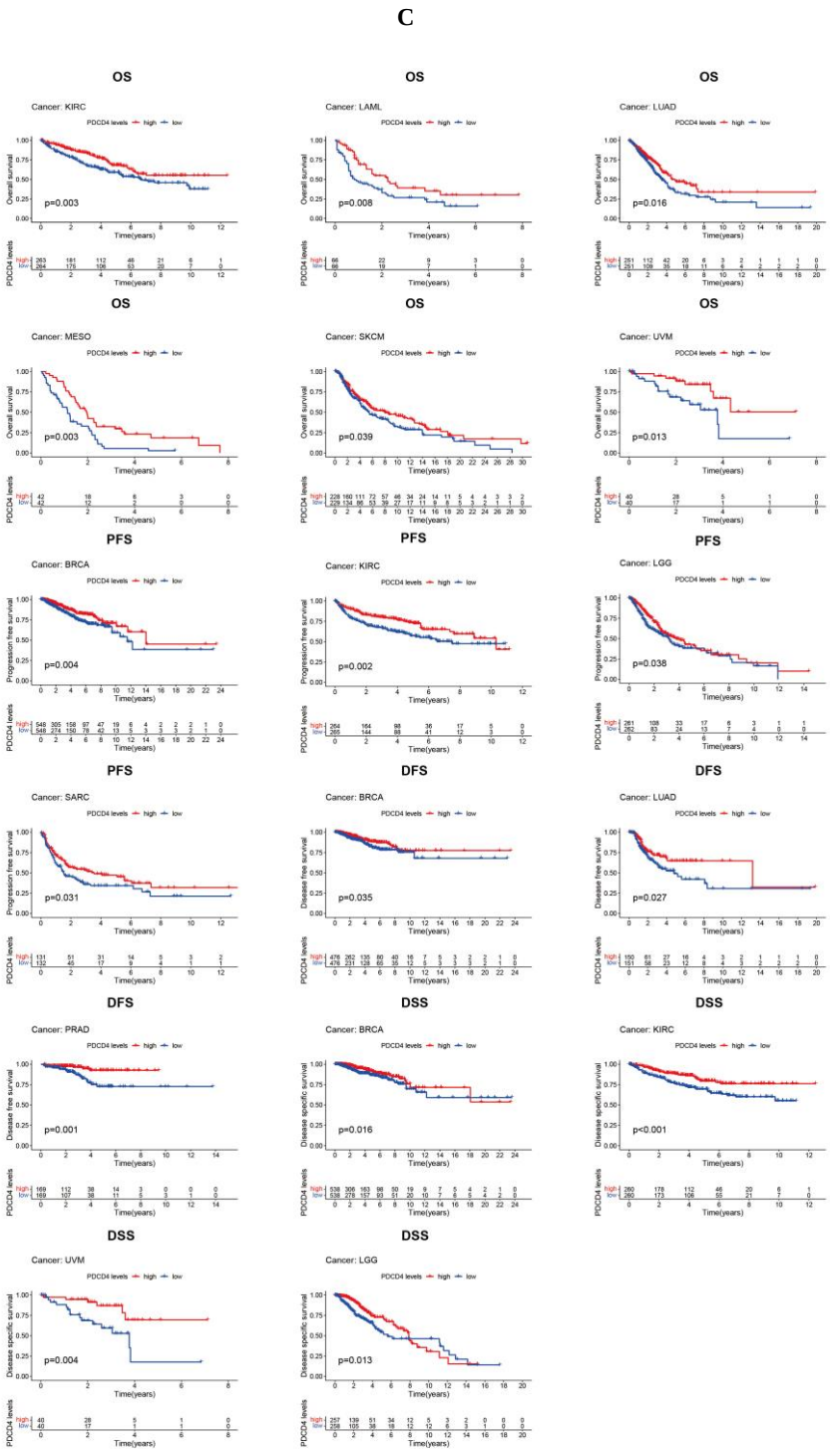


Figure 3. Diagnostic value and survival prognosis analysis of programmed cell death protein 4 (*PDCD4*) in pan-cancer: A. The correlation between *PDCD4* gene expression and cancer diagnostic efficacy was investigated by receiver operating characteristic (ROC) curve analysis. B. To investigate the association between *PDCD4* gene expression and overall survival (OS), progression-free survival (PFS), disease-free survival (DFS), and disease-specific survival (DSS) in patients with 33 types of cancer using one-way Cox regression analysis. C. To analyze the correlation between *PDCD4* gene expression and the Kaplan-Meier survival curves of the above survival indices (OS, PFS, DFS, DSS) in patients with different tumor types.

Analysis of the Correlation between *PDCD4* Gene Alterations and Tumor Progression

Tumor progression is closely associated with changes in *PDCD4* gene expression. We explored the impact of *PDCD4* variations on cancer progression using the cBioPortal. The findings revealed that among the top four cancer tissues exhibiting the highest mutation frequencies, the predominant mutation type was missense mutation, specifically in SKCM (2.93%), UCEC (2.65%), BLUC (1.46%), and COAD (1.52%). Amplification and deep deletion were also observed in these tumor tissues (Figure 4A). Furthermore, both cBioPortal and Simple Nucleotide Variation data analyses confirmed the prevalence of missense mutations in *PDCD4*. Additionally, splice site mutations were observed in LGG, COAD, lung squamous cell carcinoma (LUSC), and BRAC. COAD and LAML exhibited frameshift insertions, while GBM, BRAC, and LUSC displayed frameshift deletions (Figure 4B, C).

Leveraging the pan-cancer data housed in the UCSC database, we identified *PDCD4*'s expression patterns across 3 RNA modification categories: m1A, m5C, and m6A, relative to 44 marker genes in individual samples. *PDCD4*'s functional roles in RNA modification genes were grouped into writers, readers, and erasers. The analysis revealed a positive correlation between *PDCD4* expression and most RNA modification genes (Figure 4D).

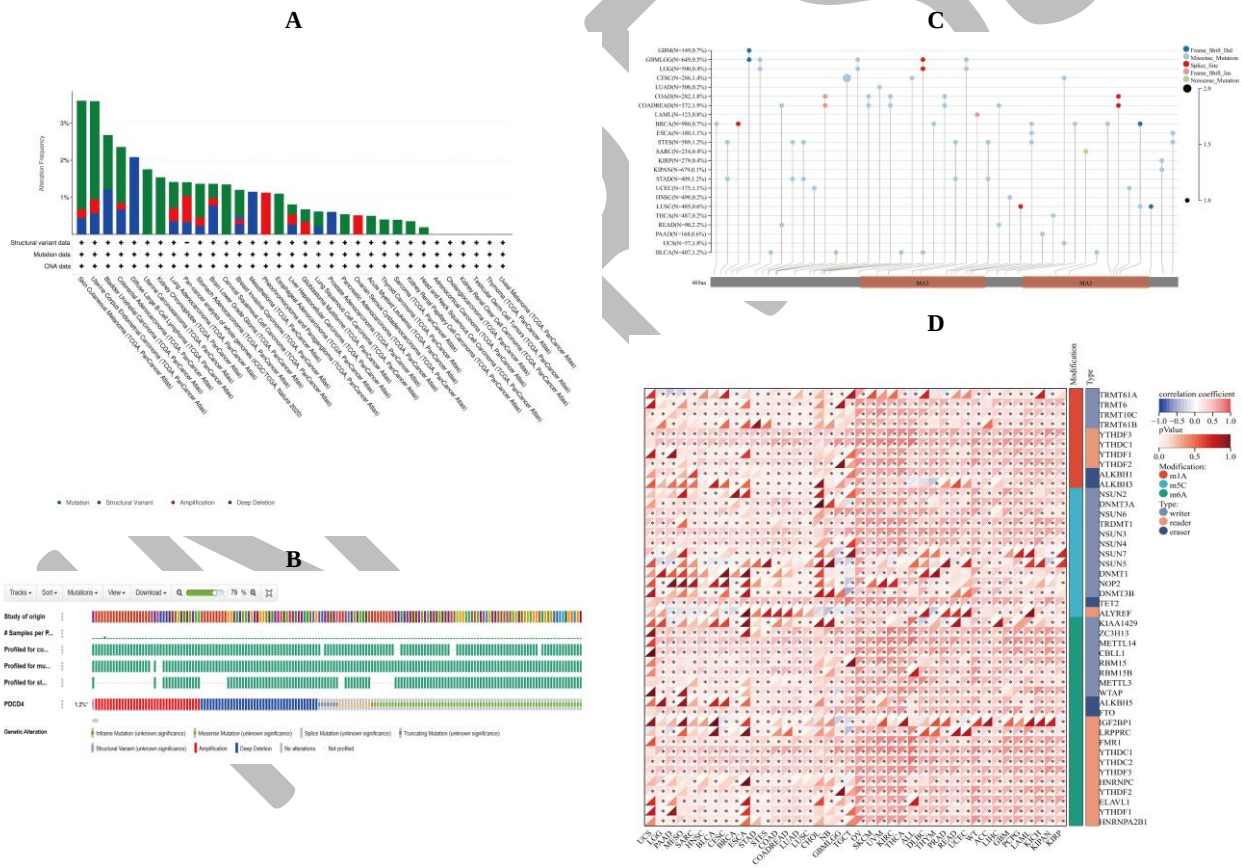


Figure 4. Genetic alterations of programmed cell death protein 4 (*PDCD4*) in pan-cancer: A. Frequency analysis of *PDCD4* gene mutation types in The Cancer Genome Atlas (TCGA) cancers by cBioPortal. B. Tumor mapping of *PDCD4* genetic alterations revealed by cBioPortal in TCGA cancers. C. Gene mutation sites of *PDCD4* in TCGA cancers demonstrated by cBioPortal. D. Correlation of PDCD4 expression with 44 class III RNA modification genes in the University of California, Santa Cruz (UCSC) database.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Cancer progression is linked to DNA methylation. Using UALCAN and MethSurv databases, we compared tumor and normal tissue methylation profiles from 23 cohorts, finding 13 significant correlations. The *PDCD4* gene showed elevated methylation in normal tissues of LIHC, LUAD, LUSC, TGCT, and UCEC. In BRCA, ESCA, HNSC, KIRC, KIRP, PAAD, PRAD, and THCA tumor tissues, the methylation level of the *PDCD4* gene was significantly increased compared to that observed in normal tissues (Figure 5A).

Five methylation probe sites linked to *PDCD4*, exhibiting differential expression patterns primarily in LIHC, CESC, LGG, and KIRC, were retrieved from the MethSurv database for analysis (Figure 5B). Subsequently, a prognostic analysis was conducted on the DNA probe sites retrieved (Figure 5C). The findings revealed distinct patterns in LIHC across the five loci. Specifically, elevated expression of cg05999324 and cg09731288 in *PDCD4* indicated a poor prognosis, whereas reduced expression of cg20032495, cg24903527, and cg26881277 suggested a favorable outcome. Elevated expression of cg05999324 was associated with a poor prognosis in COAD, UCEC, and UVM patients, whereas reduced expression positively influenced prognosis in BRCA, CESC, KICH, and KIRC.

Analysis of the Relationship between *PDCD4* Expression and Tumor Microenvironment

PDCD4 gene expression is crucial in the tumor microenvironment. We analyzed tumor purity in 37 pan-cancer tissues from the UESC database, finding significant results in 15 cohorts (Figure 6A). In a separate analysis of 33 cancer tissues with 67 immune cell types, *PDCD4* expression was negatively correlated with most immune cells (Figure 6B). Selected tumor tissues were further analyzed for immune cell infiltration (Figure 6C). We observed a positive correlation between *PDCD4* gene expression and monocyte content in GBM, as well as a positive correlation with $CD8^+$ T cell content in PAAD. However, *PDCD4* gene expression was negatively correlated with M0 macrophages in both cancers. Additionally, the resting state of mast cells in PCPG exhibited a positive correlation with *PDCD4*. Furthermore, the activity of CD4 memory T cells demonstrated an inverse correlation with PDCDA expression. Additionally, *PDCD4* expression was positively associated with M2 macrophage content in TGCT and negatively associated with gamma-delta T cell content in UVM.

We subsequently analyzed the correlation between *PDCD4* expression and 3 immunomodulators and showed the top 6 immunomodulators with the highest correlation in each section (Figure 7). Our findings indicate that *PDCD4* expression exhibits negative correlations with CTLA4, PDCDILG2, TIGIT in THCA, TGFB1 in MESO, and PVRL2 in TGCT among 25 immunosuppressants. However, a positive correlation was observed between *PDCD4* expression and CD160 in TGCT. Alternatively, among the 45 immunologists included, *PDCD4* expression displayed positive correlations with ENTPD1 in TGCT, UVM, and TNFRSF25 in TGCT. However, negative correlations were observed between *PDCD4* expression and CD80 in THCA, LTA, and TMEM173 in UVM. According to the MHC molecular level, the more prominent results of *PDCD4* expression were centered in THCA, specifically, showing negative correlations with HLA-DMA, HLA-DPA1, HLA-DPB1, HLA-DQA2, HLA-DRA, and TAP1.

Functional Enrichment Analysis of *PDCD4* Gene Expression

There is a significant relationship between *PDCD4* gene expression in tumor prognosis and immunity; therefore, we used GSEA to explore the potential biological significance and pathways of *PDCD4*. According to the obtained GO (Figure 8A), *PDCD4* showed a positive enrichment in 19 cancers. Particularly, high expression of *PDCD4* showed a positive correlation with activation of immune response and leukocyte migration in CHOL but presented opposite results in THCA. In addition, *PDCD4* gene expression was also positively enriched for activation of immune response in KIRP, SKCM, and STAD, while it was negatively enriched in READ. We also observed that leukocyte adhesion was positively correlated with *PDCD4* in UCS and negatively enriched in MESO. In addition, T-cell activation was significantly enriched with high *PDCD4* expression in both UCS and GBM. In addition, our analysis of pan-cancer tissues revealed significant enrichment in the detection of chemical stimuli, the detection of stimuli involved in sensory perception, cilia-involved processes, and microtubule-based motility (Supplementary Figure 1), which is a worthwhile aspect to be explored in drug sensitivity, metastasis, and other processes that may be involved in cancer.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

Next, concerning KEGG analysis (Figure 8B), we observed positive enrichment of cell adhesion molecules, cytokine-cytokine receptor interactions, and chemokine signaling pathways in both ACC and THCA in close correlation with high expression of *PDCD4*, whereas the above three cellular processes showed negative enrichment results in KICH and SARC. The enrichment of high expression of *PDCD4* with Ecm receptor interactions showed a positive correlation in LUSC, PRAD, and THYM, and diametrically opposite results in LIHC. High expression of *PDCD4* in LUSC showed negative enrichment results in the Toll-like receptor signaling pathway as well as in the Jak-Stat signaling pathway, while on the other hand, the Jak-Stat signaling pathway showed negative enrichment in the SARC and SKCM showed positive enrichment. Meanwhile, we found that the high expression of *PDCD4* was closely related to a variety of signaling pathways such as B-cell receptor signaling pathway, T-cell receptor signaling pathway, MAPK signaling pathway, PPAR signaling pathway and so on based on KEGG analysis in pan-cancer tissues, and the results shown in different cancer tissues were also different (Supplementary Figure 2).

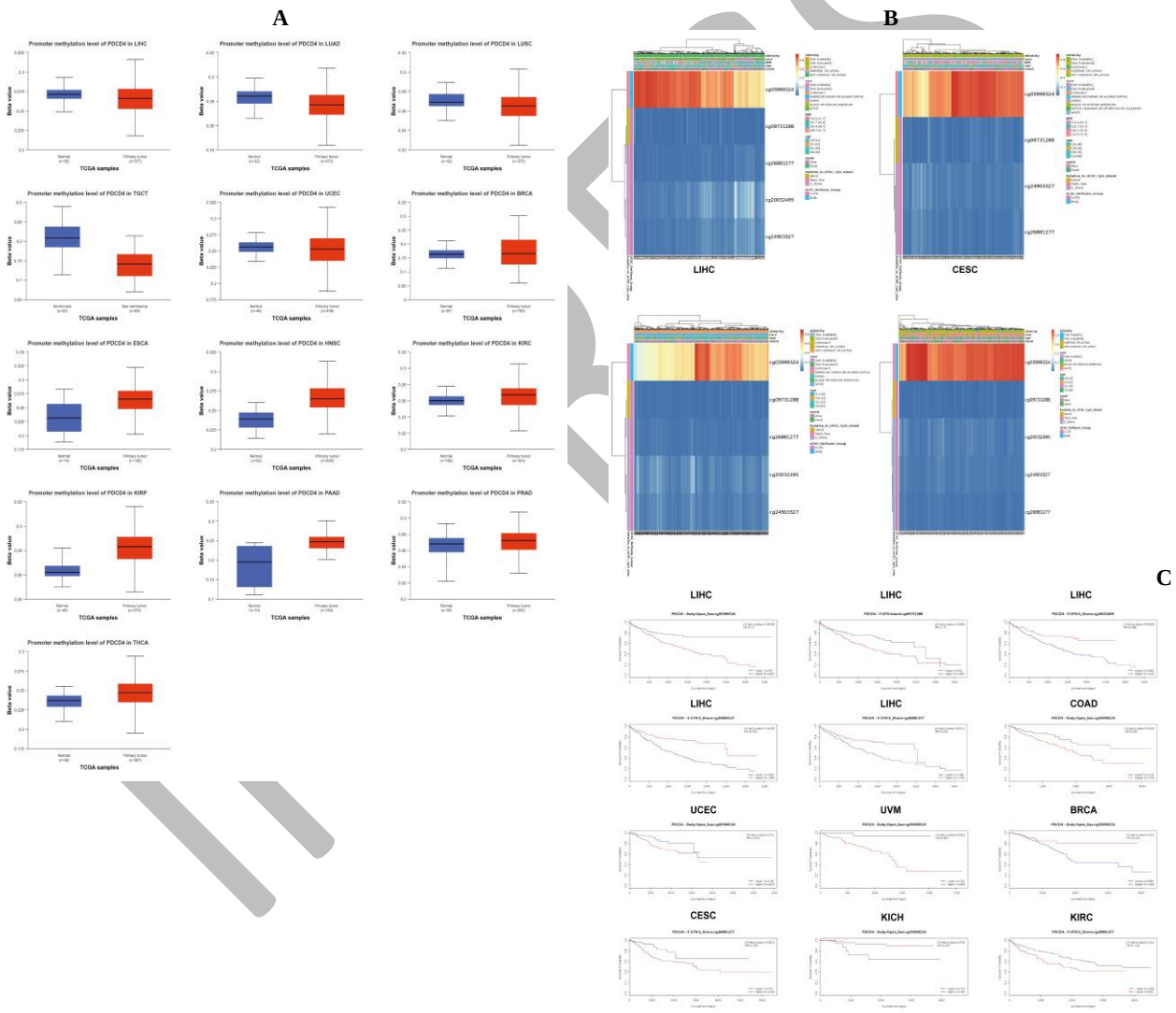


Figure 5. DNA methylation correlation analysis of programmed cell death protein 4 (*PDCD4*) in pan-cancer: A. Comparison of expression differences in DNA methylation levels of *PDCD4* between cancer patients and normal subjects using the UALCAN website. B. Study the information of 5 methylation probe sites associated with *PDCD4* in various cancers via the MethSurv website. C. To analyze the relationship between the Kaplan-Meier curves of the different probe loci of *PDCD4* and the survival prognosis of patients in different cancer types using the MethSurv website.

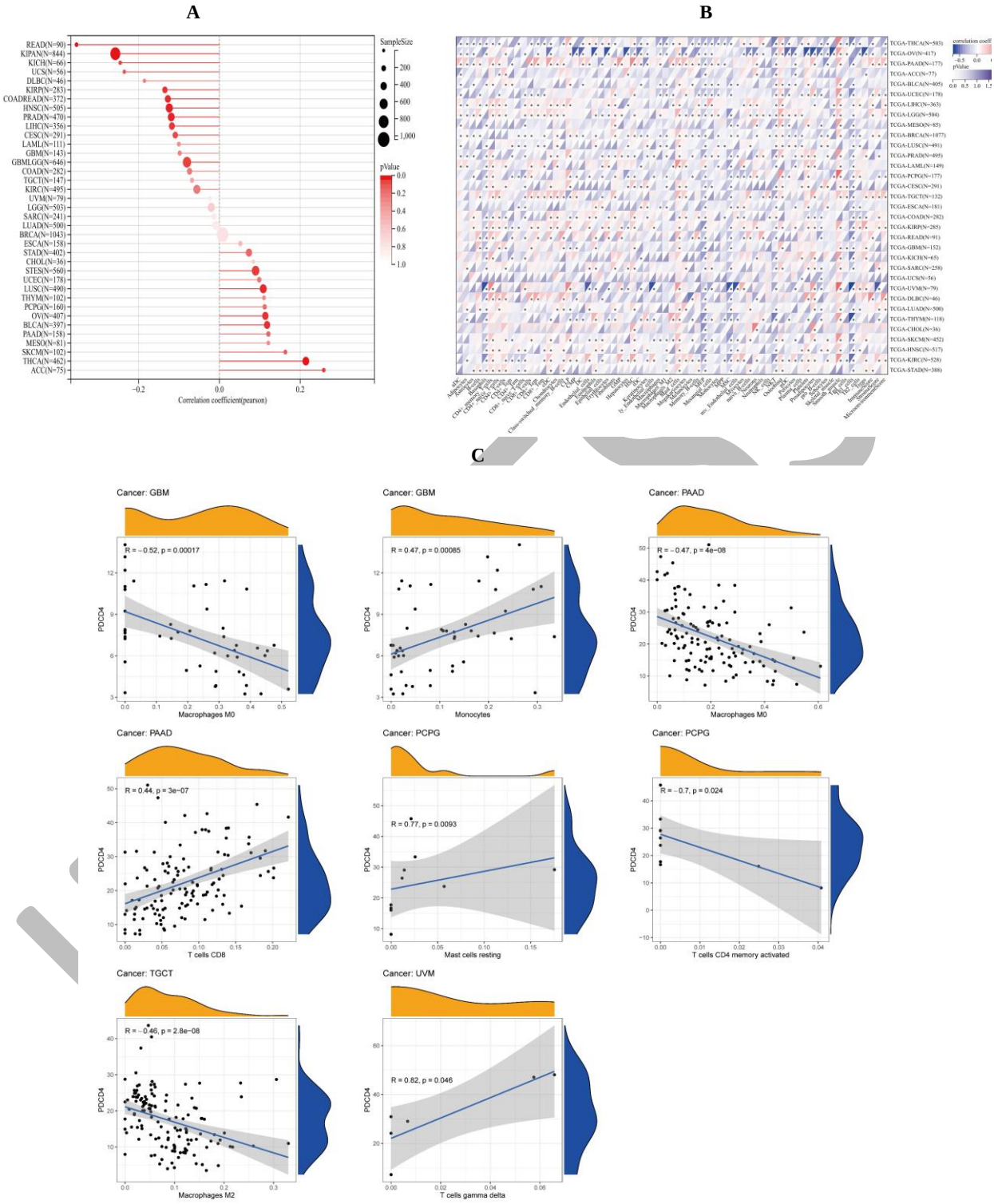
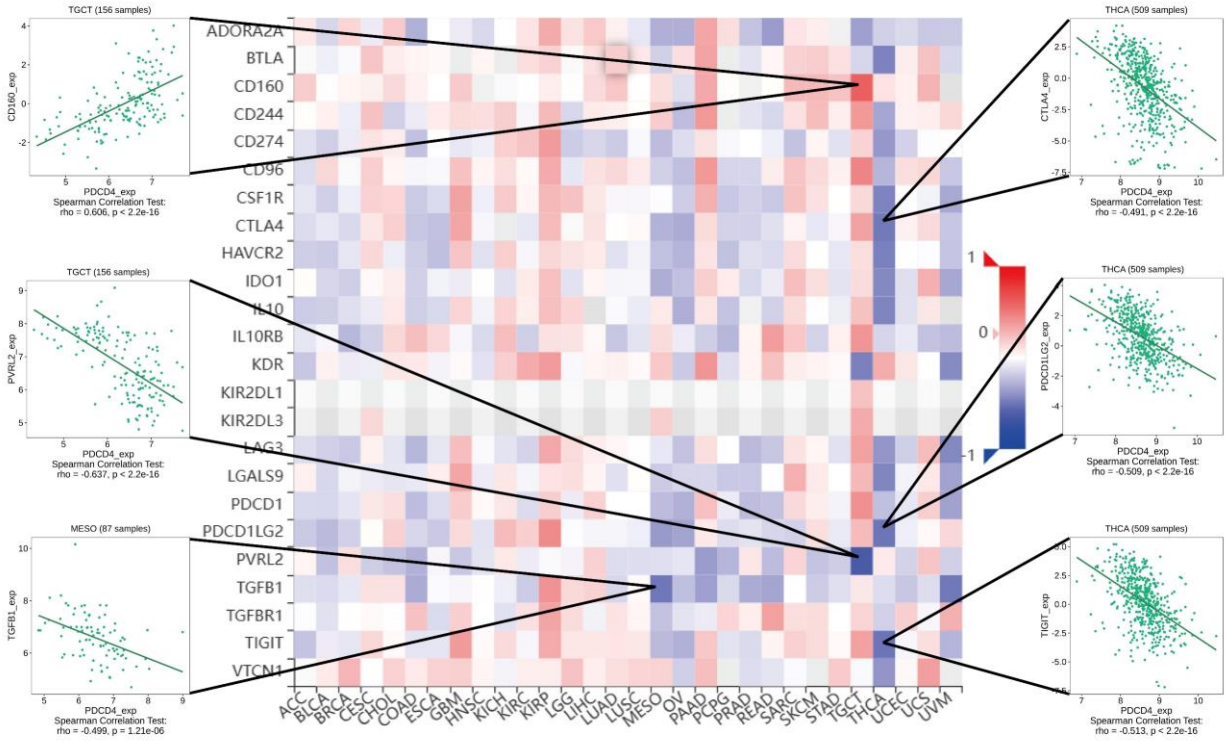


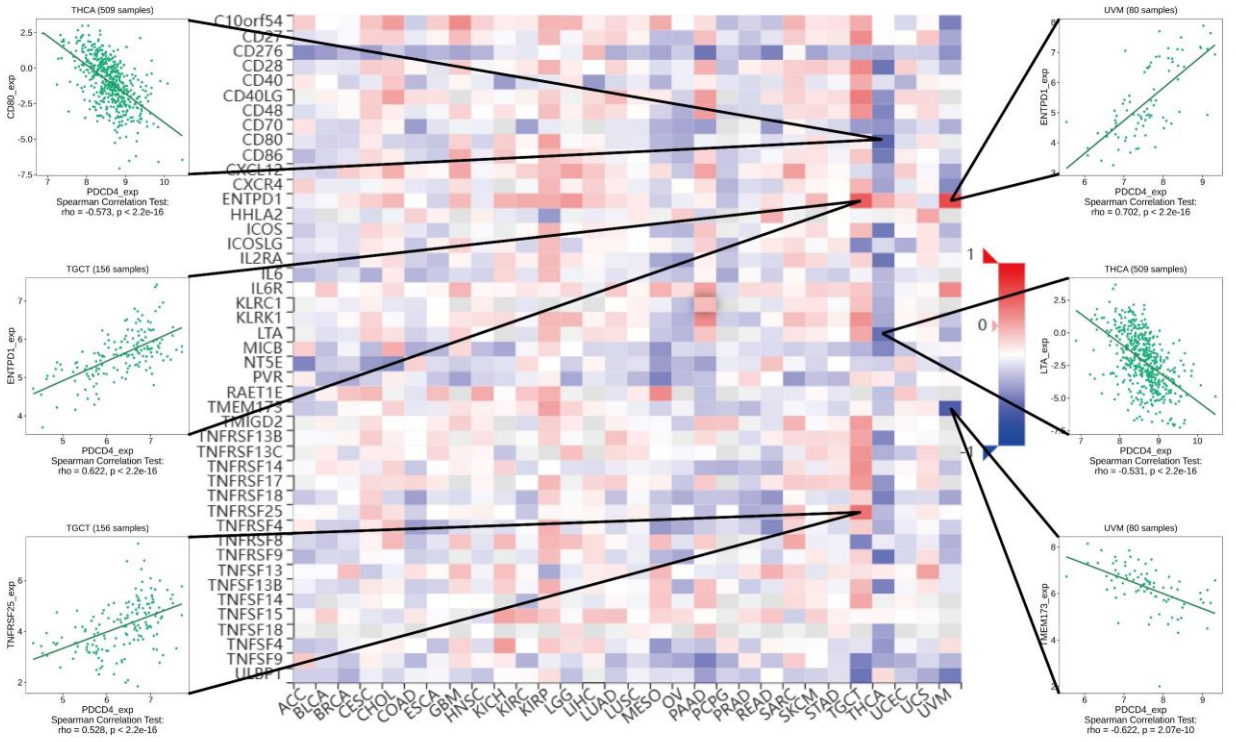
Figure 6. Association between programmed cell death protein 4 (*PDCD4*) gene expression and tumor microenvironment: A. Correlation between PDCD4 and tumor purity of different types of tumors using the University of Electronic Science and Technology of China (UESC) database information. B. Correlation between PDCD4 gene expression and 67 types of tumor immune cells. C. Regression curves of the correlation between PDCD4 and immune cell infiltration in different types of tumor tissues.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

A



B



Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

C

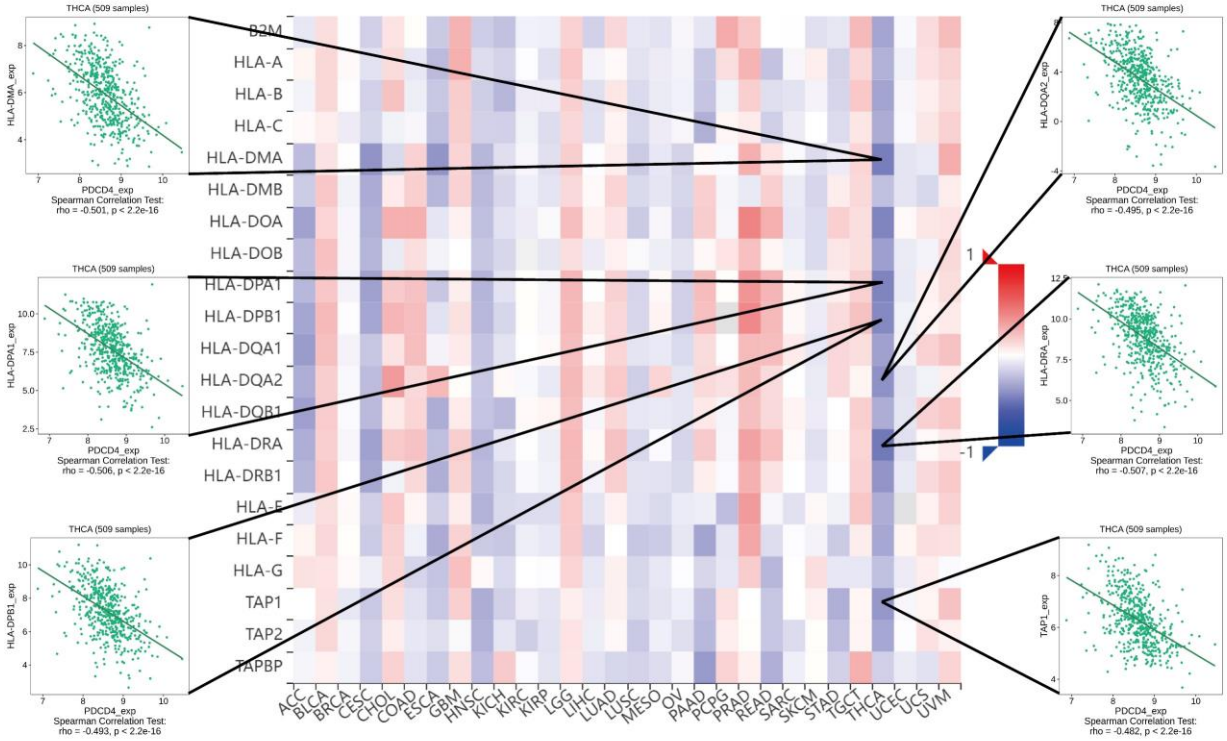


Figure 7. The correlation of programmed cell death protein 4 (*PDCD4*) with three kinds of immunomodulators across human cancers: A. Association of *PDCD4* expression with immunostimulators. B. Association of *PDCD4* expression with immunoinhibitors. C. Correlation between *PDCD4* expression and MHC molecules. Red color denotes positive correction, while blue color represents negative correlation. Dot plots are used to show the top 6 strongest associations for each section.

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

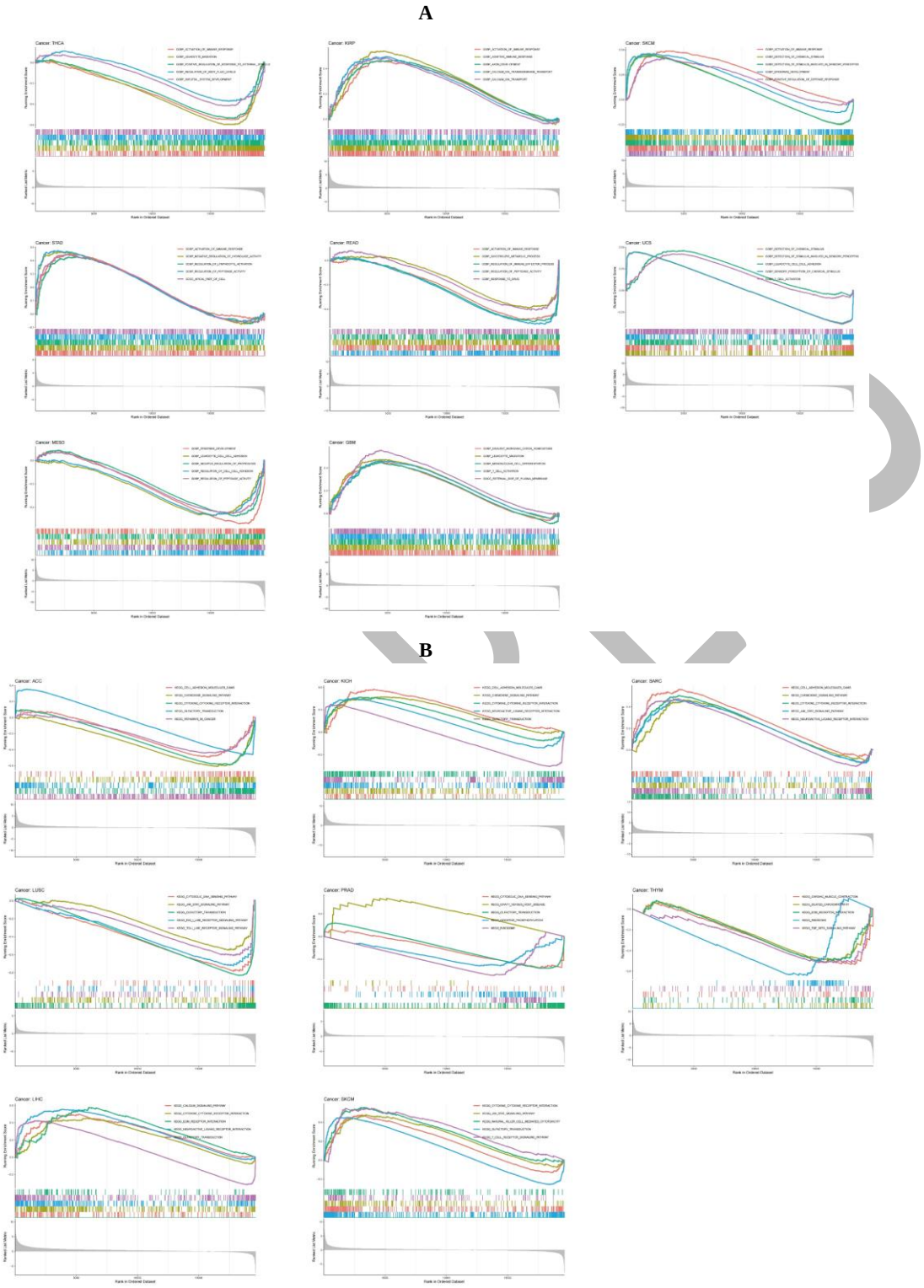


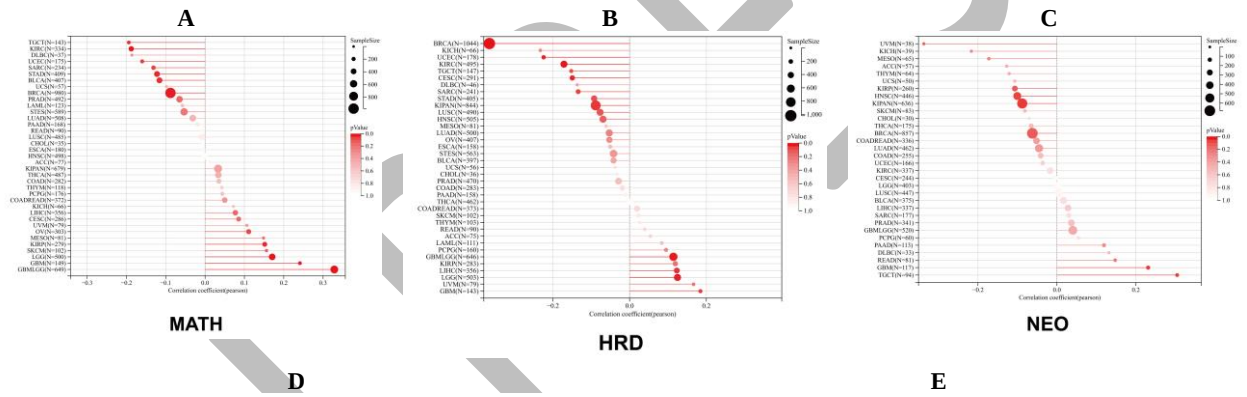
Figure 8. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of programmed cell death protein 4 (*PDCD4*) gene expression in pan-cancer: A. Gene Ontology (GO) analysis of PDCD4 in different types of cancers. B. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of PDCD4 in different types of cancers.

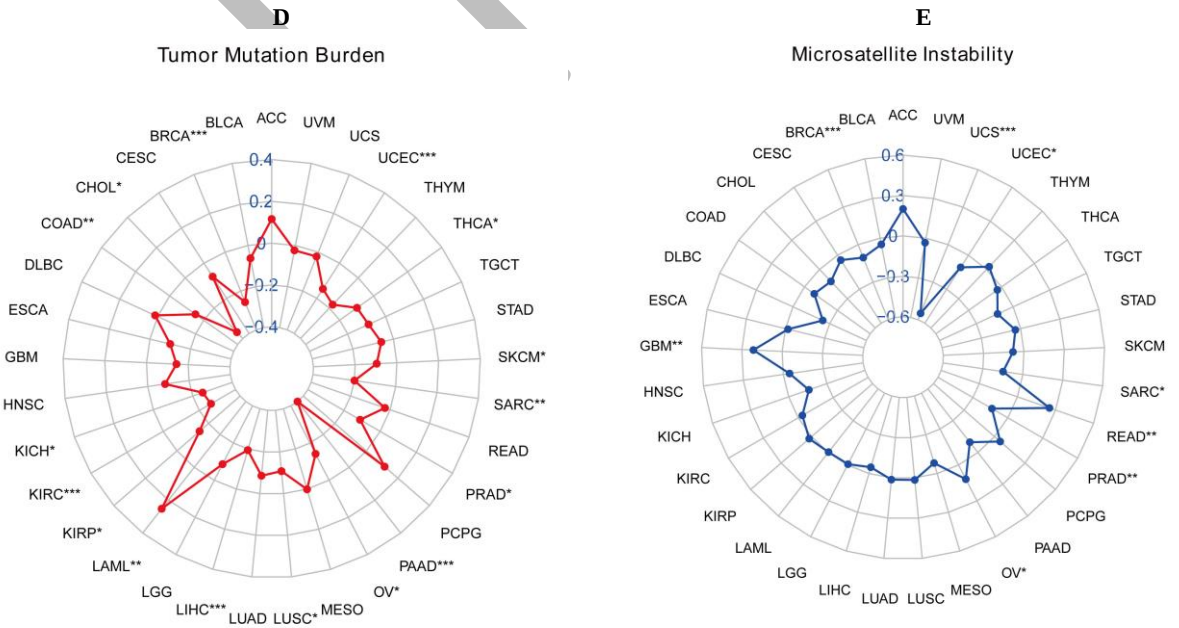
Analysis of *PDCD4* Gene Heterogeneity and Immunotherapy Response

Immunotherapy's role in cancer treatment is gaining prominence. We obtained data from UCSC for 37 cancer tissues, generating lollipop plots for genetic heterogeneity (MATH), hereditary tumor risk (HRD), and tumor neoantigens (NEO). MATH showed significance in 11 cancers (Figure 9A), with positive correlations in GBM, GBMLGG, LGG, and KIRP. HRD had 10 significant results (Figure 9B), positive in GBM, LGG, and LIHC. NEO had 4 significant results (Figure 9C), positive in GBM and TGCT. GBM showed positive correlations in all three analyses, suggesting potential for relevant tumor vaccine development.

Studying *PDCD4* gene expression's correlation with immunotherapy efficacy, we analyzed its relationship with biomarkers (TMB, MSI) predicting immune checkpoint inhibitor efficacy (Figure 9D–E). The radargrams showed significant correlations between *PDCD4* expression and 16 cancers, in which LAML showed a positive correlation in TMB. In addition, a positive correlation between *PDCD4* expression and MSI was observed in GBM, OV, and RAAD. By analyzing the results, we observed that *PDCD4* expression showed a negative correlation with TMB and MSI in BRCA, PRAD, SARC, and UCEC.

Subsequently, we investigated the relationship between *PDCD4* gene expression and immunotherapy response in three selected independent tumor immunotherapy cohorts and showed that there was no significant difference in *PDCD4* expression between the effective and ineffective groups in the three cohorts of metastatic melanoma, advanced uroepithelial carcinoma, and renal cell carcinoma treated with PD-1 (Figure 9F, G, H).





Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

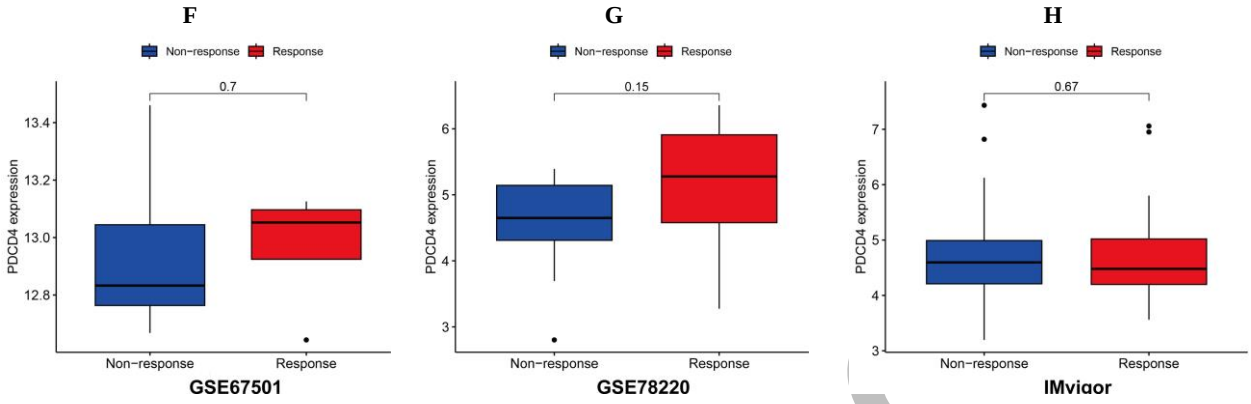


Figure 9. Correlation analysis of programmed cell death protein 4 (*PDCD4*) gene heterogeneity and immunotherapy response: A. Correlation between *PDCD4* gene expression and mutant-allele tumor heterogeneity (MATH) of different types of tumors. B. Correlation between *PDCD4* gene expression and homologous recombination deficiency (HRD) in different tumor types. C. Correlation between *PDCD4* gene expression and neoantigen load (NEO) in different types of tumors. D. Correlation between *PDCD4* gene expression and tumor mutation burden (TMB) of different types of tumors. E. Correlation between *PDCD4* gene expression and microsatellite instability (MSI) in different types of tumors. F. Correlation between *PDCD4* gene expression and immunotherapy response in Gene Expression Omnibus Study 67501 cohort (GSE67501 cohort). G. Correlation between PDCD4 gene expression and immunotherapy response in Gene Expression Omnibus Study 78220 cohort (GSE78220 cohort). H. Correlation between *PDCD4* gene expression and response to immunotherapy in IMvigor210 Study cohort (IMvigor cohort).

DISCUSSION

This study extends our understanding of *PDCD4*'s role in cancer by investigating its expression patterns in diverse tissue systems. Despite variations in subcellular localization depending on the cell type, consistently lower expression levels of *PDCD4* have been detected across multiple tumor types, suggesting a potential tumor-suppressive function. Clinically, *PDCD4* expression is inversely associated with certain clinicopathological characteristics, such as distant metastasis in patients with head and neck or urologic tumors.[6]

Furthermore, *PDCD4*'s utility as a diagnostic and prognostic biomarker has been thoroughly examined. Recognized as a major target of miR-21,[12] *PDCD4* shows strong diagnostic and prognostic relevance in gastrointestinal cancers, particularly colorectal, gastric, pancreatic, and esophageal cancers.[13] Our investigation adds to this body of knowledge by demonstrating an increased *PDCD4* expression in ESCA with promising diagnostic implications. Prior work has linked reduced *PDCD4* expression to an increase in cancer stem cells and poorer survival rates in ESCA patients.[14] In the context of colorectal adenocarcinoma (COAD), pan-cancer analysis shows decreased *PDCD4* expression and clinical evidence confirms that patients with low *PDCD4* expression in stages II and III COAD experience shorter survival periods than those with high expression,[15] reinforcing its prognostic importance. Conversely, in breast invasive carcinoma (BRCA), *PDCD4* is downregulated, and higher expression is positively correlated with improved patient outcomes in terms of progression-free survival (PFS), disease-specific survival (DSS), and disease-free survival (DFS).[16] Notably, *PDCD4*'s role in chemoresistance is highlighted by its capacity to reverse doxorubicin resistance in triple-negative breast cancer by interacting with eIF4A.[4] Moreover, *PDCD4*'s high expression predicts favorable outcomes in patients with acute myeloid leukemia (AML), as evidenced by longer overall survival (OS) following chemotherapy, supported by the Kaplan-Meier (KM) curves for AML cases.[17]

Perturbations in *PDCD4* expression at both mRNA and protein levels have pronounced consequences on cellular phenotypes. For instance, the downregulation of *PDCD4* overrides the growth-inhibitory effects of microRNA-181b-5p in thyroid cancer cells, fostering proliferation, migration, and invasion while reducing apoptosis.[18] Similarly, suppression of *PDCD4* attenuates the antitumor effects of microRNA-183-5p depletion in hepatocellular carcinoma.[19] The mRNA level and

promoter methylation status of *PDCD4* are statistically associated with HCC metastasis and differentiation, with hypermethylation serving as a predictive indicator of poor survival in HCC patients.[20] Androgens, interestingly, can induce *PDCD4* expression by demethylation, contributing to apoptosis in human ovarian granulosa cells;[21] however, hypermethylation of *PDCD4* does not appear to be a primary determinant of its altered expression in breast cancer cell lines,[22] underscoring the need for further research to clarify the complex epigenetic regulation of *PDCD4* across cancer types.

On the genetic front, *PDCD4* displays a versatile regulatory function in cancer onset and progression. Yet, its possible role in the tumor microenvironment (TME) remains an open question. Addressing this, our systematic investigation revealed a marked inverse correlation between *PDCD4* expression and immune cell infiltration across a spectrum of cancer types. Experimental data indicate that *PDCD4* suppression potently induces inflammation in macrophages,[23] and *PDCD4* also orchestrates inflammatory processes in macrophages through modulation of P13K, p38, and CK2 signaling pathways, highlighting its vital role within the TME.[24] Clinical studies in melanoma demonstrate that *PDCD4* expression correlates significantly with immune infiltrates, specifically $CD4^+$ T cells, NK cells, B cells, and mast cells, within the TME, with a strong link observed in both primary and metastatic melanomas.[25] Collectively, these findings confirm that *PDCD4* indeed plays a critical regulatory part in the TME and exerts a profound influence on cancer evolution.

While initial inquiries have illuminated *PDCD4*'s importance in immune-related regulatory mechanisms, the underlying specific functional mechanisms require deeper exploration. Intriguingly, *PDCD4*'s role in immune cells appears to implicate regulation across multiple biological processes. Researchers have found that activation of mitogen-activated protein kinase p38 leads to the degradation of *PDCD4* in macrophages and fibroblasts,[26] emphasizing the importance of understanding the upstream regulatory networks of *PDCD4* in cancer and immune cells for devising novel therapeutic strategies against cancer and inflammation. Beyond its role in inflammation, *PDCD4* is also implicated in the control of various inflammatory disease states. For instance, in a DSS-induced colitis model, *PDCD4* deletion resulted in increased IL-6 expression, subsequently activating the STAT3 pathway and promoting colitis-to-cancer transition.[27] Our study also reveals that high *PDCD4* expression in LUSC can repress the JAK/STAT signaling pathway, findings that align well with prior experimental results. Silencing the *PDCD4* gene concurrently blocks the activation of JAK/STAT and P13K/AKT pathways, thereby inhibiting lung cancer cell proliferation and metastasis, and promoting apoptosis.[28]

Although *PDCD4* expression was not significantly differentially expressed in a pan-cancer immunotherapy cohort at present, it is worth noting that targeting *PDCD4* in breast cancer cells can activate the P13K/AKT signaling pathway, consequently enhancing PD-L1 expression,[29] providing new insights and potential strategies for anti-PD-L1 antibody treatments in mouse breast cancer models.

In summary, this paper innovatively found that patients with high *PDCD4* expression have a higher survival rate; *PDCD4* serves as an important biomarker for tumor diagnosis and prognosis and has a regulatory role in tumor progression. The expression level of the *PDCD4* gene plays a key role in the tumor immune microenvironment, and there is a strong correlation between *PDCD4* expression and the biomarkers (TMB, MSI) predicting the efficacy of immune checkpoint inhibitors. *PDCD4* may serve as a potential target for tumors and immune therapy

STATEMENT OF ETHICS

Not applicable.

FUNDING

This work was supported by the Health Research Project of Anhui Province (AHWJ2023BAc20005).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The online website contains the datasets mentioned in this study. Website name and login number can be

found in the article/supplementary materials. The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

REFERENCES

1. Shibahara K, Asano M, Ishida Y, Aoki T, Koike T, Honjo T. Isolation of a novel mouse gene MA-3 that is induced upon programmed cell death. Gene. 1995;166(2):297-301.
2. Deng H, Yu B, Li Y. Tanshinone IIA alleviates acute ethanol-induced myocardial apoptosis mainly through inhibiting the expression of *PDCD4* and activating the PI3K/Akt pathway. Phytother Res. 2021;35(8):4309-23.
3. Pan J, Wang B, Pu X, Qiu C, Li D, Wu Z, et al. lncRNA GAPLINC regulates vascular endothelial cell apoptosis in atherosclerosis. Arch Med Sci. 2024;20(1):216-32.
4. Gonzalez-Ortiz A, Pulido-Capiz A, Castaneda-Sanchez CY, Ibarra-Lopez E, Galindo-Hernandez O, Calderon-Fernandez MA, et al. eIF4A/*PDCD4* Pathway, a Factor for Doxorubicin Chemoresistance in a Triple-Negative Breast Cancer Cell Model. Cells-Basel. 2022;11(24):4069.
5. Chen X, Guan Y, Zhang Y, Jia Y, Li W, Guo C, et al. Programmed cell death 4 modulates lysosomal function by inhibiting TFEB translation. Cell Death Differ. 2021;28(4):1237-50.
6. Li JZ, Gao W, Ho WK, Lei WB, Wei WI, Chan JY, et al. The clinical association of programmed cell death protein 4 (*PDCD4*) with solid tumors and its prognostic significance: a meta-analysis. Chin J Cancer. 2016;35(1):95.
7. Fan Q, Lu Q, Wang G, Zhu W, Teng L, Chen W, et al. Optimizing component formula suppresses lung cancer by blocking DTL-mediated *PDCD4* ubiquitination to regulate the MAPK/JNK pathway. J Ethnopharmacol. 2022;299:115546.
8. Huang WC, Yadav VK, Cheng WH, Wang CH, Hsieh MS, Huang TY, et al. The MEK/ERK/miR-21 Signaling Is Critical in Osimertinib Resistance in EGFR-Mutant Non-Small Cell Lung Cancer Cells. Cancers. 2021;13(23):6005.
9. Ge Y, Xia T, Feng Z, Xi Y, Hu D, Hong Y, et al. Knockdown of programmed cell death factor 4 restores erectile function by attenuating apoptosis in rats with bilateral cavernous nerve crush injury. Andrology-Us. 2024.
10. Chen Q, Lu H, Duan C, Zhu X, Zhang Y, Li M, et al. *PDCD4* Simultaneously Promotes Microglia Activation via *PDCD4*-MAPK-NF-kappaB Positive Loop and Facilitates Neuron Apoptosis During Neuroinflammation. Inflammation. 2022;45(1):234-52.
11. Zhu F, Zhang X, Wen J, Liu Y, Zhu Y. Celastrus orbiculatus extract reverses precancerous lesions of gastric cancer by inhibiting autophagy via regulating the *PDCD4*-ATG5 signaling pathway. J Pharm Pharmacol. 2024;76(3):257-68.
12. Mondin A, Bertazza L, Barollo S, Pedron MC, Manso J, Piva I, et al. Validation of miRNAs as diagnostic and prognostic biomarkers, and possible therapeutic targets in medullary thyroid cancers. Front Endocrinol. 2023;14:1151583.
13. Farasati FB, Vakili K, Fathi M, Yaghoobpoor S, Bhia M, Naimi-Jamal MR. The role of microRNA-21 (miR-21) in pathogenesis, diagnosis, and prognosis of gastrointestinal cancers: A review. Life Sci. 2023;316:121340.
14. Huckabay LM, Anderson N, Holm DM, Lee J. Cognitive, affective, and transfer of learning consequences of computer-assisted instruction. Nurs Res. 1979;28(4):228-33.
15. Kim JY, Lee H, Kim EK, Lee WM, Hong YO, Hong SA. Low *PDCD4* Expression Is Associated With Poor Prognosis of Colorectal Carcinoma. Appl Immunohisto M M. 2021;29(9):685-92.
16. Cai Q, Yang HS, Li YC, Zhu J. Dissecting the Roles of *PDCD4* in Breast Cancer. Front Oncol. 2022;12:855807.
17. Tang H, Chen Y, Zhang N, Deng J, Zhou K. Higher expression of programmed cell death 4 (*PDCD4*) in acute myeloid leukemia is associated with better prognosis after chemotherapy. Ann Hematol. 2023;102(12):3401-12.
18. Geng X, Li Y, Sun Y, Cao L, Song Z. MicroRNA-181b-5p Facilitates Thyroid Cancer Growth via Targeting Programmed Cell Death 4. Mol Biotechnol. 2024;66(5):1154-64.
19. Duan X, Li W, Hu P, Jiang B, Yang J, Zhou L, et al. MicroRNA-183-5p contributes to malignant progression through targeting *PDCD4* in human hepatocellular carcinoma. Bioscience Rep. 2020;40(10):BSR20201761.
20. Ding X, Cheng X, Gong M, Chen X, Yin F, Lai K. Hypermethylation and Expression Silencing of *PDCD4* Gene in Hepatocellular Carcinoma: A Consort Study. Medicine. 2016;95(6):e2729.
21. Qiu X, Wei Y, Liu C, Ding C, Zhao S. Hyperandrogen enhances apoptosis of human ovarian granulosa cells via up-regulation and demethylation of *PDCD4*. Gynecol Endocrinol. 2020;36(4):333-7.
22. Wen YH, Shi X, Chiriboga L, Matsahashi S, Yee H, Afonja

O. Alterations in the expression of *PDCD4* in ductal carcinoma of the breast. Oncol Rep. 2007;18(6):1387-93.
23. Yang SY, Hu Y, Zhao R, Zhou YN, Zhuang Y, Zhu Y, et al. Quercetin-loaded mesoporous nano-delivery system remodels osteoimmune microenvironment to regenerate alveolar bone in periodontitis via the miR-21a-5p/*PDCD4*/NF-kappaB pathway. J Nanobiotechnol. 2024;22(1):94.
24. Doos WG, Saravis CA, Pusztaszeri G, Burke B, Oh SK, Zamcheck N, et al. Tissue localization of zinc glycinate marker and carcinoembryonic antigen by immunofluorescence. II. Immunofluorescence microscopy. Jnci-J Natl Cancer I. 1978;60(6):1375-82.
25. Tran TT, Rane CK, Zito CR, Weiss SA, Jessel S, Lucca L, et al. Clinical Significance of *PDCD4* in Melanoma by Subcellular Expression and in Tumor-Associated Immune Cells. Cancers. 2021;13(5)
26. Braun C, Katholnig K, Kaltenecker C, Linke M, Sukhbaatar N, Hengstschlager M, et al. p38 regulates the tumor suppressor *PDCD4* via the TSC-mTORC1 pathway. Cell Stress. 2021;5(12):176-82.
27. Lai CY, Yeh KY, Liu BF, Chang TM, Chang CH, Liao YF, et al. MicroRNA-21 Plays Multiple Oncometabolic Roles in Colitis-Associated Carcinoma and Colorectal Cancer via the PI3K/AKT, STAT3, and *PDCD4*/TNF-alpha Signaling Pathways in Zebrafish. Cancers. 2021;13(21):5565.
28. Zhou J, Li H, Li N, Li X, Zhang H, Song Q, et al. MicroRNA-641 inhibits lung cancer cells proliferation, metastasis but promotes apoptosis in cells by targeting *PDCD4*. Int J Clin Exp Patho. 2017;10(8):8211-21.
29. Guo LM, Ding GF, Xu WC, Ge H, Jiang Y, Lu YF. Anti-PD-L1 Antibody Enhances T Cell Immune Responses and Reduces Resistance of Breast Cancer Cells to Radiotherapy. Oxid Med Cell Longev. 2022;2022:5938688.