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ORIGINAL RESEARCH

Comprehensive Bioinformatics Analyses and Experimental Validation of the Cell Cycle Related Protein SAPCD2 as a New Biomarker and Potential Therapeutic Target in Pancreatic Cancer

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Purpose: Pancreatic adenocarcinoma (PAAD) is a highly aggressive cancer with a poor prognosis, reliable markers are urgently needed for early detection and prognosis evaluation. *SAPCD2*, a cell cycle related gene, has been implicated in tumorigenesis and proposed as a potential therapeutic target in cancer. However, no comprehensive study has explored its expression and regulation, discussed its role in tumor prognosis and immune modulation, along with therapy response in pan-cancer until now.

Methods: SAPCD2 expression was analyzed using data from The Cancer Genome Atlas database (TCGA) and Human Protein Atlas (HPA) database. Genetic and epigenetic alterations of *SAPCD2* and the immune microenvironment were explored via NCBI, TIMER2 and cBioPortal platforms. Western blot analysis and immunohistochemistry (IHC) were performed to check SAPCD2 protein expression in PAAD cells and tissues. Cell counting kit 8 (CCK8), flow cytometry, and transwell experiments were used to evaluate the role of SAPCD2 in PAAD cell lines.

Results: Our study found that SAPCD2 is notably upregulated in various cancers, especially early-stage digestive cancers, and is linked to poor survival in most cancers like PAAD and LIHC. Gene amplification and promoter DNA hypomethylation appear to drive this upregulation. Additionally, SAPCD2 expression correlates with tumor mutation burden, microsatellite instability, and immune scores across several cancers. In PAAD, elevated SAPCD2 levels correlated with reduced immune activity, whereas in stomach cancer (STAD), its prognostic impact appeared immune-independent. In PDAC cell lines, SAPCD2 knockdown reduced proliferation and invasion, and caused reduction of G0/G1 phase. PAAD cells with high SAPCD2 expression showed increased sensitivity to DNA-PK, p38α MAPK, and Bcl-2 inhibitors.

Conclusion: SAPCD2 serves as both a prognostic marker and a potential therapeutic target in PAAD, where its low expression may enhance responsiveness to specific drugs. These findings underscore SAPCD2's dual role in cancer progression and therapy.

Keywords: SAPCD2, pan-cancer analysis, pancreatic cancer, biomarker, prognosis

Introduction

Pancreatic cancer is one of the most lethal cancers globally,[1–3] with nearly 20 million new cases and approximately 10 million deaths reported in the GLOBOCAN 2022 report.[4] The 5-year survival rate remains below 15%[3,5,6] and the median overall survival is less than one year.[7,8] The incidence of pancreatic cancer is increasing by 1% annually,[3,9] posing a

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growing global health threat. Approximately 90% of pancreatic cancer cases are classified as pancreatic ductal adenocarcinoma (PDAC).[10] Late diagnosis, aggressive tumor biology, and limited treatment options contribute to its poor outcomes. Around 80% of PAAD patients diagnosed at advanced stages,[10,11] often missing the chance for curative surgery. Chemotherapy offers only limited benefit, and immunotherapy has shown disappointing results due to the tumor's immunosuppressive microenvironment.[12,13] To improve survival and quality of life for PDAC patients, it is essential to enhance early detection, identify reliable biomarkers, and develop more effective therapeutic strategies.

Suppressor anaphase-promoting complex (APC) domain containing 2 (*SAPCD2*), also known as chromosome 9 open reading frame 140 (*C10orf140), p42.3* and *ang*, is a newly characterized cell cycle gene highly conserved among mammals.[14,15] SAPCD2 has been identified as a crucial player in several cancers, such as gastric,[15–19] colorectal,[20–23] hepatocellular,[24–26] and lung cancers,[27–30] where its elevated expression correlates with poor prognosis. Studies highlights SAPCD2's role as an oncogene, facilitating tumor cell proliferation, migration, and invasion. Despite these insights, comprehensive studies examining the regulation of the SAPCD2 gene, its methylation status, immune infiltration, and treatment response across different cancer types remain limited.

In this study, we first conducted comprehensive analyses of SAPCD2 across multiple tumor types using The Cancer Genome Atlas (TCGA) datasets, examining pan-cancer expression and prognosis relevance, genetic and epigenetic alterations, immune cell infiltration, immune gene expression, pathway enrichment, responses to chemotherapy and immunotherapy. Our findings reveal that SAPCD2 is overexpressed in multiple cancers, including digestive cancers, and its expression is associated with prognosis, clinicopathological characteristics, immune infiltration and treatment response in PDAC patients. The overexpression of SAPCD2 is likely driven by gene amplification and promoter DNA hypomethylation. Functional experiments demonstrated that SAPCD2 inhibition markedly suppressed tumor cell proliferation and invasion, while inducing a decrease in the percentage of cells in G0/G1 phase. These results offer valuable insights into the role of SAPCD2 in pan-cancer, and suggest its potential as a novel therapeutic target and prognostic biomarker for pancreatic cancer.

Materials and Methods

Genetic and Proteomic Overview of SAPCD2

The genetic and proteomic structure information of SAPCD2 were firstly obtained in several databases to comprehensively understand its potential functions. First, the chromosomal localization of *SAPCD2* gene were retrieved from the GeneCards database (https://www.genecards.org/). The conserved protein domains and corresponding RefSeq protein numbers of SAPCD2 in human and other species were gathered using the "HomoloGene" function of the national center for biotechnology information (NCBI) (https://www.ncbi.nlm.nih.gov/homologene/). Then, the RefSeq protein numbers were put into the Constraint-based Multiple Alignment Tool of NCBI (https://www.ncbi.nlm.nih.gov/tools/cobalt/), "Neighbor Joining" method and "Taxonomic Name (Sequence ID) Slanted" label was used to generate a phylogenetic tree. Additionally, evolutionary conserved protein domains related to adenomatous polyposis coli (APC) were retrieved from the cBioPortal database (www.cbioportal.org). DNA methylation probes sequences for SAPCD2 were retrieved from MEXPRESS (https://mexpress.ugent.be/), the exons, CpG island and the DNA methylation probes were mapped to the *SAPCD2* gene, providing a detailed visualization of its sequence characteristics.

Gene Expression and Protein Expression of SAPCD2 in Pan-Cancer

Bulk gene expression data was obtained from TISCH database (http://tisch.comp-genomics.org/search-gene/?genesearch=SAPCD2). Expression data of *SAPCD2* across 33 types of cancers, including clinical stages and overall survival data, were downloaded from TCGA Portal website (https://portal.gdc.cancer.gov/). The R package (version 4.2.2) was used to process the data. The rma function was applied to filter out missing and duplicated entries, and the dataset was normalized using log2(TPM+1) transformation. Protein expression levels of SAPCD2 in COAD, LIHC, PAAD, and stomach adenocarcinoma (STAD) were collected from The Human Protein Atlas (HPA) database (https://www.proteinatlas.org/). Single-cell data was also sourced from the TISCH database.

Mutation and Copy Number Variation (CNV) Analysis in cBioPortal

The genetic alterations in *SAPCD2* gene were analyzed using the cBioPortal (http://www.cbioportal.org/) database. The "PanCancer Studies" dataset, which contain 10 different tumor studies and a total of 76639 samples (till 1st August 2024), was chosen for analyzing the frequencies of copy number amplifications and gene mutations through the "Cancer Types Summary" module. Specific mutation sites and frequencies were further assessed via the "Mutations" module. The relationship between SAPCD2 expression and CNVs across different tumor types was analyzed in the GSCA database (https://guolab.wchscu.cn/GSCA/#/) via the "Mutation" module.[31]

DNA Methylation Analysis of SAPCD2 Gene

The DNA methylation level of *SAPCD2* gene were analyzed using the MEXPRESS platform (https://mexpress.ugent.be/).[32,33] Samples were sorted by the "expression" as the default and ordered according to the mRNA expression value of *SAPCD2*. The associations between the *SAPCD2* gene expression level with its methylation status of individual probes (cg15733507, cg21241219, cg21066537, cg15785720, cg03180426, cg01448891, cg14607755, cg14156314) were calculated and visualized across different tumors using MEXPRESS. Methylation levels were represented with beta value, and the results included Benjamini-Hochberg-adjusted p values and the Pearson correlation coefficient value (r value).

Survival Analysis and Cox-Regression

Gene expression profiles and clinical data from 33 cancer types were retrieved from TCGA database, with *SAPCD2* expression values normalized using log2(TPM+1). Patients with insufficient survival data were excluded. Kaplan-Meier analysis was performed to compare survival outcomes between high and low *SAPCD2* expression groups, stratified by the median expression level. The Log rank test was applied to evaluate differences in survival between the groups. Furthermore, Cox proportional-hazards regression models were used to evaluate the association between *SAPCD2* expression and survival outcomes, adjusting for covariates such as age, gender, tumor stage, and treatment status. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated, and statistical significance was defined as $p < 0.05$. All analyses were conducted using R software, employing packages such as survival and survminer for data processing and visualization.

Immune Cell Infiltration and Immune Score Analysis

Immune score data for high and low SAPCD2 expression group in PAAD and STAD were generated using the ESTIMATE algorithm. Differences in immune cell infiltration between two groups were evaluated using CIBERSORT, which includes markers for 22 immune cell types. The TIMER2.0 database was utilized to assess correlations between SAPCD2 expression and the infiltration levels of 15 immune cell types. Additionally, single-sample gene set enrichment analysis (ssGSEA) was conducted to compare enrichment scores for 13 immune cell-related functions between groups with low and high SAPCD2 expression levels. Detailed analysis methods have been described previously.

Cancer Hallmark Correlation Analysis

Cancer Hallmark Correlation Analysis was performed using Gene Set Variation Analysis (GSVA) to investigate the enrichment of cancer hallmark gene sets associated with SAPCD2 expression.

Cell Culture and Transfection

PDAC cell lines, including BxPC3, Panc-1, SW1990, and CFPAC-1, were purchased from Jennio Biotech Co.,Ltd company (Guangzhou, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (P/S). They were maintained in a 37°C incubator with 5% CO_2. The sh*SAPCD2* plasmid was constructed by cloning hairpin reverse complement DNA fragment into the PLKO.1-U6-EF1a-copGFP-T2A-puro vector. The corresponding plasmid sequence are provided in Table S1. The transfection procedures were performed as described previously.[34]

Cell Cycle

BxPC3 and Panc-1 cell lines with *SAPCD2* knockdown were harvested by trypsinization, washed, and fixed in 70% ethanol overnight at −20°C. The fixed cells were then stained with propidium iodide (PI) solution containing RNase A for 30 minutes at 37°C. Flow cytometry was employed to analyze the cell cycle distribution, and the results were processed using FlowJo software. The proportion of cells in different phases (G0/G1, S, and G2/M) was calculated to assess the impact of *SAPCD2* knockdown on cell cycle progression.

Western Blot

Cell lysates from PDAC and HPDE cell lines were prepared using RIPA buffer supplemented with protease and phosphatase inhibitors. The protein concentration was determined using the bicinchoninic acid (BCA) assay. Equal amounts of protein (30 μg) were loaded onto 10% SDS-PAGE gels and separated by electrophoresis. Proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking with 5% non-fat milk in Tris-Buffered Saline-Tween 20 (TBST) for 1 hour at room temperature, the membrane was incubated overnight at 4°C with primary antibodies against SAPCD2 (Thermo, PA560632) and GAPDH (Proteintech, 60004-1-Ig). Following extensive washing with TBST, the membrane was incubated with the appropriate secondary antibody for 1 hour at room temperature and washed with TBST buffer three times. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system, and images were captured using a digital imaging system.

CCK8 and Transwell Invasion

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8, Beyotime, China) assay. Cells were seeded in 96-well plates at a density of 2,000 cells per well and allowed to adhere overnight. Add 10 μL of CCK-8 solution to each well, and the plates were incubated for an additional 1 hour at 37°C. Absorbance was measured at 450 nm using a microplate reader. The viability percentage was calculated relative to control cells.

Transwell invasion was evaluated using chambers coated with Matrigel (Corning, USA). Cells were trypsinized and resuspended in serum-free medium at a concentration of 1 × 10^5 cells/mL. Cell suspension (200 μL) was added to the upper chamber, while 700 μL of complete medium was added to the lower chamber as a chemoattractant. After 48 hours of incubation, non-invading cells on the upper surface were gently removed with a cotton swab. Invading cells on the lower surface were fixed with 4% paraformaldehyde, stained with crystal violet, and counted under a microscope in five random fields. Results were expressed as the average number of invading cells per field.

IHC Staining

A PDAC tissue slide (D097Pa01S) was obtained from Bioaitech company (Xi'an, China) containing 90 PDAC tissue samples with corresponding TNM classification and stage information. The microarray slides were deparaffinized using xylene, followed by rehydration through a graded ethanol series (100%, 90%, 80%) into water. Antigen retrieval was performed using EDTA to expose the antigens. The slides were then incubated with 5% BSA to block nonspecific binding. After blocking, the tissues were incubated overnight with the anti-SAPCD2 (Thermo, PA5-60632) antibody. Secondary antibody incubation was followed, diaminobenzidine (DAB) and hematoxylin were used. The slide was scanned by NanoZoomer (Japan) and scored based on the proportion of positively stained cells.

Results

Structural Overview of SAPCD2 Gene and Protein

The human SAPCD2 gene is located at the 9q34.3 chromosomal locus (Figure 1A). The SAPCD2 protein consists of 394 amino-acid with a molecular weight of approximately 42.3 kDa (Figure 1B). According to the "HomoloGene" function from NCBI, SAPCD2 contains two key domains at its C-terminus: Suppressor APC domain (225–306 aa) and the Mycoplasma domain (336–375 aa) (Figure 1B). The Suppressor APC domain is derived from the N-terminus of the tumor suppressor protein adenomatous polyposis coli (APC, 128–208 aa)[35] (Figure 1C). This domain is highly conserved among species (Figure 1B), and has been associated with nuclear export functions.[35,36] In contrast, the Mycoplasma

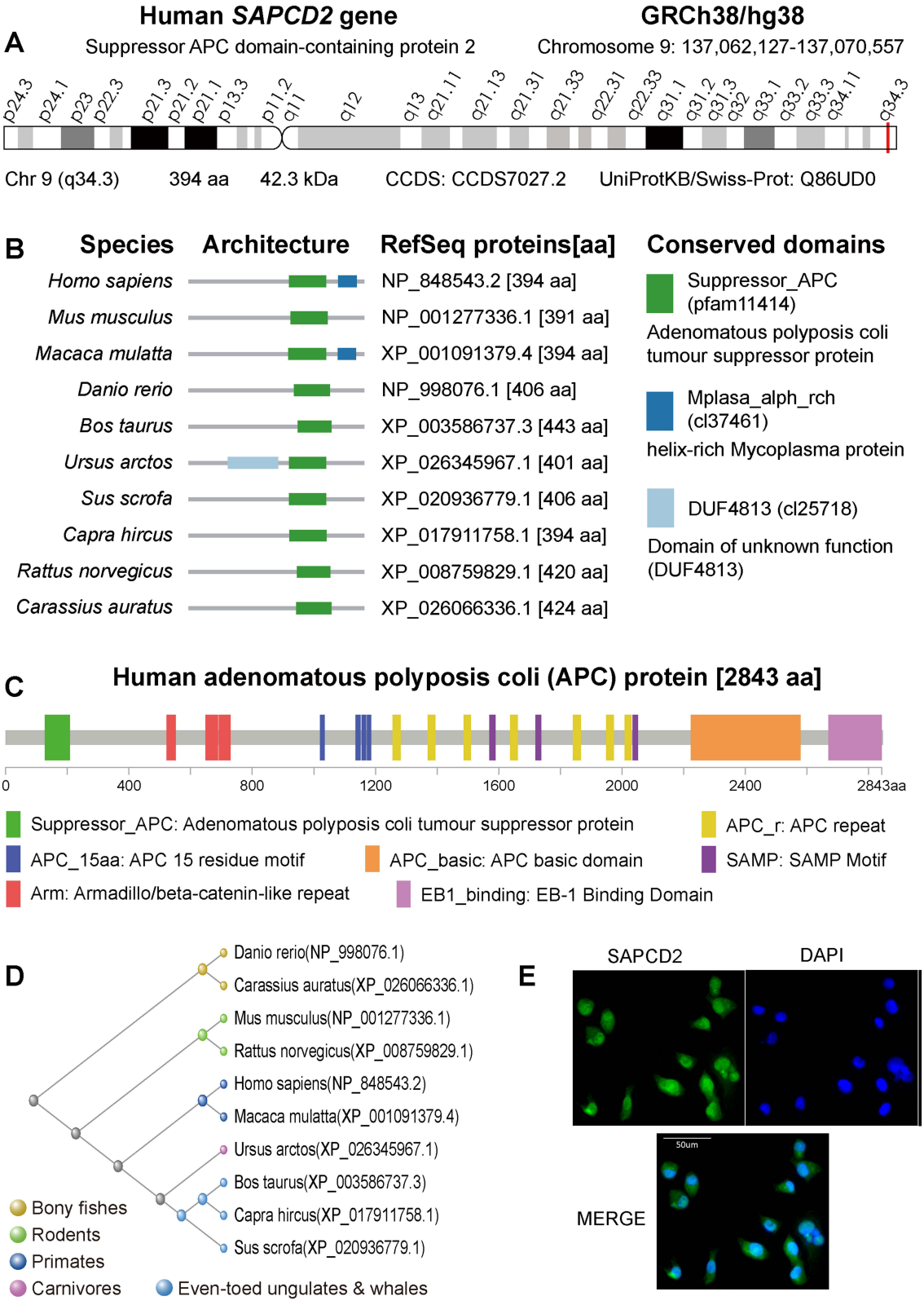

Figure 1 Chromosomal position, protein structure and subcellular protein localization of *SAPCD2* gene. (**A**) Genomic position of *SAPCD2* gene in human. The red vertical line indicates the chromosome position of human *SAPCD2* gene. (**B**) The conserved protein domain of SAPCD2 in human and several other species. (**C**) The conserved protein domain of human APC. (**D**) The phylogenetic tree of SAPCD2 protein across different species. (**E**) The subcellular localization of SAPCD2 protein in PDAC cell Panc-1. Blue, nucleus; Green, SAPCD2 protein.

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domain is derived from helix-rich Mplasa_alph_rch protein[37] and its functions remain unknown at present. The phylogenetic tree of SAPCD2 protein was drawn to illustrate its evolutionary relationship among different species (Figure 1D). We also showed that SAPCD2 was mostly localized in nuclear of tumor cells, but also expressed in the cytoplasm at lower levels in PDAC cell line Panc-1 (Figure 1E).

SAPCD2 Overexpressed in Multiple Cancers, Particularly in Digestive Cancers

The expression of SAPCD2 mRNA was analyzed in normal human tissues using the Genotype-Tissue Expression (GTEx) database (Figure 2A). High levels of SAPCD2 were observed in esophagus-muscularis, EBV-transformed lymphocytes, and brain tissues, while low expression was detected in the pancreas, liver, stomach, and colon. To evaluate SAPCD2 expression in tumors, RNA levels were analyzed using data from the TCGA database (Figure 2B). SAPCD2 was significantly upregulated in various cancers, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), brain lower-grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), STAD, testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS) (Figure 2B). Notably, significant overexpression of SAPCD2 was observed in several digestive cancers (Figure 2C), indicating its potential role as an oncogene in these cancers. Additionally, protein expression data from the Human Protein Atlas (HPA) indicated that SAPCD2 protein predominantly localized in the nucleus, with relatively lower expression in the cytoplasm (Figure 2D). SAPCD2 upregulated in several digestive cancers, including PAAD (n=20), COAD (n=22) STAD (n=16). However, no significant upregulation was observed in LIHC (n=24).

Single Cell Expression of SAPCD2 in Pan-Cancer

To understand the cellular expression patterns of SAPCD2 in various cancers, single-cell expression analysis was performed using data from the TISH database (Figure 3A). The findings revealed that SAPCD2 is upregulated in proliferating T cell (T prolif) and is widely expressed in malignant cells across most cancers.

Specifically, in PAAD using data from the GSE111672 dataset (Figure 3B), SAPCD2 expression was detected in CD8 T cells, monocyte-derived macrophages, neutrophils, fibroblasts, and malignant cells, Notably, the expression was particularly elevated in proliferating T cells. A similar expression pattern was observed in colorectal cancer (CRC) using the GSE166555 dataset (Figure 3C). In CRC, proliferating T cells and malignant cells showed particularly high SAPCD2 expression, underscoring its potential role in cancer cell proliferation and immune cell dynamics.

Genetic and Epigenetic Alterations in SAPCD2

Genetic and epigenetic alterations are critical drivers of tumorigenesis and cancer progression. The genetic modifications in *SAPCD2* gene were analyzed in various tumors using the cBioPortal database (Figure 4A and B, Supplementary Figure 1). SAPCD2 mutations were observed in 84 cases (0.11%) out of 76,639 tumor samples, with amplifications being more frequent than deep deletions and mutations. The amplification frequencies of *SAPCD2* gene were found to be higher than 4% in pancreatic neuroendocrine tumor (16.28%), ovarian epithelial tumor (10.00%), papillary stomach adenocarcinoma (9.09%), esophageal adenocarcinoma (6.61%), STAD (6.25%), tubular stomach adenocarcinoma (5.88%), serous ovarian cancer (4.67) and liposarcoma (5.00%). Interestingly, oral cavity squamous cell carcinoma exhibited both amplifications (4.76%) and deep deletions (9.52%). Distinct patterns of SAPCD2 gene regulation in different cancers may influenced by these amplifications. In contrast, SAPCD2 mutations were relatively rare and mainly observed in cutaneous melanoma (1.36%), bladder urothelial carcinoma (1.04%), renal clear cell carcinoma (0.57%), breast invasive ductal carcinoma (0.40%), pancreatic adenocarcinoma (0.14%), and lung adenocarcinoma (0.06%) (Figure 4A, Table S1). Further investigation into mutation types using the cBioPortal "mutation" module identified 9 mutations, including 6 missense mutations, 1 truncating mutation, and 2 in-frame mutations (Figure 4B).

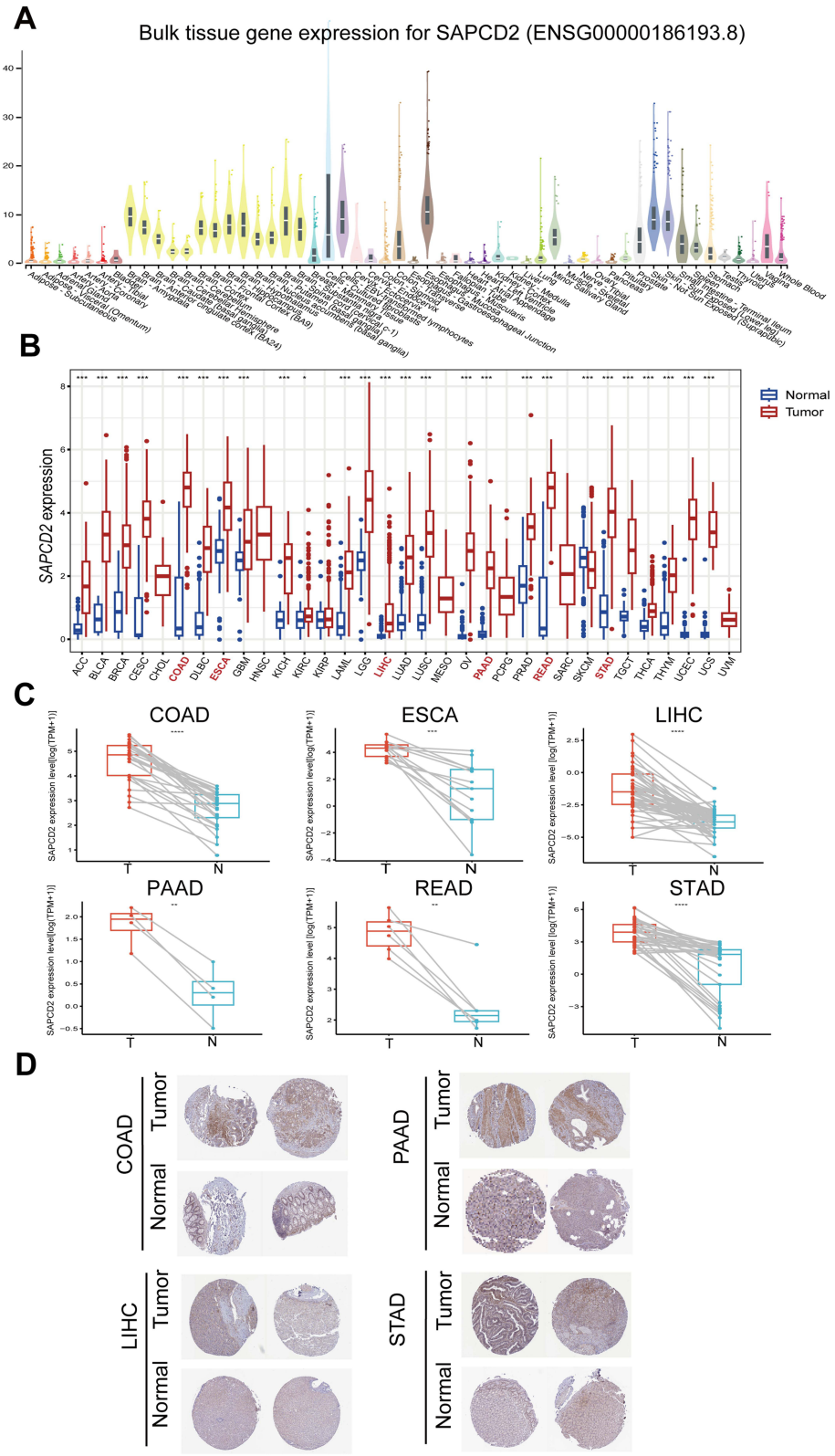

Figure 2 SAPCD2 expression across 33 cancer types. (**A**) SAPCD2 expression in normal human tissues from the GTEx database. (**B**) SAPCD2 expression across multiple cancer types from the TCGA database, with red and blue bar graphs representing tumor and normal tissues, respectively. (**C**) SAPCD2 expression in paired tumor and adjacent normal tissues in COAD, ESCA, LIHC, PAAD, READ and STAD. (**D**) Representative images of SAPCD2 protein expression in digestive cancers from HPA database. *p<0.05, **p<0.01, ***p<0.001.

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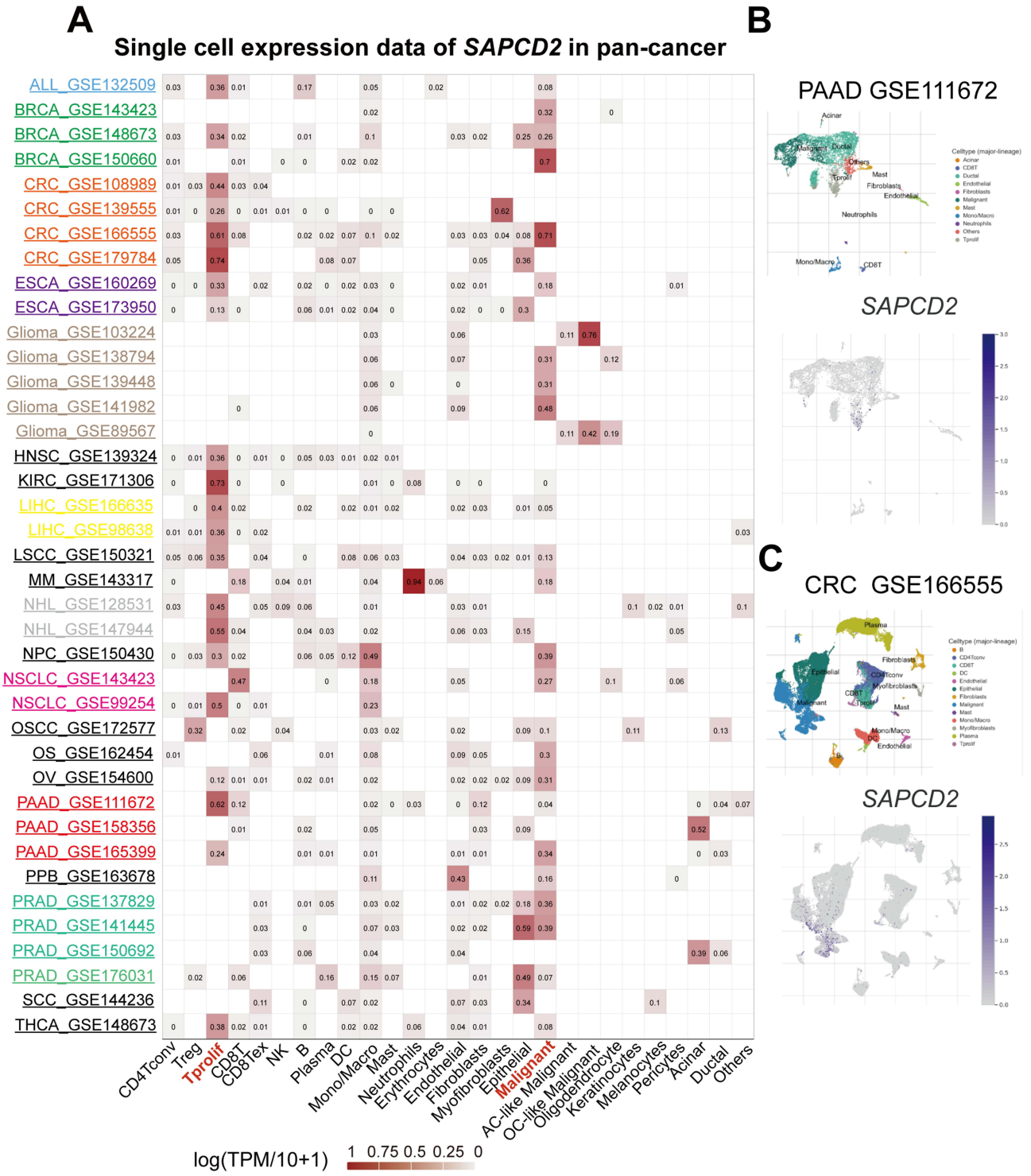

Figure 3 Single-cell expression analysis of SAPCD2 in pan-cancer. (**A**) Cluster heatmaps showing mRNA levels of SAPCD2 across various cancers using data from the TISH database. (**B**) SAPCD2 expression among different cell types in PAAD from the GSE111672 dataset. (**C**) SAPCD2 expression among different cell types in CRC from the GSE166555 dataset.

To explore CNVs and their impact on *SAPCD2* expression and prognosis, we analyzed digestive cancers using the GSCA database (Figure 4C-E). Heterozygous amplifications and deletions were the predominant CNV types, with heterozygous deletions particularly common in PAAD and LIHC (Figure 4C). *SAPCD2* gene expression was positively correlated with CNVs in COAD, READ, STAD and ESCA (Figure 4D). Moreover, survival analyses results

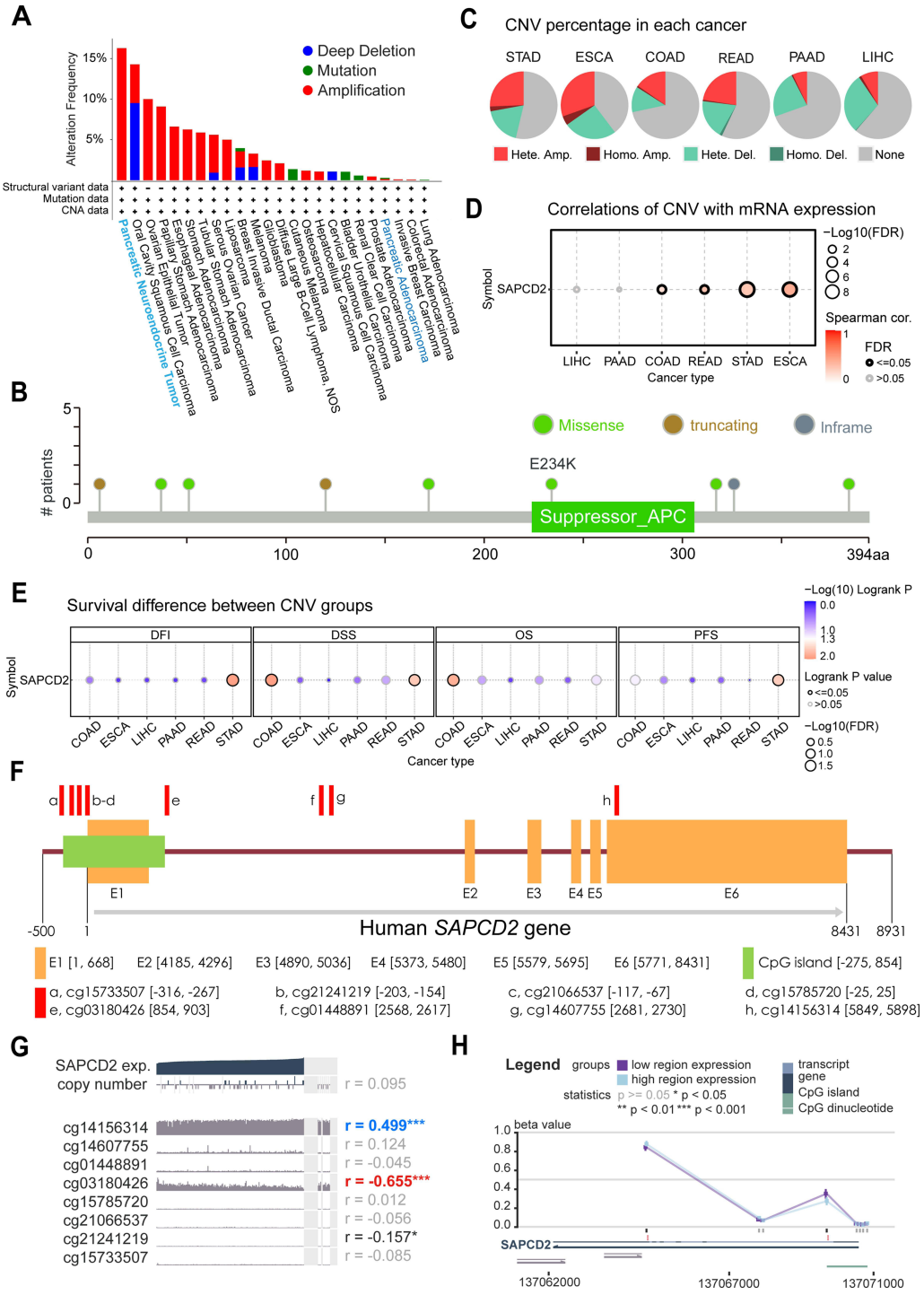

Figure 4 Genomic alterations, expression, and epigenetic regulation of SAPCD2 in gastrointestinal tumors. (**A**) The landscape of the copy number amplification and mutation frequency of *SAPCD2* gene in various tumor types. (**B**) Potential mutations sites across the SAPCD2 protein in various cancers. Green dots, missense mutations; Black dots, truncating mutations; Brown dots, in-frame mutations. The hotspot mutation (E234K) is observed in the conserved Suppressor_APC domain. (**C**) The CNV percentage in each GI tumor. (**D**) The correlation between CNV and RNA-seq derived *SAPCD2* gene expression in digestive tumors. (**E**) The differences of the patients' survival between the CNV group and the wild type group in digestive tumors. (**F**) The diagram of the genomic structure of human *SAPCD2* gene. The green box indicates the CpG island locus; the Orange boxes indicate the 6 exons' locus; the red boxes indicate the positions of the 8 probes for evaluating the DNA methylation status; the grey arrow indicates the transcription direction of *SAPCD2* gene whose cDNA is 8431 base pairs long. The width of each box indicates its relative length, and numbers within the square brackets indicates the start and end sites respectively. The 8 probes in this study are: cg15733507, cg21241219, cg21066537, cg15785720, cg03180426, cg01448891, cg14607755, cg14156314. (**G**) The summarized view of the relationship between the RNA-seq derived *SAPCD2* gene expression and DNA methylation at 8 probes sites in TCGA-PAAD cohort. Each probe (Infinium Human Methylation 450 microarray data) was indicated on the left side, and the numbers on the right side indicated the correlation coefficient or P value. *p<0.05, **p<0.01, ***p<0.001. (**H**) The detailed view of the relationship between of *SAPCD2* gene expression and DNA methylation at 8 probes sites in TCGA-PAAD cohort. The methylation level differed in *SAPCD2* gene high and low expression group at 3 probe sites: cg21241219*, cg03180426 ***, cg14156314 ***.

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demonstrated that the disease-free interval (DFI) was primarily observed in STAD, the disease-specific survival (DSS) difference were notable in both COAD and STAD. Changes in overall survival (OS) were predominantly detected in COAD, whereas disparities in progression-free survival (PFS) were most evident in STAD between patients with and without CNVs (Figure 4E).

The CpG island typically presented at around the promoter region which spans the promoter, the first exon and part of the first intron of *SAPCD2* gene, laying a solid foundation for epigenetic regulation (Figure 4F). The relationship of DNA methylation and *SAPCD2* gene expression pattern were further explored in MEXPRESS database (Figure 4G-H, Supplementary Figure 1). Overall, *SAPCD2* gene expression level inversely correlated with DNA methylation around the promoter region but positively associated with methylation in the gene body (non-promoter region) across multiple cancers. Remarkably, 21 out of 33 (63.6%) types of tumors displayed a statistically significant negative association between *SAPCD2* expression and DNA methylation of cg03180426 probe site which was located at the first intron and adjacent to the end of the CpG island (Figure 4G and H, Supplementary Figure 1). Taken together, these data indicate that *SAPCD2* promoter hypomethylation might be one of the contributors to the upregulation of *SAPCD2* gene across multiple cancers, thereby playing a role in cancer pathogenesis and progression.

Correlation Between SAPCD2 Expression and Prognosis in LIHC, PAAD and STAD

The expression of SAPCD2 was further examined across different stages of digestive cancers to investigate its correlation with disease progression (Figure 5A). In COAD, ESCA, LIHC, PAAD, READ, and STAD, SAPCD2 was demonstrated to be upregulated in stage I–IV tumor tissues when compared to the normal tissues. SAPCD2 was upregulated even in the early-stage I tumors, suggesting its potential as an early biomarker for these digestive cancers.

To explore the prognostic significance of SAPCD2, the TCGA database was utilized to assess the survival outcomes (Figure 5B). In LIHC and PAAD, lower SAPCD2 expression levels were linked to improved survival (LIHC: $p<0.001$, PAAD: $p=0.0046$). Conversely, in STAD, higher SAPCD2 expression levels were linked to prolonged survival (STAD: $p=0.002$), suggesting a cancer-specific prognostic role. However, in COAD, ESCA, and READ, no significant correlation was observed between SAPCD2 expression and patients' survival outcomes (Supplementary Figure 2A–2C).

A broader comparison of SAPCD2 expression across multiple cancer types was performed using COX regression analysis to assess its impact on OS, DSS, DFI, and progression-free interval (PFI) (Figure 5C). The hazard ratio analysis revealed that elevated SAPCD2 expression was associated with worse OS and DSS outcomes in cancers such as KIRP, KIRC, MESO, LIHC, ACC, PRAD, SARC, LUAD, and THCA. Similarly, higher SAPCD2 expression levels correlated with poorer DFI and PFI in KIRP, BRCA, LIHC, UCS, THCA, and PRAD. In contrast, SAPCD2 appeared to act as a protective factor in STAD and LGG, where it was associated with better OS, DSS, and PFI outcomes. These findings suggest a dual role for SAPCD2 in different cancer types—serving as an oncogene in KIRP, KIRC, LIHC, ACC, PAAD, MESO, and PRAD, while exhibiting a tumor-suppressive effect in STAD and LGG.

Immune Evaluation of SAPCD2 in Pan-Cancer

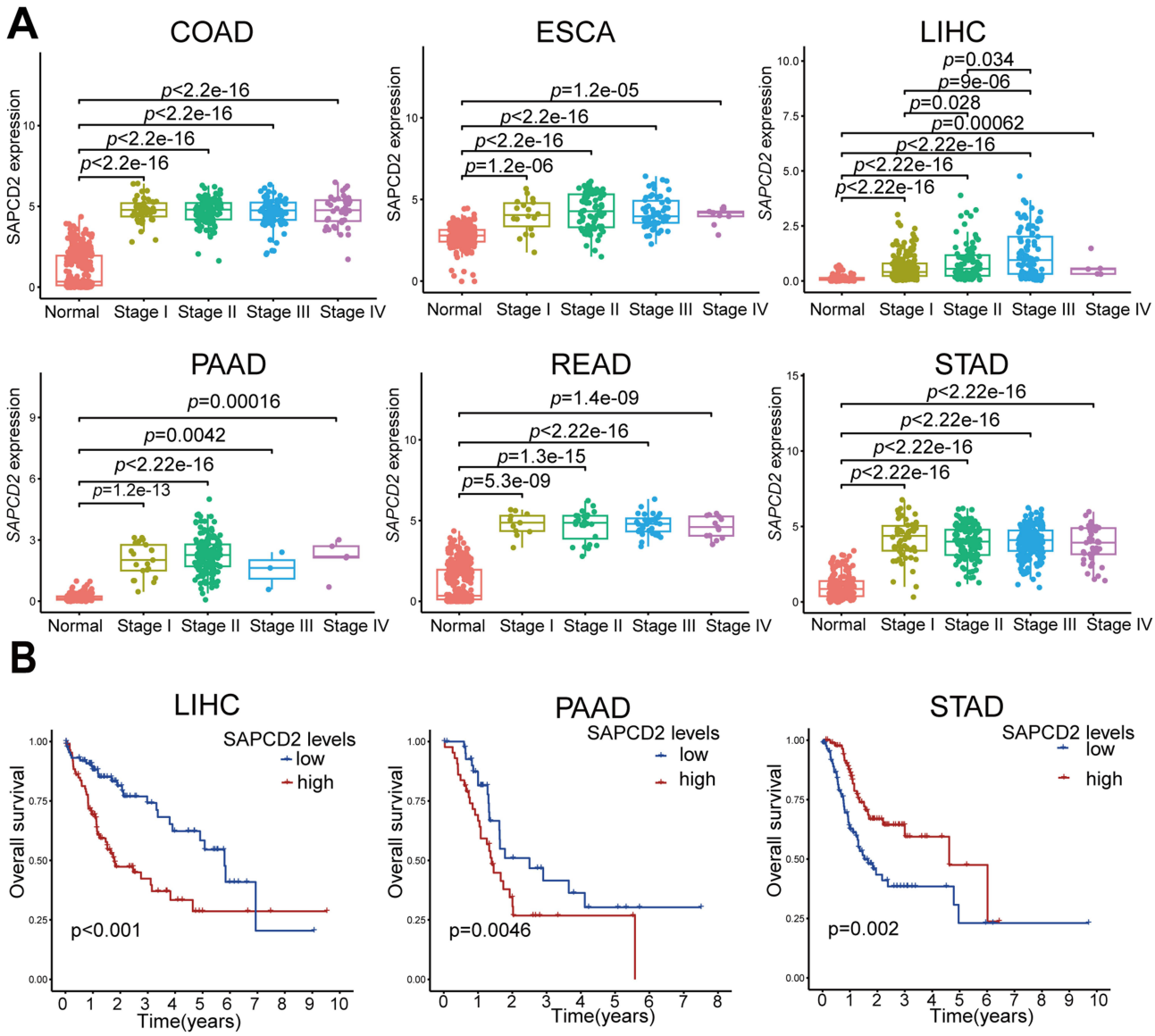
Given the dual role of SAPCD2 in different cancers, its impact on the immune landscape of tumors was analyzed. Tumor mutation burden (TMB) and microsatellite instability (MSI) across a wide spectrum of cancers were measured in CBioPortal database. A strong association was identified between SAPCD2 expression levels and TMB in several cancer types, including BRCA, BLCA, UCEC, THYM, THCA, STAD, SKCM, SARC, PRAD, PAAD, LUSC, and LUAD (Figure 6A). MSI analysis revealed a positive correlation between SAPCD2 expression and MSI in BLCA, ACC, UCEC, STAD, SARC, READ, and LUSC (Figure 6B).

Notably, in STAD, a significant correlation was observed between SAPCD2 expression and both TMB and MSI, suggesting a potential link between SAPCD2 levels, genomic instability and immune response. However, in PAAD, SAPCD2 expression was only associated with TMB, with no significant relationship with MSI. This discrepancy may be due to the low incidence of MSI in PAAD, present in only 1–3% of cases, compared to 10% to 20% of cases in STAD,[38] more samples are needed to reach a reliable conclusion.

Furthermore, to elucidate the differing prognostic roles of SAPCD2 in STAD and PAAD, we assessed immune and stromal scores in relation to SAPCD2 expression (Figure 6C and D). The immune score reflects the presence of

infiltrating immune cells within tumor tissues, while the stroma score indicates the proportion of stroma cells in the tumor microenvironment, providing insight into immune function and the tumor microenvironment. In PAAD, there is a negative correlation between SAPCD2 expression and both the immune score (p=3.2x10-6) and stromal score (p=8.6x10-8). Similarly, in STAD, SAPCD2 expression exhibited a negative correlation with the immune score (p=3.2x10-6) and stromal score (p=8.6x10-8). In COAD, ESCA, and READ, SAPCD2 expression also showed a negative correlation with the immune score and stromal score (Supplementary Figure 2D–2F).

These observations suggest that lower SAPCD2 expression levels in PAAD may potentially activate immune responses, contributing to a more favorable outcome. In STAD, the immune assessment does not align with favorable prognosis linked to high SAPCD2 expression. This indicates that SAPCD2's influence in STAD may not be immune-mediated but rather involve alternative signaling pathways or interactions within the tumor microenvironment.



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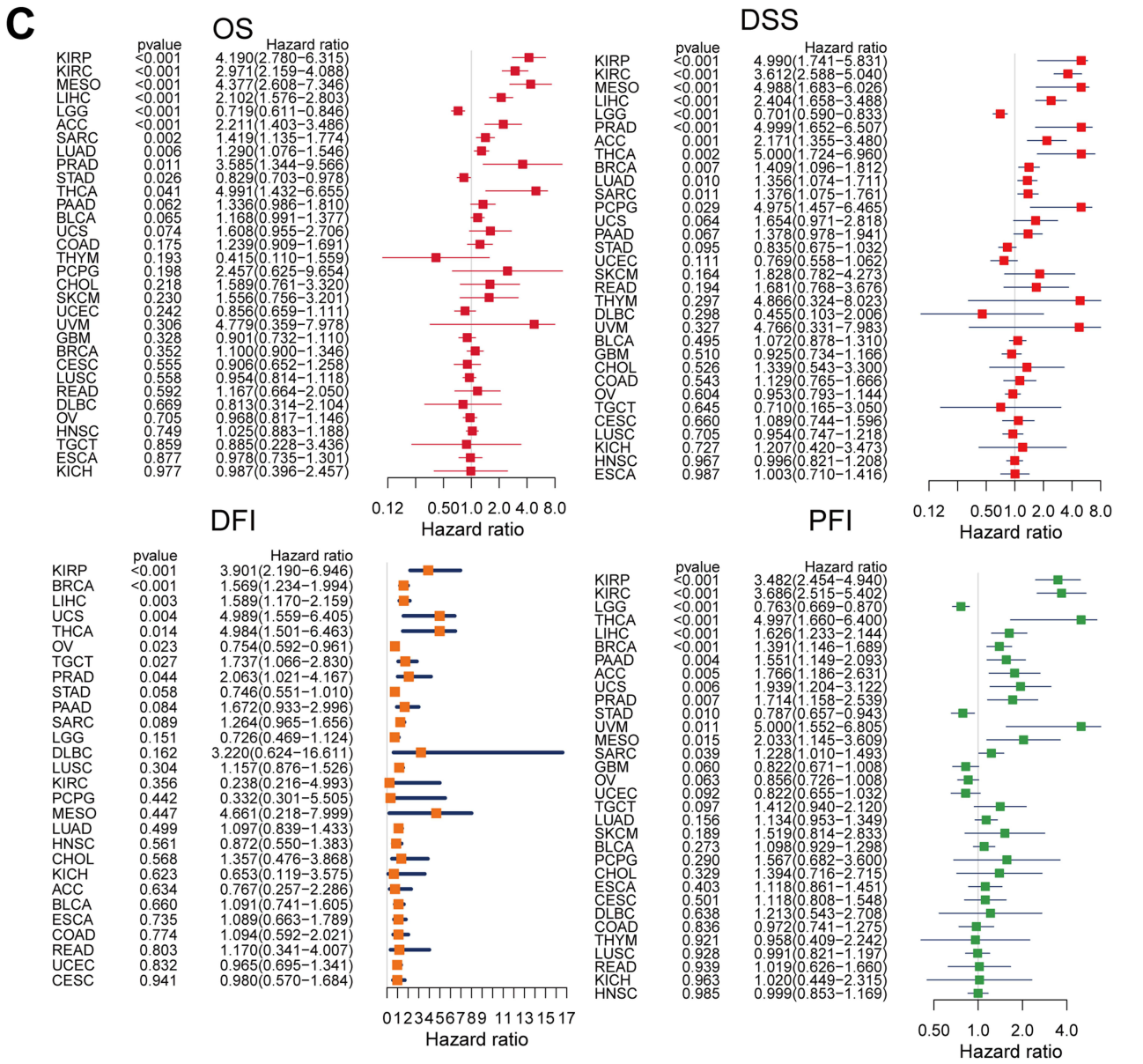

Figure 5 Prognostic impact of SAPCD2 expression across digestive cancers. (**A**) SAPCD2 expression levels across different stages of digestive cancers. (**B**) Kaplan Meier survival analysis of SAPCD2 expression in LIHC, PAAD and STAD. (**C**) The prognostic impact of SAPCD2 expression on OS, DSS, DFI and PFI in different cancer types. **Abbreviations**: OS, overall survival; DSS, disease-specific survival; DFI, disease-free interval; PFI, progression-free interval.

Correlation of SAPCD2 Expression With the Tumor Immune Cell Infiltration

Given the differing immune roles and prognostic implications of SAPCD2 in PAAD and STAD, we further investigated the correlation between SAPCD2 expression and the tumor immune microenvironment in both cancers (Figure 6E and F). In PAAD, elevated SAPCD2 expression exhibited a positive correlation with memory B cells, macrophages M0 and activated dendritic cells, while showing a negative correlation with CD8 T cells, activated memory CD4 T cells, monocytes, and resting mast cells. Conversely, in STAD, decreased SAPCD2 expression correlated positively with T cells CD4 memory resting, monocytes, resting dendritic cells, resting mast cells, and eosinophils, but negatively with activated CD4 memory T cells, follicular T helper cells, activated NK cells, M0 macrophages, and M1 macrophages.

In PAAD, lower SAPCD2 expression level displayed a significant correlation with the infiltration of CD4 T memory T cells and CD8 T cells (Figure 6G). While in STAD, higher SAPCD2 correlated with increased presence of macrophages and activated CD4 T cells (Figure 6H). These findings suggest that lower SAPCD2 levels in PAAD may relate to a more robust

T cell immune response. In contrast, in STAD, higher SAPCD2 expression is appears to support the recruitment of M1 macrophages and activated CD4 T cells. SAPCD2 appears to play distinct immune role in PAAD and STAD.

Evaluation the Role of SAPCD2 on Immune Modulation

The enrichment scores of 13 immune-related functions were compared between low and high SAPCD2 expression groups using the single-sample gene set enrichment analysis (ssGSEA). In PAAD, lower SAPCD2 was significantly correlated with several key immune functions, including chemokine receptors (CCR), checkpoint activity, cytolytic activity, human leukocyte antigen (HLA) expression, inflammation promotion, major histocompatibility complex class I (MHC-I), and Type II interferon (IFN) response (Figure 6I). These findings suggest that lower SAPCD2 levels are correlated with a more activate immune system. Furthermore, intriguingly, reduced SAPCD2 levels were also associated with both T cell co-stimulation and inhibition, indicating SAPCD2 plays a complex role in immune modulation in PAAD microenvironment.

In STAD, decreased SAPCD2 was found to correlate with APC co-stimulation, CCR, check point activity, HLA expression, proinflammation, T cell co-stimulation, type I–IFN response, and type II IFN response (Figure 6J). These results suggest that lower SAPCD2 levels may trigger immune system activation in STAD. However, this immune activation contrasts with the prolonged survival associated with SAPCD2 in STAD, implying SAPCD2 might not

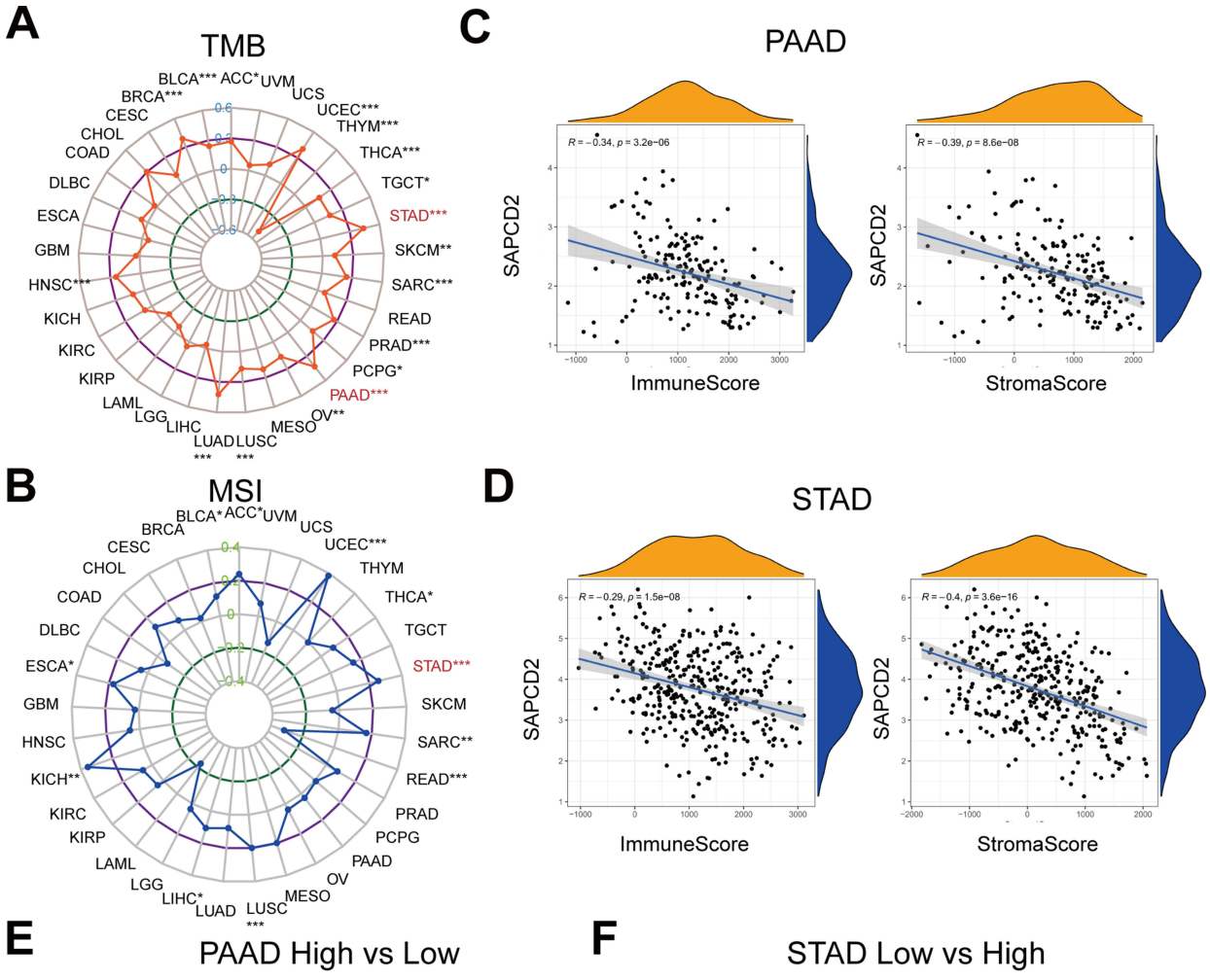

Figure 6 Continued.

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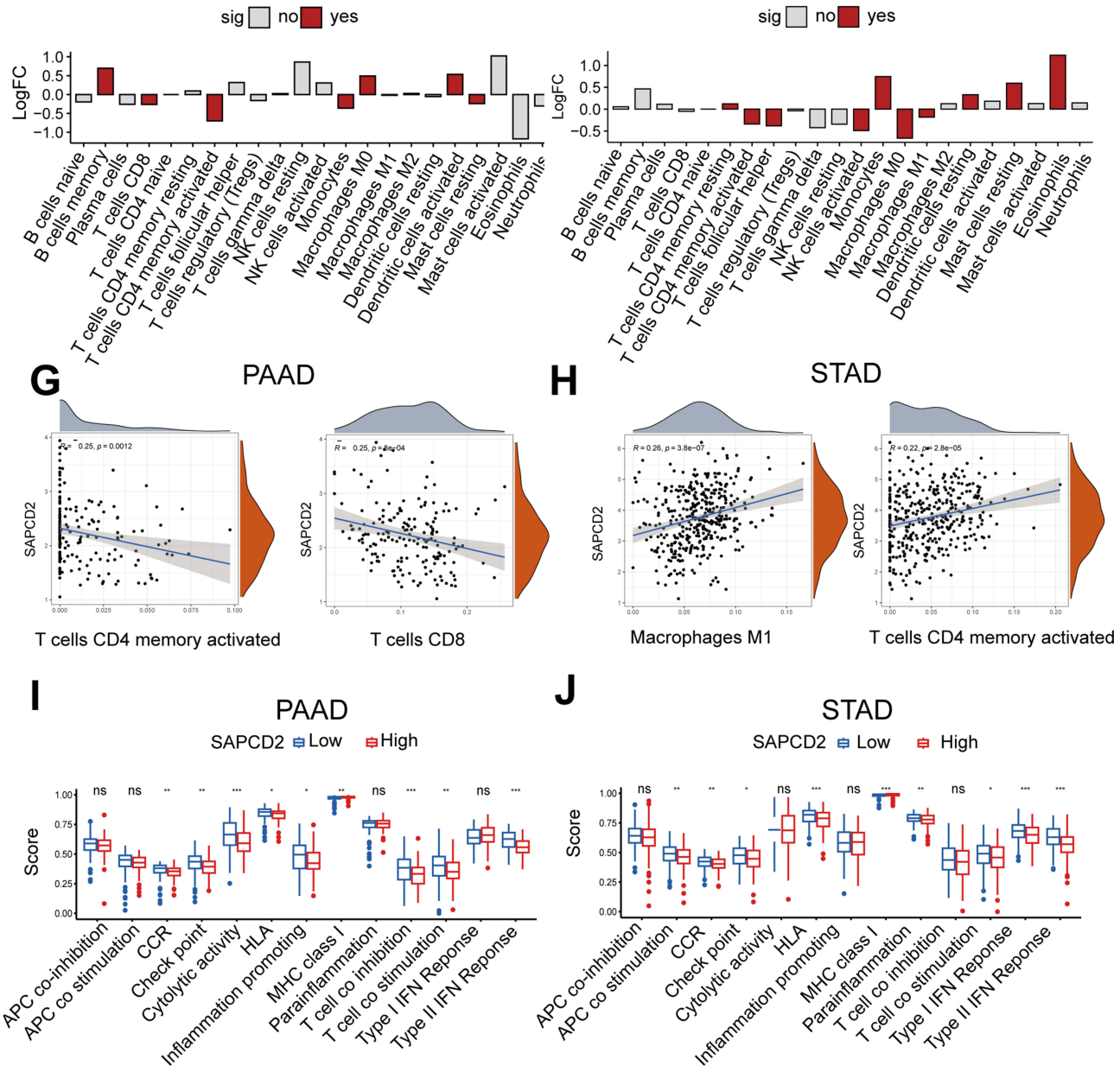

Figure 6 Comprehensive analysis of SAPCD2 expression and immune infiltration markers across multiple cancer types. (**A** and **B**) Radar plots represent the correlation between SAPCD2 expression and immune infiltration markers, including TMB and MSI, across various cancers. (**C** and **D**) Analysis of SAPCD2 expression in relation to immune and stromal scores in PAAD and STAD. (**E** and **F**) Correlation of SAPCD2 expression with immune cell types in PAAD and STAD. (**G** and **H**) Detailed analysis of SAPCD2's impact on specific immune cell populations, with a focus on CD4 and CD8 T cells in PAAD, and macrophages and activated CD4 T cells in STAD. (**I** and **J**) ssGSEA analysis comparing immune-related cell proportion between low and high SAPCD2 expression levels in PAAD and STAD. *p<0.05, **p<0.01, ***p<0.001.

primarily exert its effects through immune-related mechanisms, involving alternative pathways or functions beyond immune modulation.

SAPCD2 Immune Response

We further investigated the cancer hallmark gene enrichment associated with SAPCD2 using Gene Set Variation Analysis (GSVA) (Figure 7A). In most of the cancers, except uterine carcinosarcoma (UCS), SAPCD2 showed a strong correlation with cell cycle processes, particularly emphasizing the G2/M checkpoint. SAPCD2 plays a crucial role in the mitotic spindle function during cell proliferation and engages in key signaling pathways such as MYC, E2F, and mTORC1 highlight its importance in regulating cell division. In comparison to its involvement in cell proliferation mechanisms,

SAPCD2 appears to exhibit lower involvement in immune pathways, such as interferon gamma responses, inflammation response, as well as IL6 JAK-STAT3 signaling. Notably, SAPCD2 expression levels did not correlate with bile acid metabolism but was associated with glycolysis. In PAAD, SAPCD2 expression correlated with cell proliferation, interferon alpha response and P53 pathway. While in STAD, SAPCD2 expression also correlated with cell cycle G2M check point, mTORC1 pathway, and was involved in unfolded protein response, suggesting a potential role for SAPCD2 in regulating cellular stress responses related to protein folding within the endoplasmic reticulum in STAD.

A comprehensive analysis of SAPCD2 expression across immune checkpoint marker in 20 different cancers revealed strong correlations with immune-suppressive checkpoints such as CD276, TNFRSF9, TNFRSF18, and TNFRSF14. In contrast, weaker correlations were observed with T cell activation markers such as CD28, CD40LG, and TNFSF14 (Figure 7B). Specifically, in PAAD, SADCP2 exhibited positive correlations with immune suppressive gene checkpoints like TNFSF9 and TNFRSF14, as well as with immune-stimulatory genes TNFRSF25, TNFRSF18, and TNFSF15. Notably, SAPCD2 expression exhibited negative correlations with immune checkpoints PDCD1, CTLA4, TIGIT, BTLA, and CD160, no correlation with CD274 (PD-L1). In STAD, SAPCD2 showed positive correlations with immune-suppressive markers IDO1 and TNFSF9, along with immune-stimulatory markers TNFRSF25, TNFRSF4, TNFRSF18. However, there was no correlation with PDCD1, CD274, CTLA4, LAG3 or TIGIT, and a low correlation with BTLA. Interestingly, SAPCD2 expression in LIHC was highly correlated with immune markers, showing positive relationships with checkpoints CD274, PDCD1, CTLA4, TIGIT, HAVCR2 (TIM3), VSIR, CD276, LAG3.

Further analysis of immune pathways involving SAPCD2 (Figure 7C and D) revealed differential gene expression patterns in PAAD that were correlated with various immune processes. These genes were linked to adaptive immune responses, antigen binding, B cell receptor signaling, complement activation, immunoglobulin complexes, receptor binding, and phagocytosis recognition. Additionally, the differential gene expression profile indicated enrichment in genes related to the immune microenvironment, including components such as collagen-containing extracellular matrix, contractile fibers, extracellular matrix components, muscle contraction, and the T cell receptor complex within the context of PAAD. These findings shed light on the potential impact of SAPCD2 expression levels on immune responses and the tumor microenvironment in pancreatic cancer, suggesting avenues for further exploration and potential targeted therapeutic interventions in PAAD.

SAPCD2 Expression and Function in PDAC

The expression levels and functions of SAPCD2 in PDAC were investigated. SAPCD2 expression was assessed in PDAC cell lines (BxPC3, SW1990, CFPAC-1 and Panc-1) and immortal human pancreatic duct epithelial cells (HPDE). Results indicated that SAPCD2 was upregulated in BxPC3 and SW1990 compared to HPDE cells (Figure 8A). Subsequent knockdown experiments targeting SAPCD2 in Panc-1 and BxPC3 cells (Figure 8B and 8D) unveiled that reduced SAPCD2 levels led to inhibited PDAC cancer cells proliferation (Figure 8C and E). Additionally, a correlation between SAPCD2 and cell cycle function was explored through flow cytometry (Figure 8F and G). The findings revealed that knocking down SAPCD2 in the Panc-1 and BxPC3 cell line resulted in a decrease in the percentage of cells in G0/G1 phase, a slight upregulation of cells in the S phase, with G2/M phase remaining unchanged. Knock down of SAPCD2 also impaired invasion ability of PDAC cells (Figure 8H and I). These findings highlight SAPCD2's involvement in both cell cycle progression and tumor invasion.

SAPCD2 protein expression was validated in our PDAC tissue array samples (n=80), the expression levels varied among patients, categorized based on staining intensity as low expression (25-50% positive expression rate), or high expression (51-75% positive expression rate) (Figure 9A). Our results showed that high SAPCD2 expression was significantly associated with poor prognosis (Figure 9B), while low SAPCD2 expression correlated with better prognosis. SAPCD2 expression was significantly correlated with tumor T and N stages (Figure 9C, Log rank test, $P<0.05$). Notably, SAPCD2 was expressed in both the cytoplasm and nucleus, with higher expression in the cytoplasm than in the nucleus (Figure 9A).

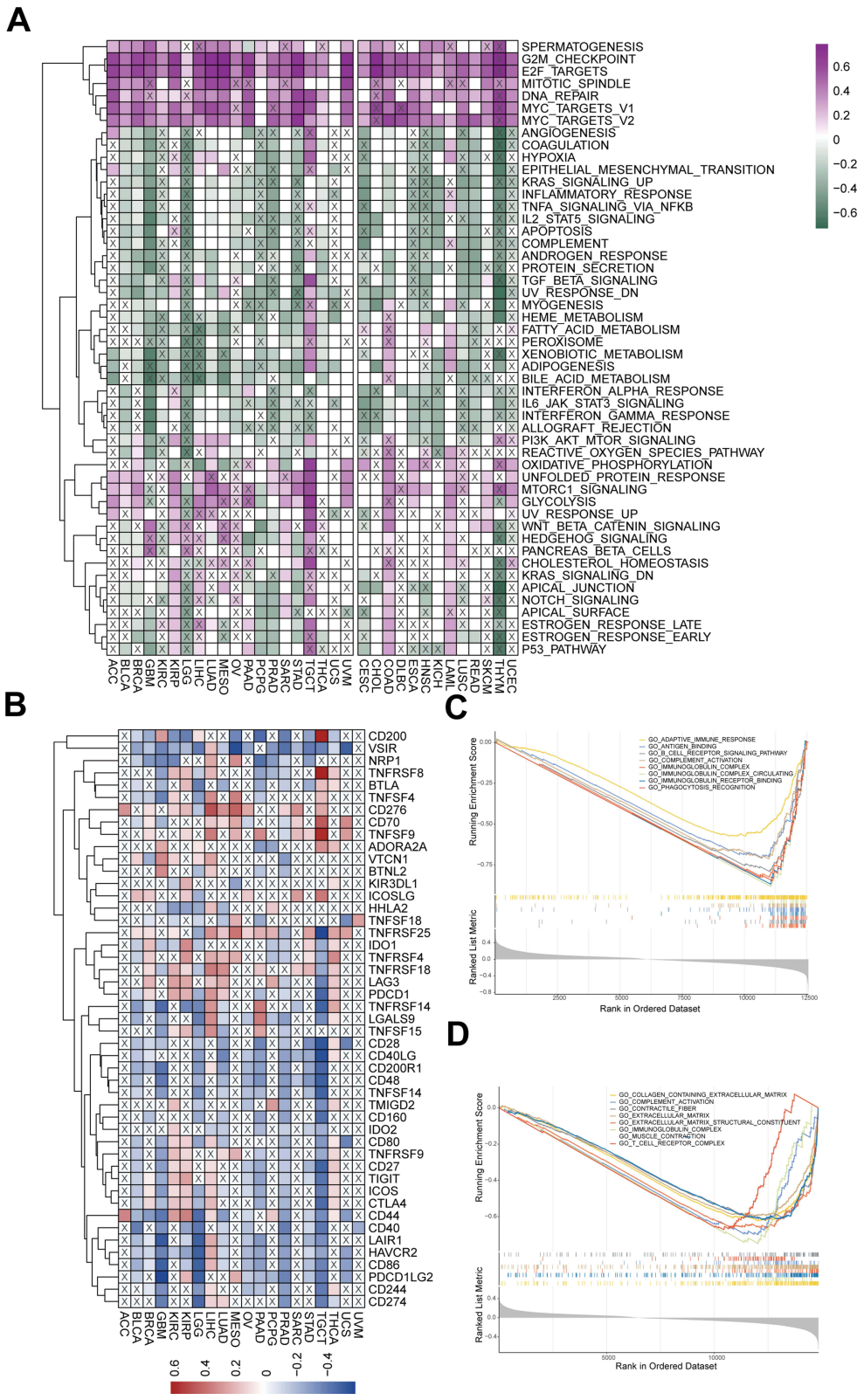

Figure 7 SAPCD2 immune response and cancer hallmarks. (**A**) Association between SAPCD2 expression and cancer hallmarks in pan-cancer. (**B**) SAPCD2 expression and immune checkpoint marker gene analysis. (**C** and **D**) SAPCD2 involvement in immune pathway analysis.

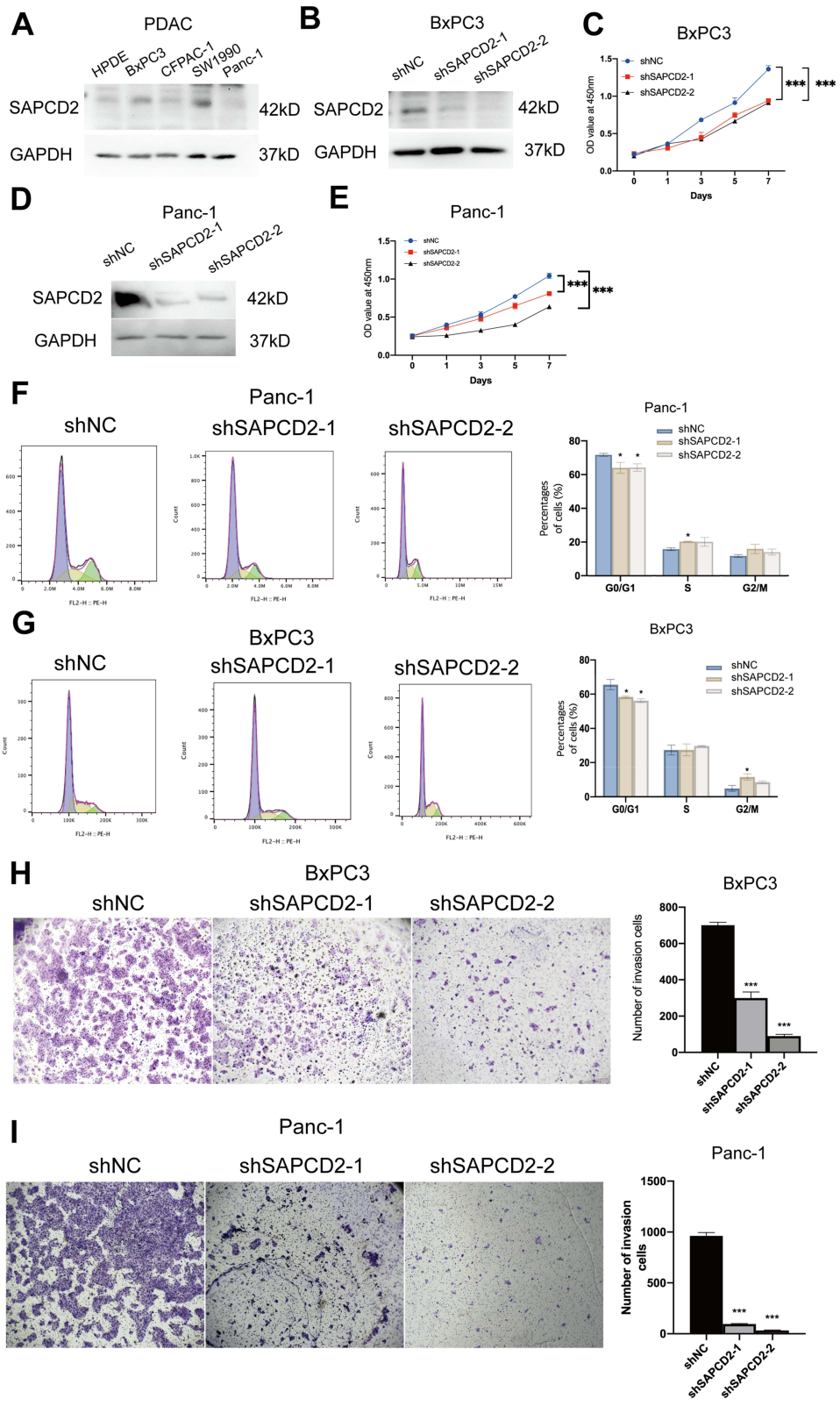

Figure 8 SAPCD2 expression and cell function in PDAC. (**A**) SAPCD2 protein expression level were analyzed in PDAC cell lines and HPDE. (**B** and **D**) SAPCD2 knockdown experiments were performed in Panc-1 and CFPAC-1 cell lines. (**C** and **E**) The knockdown of SAPCD2 significantly inhibited cell proliferation in both Panc-1 and CFPAC-1 cell lines. (**F** and **G**) Cell cycle analysis of Panc-1 and BxPC3 cells with SAPCD2. (**H** and **I**) Knock down of SAPCD2 inhibited cell invasion in Panc-1 and CFPAC-1 cell lines. *p<0.05, ***p<0.001.

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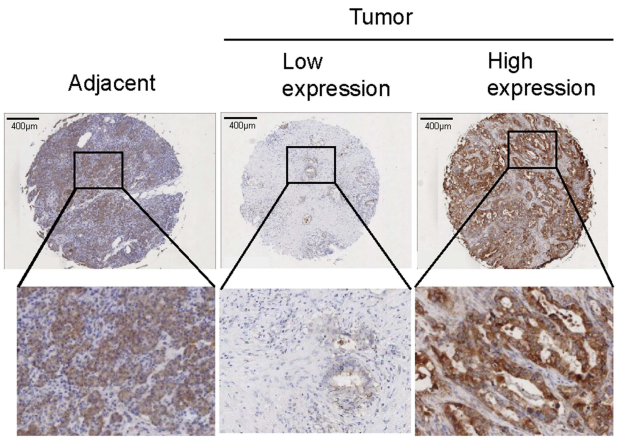
A



B

Charitcristic	high expression	low expression	P value
n	33	37	
Tumor size			0.719
≤2cm	6 (18.2%)	8 (21.6%)	
>2cm	27 (81.8%)	29 (78.4%)	
T stage,n(%)			0.034
1	9 (27.3%)	4 (10.8%)	
2	17 (51.5%)	13 (35.1%)	
3	6 (18.2%)	15 (40.5%)	
4	1 (3.0%)	5 (13.5%)	
N stage,n(%)			0.013
0	23 (69.7%)	17 (45.9%)	
1	8 (24.2%)	20 (54.1%)	
2	2 (6.1%)	0 (0.0%)	
M stage,n(%)			1
0	29 (87.9%)	33 (89.2)	
1	4 (12.1%)	4 (10.8%)	
Pathologic stage,n(%)			0.073
Well-differentiated	2 (6.1%)	1 (2.7%)	
Moderately-differentiated	19 (57.6%)	13 (35.1%)	
Poorly differentiated	12 (36.4%)	23 (62.2%)	
Vascular invasion			0.744
No	20 (60.6%)	21 (56.8%)	
Yes	13 (39.4%)	16 (43.2%)	
n	28	33	
CA125			0.375
≤35U/ml	20(71.4%)	20(60.6%)	
>35U/ml	8(28.6%)	13(39.4%)	
CA199			0.375
<37U/ml	8 (28.6%)	13 (39.4%)	
>37U/ml	20 (71.4%)	20 (60.6%)	
CEA			0.726
<5ng/ml	21(75.0%)	26(78.8%)	
>5ng/ml	7(25.0%)	7(21.2%)	

C

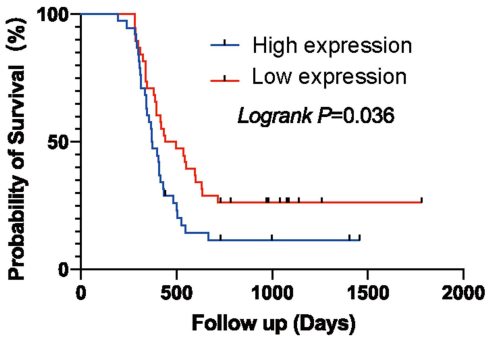


Figure 9 Validation of SAPCD2 expression in PDAC microarray samples. (**A**) Representative images showing high and low SAPCD2 expression in PDAC tumors and adjacent tissues. (**B**) Correlation analysis between SAPCD2 expression and clinical characteristics of PDAC tissue. (**C**) Kaplan-Meier survival analysis of 80 PDAC patients based on high and low SAPCD2 expression.

Correlation of SAPCD2 Expression With Drug Sensitivity in PAAD

In our study focusing on PAAD, drug sensitivity based on SAPCD2 expression was evaluated using the pRRophetic package. The results showed that PAAD cells with higher SAPCD2 expression were more sensitive to the p38α MAPK inhibitor VX-702 (Figure 10A), the DNA-PK inhibitor NU7441 (Figure 10B), and the Bcl-2 family protein inhibitor ABT-263 (Figure 10C). Conversely, cells with lower SAPCD2 expression were more sensitive to the kinesin Eg5 inhibitor S-Trityl-L-cysteine (Figure 10D), the antineoplastic agent Paclitaxel (Figure 10E), and the Raf inhibitor Sorafenib (Figure 10F). These finding suggests that reduced SAPCD2 expression could render cells more susceptible to drugs targeting microtubule dynamics and Raf signaling pathways, offering an alternative therapeutic strategy for PAAD with low SAPCD2 expression. Collectively, these results underscore the potential of SAPCD2 as a biomarker to guide personalized therapeutic approaches in pancreatic cancer.

Discussion

SAPCD2 is highly expressed in various tumor tissues and cells. Notably, compared to adjacent normal tissues, SAPCD2 is significantly upregulated in cancers, like gastric cancer, colorectal cancer, hepatocellular carcinoma, lung cancer, breast cancer, prostate cancer, nasopharyngeal carcinoma, and gliomas. Our study found SAPCD2 also upregulated in digestive cancers, high SAPCD2 expression is associated with poor prognosis, including LIHC and PAAD. However, in the TCGA-STAD dataset, higher SAPCD2 expression correlates with better prognosis, and shows no significant association with survival in colorectal cancer.[20] Previous studies showed SAPCD2 expression is closely associated with several

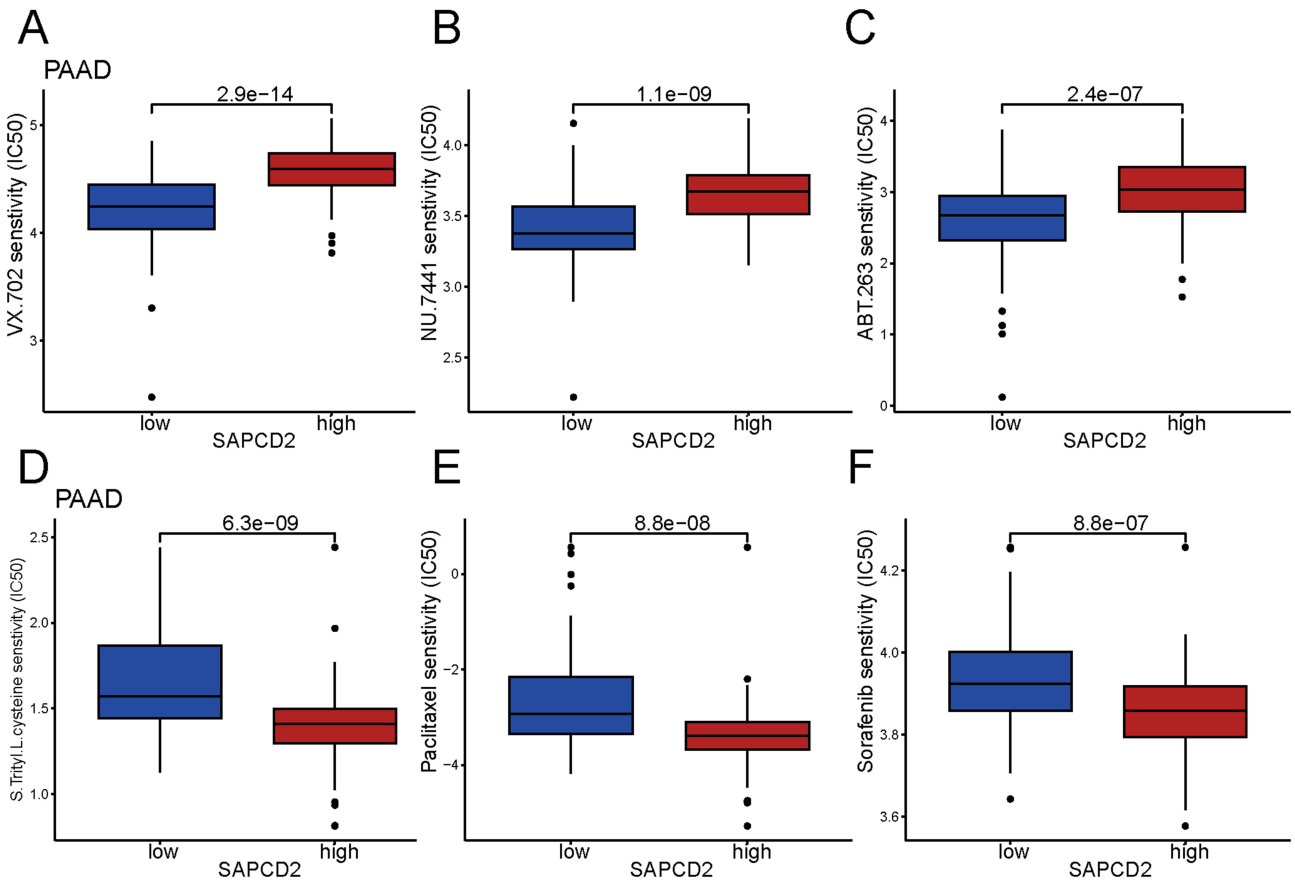


Figure 10 Correlation of SAPCD2 expression with drug sensitivity in PAAD. (**A–F**) Drug sensitivity analysis using the pRRophetic package in PAAD cells demonstrated distinct responses based on SAPCD2 expression levels.

clinicopathological characteristics,[39] such as tumor size,[40] TNM stage,[28,40] clinical stage,[28,30,41] histological grade,[21,22,26,30] metastatic status,[40,42] but not related to age and gender.[20,22,30,40,41] Some studies did not find the association of SAPCD2 expression with metastasis,[20,22] TNM stage.[19,20,22,26] In our study, we found that SAPCD2 expression correlated with T stages and was primarily located in the cytoplasm (Figure 9B). No significant correlation was observed with M stages, nor with overall pathological stages.

Until now, only limited researches have explored the genetic and epigenetic alterations of SAPCD2 gene in tumors. In prostate cancer, the DNA methylation level of SAPCD2 gene was reported to be decreased in tumor tissues,[43] and negatively correlates with the patients' survival.[43] The DNA methylation level of some CpG loci of SAPCD2 was higher in LUAD when compared to LUSC,[30] indicating that aberrant DNA methylation may contribute to the disordered SAPCD2 expression. Our methylation analysis revealed that SAPCD2 expression is negatively correlated with DNA methylation at the promoter region across multiple tumor types. Specifically, hypomethylation at CpG sites around the promoter likely contributes to the upregulation of SAPCD2. A strong inverse relationship between SAPCD2 expression and methylation was observed in 63.6% of the types of tumors, underscoring the potential role of promoter hypomethylation in driving the SAPCD2 overexpression.

Regarding to the immune aspect, some studies have explored the association of SAPCD2 with immunity. In inflammatory bowel disease (IBD), Frenkel found SAPCD2 gene amplifications in the blood samples. In tumors, He et al found that expression of SAPCD2 was positively associated with B and T lymphocyte attenuator (BTLA), V-domain Ig suppressor of T cell activation (VISTA), but negatively associated with PVR/CD155.[16] In our study, we found SAPCD2 expression was negatively correlated with immune checkpoints VISTA, PDCD1, CTLA4, TIGIT, BTLA, and CD160, and showed no correlation with CD274 (PD-L1) in PAAD. High SAPCD2 expression

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in PAAD tissues was associated with increased infiltration of CD8 T cells, monocyte-derived macrophages, neutrophils, fibroblasts, and malignant PAAD cells, reduced SAPCD2 levels were associated with T cell stimulation in PAAD. In contrast, in STAD, SAPCD2 might not primarily involved in immune-related mechanisms.

SAPCD2 may promote the tumor cell proliferation via upregulating the expression of downstream target proteins and activating several pathways, and plays a pivotal role in tumor occurrence and progression. SAPCD2 expression was reported highly correlated with the cell cycle.[15,44–46] We observed in PAAD that SAPCD2 is involved in mitotic spindle function during cell proliferation and participates in key signaling pathways such as MYC, E2F, and mTORC1, highlighting its critical role in regulating cell division. Previous studies have reported that downregulation of SAPCD2 decreased the G1 phase cells with an increased G2/M phase cells in gastric cancer,[15] or unchanged G2/M phase cells in neuroblastoma,[47] colorectal cancer.[20] However, Some others obtained different results, they found that downregulation of SAPCD2 increased G1 phase cells while decreased G2/M phase cells in bladder cancer[48] and gastric cancer.[19] In our experiment, silencing SAPCD2 led to suppressed proliferation of PDAC cells, a decline in the proportion of cells in the G0/G1 phase, a slight increase in cells in the S phase, with G2/M phase remaining unaffected. The reasons underlying this inconsistency remains unclear, it may infer that SAPCD2 have different effects on cell cycle. It's believed that cell cycle disturbances are closely associated with pathogenesis and progression of tumor, SAPCD2 may promote malignant transformation via accelerating the M phase, increasing the mis-segregation of chromosomes, eliciting chromosomal instability and inducing the cells entering the next cell cycle.[45] Actually, SAPCD2 overexpression evidently increased the level of γ-H2A.X, a marker of DNA damage response.[45] SAPCD2 also elevated the expression of cyclin B1 and Chk2,[15] two key proteins that are essential for cell cycle regulation.

Furthermore, the knockdown of SAPCD2 hampered the invasiveness of PDAC cells. Previous researches demonstrated that SAPCD2 overexpression decreased the expression of E-cadherin and increased the expression of N-cadherin and vimentin,[18,21,49] indicating that SAPCD2 promotes the tumor invasion and migration ability via accelerating the EMT progress. In addition, it's proposed that SAPCD2 may promote the tumor metastasis and EMT progression via Wnt, MAPK, and PI3K/AKT signaling pathways.[41,49]

Previous studies have shown that SAPCD2 expression is significantly repressed upon GSK-J4 treatment, a newly identified inhibitor of histone demethylase KDM6B, which has demonstrated high sensitivity in neuroblastoma.[47] Additionally, downstream regulatory network analysis found that the SAPCD2 is a responsive gene in organoids derived from esophageal adenocarcinoma tissues.[50] Our findings suggests that reduced SAPCD2 expression could render cells more susceptible to agents affecting microtubule dynamics and Raf signaling pathways, presenting an alternative therapeutic strategy for PAAD with low SAPCD2 expression. SAPCD2 emerges as a potentially valuable biomarker for predicting drug sensitivity in PAAD, providing insights that could inform personalized treatment strategies. The differential drug responses observed between cells with high and low SAPCD2 expression suggest that SAPCD2 might modulate key signaling pathways involved in pancreatic cancer progression and treatment response. However, the predictive efficacy of SAPCD2 in chemotherapy requires evidences.

Conclusion

In conclusion, our study demonstrated the overexpression of SAPCD2 across various cancers, highlighting its genetic and epigenetic alterations. We evaluated its prognostic significance, its impact on the immune microenvironment, and its predictive value in chemotherapy response, with a particular focus on PAAD. Additionally, we verified its expression and functional roles in PAAD. These findings suggest that SAPCD2 may serve as both a biomarker and a potential therapeutic target in PAAD.

Abbreviations

PAAD, pancreatic adenocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; LIHC, liver hepatocellular carcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; TMB, tumor mutation burden; MSI, microsatellite instability; PDAC, pancreatic ductal adenocarcinoma; SAPCD2, suppressor anaphase-promoting complex domain containing 2; C10orf140, chromosome 9 open reading frame 140; APC, adenomatous polyposis coli; CNV, copy number variation; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast

invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; DLBC, diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower-grade glioma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PRAD, prostate adenocarcinoma.

Ethics Statements

Most of the clinical data used in this study were retrieved from publicly available databases. Human clinical samples used for immunohistochemistry were commercially sourced from Bioaitech Company (Xi'an, China). In accordance with the Article 32, specifically Item 1 and 2, of the *Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects* (dated February 18, 2023, China), this study was exempted from ethical review. The exemption was approved by the Research Ethics Committee of The Seventh Affiliated Hospital of Sun Yat-sen University, as per the Exemption of Ethical Review Decision Notification (KY-M-2024-005-01).

Consent for Publication

All the authors consent for publication of this manuscript.

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Disclosure

The authors report no conflicts of interest in this work.

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