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ORIGINAL RESEARCH

Comprehensive Analysis Reveals the Potential Roles of CDKN3 in Pancancer and Verification in Endometrial Cancer

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Background: Cyclin-dependent kinase inhibitor 3 (CDKN3) has been studied in many cancers. However, the comprehensive and systematic pancancer analysis of CDKN3 genes is still lacking.

Methods: Data were downloaded from online databases. R was used for analysis of the differential expression and gene alteration of CDKN3 and of the associations between CDKN3 expression and survival, signaling pathways, and drug sensitivity. Clinical samples and in vitro experiments were selected for verification.

Results: CDKN3 expression was higher in most types of cancers, and this phenotype was significantly correlated with poor survival. CDKN3 showed gene alterations and copy number alterations in many cancers and associated with some immune-related pathways and factors. Drug sensitivity analysis elucidated that CDKN3 could be a useful marker for therapy selection. Clinical samples elucidated CDKN3 expressed high in endometrial cancer tissue. In vitro studies showed that CDKN3 induced pro-tumor effect in immune environment and facilitated endometrial cancer cell proliferation and G1/S phase transition.

Conclusion: CDKN3 has been shown to be highly expressed in most types of cancers and promoted cancer cell progression. CDKN3 may serve as a novel marker in clinical diagnosis, treatment, and prognosis prediction in future.

Keywords: CDKN3, pancancer analysis, diagnosis, prognosis, therapeutics, immunity, endometrial cancer

Introduction

The overall mortality rate of cancer has decreased by 33% since 1991 due to smoking prohibition, screening, vaccination, and the use of adjuvant therapies such as targeted therapy and immunotherapy, but the number of predicted cancer-related deaths and new cases for 2023 exceeds 0.6 million and nearly 2 million, respectively, in America.[1] Cancer has become a prime cause of death in the Chinese population, and lung carcinoma, gastric cancer, colorectal cancer, liver cancer and breast cancer are the most prevalent cancers in China.[2] According to Global Cancer Statistics 2020, the global expected cancer burden in 2040 will be nearly 1.5-fold higher than that in 2020.[3]

CDKN3, also known as CDI1, cyclin-dependent kinase-associated protein phosphatase (KAP), CIP2 and KAP1, is a gene that encodes one dual-specificity protein phosphatase family protein. CDKN3 regulates the cell cycle as a cyclin-dependent kinase inhibitor; it inactivates two eukaryotic cell cycle regulator genes: it dephosphorylates cyclin-dependent kinase 1 (CDK1), which is essential for the cell cycle, at Thr-161; it can also dephosphorylate cyclin-dependent kinase 2 (CDK2) at Thr-160.[4,5] Many researchers have been keenly aware of the role of CDKN3 and started research on a variety

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of diseases. The relationship between CDKN3 and cancers has been previously presented in many publications. The expression of CDKN3 between tumor tissues and normal tissues or paratumor tissues is different in most types of cancers according to clinicopathologic feature analyses of surgical patients.[6–8] CDKN3 commonly regulates tumor cells proliferation, invasion, migration, and apoptosis,[6,8–12] and it has been found to be significantly correlated with tumor prognosis in studied cancers.[8,9,13] However, the specific roles of CDKN3 are not identical across cancers, and the role of CDKN3 even within the same cancer is controversial. On the one hand, CDKN3 has been described as a tumor suppressor in many types of malignant tumors,[14] including glioblastoma,[6,15] gastric cancer,[16] Bcr-Abl-mediated chronic myelogenous leukemia,[17] and hepatocellular carcinoma.[18] On the other hand, CDKN3 also acts as a tumor promoter in most types of cancer, such as epithelial ovarian cancer,[19] breast cancer,[20] esophageal cancer,[11] gastric cancer,[21] lung cancer,[22] adrenocortical cancer,[23] and hepatocellular carcinoma.[24] Moreover, research on CDKN3 has not covered all cancers, such as endometrial cancer, which is the sixth most common cancer in women.[25] At present, a comprehensive and systematic analysis of CDKN3 genes across cancers is still lacking.

With the establishment of a variety of databases, including the cancer genome atlas (TCGA), genotype-tissue expression (GTEx), gene expression omnibus (GEO), cancer cell line encyclopedia (CCLE), the human protein atlas (HPA), an integrated repository portal for tumor-immune system interactions (TISIDB), and tumor immune estimation resource (TIMER), we can efficiently and accurately identify meaningful biomarkers to detect disease, predict prognosis or guide treatment.

In this study, we performed a comprehensive analysis of the role of CDKN3 across cancers and verified the role of CDKN3 in endometrial cancer. First, we explored the differential expression and prognostic significance of CDKN3 in multiple cancers. We found that CDKN3 had higher expression levels in most types of cancers and showed a significant correlation with poor survival, especially in sarcoma (SARC), uterine corpus endometrial carcinoma (UCEC), liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), and kidney renal papillary cell carcinoma (KIRP). Second, we further probed the aberrant expression spectrum of CDKN3 and found that the alteration type was different in distinct tumors, with very few abnormal changes and a rare correlation with methylation. Third, we explored the correlation between CDKN3 expression levels and pathway enrichment and found that CDKN3 is associated with some immune-related pathways (in addition to the previously recognized cell cycle-related pathways). Subsequently, we assessed the correlation of CDKN3 expression with drug sensitivity, and we found some drugs that were more effective when CDKN3 expression was high/low. Finally, clinical samples verified high CDKN3 expression in endometrial cancer by qRT-PCR; meanwhile, in vitro experiments elucidated that CDKN3 induce a pro-tumor effect in the immune environment and facilitates endometrial cancer cell proliferation and G1/S phase transition. In conclusion, our study is the first comprehensive analysis of CDKN3 across cancers, and our findings may provide new insight into the role of CDKN3 in the clinical diagnosis, treatment, and prognosis prediction of different cancers and provide new research directions.

Material and Methods

Expression Analysis and Survival Analysis

The differential expression between tumor tissues and normal tissues was assessed via the GTEx and TCGA databases. A *t* test was used for statistical analysis, and $p<0.05$ denoted a significant difference. The survival analysis was performed with the R package "survminer". Cox regression analysis was performed to assess the prognostic significance of CDKN3 across cancers. R version 4.1.2 was used to conduct the analysis.

Gene Mutation and Methylation Analysis

The cBioPortal website (https://www.cbioportal.org/) was used to perform mutation analysis. The copy number alteration (CNA) and methylation (including CpG methylation) data were acquired from the cBioPortal database. The correlations between CDKN3 expression and CNA, methylation and CpG methylation were calculated using Spearman correlation coefficients. R version 4.1.2 was used to conduct the analysis.

Pathway Analysis and Gene Set Enrichment Analysis

Molecular signatures database (MSigDB), which contains 50 hallmark pathway datasets, and the gene set variation analysis (GSVA) package, which employs a single-sample gene set enrichment analysis algorithm, were used to quantify the tumor hallmark score. The immune-related parameters were calculated by the "estimation of stromal and immune cells in malignant tumour tissues using expression data (ESTIMATE)" R package, and the list of immunostimulators, immunoinhibitors, major histocompatibility complex (MHC) molecules, etc., was obtained from the TISIDB portal. TIMER (https://cistrome.shinyapps.io/timer/) was used to quantify the correlation between CDKN3 expression and the levels of six tumor-infiltrating immune cells (TIICs). The tumor microenvironment pathway scores were calculated by the "prcomp" package, and infiltrating immune cell scores were acquired from the TCGA pancancer dataset through the immune cell abundance identifier (immuCellAI) database (http://bioinfo.life.hust.edu.cn/ImmuCellAI). The "corrplot" R package was used to calculate the correlation between CDKN3 and immune-related scores and immune-related factor expression. The "clusterProfiler" package was used to probe the biological function of CDKN3.

The "ggplot2" package was used to draw the heatmap, and the "ggridges" package was used to draw the gene set enrichment analysis (GSEA) plots in the form of a mountain map. R version 4.1.2 was used to conduct the analysis.

Drug Sensitivity Analysis

CellMiner is an in vitro drug discovery tool that has been used as a screening tool supporting cancer research based on the NCI-60 panel of cancer cell lines;[26] it was employed to explore the correlation between CDKN3 expression and drug sensitivity. Spearman correlation coefficients were calculated to investigate the correlation, and the different sensitivities between the CDKN3 high/low expression groups were assessed by the Wilcoxon rank-sum test. $p<0.05$ denoted significance. The "ggplot2" and "ggpubr" packages were used to draw the scatterplot and boxplot, respectively. R version 4.1.2 was used to conduct the analysis.

Samples Collection, RNA Preparation, and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Thirty-five cases of endometrial cancer tissues and twenty-five cases of normal endometrial tissues from uterine prolapse or uterine myoma were collected from the Department of Gynecology and Obstetrics, Tianjin Medical University General Hospital, for qRT-PCR. The study was authorized by the Ethics Committee of Tianjin Medical University General Hospital (batch number: IRB2023-YX-093-01) and written informed consent was obtained from all patients.

Collected fresh samples were stored in a −80 °C freezer for later extraction. Tissue grinding and total RNA extraction were performed using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA). StarScript III All-in-one RT Mix with gDNA Remover (GenStar, Beijing, China) was used to reverse transcribe RNA into cDNA. The qPCR assay was conducted on an ABI 7500 real-time PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA) using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. The PCR primers were as follows: CDKN3-F: 5'-GGACTCCTGACATAGCCAGC-3', CDKN3-R: 5'-CTGTATTGCCCCGGATCCTC-3'; GAPDH-F: 5'-GGTG GTCTCCTCTGACTTCAACA-3', GAPDH-R: 5'-GTTGCTGTAGCCAAATTCGTTGT-3'. Relative quantification of RNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. A *t* test was used for statistical analysis, and $p<0.05$ denoted a significant difference. GraphPad Prism version 9.5.1 was used to conduct the analysis.

Cell Culture and Transfection

Two kinds of endometrial cancer cell lines (HEC-1-A and HEC-1-B) were obtained from the American Tissue Culture Collection (Manassas, VA, USA). HEC-1-A and HEC-1-B were cultured under a humidified 5% CO_2 atmosphere at 37 °C with McCoy's 5A (Procell, Wuhan, China) and 1640 medium (Gibco, USA), respectively. The medium was supplemented with 10% fetal bovine serum.

Small interfering RNA (siRNA) (GenePharma, Suzhou, China) was transfected into cells to knock down the CDKN3 expression according to the manufacturer's protocol. Four CDKN3-specific siRNA and negative control siRNA sequences were used: siRNA-1 (CDKN3-Homo-120) with sense 5'- GCCGCCCAGUUCAAUACAATT-3' and antisense 5'-

UUGUAUUGAACUGGGCGGCTT-3' sequences, siRNA-2 (CDKN3-Homo-463) with sense 5'-GCCAGCUGCUGUGAAAUAATT −3' and antisense 5'- UUAUUUCACAGCAGCUGGCTT −3' sequences, siRNA-3 (CDKN3-Homo-565) with sense 5'- GCUGCUUGUCUCCUACUAUTT −3' and antisense 5'- AUAGU AGGAGACAAGCAGCTT −3' sequences, siRNA-4 (CDKN3-Homo-693) with sense 5'- GGACAA AUUAGCUGCACAUTT-3' and antisense 5'- AUGUGCAGCUAAUUUGUCCTT-3' sequences, and negative control (siRNA NC) with sense 5'- UUCUCCGAACGUGUCACGUTT −3' and antisense 5'- ACGUGACACGUUCGGAGAATT −3' sequences.

Western Blot

Western blotting was performed according to the manufacturer's protocol. In brief, total protein was extracted from cells using RIPA lysis buffer. Protein extracts were run on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10%) and transferred to the PVDF membranes. The membranes were blocked with 5% blocking buffer (Elabscience, Wuhan, China) at room temperature and then incubated with rabbit anti-human CDKN3 polyclonal antibody (1:1000, bs-5743R, Bioss, Beijing, China) and with mouse β-actin monoclonal antibody (1:1000, TA-09, ZSGB-BIO, Beijing, China) overnight at 4 °C. Next, the membranes were washed with 1×TBST three times for 10 min and then incubated with rabbit/mouse secondary antibodies at room temperature. ECL reagents (BL520A, biosharp, Hefei, China) were used to visualize the protein bands on the blots.

Cell Proliferation Assay

After siRNA transfection, endometrial cancer cells (4000/well) were placed on 96-well plates (100 μL/well) and then cultured in an incubator with 5% CO_2 at 37 °C. After 0 d, 1 d, 2 d, 3 d, and 4 d of incubation, 10 μl of CCK-8 reagents (GK10001, Glpbio, Montclair, CA, USA) was added to each well and then incubated for 2 h. OD 450 nm values were measured using a plate reader. GraphPad Prism version 9.5.1 was used to conduct the analysis.

Cell Cycle Assay

Seventy-two hours after siRNA transfection, cells were harvested and fixed with 70% ethanol at 4 °C overnight. Then, fixed cells were recovered by centrifugation. After washing with PBS, the cells were incubated with 100 μL RNase A (CA1510, Solarbio, Beijing, China) at 37 °C for 30 min. After staining in the dark with 400 μL PI (CA1510, Solarbio, Beijing, China) at 4 °C for 30 min, a BD LSRFortessa flow cytometer (BD Biosciences, San Jose, CA, USA) was used for measurement. Flowjo version 10.6.2 and GraphPad Prism version 9.5.1 were used to conduct the analysis.

Human PBMCs Isolation, Co-Culture and LDH Release Assay

Peripheral blood was obtained from endometrial cancer patients who all signed informed consent forms. Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll reagents (LST1077, TBDscience, Tianjin, China) and then cultured in advanced RPMI 1640 medium (Gibco) with 10% fetal bovine serum under a humidified 5% CO_2 atmosphere at 37 °C for 24 h. Twenty-four hours after siRNA transfection, endometrial cancer cells (5000/well) were placed on 96-well plates, and after 4 h culture, PBMCs were co-cultured with endometrial cancer cells at an effector/target ratio of 20:1 (100μL/well).

The lactate dehydrogenase (LDH) release assay was used to evaluate cell damage by a Cytotoxicity LDH Assay Kit (GK10003, Glpbio, Montclair, CA, USA). Forty-eight hours after cancer-PBMCs co-culture, the 96-well plate was centrifuged and the supernatants (50 μL) were placed on another 96-well plate. Then, 50 μL of working solution was added to each well and incubated in the dark at room temperature for 30 min. Next, 50 μL of stop solution was added to each well and OD values were immediately measured using a plate reader at 490 nm. Cytotoxicity (%) was calculated as follows: (experimental LDH release - spontaneous LDH release) / (total release - spontaneous LDH release).

Results

Differential Expression of CDKN3 Across Cancers

We analyzed the differential expression level of CDKN3 across 33 cancers in the TCGA database. First, we analyzed the mRNA expression level of CDKN3 between tumor and normal tissue. We found that 30 tumor tissues had marked

differential expression of CDKN3 compared to corresponding normal tissues. The mRNA expression level of CDKN3 was upregulated in 28 distinct types of tumors, including cervical squamous cell carcinoma (CESC), SARC, head and neck squamous cell carcinoma (HNSC), bladder urothelial carcinoma (BLCA), ovarian serous cystadenocarcinoma (OV), stomach adenocarcinoma (STAD), lung squamous cell carcinoma (LUSC), breast invasive carcinoma (BRCA), UCEC, colon adenocarcinoma (COAD), cholangiocarcinoma (CHOL), skin cutaneous melanoma (SKCM), LIHC, lung adenocarcinoma (LUAD), pheochromocytoma and paraganglioma (PCPG), esophageal carcinoma (ESCA), rectum adenocarcinoma (READ), kidney renal clear cell carcinoma (KIRC), uterine carcinosarcoma (UCS), glioblastoma multiforme (GBM), brain lower grade glioma (LGG), PAAD, prostate adenocarcinoma (PRAD), adrenocortical carcinoma (ACC), KIRP, thymoma (THYM), kidney chromophobe (KICH), and lymphoid neoplasm diffuse large B cell lymphoma (DLBC), whereas it was downregulated in 2 distinct types of tumors, testicular germ cell tumors (TGCT) and acute myeloid leukemia (LAML); all of the p values met the criteria for statistical significance (Figure 1A). Subsequently, analysis of CDKN3 expression in tumor tissues vs corresponding normal tissues for 22 cancers was performed (Supplementary Figure S1). We found that CDKN3 was upregulated in 17 kinds of tumors, including HNSC, BLCA, STAD, LUSC, BRCA, UCEC, COAD, CHOL, LIHC, LUAD, ESCA, READ, KIRC, PRAD, KIRP, KICH, and thyroid carcinoma (THCA), and all of the p values indicated statistical significance (Figure 1B). Downregulated CDKN3 expression was not found in any of the cancers in the paired sample expression analysis.

The Prognostic Significance of CDKN3 Across Cancers

Since we observed the differential expression of CDKN3 between almost all tumor and paired normal tissues, we investigated the prognostic significance of CDKN3 across cancers. We used univariate Cox regression to analyze overall survival (OS) (Figure 2A), disease-specific survival (DSS) (Figure 2B), disease-free interval (DFI) (Figure 2C), and progression-free interval (PFI) (Figure 2D). Factors with a hazard ratio (HR) greater than 1 were considered to be prognostic risk factors.

In terms of OS, CDKN3 was an independent risk factor in PCPG, KIRP, mesothelioma (ACC), MESO, KICH, PRAD, KIRC, LGG, PAAD, LUAD, UCEC, LIHC, HNSC, SARC, and LUSC. In terms of DSS, CDKN3 was an independent risk factor in PCPG, KIRP, PRAD, KICH, ACC, MESO, KIRC, LGG, ESCA, PAAD, LIHC, LUAD, UCEC, HNSC, and DARC. In terms of DFS, CDKN3 was an independent risk factor in KIRP, TGCT, THCA, ESCA, PAAD, PRAD, SARC, BRCA, UCEC, and LIHC. Finally, in the analysis of PFS, CDKN3 was an independent risk factor in KIRP, KICH, TGCT, ACC, PCPG, ESCA, PRAD, THCA, KIRC, PAAD, MESO, LGG, UCEC, SARC, LIHC, BLCA, HNSC, BRCA, LUAD, and STAD. All HR values were greater than 1, and the p values met the threshold for statistical significance.

CDKN3 was found to have marked prognostic significance in 21 of 33 cancers by univariate Cox regression analysis of OS, DSS, DFI, or PFI. For example, CDKN3 was significantly correlated with prognosis in all four analyses of SARC (OS: HR (95% CI) = 1.3 (1−1.6), p =0.032; DSS: HR (95% CI) = 1.3 (1−1.7), p =0.036; DFI: HR (95% CI) = 1.5 (1.1−2), p =0.0059; PFI: HR (95% CI) = 1.4 (1.1−1.7), p =0.0019), UCEC (OS: HR (95% CI) = 1.5 (1.1−2.1), p =0.014; DSS: HR (95% CI) = 1.6 (1.1−2.3), p =0.026; DFI: HR (95% CI) = 1.5 (1−2.2), p =0.04; PFI: HR (95% CI) = 1.5 (1.1−1.9), p =0.0054), LIHC (OS: HR (95% CI) = 1.4 (1.2−1.7), p =0.00037; DSS: HR (95% CI) = 1.6 (1.3−2), p =0.00017; DFI: HR (95% CI) = 1.2 (1−1.4), p =0.021; PFI: HR (95% CI) = 1.3 (1.1−1.5), p =0.00057), PAAD (OS: HR (95% CI) = 1.9 (1.3−2.8), p =0.00079; DSS: HR (95% CI) = 1.8 (1.2−2.8), p =0.0046; DFI: HR (95% CI) = 2.2 (1.1−4.3), p =0.028; PFI: HR (95% CI) = 2 (1.4−2.8), p =0.00025), and KIRP (OS: HR (95% CI) = 4.7 (3.1−7.3), p <0.0001; DSS: HR (95% CI) = 8.9 (5.3−15), p <0.0001; DFI: HR (95% CI) = 6.2 (3.4−11), p <0.0001; PFI: HR (95% CI) = 5.1 (3.5−7.4), p <0.0001).

Gene Alteration and Methylation Analysis of CDKN3 Across Cancers

Cancer driver gene mutations play a significant role in tumorigenesis, while DNA methylation is usually abnormal in tumors.[27,28] Therefore, we tried to identify the gene mutation and methylation of CDKN3 in multiple cancers.

Primarily, we explored the alteration frequency of CDKN3 across cancers. The results showed that the alteration type of CDKN3 is different in distinct tumors, and the highest alteration frequency of CDKN3 was found in DLBC, in which the main alteration type was amplification, followed by UCEC, LUAD, MESO, etc. (Figure 3A).

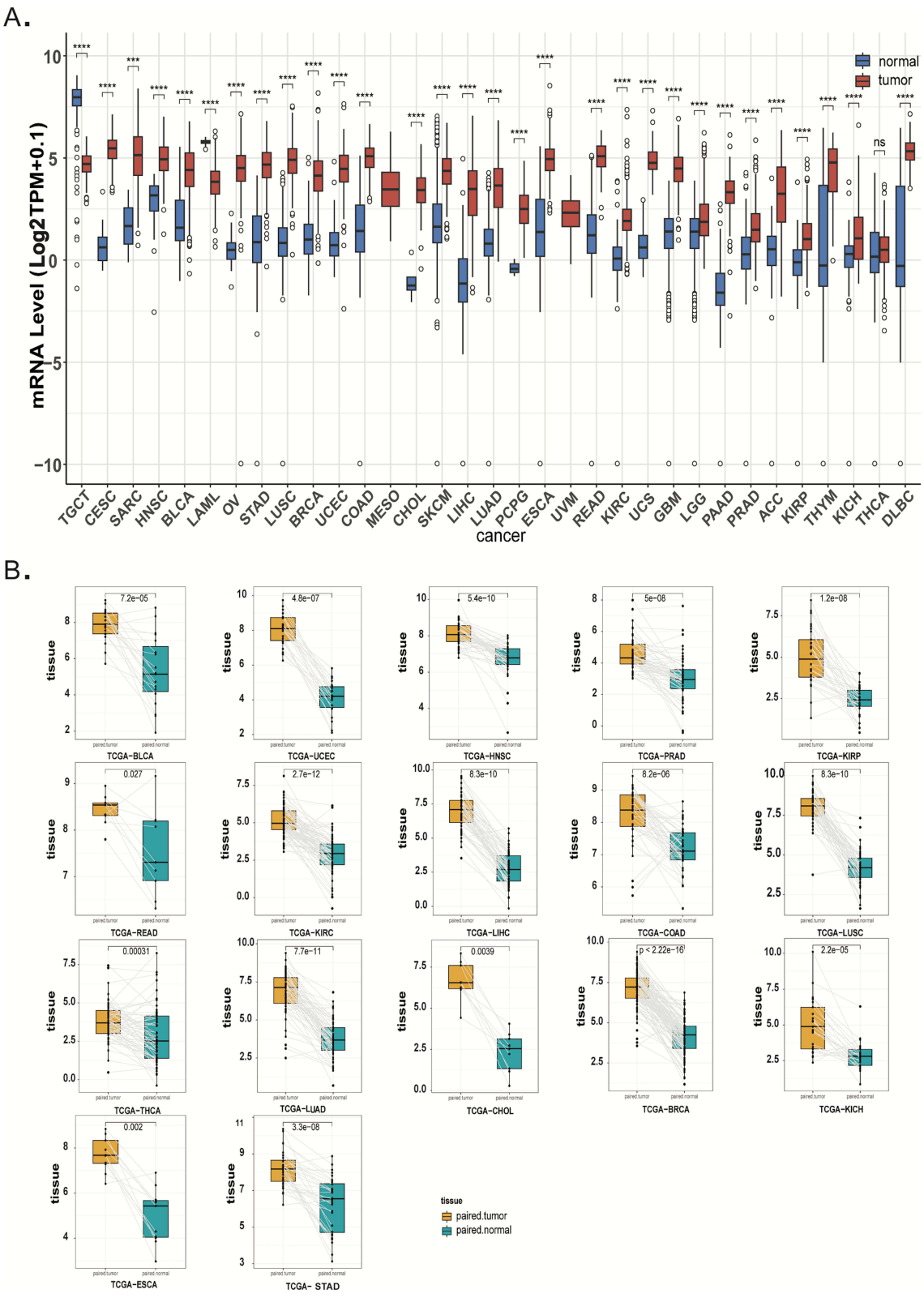

Figure 1 The differential expression of CDKN3. (**A**) CDKN3 expression in normal and cancer tissues according to the GTEx database and TCGA database. (**B**) CDKN3 expression in corresponding tumors and normal tissues according to the TCGA database. ***p < 0.001, ****p < 0.0001.

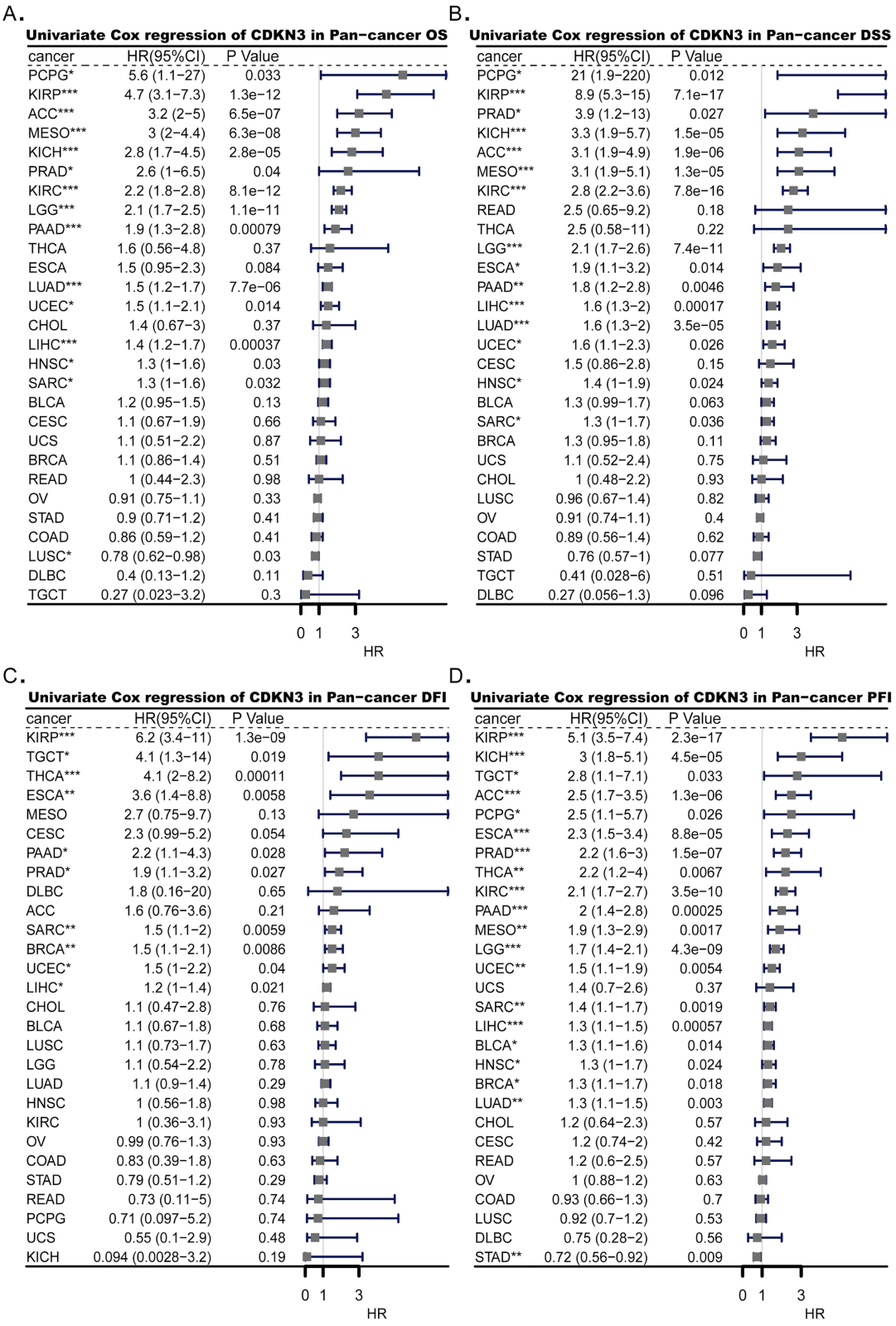

Figure 2 The prognostic significance of CDKN3. Univariate Cox regression analysis was performed, and forest maps are shown for (**A**) OS, (**B**) DSS, (**C**) DFI, and (**D**) PFI. *p < 0.05, **p < 0.01, ***p < 0.001.

Subsequently, we analyzed the association between CDKN3 expression and CNA across cancers. The results showed that CDKN3 expression showed a strong positive correlation with CNA in 13 distinct types of tumors, including CHOL, LUSC, COAD, TGCT, UCS, CESC, SARC, BLCA, PCPG, HNSC, DLBC, ESCA, and GBM (Spearman's R: 0.3–0.5, and Spearman's p values<0.05), a weak positive correlation with the other 8 kinds of tumors and a weak negative correlation with KIRP (Figure 3B).

Finally, we explored the correlation between CDKN3 expression and methylation of the promoter region and CpG region in multiple cancers since the methylation of CpG sites of the promoter regulates gene expression.[29] The results demonstrated that CDKN3 exhibited a weak positive correlation with promoter methylation in CESC and a weak negative correlation in PRAD (Spearman's R<0.3, and Spearman's p values<0.05) (Figure 3C). CDKN3 showed a weak negative correlation with CpG methylation in LGG, STAD, THCA, and MESO (Spearman's R<0.3, and Spearman's p values<0.05) (Figure 3D).

Correlation Analyses Between CDKN3 and Hallmark Pathways in Pancancer

Herein, we found differences in the expression levels, prognostic significance and gene alterations of CDKN3 across cancers. Next, we analyzed the correlation between the expression level of CDKN3 and the hallmark pathways in distinct types of cancers.

We found that CDKN3 exhibited a positive correlation with the pathways of the cell cycle, signal transduction, and metabolism, which are all associated with the occurrence and development of cancer (Figure 4).

The cell cycle-associated pathways, including the G2M checkpoint, E2F targets, DNA repair, and mitotic spindle, showed a strong positive correlation with CDKN3 expression in almost all types of cancer. The signal transduction-associated pathway, metabolism-associated pathway and proliferation-associated pathways, including MTORC1 signaling, MYC targets V1, MYC targets V2, and glycolysis, also showed a relatively strong positive correlation.

Moreover, the results showed that CDKN3 is negatively correlated with bile acid metabolism, heme metabolism, adipogenesis, apoptosis, etc.

Correlation Analysis of CDKN3 and Microenvironment-Related Pathways Across Cancers

Subsequently, we tried to determine the relationship between the expression of CDKN3 and tumor microenvironment-related pathways (Figure 5A).

The results showed that CDKN3 was strongly positively associated with the cell cycle pathway and genetic information processing in all cancers; the related pathways included the cell cycle, DNA replication, and base excision repair pathways. Other cell cycle pathways and genetic information processing pathways, including cell cycle regulation and histones, showed a positive correlation with CDKN3 expression in most cancers.

We further explored the association of multiple pathways, especially cell cycle-related pathways, with CDKN3 expression across cancers. The top 20 related pathways are presented in the form of a mountain map, and cell cycle-related pathways, which basically covered all of the top 20 pathways, are marked. Here, we listed information for 5 types of cancers: UCEC, SARC, PAAD, LIHC, and KIRP (Figure 5B). As mentioned above, all cancers had different expression levels of CDKN3 between tumor and normal tissues and/or paired tissues; CDKN3 was significantly related to prognosis according to univariate Cox regression analysis of OS, DSS, DFI, and PFI. For example, the expression of CDKN3 in UCEC was associated with cell cycle DNA replication, nuclear DNA replication, DNA replication initiation, negative regulation of metaphase/anaphase transition of the cell cycle, spindle checkpoint signaling, negative regulation of nuclear division, etc. The mountain map of the other 28 kinds of cancers is shown in Supplementary Figure S2.

Correlation Between CDKN3 and Immune-Related Factors Across Cancers

Immunotherapy plays a significant role in decreasing the overall mortality rate of cancers.[1] A previous study revealed that cell cycle-targeted therapy plus immunotherapy led to promising therapeutic benefits;[30] DNA replication could also affect antitumor immunity.[31] As mentioned above, CDKN3 was strongly positively associated with the cell cycle and

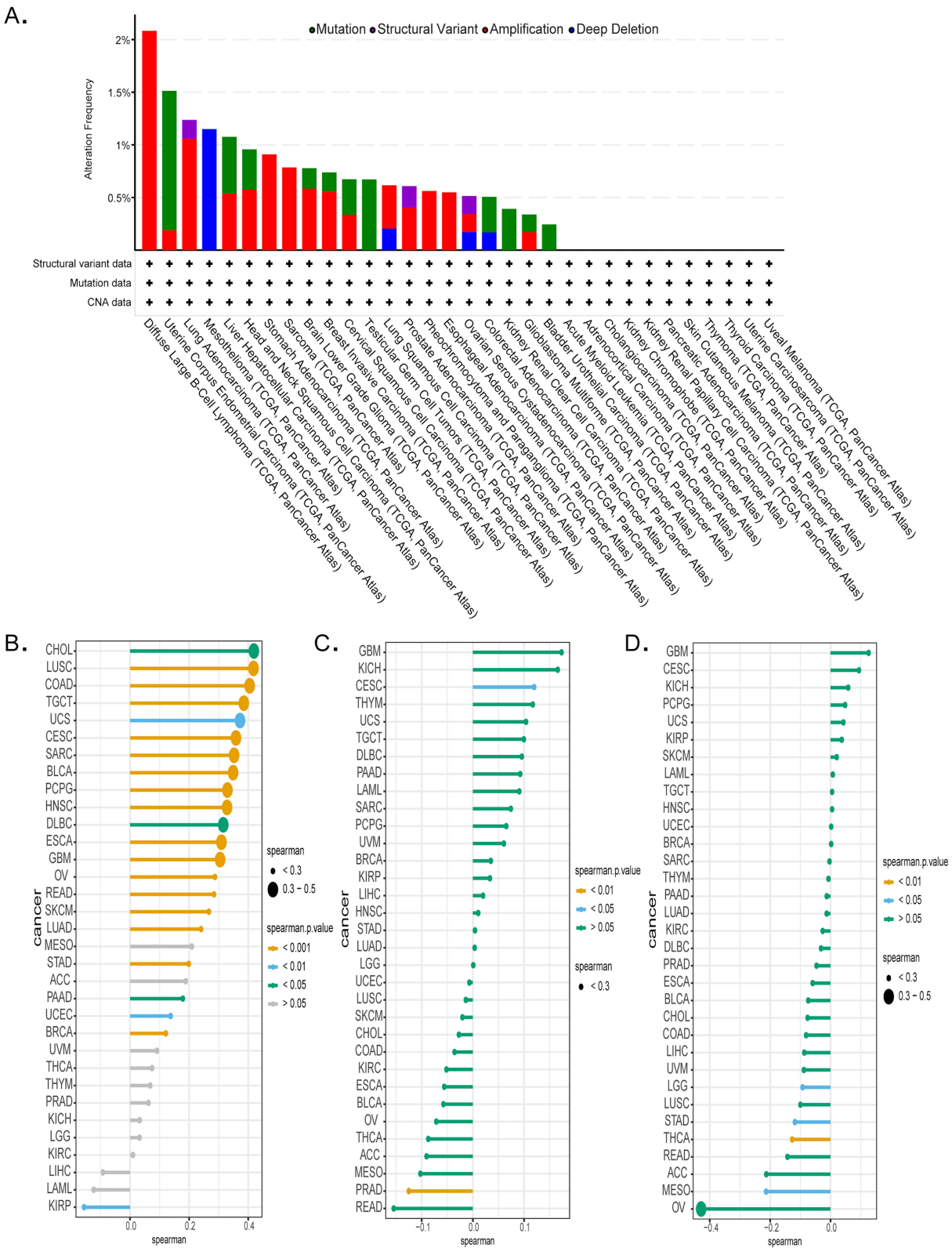

Figure 3 Gene mutation and methylation analysis of CDKN3. (**A**) Alteration frequency. (**B**) Correlations between CDKN3 and CNA. The green, blue and Orange colors indicate significant cancer types ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). (**C**) Correlations between CDKN3 and DNA promoter methylation. Blue and Orange indicate significant cancer types ($p < 0.05$ and $p < 0.01$, respectively). (**D**) Relationships between CDKN3 and CpG methylation. Blue and Orange indicate significant cancer types ($p < 0.05$ and $p < 0.01$, respectively).

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Figure 4 Correlation between CDKN3 and the hallmark pathway in the form of a heatmap. *p < 0.05, **p < 0.01.

DNA replication. Therefore, we further explored the correlation between CDKN3 expression level and immune scores and immune-associated genes.

Primarily, we explored the correlation between the expression level of CDKN3 and immune scores across cancers. The results showed that the CDKN3 expression level of more than half of cancers was significantly associated with immune scores (Figure 6A). The CDKN3 gene expression of most cancers showed a positive association with tumor purity and a negative association with stromal score, immune score, and ESTIMATE score in addition to KIRC and THCA, which showed opposite results. Further analysis of tumor-infiltrating immune cells and CDKN3 expression levels revealed that several tumors showed correlations between CDKN3 and infiltrating immune cells (Figure 6B). The expression level of CDKN3 was positively associated with the levels of six infiltrating immune cells in LIHC and THCA and positively correlated with the levels of most infiltrating immune cells in BLCA, BRCA, COAD, HNSC, KIRC, LGG, MESO, PRAD, SARC, SKCM, and THYM. Moreover, we found that the levels of several infiltrating immune cells showed a negative correlation with CDKN3 in many tumors. For instance, the levels of neutrophils were

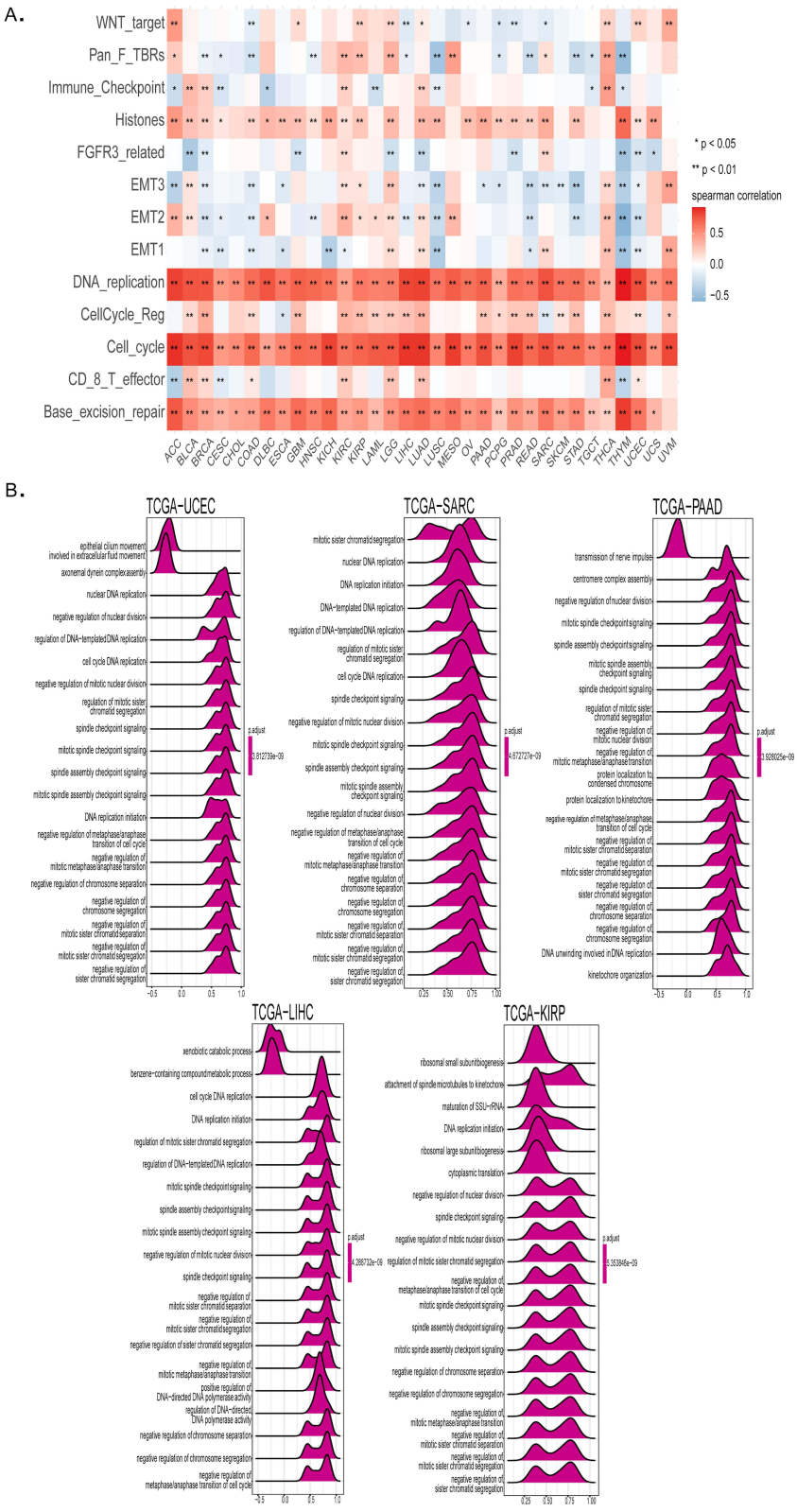

Figure 5 Correlation analysis of CDKN3 and microenvironment-related pathways. (**A**) The correlations between CDKN3 and microenvironment-related pathway scores. *p < 0.05, **p < 0.01 (**B**) The top 20 GSEA-related pathways in UCEC, SARC, PAAD, LIHC, and KIRP are exhibited in the form of a mountain map.

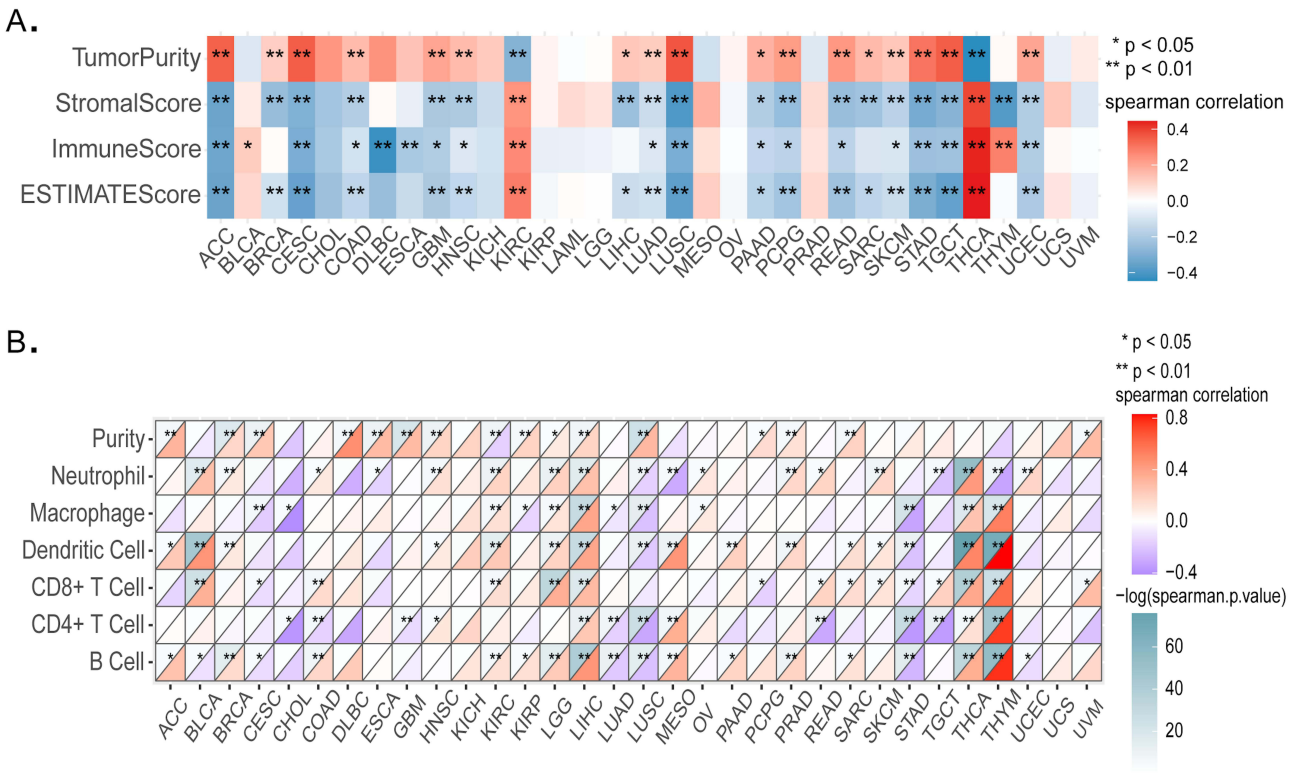

Figure 6 (**A**) The correlations between CDKN3 expression and stromal score, immune score, and ESTIMATE score. (**B**) The correlations between CDKN3 expression and the levels of TIICs. *p < 0.05, **p < 0.01.

negatively associated with CDKN3 in ESCA, LUSC, MESO, TGCT, and THYM; the levels of macrophages were negatively associated with CDKN3 in CESC, CHOL, KRIP, LUAD, LUSC, and STAD; the levels of dendritic cells were negatively associated with CDKN3 in LUSC and STAD; the levels of CD8+ T cells were negatively associated with CDKN3 in CESC, PCPG, and STAD; the levels of CD4+ T cells were negatively associated with CDKN3 in CHOL, COAD, GBM, LUAD, LUSC, READ, STAD, and TGCT; and the levels of B cells were negatively associated with CDKN3 in BLCA, CESC, LUAD, LUSC, STAD, and UCEC. Overall, CDKN3 expression showed a correlation with tumor-infiltrating immune cells in more than half of tumors.

Subsequently, we further probed the correlation between the CDKN3 gene level and immune cell-associated factors across cancers. We explored the correlation between CDKN3 gene expression and chemokines (Figure 7A), chemokine receptors (Figure 7B), MHC (Figure 7C), immunostimulators (Figure 7D), and immunoinhibitors (Figure 7E). The results showed that the correlation of CDKN3 and immune-associated factors in different cancers is different. CDKN3 expression had a significant positive correlation with chemokines, chemokine receptors, MHC molecules, immunostimulators, and immunoinhibitors in BLCA, KIRC, LGG, LIHC, and THCA and a negative correlation with these factors in most other cancers. From another perspective, we found that some immune factors showed similar correlation patterns in nearly all cancers. For instance, the chemokines C-X-C motif chemokine ligand 8 (CXCL8), C-X-C motif chemokine ligand 10 (CXCL10), C-X-C motif chemokine ligand 11 (CXCL11), C-X-C motif chemokine ligand 13 (CXCL13), C-C motif chemokine ligand 7 (CCL7), C-C motif chemokine ligand 8 (CCL8), and C-C motif chemokine ligand 26 (CCL26); the immunostimulators UL16 binding protein 1 (ULBP1), PVR, and MHC class I polypeptide-related sequence B (MICB); and the immunoinhibitors transforming growth factor beta receptor 1 (TGFBR1), lymphocyte activating 3 (LAG3), and indoleamine 2,3-dioxygenase 1 (IDO1) showed positive correlations with CDKN3; the chemokines C-C motif chemokine ligand 14 (CCL14) and C-C motif chemokine ligand 16 (CCL16); the MHC molecules major histocompatibility complex, class I, E (HLA-E), the immunostimulators TNF superfamily member 13 (TNFSF13); and the chemokine receptors C-X-C motif chemokine receptor 1 (CXCR1) and C-X3-C motif chemokine receptor 1

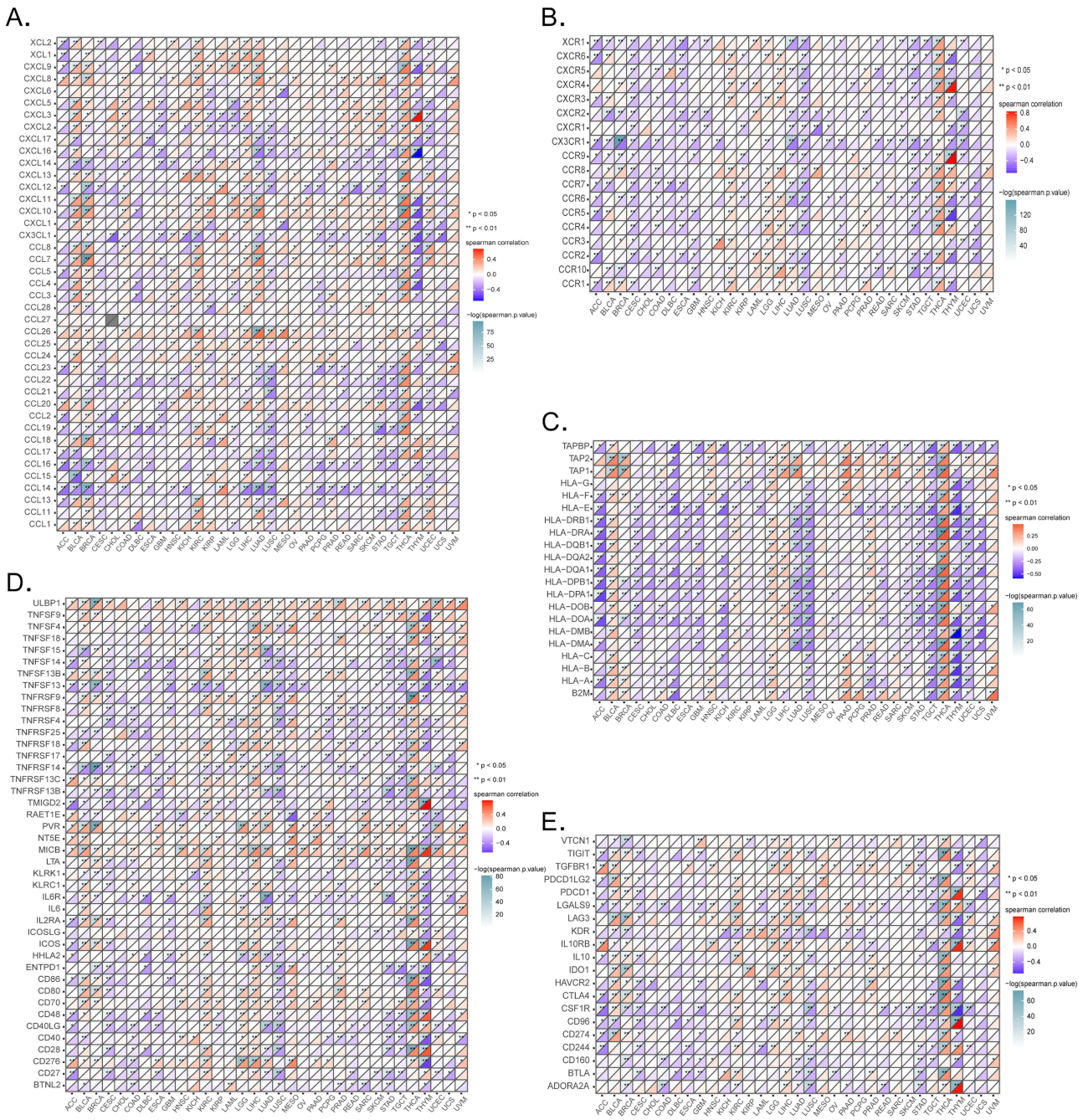

Figure 7 Correlation between CDKN3 and immune-related genes. (**A**) Chemokines. (**B**) Chemokine receptors. (**C**) MHC molecules. (**D**) Immunostimulators. (**E**) Immunoinhibitors. *p < 0.05, **p < 0.01.

(CX3CR1); and the immunoinhibitors colony stimulating factor 1 receptor (CSF1R), CD244, CD160 and kinase insert domain receptor (KDR) showed negative correlations.

Correlation Between Drug Sensitivity and CDKN3 in Cancers

In previous studies, CDKN3 was found to play an important role in some cisplatin-resistant cancers.[7,32,33] Therefore, we explored the correlation between drug sensitivity and CDKN3 expression by using CellMiner; this database includes information on drug sensitivity for the NCI-60 panel of 60 kinds of cancer cell lines from 9 distinct organs. The complete findings of the correlation between the half maximal inhibitory concentration (IC50) values for 408 food and drug

administration (FDA)-approved drugs or clinical trial drugs and CDKN3 expression levels are shown in Supplementary Table S1. Here, we showed the top 8 drugs with a strong negative correlation and the top 4 drugs with a strong positive correlation (Figure 8A); we also showed the different IC50 values of the corresponding drugs in different CDKN3 expression groups (Figure 8B).

As a result, we found that the IC50 values of norvir (Cor=−0.336, p=0.009), vemurafenib (Cor=−0.320, p=0.013), PLX−4720 (Cor=−0.317, p=0.013), bosutinib (Cor=−0.303, p=0.019), denileukin diftitox (Cor=−0.297, p=0.021), tegafur (Cor=−0.273, p=0.035), RAF−265 (Cor=−0.272, p=0.035), and caffeic acid (Cor=−0.269, p=0.038) had negative correlations with CDKN3 expression. In addition, the IC50 values of procarbazine (Cor=0.318, p=0.013), BMS-754807 (Cor=0.311, p=0.015), nelarabine (Cor=0.300, p=0.020), and TAE-684 (Cor=0.287, p=0.026) showed positive correlations with CDKN3 expression.

Moreover, we found that most of the drugs with negative correlations (except caffeic acid) were more effective (lower IC50 value) in the high CDKN3 expression groups. Conversely, most of the drugs with positive correlations (except procarbazine) were less effective (higher IC50 value) in the high CDKN3 expression groups.

Among the drugs, norvir, vemurafenib, bosutinib, denileukin diftitox, tegafur, procarbazine and nelarabine have been used as anticancer drugs in clinical treatment.

We further explored the drug sensitivity of some common clinical chemotherapeutic drugs. However, the correlation between CDKN3 expression level and IC50 value was nonsignificant for all drugs; and the IC50 value in different CDKN3 expression groups was nonsignificant except for fluorouracil. All of the findings are shown in Supplementary Figure S3.

The Clinical Verification of High CDKN3 Expression by qRT-PCR in Endometrial Cancer (UCEC)

To further clarify the differential expression of CDKN3 in tumor tissues and normal tissues. Thirty-five endometrial cancer samples (Figure 9A) and twenty-five normal endometrial samples (Figure 9B) were selected for qRT-PCR analysis. The relative quantification of RNA expression demonstrated that CDKN3 was significantly overexpressed in UCEC compared with normal endometrial tissues (Figure 9C). The overexpression of CDKN3 in tumors increased the credibility of CDKN3 as a promising diagnostic and screening biomarker for these tumors.

The Experimental Verification of CDKN3 in Endometrial Cancer (UCEC)

The knockdown efficiencies of CDKN3 were tested by Western blot and qRT-PCR (Figure 9D and G). According to the results, we selected HEC-1-A cells transfected with siRNA-2 and siRNA-3 and HEC-1-B cells transfected with siRNA-3 and siRNA-4 for the following experiment.

Primarily, we explored the difference in cell proliferation between the negative control and CDKN3 knockdown groups. The results showed that CDKN3 knockdown significantly weakened the cell proliferation of endometrial cancer cells after 3 days of siRNA transfection (Figure 9E and H).

Subsequently, we tested the universally accepted function of CDKN3 in the cell cycle. Seventy-two hours after CDKN3 transfection, CDKN3 knockdown significantly enriched G1 phase cells and weakened S and G2 phase cells in common endometrial cancer cell lines (HEC-1-A and HEC-1-B) (Figure 9J and K). The decrease in G1 phase cells and increase in S phase cells indicated that CDKN3 induces the G1/S transition.

Finally, we tried to prove the immunity correlation of CDKN3 in pancancer, which is the novel finding in this paper. Forty-eight hours after the cancer-PBMCs co-culture system with an effector/target ratio of 20:1, the cytotoxicity of the CDKN3 knockdown groups was significantly higher than that of the negative control groups (Figure 9F and I). The results suggested that CDKN3 acts as a tumor promoter in endometrial cancer when co-cultured with PBMCs.

Discussion

With the development of genetic and genomic research, an increasing number of cancer-related genes have been discovered. Compared with traditional research methods, bioinformatics analysis undoubtedly provides a more rapid,

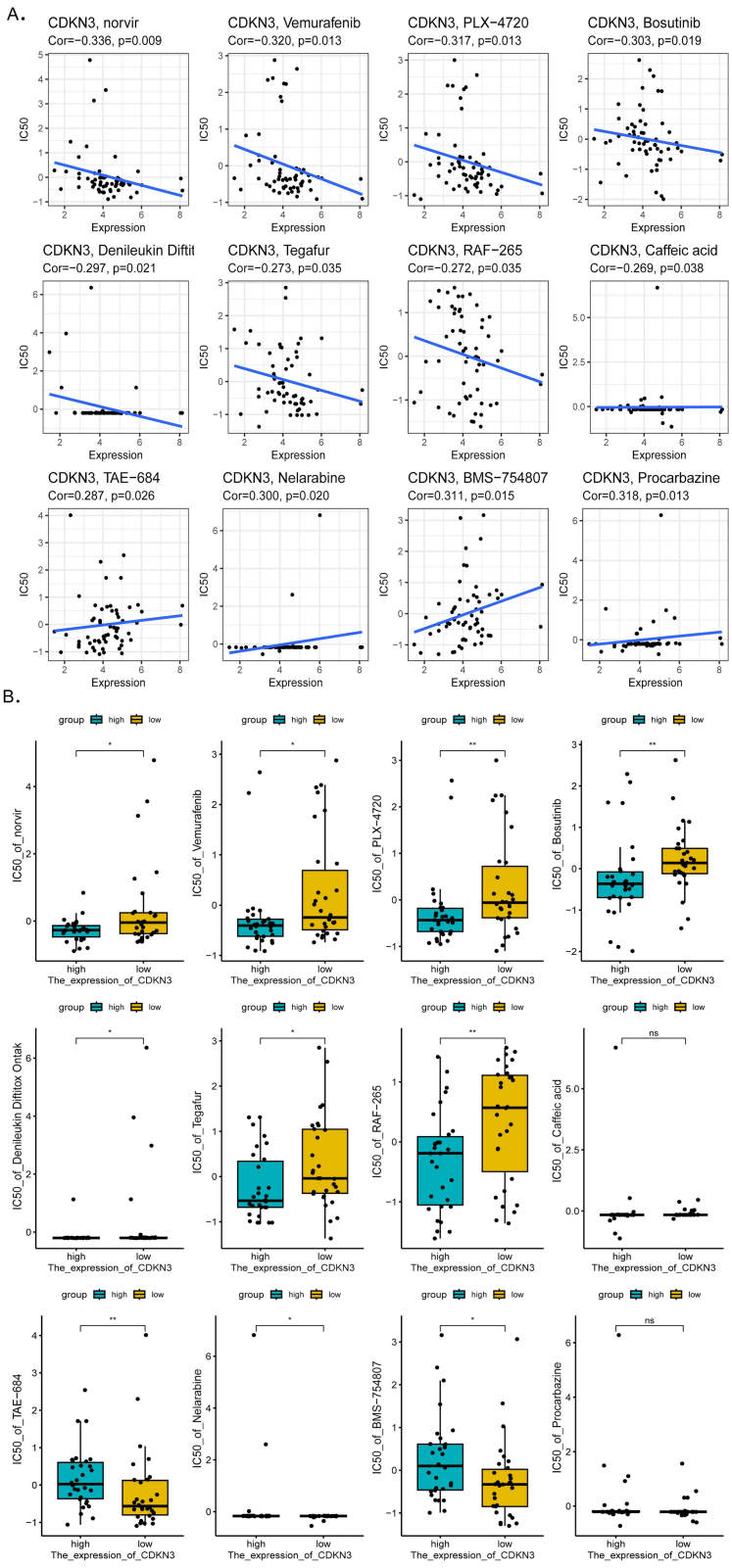

Figure 8 Drug sensitivity analysis. (**A**) Correlation between drug sensitivity and CDKN3 expression. Spearman correlation coefficients were calculated to investigate the correlation. (**B**) The different IC50 values of the corresponding drugs in different CDKN3 expression groups. *p < 0.05, **p < 0.01.

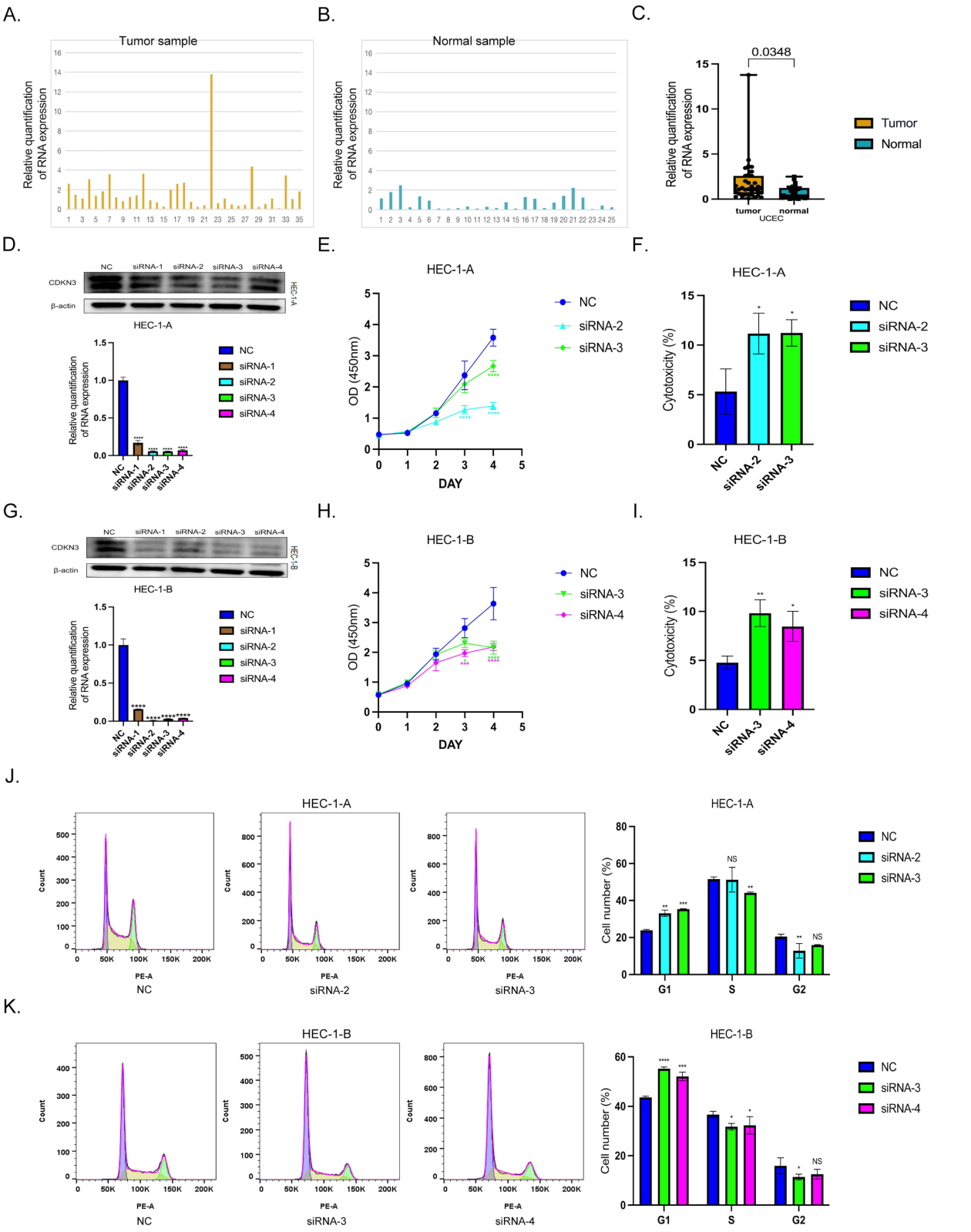

Figure 9 Verification in endometrial cancer (UCEC). (**A**–**C**) CDKN3 expression between endometrial cancer tissues and normal endometrial tissues by qRT-PCR analysis of clinical samples. (**D**) The knockdown efficiencies of CDKN3 in HEC-1-A cells were tested by Western blot and qRT-PCR. (**E**) Comparison of cell proliferation between the negative control and CDKN3 knockdown groups of HEC-1-A cells. (**F**) The cytotoxicity between the negative control and CDKN3 knockdown groups in the HEC-1-A cell cancer-PBMCs co-culture system with an effector/target ratio of 20:1. (**G**) The knockdown efficiencies of CDKN3 in HEC-1-B cells were tested by Western blot and qRT-PCR. (**H**) Comparison of cell proliferation between the negative control and CDKN3 knockdown groups of HEC-1-B cells. (**I**) The cytotoxicity between the negative control and CDKN3 knockdown groups in HEC-1-B cell cancer-PBMCs co-culture system with an effector/target ratio of 20:1. (**J**) Cell cycle analysis between the negative control and CDKN3 knockdown groups in HEC-1-A cells. (**K**) Cell cycle analysis between the negative control and CDKN3 knockdown groups in HEC-1-B cells. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

accurate and cost-effective screening method for disease-related genes. A high-value disease-related gene/genome or gene/genomic alteration is of great significance for disease prevention, diagnosis, treatment and prognosis. In the present study, we explored the comprehensive role of CDKN3 using TCGA pancancer data.

CDKN3 has always been described as a gene that encodes a cell cycle regulator protein. CDKN3 interacts with cyclin-dependent kinases (CDKs) through a key interface, which is formed by the C-terminal helix of CDKN3 and the C-terminal lobe of CDKs, so CDKN3 strongly interacts with CDK1 and cyclin-dependent kinase 3 (CDK3) and relatively weakly interacts with CDK2 but does not interact with cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 7 (CDK7).[34–38] CDKN3 affects CDK1 through its phosphatase activity and does not directly dephosphorylate CDK1.[6] To inactivate CDK2, CDKN3 dephosphorylates Thr160 in the absence of cyclin, and Thr160 is a conserved threonine that is required for the activation of CDKs through phosphorylation by CDK-activating kinase (CAK).[39–45] CDKN3 has been confirmed to be expressed in both the nucleus in normal tissues and the cytoplasm in tumor tissues.[34,46] Nalepa et al further detected that the location of CDKN3 in cells varies as the cell cycle progresses: in interphase, CDKN3 is mostly expressed in nuclei, with little expression in the centrosome; in anaphase, CDKN3 is primarily in the centrosome, while in telophase, CDKN3 is predominantly expressed in newly formed nuclei; subsequently, a new cycle begins.[15] A decrease in CDKN3 promoted G1 phase cell cycle arrest,[15,20,47] which might be due to decreasing the expression of CDK2,[10] while an increase in CDKN3 significantly facilitated G1/S transition, S phase arrest, and G2/M transition.[9,11,48–52] In this paper, the in vitro experiment showed that CDKN3 significantly facilitated the G1/S transition. CDKN3 has also been found to regulate the cell cycle-associated proteins cyclin D1, CDK4, pAKT, P27, P53 and P21.[9,11,53,54] Overall, CDKN3 is seemingly a well-studied gene that may be involved in cancer by regulating the cell cycle, and there are many studies on CDKN3 and tumors. However, all of them focused on one type of cancer and did not involve some common cancers. Therefore, we tried to further explore the role of CDKN3 across cancers.

In previous studies, CDKN3 was found to be upregulated and associated with poor prognosis in many cancers, which is similar to our results, especially in five cancers, UCEC, SARC, PAAD, LIHC, and KIRP. The reason for CDKN3 upregulation is controversial. Considering the low amplification of CDKN3, Cress et al thought the increase in CDKN3 is based on the massive mitosis of the carcinoma.[4] This seems to indicate that the increase in CDKN3 is a consequence of cancer. However, Lee et al considered that CDKN3 serves as an oncogene involved in tumorigenesis.[34] Islam et al regarded CDKN3 as one of the core genes to diagnose colorectal cancer.[55] In this paper, in vitro experiment showed that CDKN3 significantly facilitated endometrial cancer cell proliferation. These results strongly suggest that CDKN3 is a tumor promoter in most cancers and could be regarded as a diagnostic and prognostic marker.

Gene mutations play a notable role in tumorigenesis. However, the mutation frequency of CDKN3 may be very low.[22] Yang et al found that the mutation frequency was only 1.2% in lung cancer.[56] Barrón et al did not find mutated or aberrant CDKN3 transcripts in cervical cancer cell lines and samples.[8] In our results, the highest alteration frequency of CDKN3 (slightly more than 2%) occurred in DLBC. However, Yu et al found an aberrant splicing, variant d, in malignant astrocytomas; it is a dominant negative CDKN3 variant resulting in increased proliferation; they also found that a full-length transcript (variant a) and a truncated transcript lacking exon 2 (variant c) exist in normal and tumor tissue.[6] Yang et al found that the amplification mechanism of CDKN3 before tumor cell dissemination is a breakage-fusion-bridge cycle mechanism, which is an important cancer gene amplification mechanism.[57] DNA methylation is usually abnormal in tumors.[28] However, our results did not reveal a significant correlation between CDKN3 levels and promoter or CpG methylation in most types of cancers. Qi et al also found no significant DNA promoter methylation in upregulated CDKN3.[58] Many previous studies have found that CDKN3 is upregulated/downregulated and hypomethylated/hypermethylated in many cancers.[59,60] CDKN3 has also been revealed to have a CpG island in promoter and exon 1, and the increased gene and protein levels of CDKN3 are usually accompanied by promoter hypomethylation.[29,61,62] Yaqinuddin et al considered that the methylation status of CDKN3 might be regulated by DNMT3b, which is a DNA methyltransferase.[63] Therefore, the specific correlation between CDKN3 levels and methylation needs more research.

As a generally acknowledged cell cycle-related gene, we found that CDKN3 is correlated with cell cycle-associated pathways in almost all types of cancers. For example, E2F, MYC, and mTOR, which have been found to regulate cell cycle progression and cell proliferation,[64–66] were strongly positively correlated with CDKN3 in our results. However,

we also found some new noncell cycle-correlated pathways. Previously, Mori et al thought CDKN3eRNA was a cell cycle-unassociated RNA regulator for CDKN3.[24] CDKN3 has been described as a metabolism-related gene,[67,68] a hypoxia-related gene,[69,70] a paraptosis-related gene,[71] an anti-apoptotic gene,[72] and a NO-sensitive gene,[73] Some studies have found that CDKN3 is involved in oxidative stress regulation, angiogenesis, DNA damage repair, cholesterol metabolism, fatty acid metabolism, hematopoiesis, neurocyte dendritic function regulation, and spermatogenesis.[74–78] Thus, CDKN3 might play a role in pathways other than the cell cycle.

Furthermore, some researchers have explored the association between CDKN3 and immune cells in one type of cancer. In lung adenocarcinoma, CDKN3 has been found to be negatively associated with the levels of four immune cells, including CD4+ T cells, B cells, macrophages and dendritic cells, but positively associated with the levels of two immune cells, CD8+ T cells and neutrophils.[56] One multicenter study confirmed that CDKN3 is a marker of response to immune checkpoint inhibitors in non-small cell lung cancer.[79] CDKN3 has also been found to be involved in the infiltration of M0 macrophages, activated memory CD4+ T cells, and neutrophils and to show a better response to immune checkpoint inhibitors in lung cancer.[68] A comparative study confirmed that CDKN3 is dysregulated in T cells from chronic hepatitis B infection patients and contributes to T-cell functional exhaustion by regulating the proliferation of CD4+ T cells but not CD8+ T cells.[80] Chen et al found that CDKN3 is strongly related to the abundance of neutrophils, B cells, and dendritic cells but associated with the markers of most immune cells, especially CD163, v-set and immunoglobulin domain containing 4 (VSIG4) and membrane spanning 4-domains A4 (MS4A4) of M2 macrophages.[81] Therefore, we further explored the correlation between CDKN3 and the immune environment. In the present study, we found that the CDKN3 expression level is correlated with immune scores and the levels of immune-infiltrating cells, which provides evidence supporting previous studies. In addition, we further elucidated the correlation between CDKN3 levels and immune-associated factors. Immunotherapy is a promising treatment for cancer; however, the efficiency in most types of solid tumors is less than 30%.[82] Tumor purity and TIICs might be potential factors guiding patient selection for immunotherapy treatment.[83,84] In our study, CDKN3 was associated with tumor purity and immune scores in more than half of tumors, and further analysis of TIICs and immunomodulatory factors provided more evidence that CDKN3 is associated with the immune infiltration environment. The proinflammatory chemokines CXCL8, CXCL10, and CXCL11; monocyte/macrophage chemokines CCL7 and CCL8; and eosinophil chemokine CCL26 showed a positive correlation with CDKN3 in almost all types of cancers. CXCL8, CXCL10, and CXCL11 are all involved in angiogenesis. In addition, CXCL8 recruits and induces the accumulation of mesenchymal stem cells (MSCs), tumor-associated macrophages (TAMs), tumor-associated neutrophiles (TANs), and myeloid-derived suppressor cells (MDSCs), and CXCL10 and CXCL11 recruit and induce the accumulation of TILs and Tregs in the tumor niche.[85] CCL7 has been found to amplify inflammatory processes,[86] CCL8 can induce monocyte differentiation into macrophages,[87] and CCL26 is involved in TAM recruitment and angiogenesis; these chemokines are extensively involved in cell proliferation and migration.[88] Some chemokine receptors (CXCR1 and CX3CR1) showed a negative correlation with CDKN3 in almost all types of cancers, while other chemokine receptors showed a negative correlation with CDKN3 in most cancers. CX3CR1 recruits MDSCs into the tumor, which can significantly inhibit the immune cell response.[88] The immunostimulator TNFSF13 belongs to the tumor necrosis factor (TNF) superfamily, which can inhibit tumor cell functions, and plays specialized roles in B-cell maturation and function.[89] The other immunostimulator belonging to the TNF superfamily generally showed a negative association with CDKN3 in most types of cancers. Approximately half of cancers showed a negative correlation between CDKN3 and MHC and immunoinhibitors. In this paper, in vitro experimental results also showed that CDKN3 significantly induced robust pro-tumor effects in endometrial cancer when co-culture with PBMCs. Overall, these results illustrate the correlation of CDKN3 with immune features and indicate that CDKN3 may play a potential role in patient selection for immunotherapy treatment.

Previous studies found that CDKN3 may play a significant role in tumor treatment because it is druggable.[90,91] CDKN3 might promote cisplatin resistance in colorectal cancer (CRC) and esophageal cancer.[7,32] CDKN3 downregulation can solve cisplatin resistance in bladder cancer by decreasing glycolysis by inhibiting lactate dehydrogenase A (LDHA) expression.[33] Yang et al found that CDKN3 could facilitate resistance to denileukin diftitox.[56] CDKN3 has been found to be the target of several drugs and plant extracts, including ionomycin, resveratrol, cryptolepine, lucanthone, and *Anacardium occidentale*, especially irinotecan, etoposide, and vorinostat, which all target only

CDKN3.[81,92,93] Wang et al even found that a decrease in CDKN3 promoted radiosensitivity.[12] CDKN3 can also serve as a treatment progress marker for some drugs, including neratinib and paclitaxel.[94,95] We found a correlation between drug sensitivity and CDKN3 for several drugs that have been FDA approved or studied in clinical trials. We found that high levels of CDKN3 indicate sensitivity to several drugs (high levels of CDKN3 along with low IC50), which means they are more effective in the patient subgroup with high levels CDKN3; several drugs were more effective in the group with low levels of CDKN3. The results provide more insight into precision clinical treatment.

We tried to explore the protein expression of CDKN3 because several studies found that an increase in CDKN3-related transcripts might be associated with a decrease in CDKN3-related proteins in some cancers.[6,29] Unfortunately, we failed to find relevant information on CDKN3 protein expression in the HPA database. However, we demonstrated the overexpression of CDKN3 in endometrial cancer by qRT-PCR analysis of selected clinical samples.

The role of CDKN3 as a tumor suppressor or tumor promoter is controversial, and different descriptions have even emerged within the same cancer. This might result from a dual function of CDKN3 in cell cycle regulation; on the one hand, it can inhibit CDK2 activity; on the other hand, it binds with MDM2 and P53, resulting in accelerated cell cycle progression.[96] An increasing number of studies have contradicted the conclusion that CDKN3 is a tumor suppressor;[4] however, we believe that these findings are a great illustration of the diversity of CDKN3 functions in different cancers, and we believe that CDKN3 acts as a tumor promoter in most types of malignant tumors.

Conclusion

To the best of our knowledge, this study is the first analysis of the role of CDKN3 across cancers. Compared with previous studies, we elucidate that CDKN3 is a tumor promoter in numerous cancers, and may serve as a marker for diagnosis, treatment and prognosis evaluation. Future studies need to be performed to identify the specific molecular mechanisms of CDKN3 in cancers.

Abbreviations

CDKN3, cyclin-dependent kinase inhibitor 3; CDK1, cyclin-dependent kinase 1; CDK2, cyclin-dependent kinase 2; TCGA, the cancer genome atlas; GTEx, genotype-tissue expression; GEO, gene expression omnibus; CCLE, cancer cell line encyclopedia; HPA, the human protein atlas; TISIDB, an integrated repository portal for tumor-immune system interactions; TIMER, tumor immune estimation resource; SARC, sarcoma; UCEC, uterine corpus endometrial carcinoma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; KIRP, kidney renal papillary cell carcinoma; CNA, copy number alteration; MSigDB, molecular signatures database; GSVA, Gene Set Variation Analysis; ESTIMATE, Estimation of stromal and immune cells in malignant tumour tissues using expression data; MHC, major histocompatibility complex; TIICs, tumor-infiltrating immune cells; immuCellAI, immune cell abundance identifier; GSEA, gene set enrichment analysis; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; siRNA, small interfering RNA; PBMC, Peripheral blood mononuclear cell; CESC, cervical squamous cell carcinoma; HNSC, head and neck squamous cell carcinoma; BLCA, bladder urothelial carcinoma; OV, ovarian serous cystadenocarcinoma; STAD, stomach adenocarcinoma; LUSC, lung squamous cell carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; CHOL, cholangiocarcinoma; SKCM, skin cutaneous melanoma; LUAD, lung adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; ESCA, esophageal carcinoma; READ, rectum adenocarcinoma; KIRC, kidney renal clear cell carcinoma; UCS, uterine carcinosarcoma; GBM, glioblastoma multiforme; LGG, brain lower grade glioma; PRAD, prostate adenocarcinoma; ACC, adrenocortical carcinoma; THYM, thymoma; KICH, kidney chromophobe; DLBC, lymphoid neoplasm diffuse large B cell lymphoma; TGCT, testicular germ cell tumors; LAML, acute myeloid leukemia; THCA, thyroid carcinoma; OS, overall survival; DSS, disease-specific survival; DFI, disease-free interval; PFI, progression-free interval; HR, hazard ratio; MESO, mesothelioma; CXCL8, C-X-C motif chemokine ligand 8; CXCL10, C-X-C motif chemokine ligand 10; CXCL11, C-X-C motif chemokine ligand 11; CXCL13,C-X-C motif chemokine ligand 13; CCL7, C-C motif chemokine ligand 7; CCL8, C-C motif chemokine ligand 8; CCL26, C-C motif chemokine ligand 26; ULBP1, UL16 binding protein 1; MICB, MHC class I polypeptide-related sequence B; TGFBR1, transforming growth factor beta receptor 1; LAG3, lymphocyte activating 3; IDO1, indoleamine 2,3-dioxygenase 1; CCL14, C-C motif chemokine ligand 14; CCL16, C-C motif

chemokine ligand 16; HLA-E, major histocompatibility complex, class I, E; TNFSF13, TNF superfamily member 13; CXCR1, C-X-C motif chemokine receptor 1; CX3CR1, C-X3-C motif chemokine receptor 1; CSF1R, colony stimulating factor 1 receptor; KDR, kinase insert domain receptor; IC50, the half maximal inhibitory concentration; FDA, food and drug administration; CDK3, cyclin-dependent kinase 3; CDK4, cyclin-dependent kinase 4; CDK7, cyclin-dependent kinase 7; CAK, CDK-activating kinase; VSIG4, v-set and immunoglobulin domain containing 4; MS4A4, membrane spanning 4-domains A4; MSC, mesenchymal stem cell; TAM, tumor-associated macrophage; TAN, tumor-associated neutrophile; MDSC, myeloid-derived suppressor cell; TNF, tumor necrosis factor; CRC, colorectal cancer; LDHA, lactate dehydrogenase A.

Data Sharing Statement

The original manuscript contained is included in the article/Supplementary Materials. Further inquiries can be acquired directly to the corresponding author.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Tianjin Medical University General Hospital (batch number: IRB2023-YX-093-01).

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Disclosure

The authors report no conflicts of interest in this work.

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