Am J Transl Res 2023;15(11):6437-6450
www.ajtr.org /ISSN:1943-8141/AJTR0151849

Original Article

Association of ACP5 with the tumor immune microenvironment and its role in cell proliferation and metastasis in pancreatic cancer

Jianbo Shen[1*], Min Shi[1*], Aqian Song[2*], Yue Ming[3], Xiaolin Zhu[1], Yun Ruan[1], Tingting Zhang[4], Guoxiong Zhou[5]

[1]Department of Gastroenterology, Affiliated Hospital of Nantong University, Medical School of Nantong University, Nantong 226001, Jiangsu, China; [2]Department of Gastroenterology, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China; [3]Department of Clinical Examination, People's Hospital of Dongxihu District, Wuhan 430040, Hubei, China; [4]Department of Gastroenterology, Dongtai People's Hospital, Yancheng 224200, Jiangsu, China; [5]Department of Gastroenterology, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China. [*]Equal contributors.

Received June 21, 2023; Accepted October 23, 2023; Epub November 15, 2023; Published November 30, 2023

Abstract: Background: Tartrate-resistant acid phosphatase (ACP5) has been implicated in the progression of most malignant tumors, but its role in pancreatic cancer (PC) remained unclear. Thus, this study aimed to elucidate the role and function of ACP5 in PC progression. Methods: The expression of ACP5 in PC samples was assessed via R programming, TNM plot, and Gene Expression Profiling Interactive Analysis (GEPIA). Western blotting and immunohistochemistry (IHC) were performed to detect ACP5 expression in cells and tissues. The correlation between ACP5 and methylation was analyzed using the University of ALabama at Birmingham Cancer data analysis Portal (UALCAN) and cBio Cancer Genomics Portal (cBioPortal). The Database for Annotation, Visualization and Integrated Discovery (DAVID) and Gene Set Enrichment Analysis (GSEA) were used for the enrichment of ACP5 in PC. Subsequently, Cell Counting Kit-8 (CCK8), clonogenic, and wound healing assays were used to investigate the role of ACP5 in PC. Finally, Tumor Immune Estimation Resource (TIMER) and R programming was utilized in evaluating the association between ACP5 and immune cell infiltration in PC. Results: The analyses confirmed that ACP5 was highly expressed in PC samples. According to UALCAN and cBioPortal analysis, ACP5 expression, and methylation levels were negatively correlated in PC. The enrichment analysis also revealed that ACP5 was enriched in the proliferation and migration pathways. Meanwhile, ACP5 knockout reduced PC cell proliferation and migration and impaired the cells' independent viability. This gene also positively correlated with immune cell infiltration in PC, particularly regulatory T cells (Tregs). Conclusion: ACP5 is crucial for proliferation, migration, and immune cell infiltration in PC. Therefore, ACP5 may be a valuable target for future PC treatment.

Keywords: Pancreatic cancer, ACP5, proliferation, migration, immunity

Introduction

Pancreatic cancer (PC) is a malignant tumor of the digestive system with high metastatic potential [1, 2]. The morbidity and mortality rates of PC patients are increasing, with a 5-year survival rate of approximately 11% [3]. PC is ranked fourth as a cause of cancer death in the United States [3] and the sixth chief cause of cancer death in China [4]. PC is hard to diagnose due to the lack of early clinical symptoms and is often detected at the advanced stage accompanied by metastasis. Currently, only 10% to 20% of patients with localized and non-metastatic tumors can be treated by surgery [5]. The poor survival status of PC patients makes it imperative to improve their survival rate. Therefore, there is an urgent need to explore new treatment for PC.

Tartrate-resistant acid phosphatase (ACP5) is a metalloprotease located on chromosome 19, belonging to the acid phosphatase family, which can dephosphorylate proteins [6-9]. Previously, ACP5 was recognized as a marker of osteoclasts [10] and was later linked to immune

system function and the development of autoimmune diseases [11-13]. Furthermore, ACP5 was recently identified as an oncoprotein in several malignant tumors [8, 14-17], contributing to tumor cell proliferation, invasion, migration, and chemotherapy resistance [14]. This biomarker also indicates the severity of bone metastasis in malignant tumors [18, 19]. Nevertheless, the role of ACP5 in PC progression has not been revealed.

The current study discovered that ACP5 was highly expressed in PC based on Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) data analysis and immunohistochemistry (IHC). The upregulation of ACP5 expression in tumors was attributed to decreased methylation promoters. An enrichment analysis indicated that ACP5 primarily influences the proliferation and migration pathways in PC, which was validated in PANC-1 cells. The bioinformatic analysis also identified the association between ACP5 and immune cell infiltration. In summary, these findings suggest that ACP5 is critical for PC progression and immune microenvironment.

Materials and methods

Gene expression analysis

ACP5 expression from GTEx and TCGA-PAAD (Pancreatic adenocarcinoma) samples was evaluated using R software and visualized in a ggplot2. The TNMplot (https://tnmplot.com/analysis/) can compare the differential expression of ACP5 between normal, orthotopic, and metastatic tumor samples. GEPIA (http://gepia.cancer-pku.cn/) was used to analyze ACP5 expression in different stages of PC. The GEO series (GSE16515, GSE211398, and GSE183795) were obtained from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) and analyzed by the online easyGEO tool (https://tau.cmmt.ubc.ca/eVITTA/easyGEO/). The results were downloaded from easyGEO and visualized using GraphPad Prism.

DNA promoter methylation

The cBioPortal (https://www.cbioportal.org/) is a data mining and integration platform. A scatter plot was generated to illustrate the correlation between ACP5 expression and methylation levels in PC. Meanwhile, the UALCAN database (http://ualcan.path.uab.edu) mainly analyzed the TCGA cancer omics data and the promoter methylation of ACP5 in normal and tumor samples from TCGA-PAAD.

Gene correlation enrichment analysis

The ACP5 co-expressed genes were first predicted by cBioPortal. Subsequently, an online tool, DAVID 6.8 (http://david.abcc.ncifcrf.gov/), was utilized for Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The biological process (BP), cellular component (CC), molecular function (MF), and KEGG results were visualized by EXCL. In addition, the TCGA-PAAD samples were divided into low and high-expression groups based on the ACP5 levels, and GSEA V3.2 software was used to analyze the signature pathway of ACP5. The pathways were considered significant when the FDR $q < 0.25$, NOM $P < 0.05$, and $|NES| > 1$.

Immune infiltration

Immune cell infiltration in different tumors was systematically analyzed using TIMER (https://cistrome.shinyapps.io/timer). TIMER analyzed the correlation between ACP5 expression and six specific immune cells infiltrating the tumor in TCGA-PAAD samples. Tdifferences in the infiltration of 24 immune cells between the ACP5 high and low expression groups were calculated by ssGSEA using R software. Likewise, the correlation of ACP5 expression with stromal score, immune score, and estimated score in PC, correlation between ACP5 expression and the infiltration of 24 immune cells were calculated by ssGSEA with R software. Finally, the results were visualized using ggplot2.

Pancreatic cancer tissue samples

This research was approved by the Ethics Committee of Affiliated Hospital of Nantong University.

Cancerous and para-cancerous tissues were collected from 20 PC patients who have consented to the study from 2020 to 2022, consisting of thirteen male and seven female patients. The patients underwent surgery without treatment at Affiliated Hospital of Nantong University. Subsequently, the tissues were

embedded in paraffin after immediate fixation in 4% paraformaldehyde.

Immunohistochemistry

Tissue blocks from three patients were randomly selected and sectioned (4 μm) onto poly-L-lysine slides. The slides were then immersed in xylene for wax removal, followed by antigen retrieval using sodium citrate. After the blocking step to remove non-specific binding, the tissue was capped with ACP5 antibody (1:100, A2528; ABclonal) and incubated overnight at 4°C. Subsequently, the sections were incubated with HRP-antibody (1:50; A0216, Beyotime, Shanghai, China) after rinsing with phosphate-buffered saline (PBS). Once the tissues were stained with diaminobenzidine (DAB), the sections were counter-stained using hematoxylin. Finally, the slides were examined under an upright optical microscope (Olympus Corporation, Tokyo, Japan), and the images were obtained.

Cell lines and cell culture

Normal pancreatic epithelial cells (hTERT-HPNE) and PC cell lines (PANC-1, BXPC3, CFPAC-1, ASPC-1) were obtained from BeNa Culture Collection (Beijing, China). First, cells were cultured in DMEM or RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, MA, USA), streptomycin (100 μg/mL) and penicillin (100 U/ml). The cell culture flasks were then placed in an incubator with 5% carbon dioxide (CO_2) at 37°C (Thermo Fisher Scientific, MA, USA).

Cell transfection

Human ACP5 sgRNA (5'-GCCCTGCGCTTTGTAGCCGT-3') primer was synthesized by Sangon and integrated into PAX459 vector to construct plasmid sgACP5. The sgACP5 plasmid and the sgVector control plasmid were transfected into PANC-1 cells with Lipo3000 (Thermo Fisher Scientific, MA, USA). The PANC-1 cells with a stable knockout of ACP5 were screened with puromycin (APExBio, TX, USA).

Western blotting

The cells were lysed on ice with a mixture of RIPA lysis buffer and protease phosphatase inhibitor (New Cell & Molecular Biotech Co., Ltd., Suzhou, China). The cell samples were centrifuged at 12,000 rpm at 4°C, and the supernatant was aspirated. Then, bicinchoninic acid assay (BCA, New Cell & Molecular Biotech Co., Ltd., Suzhou, China) was utilized to determine the protein concentration. The total protein from each sample was subjected to gel electrophoresis on SDS-PAGE gel (GenScript Corporation, Nanjing, China) and transferred to a polyvinylidene fluoride membrane (PVDF, Millipore Corporation, MA, USA). After blocking with 5% skim milk, the PVDF was incubated with primary antibody overnight at 4°C. Subsequently, and the PVDF was incubated with HRP-antibody (Sangon, Shanghai, China) at room temperature after washing off the primary antibody with tris buffered saline containing 0.5% (v/v) Tween 20 (TBST) buffer. After another round of washing, the bands on the PVDF were detected using an enhanced chemiluminescence reagent (ECL, New Cell & Molecular Biotech Co., Ltd., Suzhou, China).

CCK8

The cells were injected into 96-well plates. Their growth rate was measured by the amounts of viable cells, reflected by the optical density (OD) value of CCK8 solution (Vazyme, Nanjing, China). The CCK8 and 1640 mixture was prepared at a ratio of 1:9. Once the medium was removed by washing, the CCK8 mixture (100 ul) was pipetted into each well and incubated for two hours in the incubator with 5% CO_2 at 37°C. Finally, The OD value at 450 nm was detected by a 96-well microplate reader (Thermo Fisher Scientific, MA, USA).

Clonogenic assay

After the sgVector and sgACP5 cells were digested and counted, 1500 cells per well were injected into cell culture plates and incubated with 5% CO_2 at 37°C for 14 days. The cells were later stained with 0.1% crystal violet after fixation with 4% paraformaldehyde. Subsequently, the colonies were analyzed using Image J after their images had been obtained.

Wound healing assay

After the sgVector and sgACP5 cells were digested and counted, they were injected into the cell culture plate (2×10^6 cells/well). When

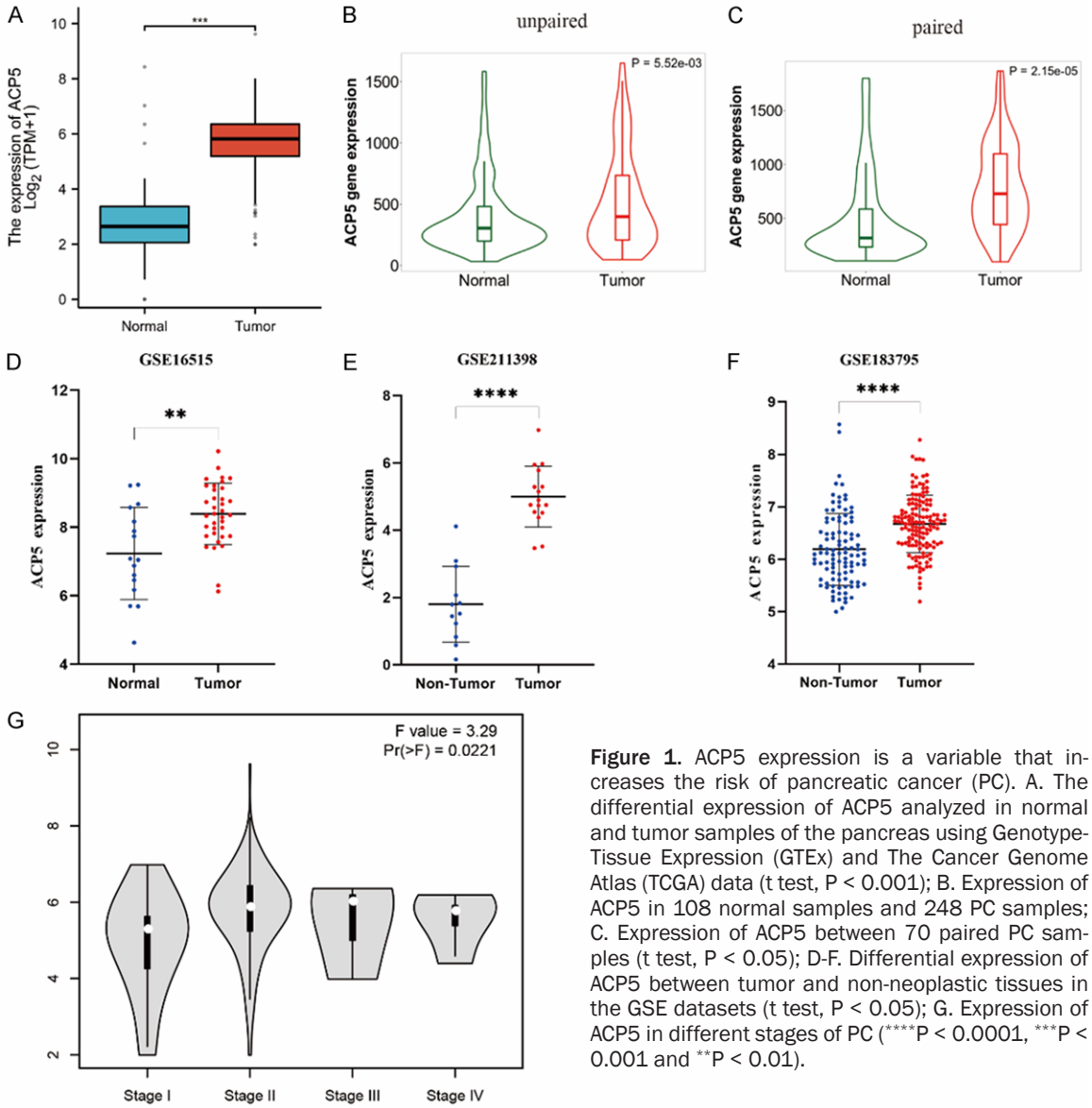


Figure 1. ACP5 expression is a variable that increases the risk of pancreatic cancer (PC). A. The differential expression of ACP5 analyzed in normal and tumor samples of the pancreas using Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) data (t test, $P < 0.001$); B. Expression of ACP5 in 108 normal samples and 248 PC samples; C. Expression of ACP5 between 70 paired PC samples (t test, $P < 0.05$); D-F. Differential expression of ACP5 between tumor and non-neoplastic tissues in the GSE datasets (t test, $P < 0.05$); G. Expression of ACP5 in different stages of PC ($^{****}P < 0.0001$, $^{***}P < 0.001$ and $^{**}P < 0.01$).

the cells reached 100% confluence, the medium was aspirated. The cells were scraped with a 100 μL sterile pipette tips, and three tracks were drawn in each well. The cells were then rinsed with PBS and cultured in 1% FBS medium before being incubated with 5% CO_2 at 37°C. The cell scratch images were acquired at 0, 24, 48, and 72 hours under an inverted optical microscope (Olympus Corporation, Tokyo, Japan).

Statistical analysis

All statistical analyses were performed using SPSS 22.0 and GraphPad Prism 8.2, and the data were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) or Student's t-test was used to determine the differences between the two groups. Statistical significance was set at $P < 0.05$ ($^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$).

Results

Upregulation of ACP5 in PC

The GTEx and TCGA data analysis demonstrated that ACP5 was highly expressed in PC samples (**Figure 1A**). Furthermore, the TNM plot confirmed that ACP5 was significantly higher in tumor tissues from paired and unpaired PC samples (**Figure 1B**, **1C**). In addition, the GEO dataset analysis also exhibited that the ACP5

level was significantly lower in non-neoplastic tissues (**Figure 1D-F**). The GEPIA analysis revealed that ACP5 was highly expressed in advanced-stage PC (**Figure 1G**). Thus, ACP5 is a possible variable that increases the risk of PC.

Western blotting evaluated the ACP5 protein expression in human PC cell lines (PANC-1, BXPC3, CFPAC-1, ASPC-1) and normal pancreatic epithelial cell hTERT-HPNE. This study discovered that ACP5 was highly expressed in cancer cells (**Figure 2A**, **2B**). Meanwhile, IHC staining indicated that ACP5 was highly expressed in cancerous tissues compared to para-cancerous tissues (**Figure 2C**). The experiment's findings revealed that staining of ACP5 in the para-cancerous tissues predominantly exhibited light yellow and tan hues, whereas in PC tissues it was primarily tan and brown, suggesting a more pronounced expression of ACP5 in PC. ImageJ software was employed to quantify the percentage of DAB-colored regions in both the para-cancerous tissues and PC tissues, and it was observed that the para-cancerous group exhibited a significantly lower percentage of DAB staining area compared to the PC group, indicating a higher abundance of ACP5 in PC. These results were subjected to statistical analysis using a t test, with a significance level set at $P < 0.05$. Based on the aforementioned data, it can be inferred that the expression of ACP5 in PC is notably elevated compared to para-cancerous tissues, and this disparity holds statistical significance. These findings suggest that ACP5 is elevated in the cells and tissues of PC.

Furthermore, the clinical data analysis showed a clear distinction between ACP5 levels in the T stage and pathologic stage; the group with a higher ACP5 expression exhibited a more extensive malignancy rate. Moreover, ACP5 expression correlated with the location of PC, such that tumors with high ACP5 expression were often found at the head of the pancreas (**Table 1**).

ACP5 promoter hypomethylation in PC

Epigenetic modifications play a crucial role in governing gene expression and overseeing various cellular processes in both normal and cancerous cells. Abnormal gene methylation often leads to cancer, manifested primarily through the silencing of tumor suppressor genes by hypermethylation and the activation of oncogenes by hypomethylation [20, 21]. Therefore, ACP5 upregulation may be linked to the promoter methylation promotion. The UALCAN analysis found low levels of methylated ACP5 in tumors compared to normal tissue (**Figure 3A**). Subsequently, further investigation was conducted on the different clinicopathologic subgroups of TCGA-PAAD samples in UALCAN. The promoter methylation of ACP5 was lower in tumor samples in terms of node metastasis (**Figure 3B**), gender (**Figure 3C**), age (**Figure 3D**), smoking (**Figure 3E**), tumor grade (**Figure 3F**), cancer stages (**Figure 3G**), and P53 mutation (**Figure 3H**). It was observed that the methylation level of ACP5 was reduced in PC tissues that exhibited lymph node metastasis, in comparison to normal tissues (**Figure 3B**). Further stratification by gender revealed that female patients displayed significantly lower levels of ACP5 methylation compared to the normal group (**Figure 3C**). Additionally, age stratification demonstrated a significant decrease in ACP5 methylation levels among patients aged 20-80 years (**Figure 3D**). When stratified by smoking status, it was observed that patients who smoked exhibited significantly lower levels of ACP5 methylation compared to the normal group (**Figure 3E**). Furthermore, it was found that ACP5 methylation levels were lower in high-grade tumors (**Figure 3F**, **3G**), regardless of the presence of P53 mutations in PC (**Figure 3H**).

In addition, the cBioPortal analysis demonstrated that ACP5 expression was negatively correlated with promoter methylation in PC (**Figure 3I**), thus, indicating that the decline in promoter methylation was responsible for the high ACP5 expression in PC.

ACP5-related enrichment analysis

GO annotations and KEGG pathway of ACP5 co-regulated genes were analyzed by DAVID. The GO annotations results revealed that the co-expressed genes were mainly located on various membranes, could bind to proteins and regulate receptor activity, and were involved in the inflammatory response, antigen presentation, immune response, and receptor signal transduction (**Figure 4A-C**). Meanwhile, the KEGG pathway analysis revealed that the co-regulated genes were enriched in rheumatoid arthritis, hematopoietic lineage, osteoclast differentiation, and cell adhesion molecules (**Figure 4D**).

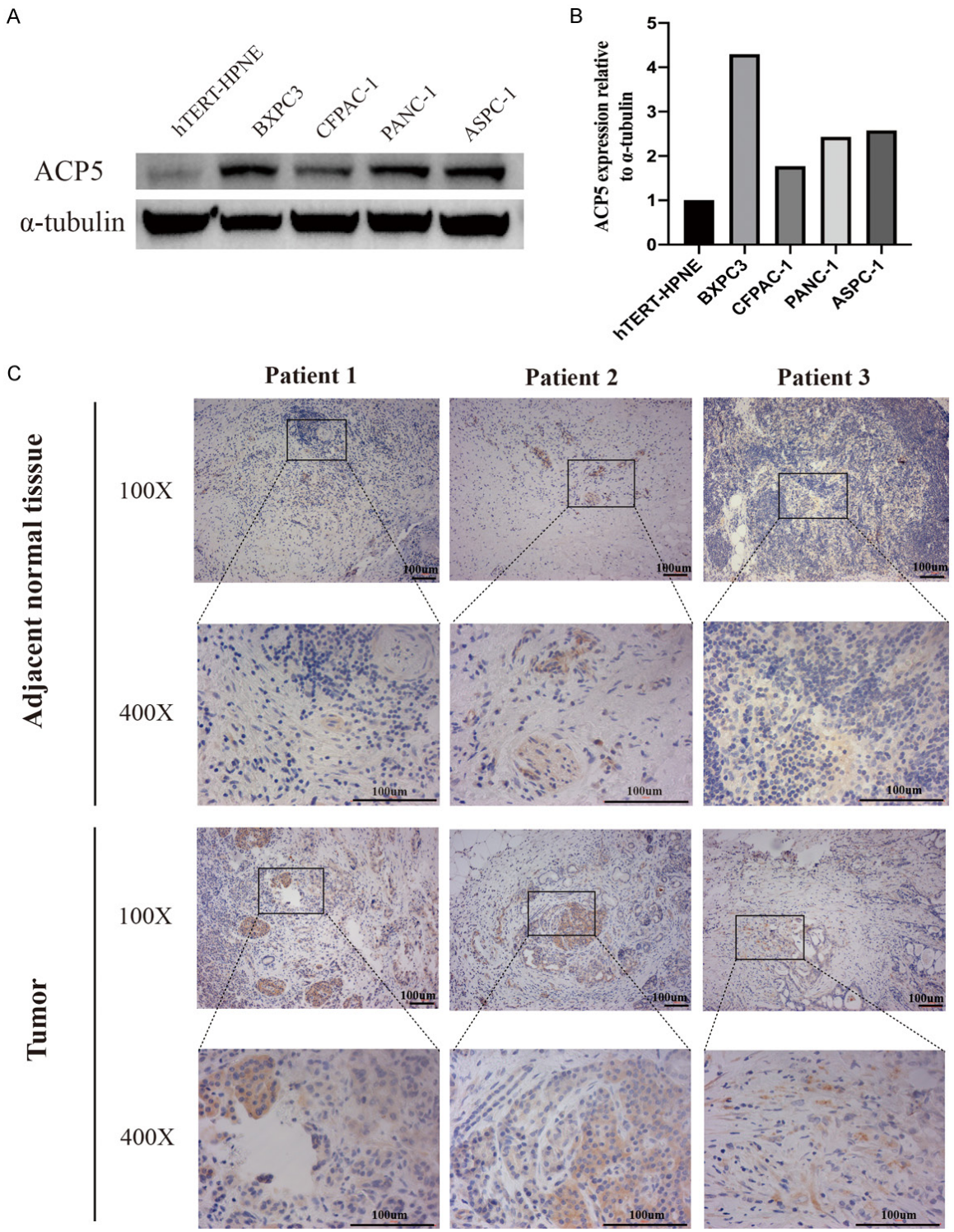


Figure 2. The ACP5 level is elevated in the cells and tissues of pancreatic cancer (PC). A, B. Western blotting of ACP5 in PC cells; C. Immunohistochemistry (IHC) analysis of ACP5 in the tissues of three PC patients.

The specific ACP5 function in PC was investigated using GSEA. Several signaling pathways associated with proliferation and migration were enriched in patient samples with high ACP5 expression (**Figure 4E-J**), such as calcium signaling pathway, cell cycle, JAK-STAT signal-

Table 1. Clinicopathologic characteristics in relation to ACP5 expression status in TCGA cohort

Characteristic	Group	Low expression	High expression	*P*
Age	≤ 65 (n=93)	51	42	0.230
	> 65 (n=85)	38	47	
Gender	Female (n=80)	38	42	0.651
	Male (n=98)	51	47	
T stage	T1 (n=7)	7	0	0.025
	T2 (n=24)	13	11	
	T3 (n=142)	67	75	
	T4 (n=3)	1	2	
Pathologic stage	Stage I (n=21)	16	5	0.013
	Stage II (n=146)	66	80	
	Stage III (n=3)	1	2	
	Stage IV (n=5)	4	1	
Histologic grade	G1 (n=31)	20	11	0.209
	G2 (n=95)	42	53	
	G3 (n=48)	25	23	
	G4 (n=2)	1	1	
Anatomic neoplasm subdivision	Head of Pancreas (n=138)	63	75	0.048
	Other (n=40)	26	14	
History of chronic pancreatitis	No (n=128)	63	65	1.000
	Yes (n=13)	6	7	

ing pathway, insulin signaling pathway, MAPK, and WNT signaling pathways.

Roles of ACP5 in PC progression

Based on the bioinformatic analysis, ACP5 was enriched in the proliferation and migration pathways. The role of ACP5 in PC proliferation and migration was validated by transfecting the plasmids into PANC-1 cells to knock out ACP5. The resulting PANC-1 cells transfected with control plasmid were referred to as sgVector cells, and those transfected with sgACP5 plasmid were sgACP5 cells. Puromycin screening was conducted to identify stable PANC-1 cells with ACP5 knockout. Resultantly, the ACP5 protein expression was significantly reduced in sgACP5 cells compared to sgVector cells (**Figure 5A**, **5B**). The sgVector and sgACP5 cells were utilized in the following experiments.

SgVector and sgACP5 cells were detected using CCK8 to reflect their proliferation rates. The proliferation rate of sgACP5 cells was significantly lower than sgVector cells (**Figure 5C**). Meanwhile, sgACP5 cells formed smaller and fewer colonies than sgVector cells (**Figure 5D**, **5E**). Thus, the findings indicated that ACP5 may be involved in the proliferation and independent viability of PC cells.

The TNMplot illustrated that ACP5 was highly expressed in metastatic PC compared to orthotopic PC (**Figure 5F**). In addition, a wound-healing assay was performed to evaluate the role of ACP5 in PC migration. The sgACP5 cells healed slowly compared to sgVector cells (**Figure 5G**, **5H**), indicating that ACP5 could promote the proliferation and migration of PC cells.

Association between ACP5 and immune microenvironment in PC

The role of ACP5 in the immune system and participation in immune disease progression has been previously reported [11]. In this study, the GO annotation demonstrated a strong correlation between ACP5 and immune function in PC (**Figure 4A** and **4C**). Meanwhile, the KEGG and GSEA indicated ACP5 involvement in several immune-related pathways (**Figures 4D** and **6A**). This study also evaluated a differential infiltration of immune subpopulation cells between the high and low ACP5 expression groups in TCGA-PAAD samples to assess the association between ACP5 and

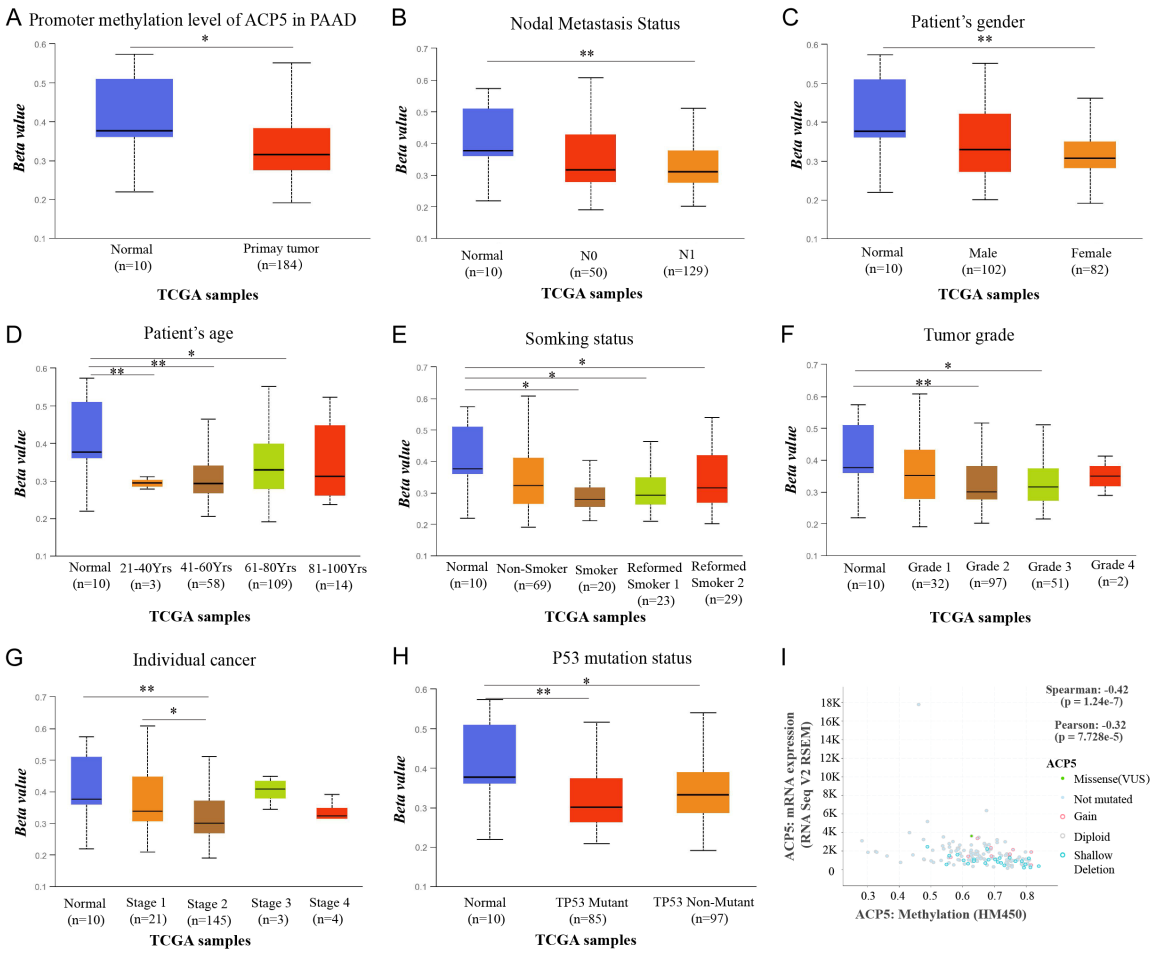


Figure 3. The expression of ACP5 and promoter methylation are negatively correlated. (A) University of Alabama at Birmingham Cancer data analysis Portal (UALCAN) analysis of ACP5 promoter methylation in The Cancer Genome Atlas-pancreatic adenocarcinoma (TCGA-PAAD); analysis of ACP5 methylation level according to (B) node metastasis, (C) gender, (D) age, (E) smoking, (F) tumor grade, (G) cancer stages, (H) P53 mutation, (I) The relation between ACP5 expression and promoter methylation in TCGA-PAAD (***P < 0.001, **P < 0.01 and *P < 0.05).

immune microenvironment in PC (**Figure 6B**). Furthermore, the TIMER analysis revealed that B cell, CD8 T cell, CD4 T cell, macrophage, neutrophil, and dendritic cell infiltration in PC increased with ACP5 upregulation, thus reducing the tumor purity (**Figure 6C**).

Further evaluation of the relationship between ACP5 expression and stromal, immune, and estimate scores indicated a positive correlation between ACP5 expression and increased non-neoplastic cells in PC (**Figure 6D**). In addition, the correlation between ACP5 and immune subpopulation cells was evaluated by ssGSEA. The results revealed that immune subpopulation cells were significantly correlated with ACP5 expression (**Figure 6E**). Precisely, the positive correlation between ACP5 and Treg cells was extremely high. These cells resist immunotherapy, possibly contributing to the immunosuppressive microenvironment in PC. Therefore, ACP5 may be a valuable target for future PC immunotherapy.

Discussion

Societal development, changes in eating habits, poor quality lifestyle, and increased work pressure have increased the morbidity of PC [22]. The pathogenesis of PC remains unclear, while genetic factors, immune status, and external mutagens have been identified as essential components of this disease [23, 24]. Currently, surgery and chemotherapy are the

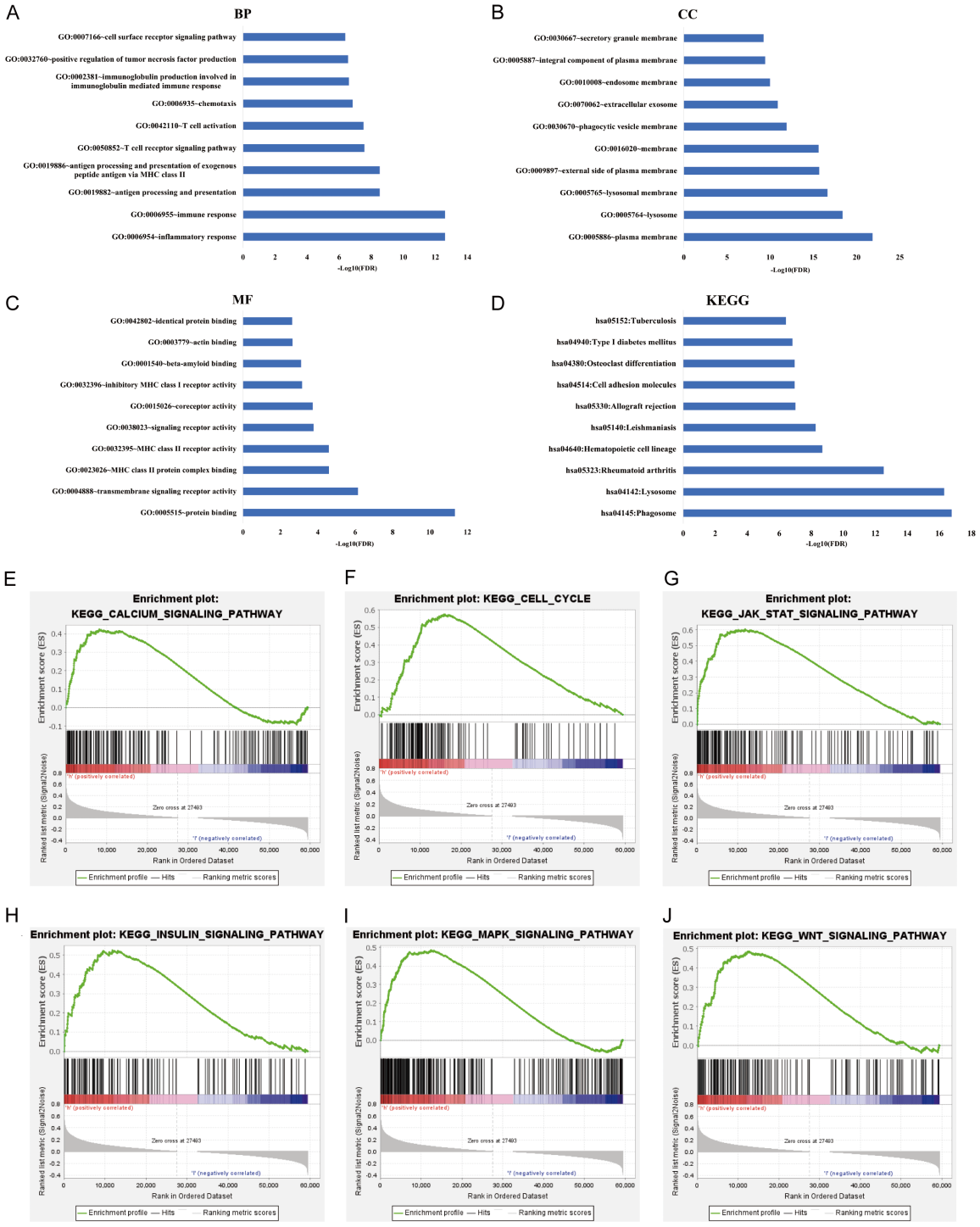


Figure 4. The enrichment of ACP5 in pancreatic cancer (PC). (A) Biological process (BP), (B) Cellular component (CC), (C) Molecular function (MF), and (D) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of ACP5 in PC, the enrichment of (E) calcium signaling pathway, (F) cell cycle, (G) JAK-STAT signaling pathway, (H) insulin signaling pathway, (I) MAPK signaling pathway and (J) WNT signaling pathway in high ACP5 group.

primary clinical treatments for PC [25]. Precisely, surgery is the first-line treatment for this disease, with chemotherapy as an adjunct [26]. This study is the first to demonstrate the link between ACP5 and PC development. As an oncogene, ACP5 reportedly participates in sev-

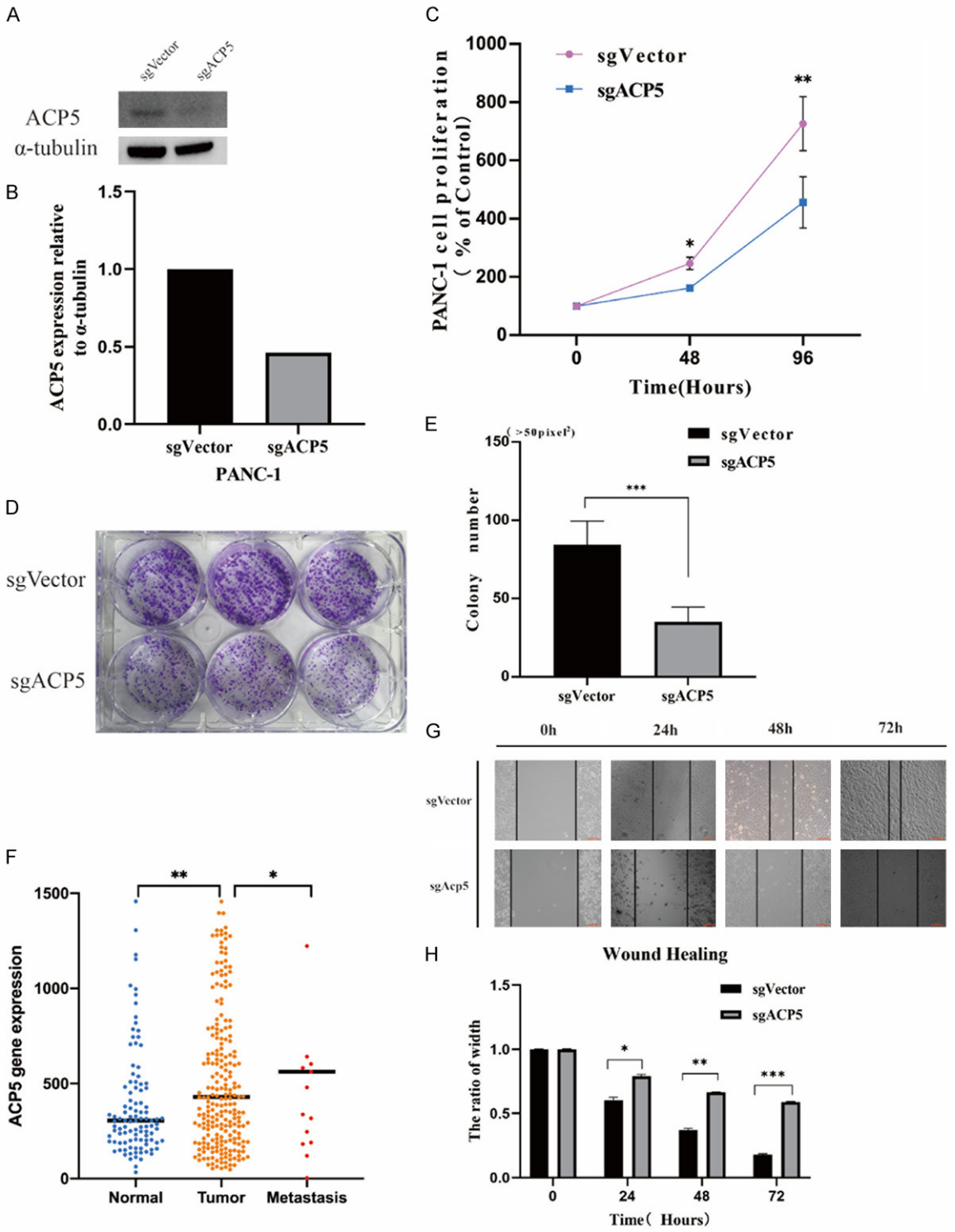
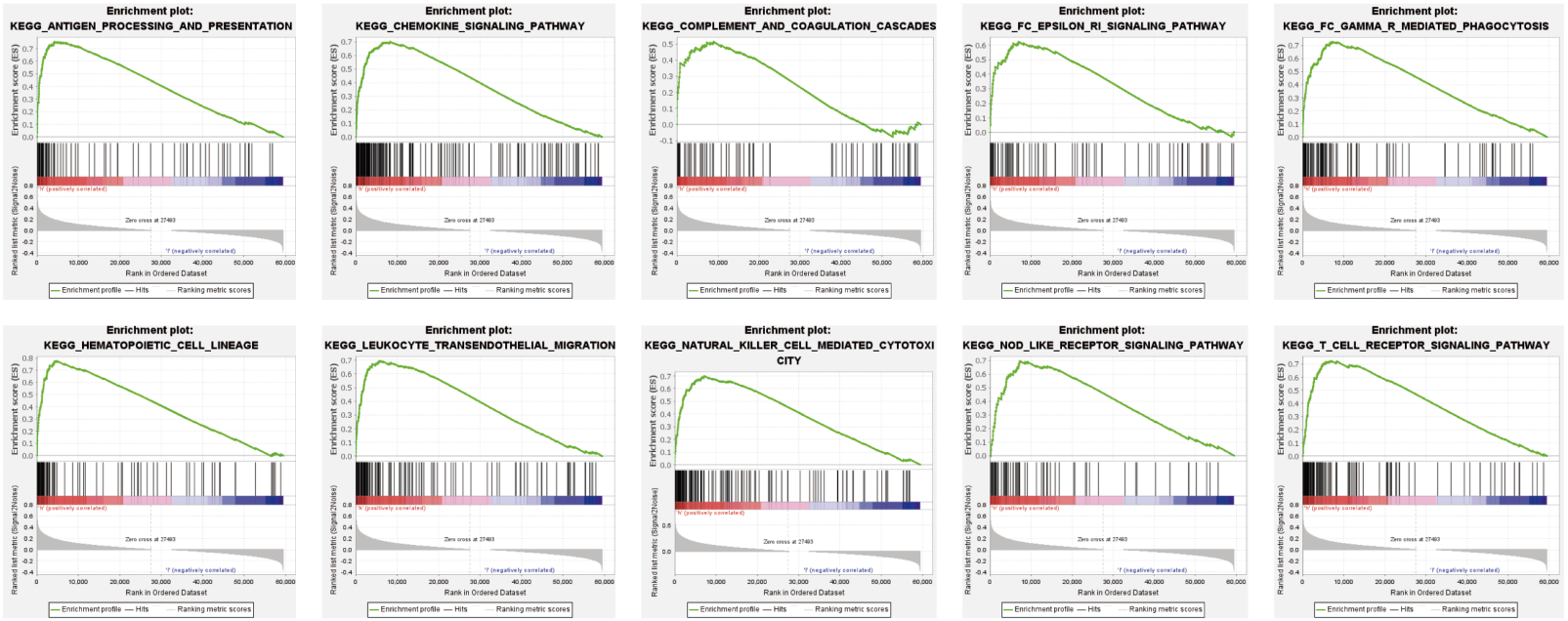
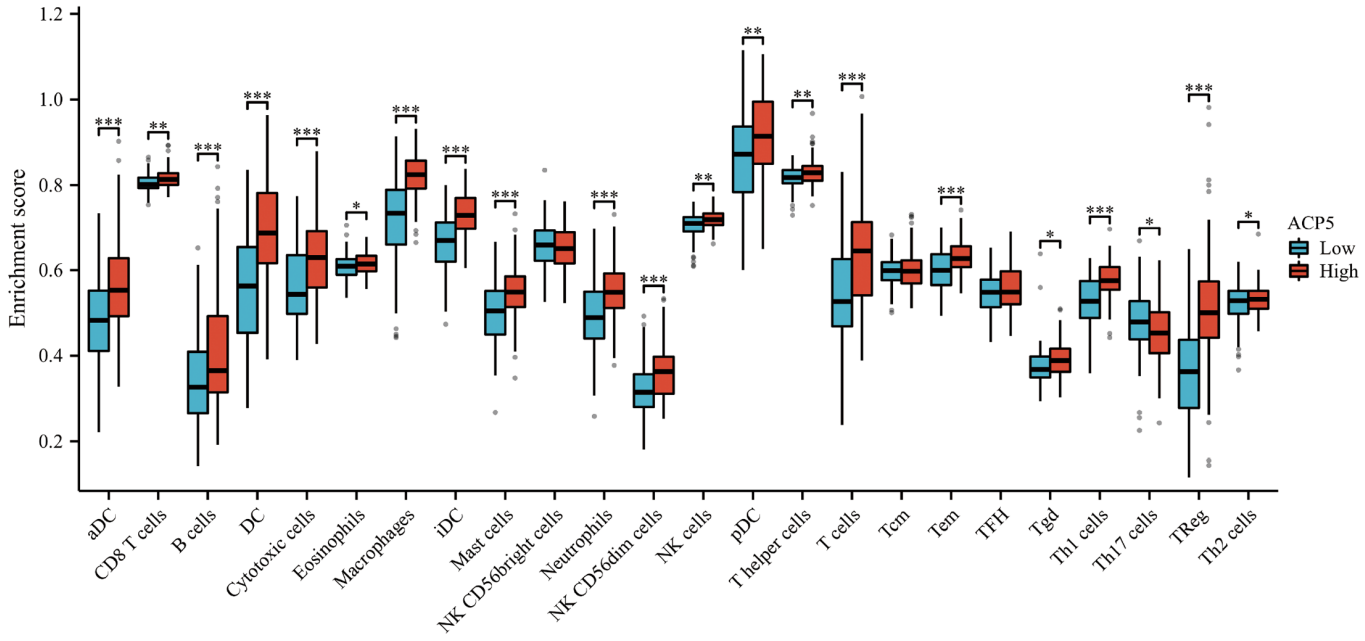


Figure 5. The ACP5 promotes pancreatic cancer (PC) cell proliferation and migration and enhances clone formation. (A, B) Western blotting of the knockout of ACP5 in PANC-1 cell, (C) Proliferation of sgVector and sgACP5 cells performed by Cell Counting Kit-8 (CCK8) (t test, $P < 0.05$), (D) Clone formation of sgVector and sgACP5 cells performed in the six-well plate, (E) Relative clone numbers of sgVector and sgACP5 cells (t test, $P < 0.001$), (F) The differential expression of ACP5 in normal, orthotopic, and metastatic tumor samples, (G) Wound images of sgVector and sgACP5 cells at 0, 24, 48, and 72 hours. The wound in the middle of the vertical lines: (H) Relative wound conditions at 0, 24, 48, and 72 hours, calculated by wound area (t test, $P < 0.05$) (***$P < 0.001$, *$P < 0.01$ and *$P < 0.05$).

A


Enrichment plot:
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
Enrichment plot:
KEGG_CHEMOKINE_SIGNALING_PATHWAY
Enrichment plot:
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES
Enrichment plot:
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY
Enrichment plot:
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS
Enrichment plot:
KEGG_HEMATOPOIETIC_CELL_LINEAGE
Enrichment plot:
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
Enrichment plot:
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY
Enrichment plot:
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY
Enrichment plot:
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY
Enrichment score (ES)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

B


Enrichment score
ACP5
Low
High
aDC
CD8 T cells
B cells
DC
Cytotoxic cells
Eosinophils
Macrophages
iDC
Mast cells
NK CD56bright cells
Neutrophils
NK CD56dim cells
NK cells
pDC
T helper cells
T cells
Tcm
Tem
TFH
Tgd
Th1 cells
Th17 cells
TReg
Th2 cells

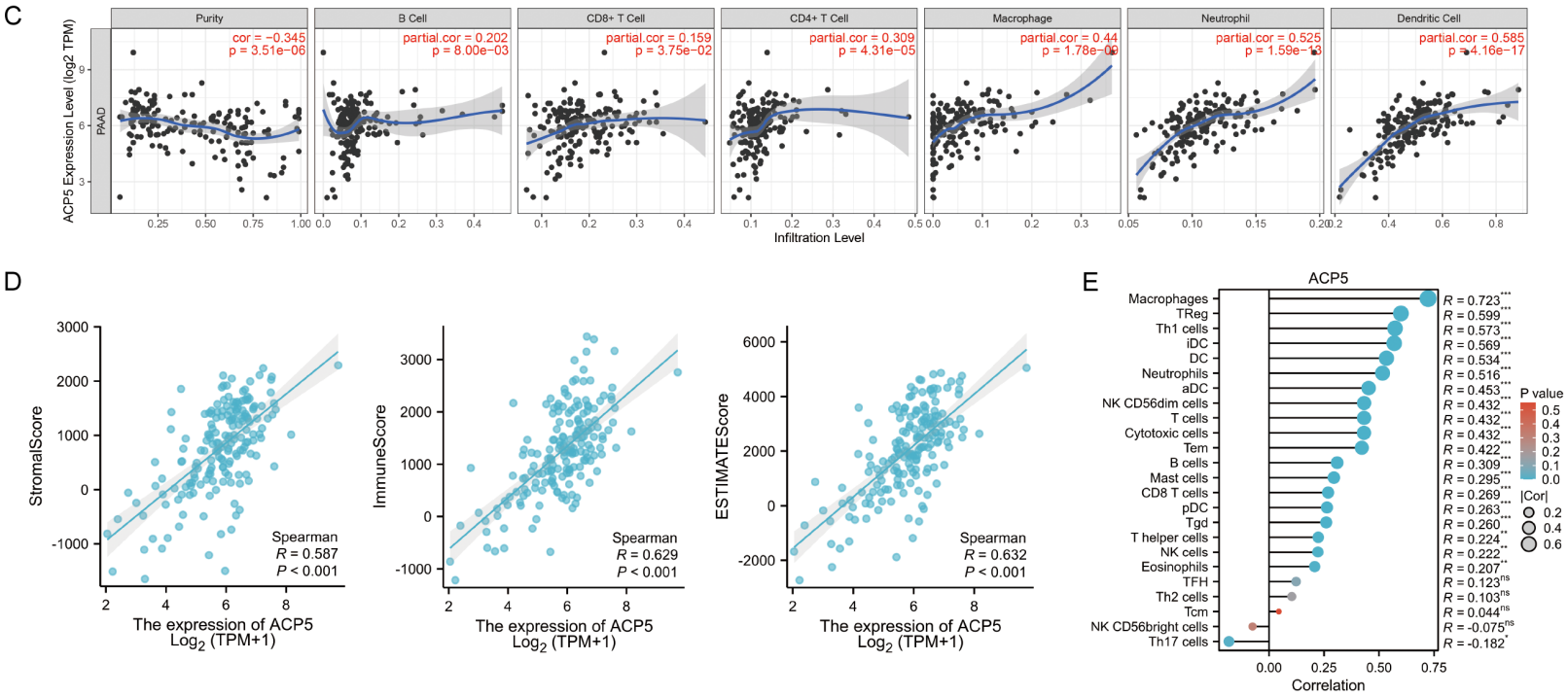


Figure 6. The ACP5 expression positively correlates with non-neoplastic cell infiltration in pancreatic cancer (PC). (A) The enrichment of immune pathways in ACP5 high expression group, (B) The infiltration of immune subpopulation cells between high and low ACP5 groups, (C) The correlation between six specific immune cells and ACP5 in PC, (D) The correlation between ACP5 and stromal, immune and estimate score, (E) ssGSEA analysis to determine the association between ACP5 and immune subpopulation cells (***P < 0.001, **P < 0.01 and *P < 0.05).

eral tumor processes. For instance, the high ACP5 expression in gastric cancer leads to tumor metastasis and peritoneal spread [15]. Meanwhile, high FoxM1 expression promotes hepatocellular carcinoma metastasis and invasion, and ACP5 is a key node in this process [27]. Highly expressed ACP5 also promotes breast cancer proliferation, migration, and invasion [8].

This study confirmed that ACP5 was upregulated in PC and related to different stages, grades, and locations. The TCGA data analysis indicated that the ACP5 expression varied according to the American Joint Committee on Cancer (AJCC) tumor stage (P=0.025), pathologic grade (P=0.013), and anatomic site (P < 0.048). Furthermore, ACP5 could promote PC proliferation and migration, thus, a valuable therapeutic target for PC. A small molecule inhibitor of ACP5, 5-phenylnicotinic acid, is a potential target for PC treatment [28].

PC often has an immunosuppressive tumor microenvironment (TME) with few immune effector cells [29]. The TME also contains numerous stromal cells, including cancer-associated fibroblasts (CAFs) [30]. The CAFs form a suppressive immune microenvironment with immunosuppressive cells [31, 32], which limits immune effector cell infiltration, leading to the failure of PC to respond positively to immunotherapy. Tumor immunotherapy offers the prospect of new therapeutic modalities for treating PC to overcome the suppressive immune microenvironment in PC. The ACP5 expression positively correlated with Treg cell infiltration in PC, which could be reduced by suppressing ACP5 expression. This strategy could improve the suppressive immune microenvironment, possibly enhancing immunotherapy response in PC patients.

In summary, this study identified ACP5 as a vital gene and a possible prognostic marker in PC. The upregulation of ACP5 significantly affected cell proliferation, migration, and other behaviors in PC. In addition, ACP5 is a critical immune regulatory gene in PC. These findings offer insight for developing future PC treatments.

Acknowledgements

We thank Home for Researchers editorial team (https://www.home-for-researchers.com) for language editing service and Xiantao Academic for providing R package. This research is supported by Postgraduate Research & Practice Innovation Program of Jiangsu Province, KYCX20_2801.

Written informed consent was obtained from each participant.

Disclosure of conflict of interest

None.

Address correspondence to: Guoxiong Zhou, Department of Gastroenterology, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu, China. E-mail: zhougx@ntu.edu.cn

References

[1] Hidalgo M, Cascinu S, Kleeff J, Labianca R, Lohr JM, Neoptolemos J, Real FX, Van Laethem JL and Heinemann V. Addressing the challenges of pancreatic cancer: future directions for improving outcomes. Pancreatology 2015; 15: 8-18.

[2] Keleg S, Buchler P, Ludwig R, Buchler MW and Friess H. Invasion and metastasis in pancreatic cancer. Mol Cancer 2003; 2: 14.

[3] Siegel RL, Miller KD, Fuchs HE and Jemal A. Cancer statistics, 2022. CA Cancer J Clin 2022; 72: 7-33.

[4] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[5] Strobel O, Neoptolemos J, Jager D and Buchler MW. Optimizing the outcomes of pancreatic cancer surgery. Nat Rev Clin Oncol 2019; 16: 11-26.

[6] Lamp EC and Drexler HG. Biology of tartrate-resistant acid phosphatase. Leuk Lymphoma 2000; 39: 477-484.

[7] Leach RJ, Reus BE, Hundley JE, Johnson-Pais TL and Windle JJ. Confirmation of the assignment of the human tartrate-resistant acid phosphatase gene (ACP5) to chromosome 19. Genomics 1994; 19: 180-181.

[8] Reithmeier A, Panizza E, Krumpel M, Orre LM, Branca RMM, Lehtio J, Ek-Rylander B and Andersson G. Tartrate-resistant acid phosphatase (TRAP/ACP5) promotes metastasis-related properties via TGFbeta2/TbetaR and CD44 in MDA-MB-231 breast cancer cells. BMC Cancer 2017; 17: 650.

[9] Ren X, Shan WH, Wei LL, Gong CC and Pei DS. ACP5: its structure, distribution, regulation and novel functions. Anticancer Agents Med Chem 2018; 18: 1082-1090.

[10] Minkin C. Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif Tissue Int 1982; 34: 285-290.
[11] An J, Briggs TA, Dumax-Vorzet A, Alarcon-Riquelme ME, Belot A, Beresford M, Bruce IN, Carvalho C, Chaperot L, Frostegard J, Plumas J, Rice GI, Vyse TJ, Wiedeman A, Crow YJ and Elkon KB. Tartrate-resistant acid phosphatase deficiency in the predisposition to systemic lupus erythematosus. Arthritis Rheumatol 2017; 69: 131-142.
[12] Esfandiari E, Bailey M, Stokes CR, Cox TM, Evans MJ and Hayman AR. TRACP influences Th1 pathways by affecting dendritic cell function. J Bone Miner Res 2006; 21: 1367-1376.
[13] Sacri AS, Bruwier A, Baujat G, Breton S, Blanche S, Briggs TA and Bader-Meunier B. Childhood-onset autoimmune cytopenia as the presenting feature of biallelic ACP5 mutations. Pediatr Blood Cancer 2017; 64: 306-310.
[14] Bian ZQ, Luo Y, Guo F, Huang YZ, Zhong M and Cao H. Overexpressed ACP5 has prognostic value in colorectal cancer and promotes cell proliferation and tumorigenesis via FAK/PI3K/AKT signaling pathway. Am J Cancer Res 2019; 9: 22-35.
[15] Kawamura M, Tanaka K, Toiyama Y, Okugawa Y, Okigami M, Yasuda H, Saigusa S, Ohi M, Inoue Y, Uchida K, Mohri Y and Kusunoki M. Clinical significance of tartrate-resistant acid phosphatase type-5 expression in human gastric cancer. Anticancer Res 2014; 34: 3425-3429.
[16] Scott KL, Nogueira C, Heffernan TP, van Doorn R, Dhakal S, Hanna JA, Min C, Jaskelioff M, Xiao Y, Wu CJ, Cameron LA, Perry SR, Zeid R, Feinberg T, Kim M, Vande Woude G, Granter SR, Bosenberg M, Chu GC, DePinho RA, Rimm DL and Chin L. Proinvasion metastasis drivers in early-stage melanoma are oncogenes. Cancer Cell 2011; 20: 92-103.
[17] Xia L, Huang W, Tian D, Chen Z, Zhang L, Li Y, Hu H, Liu J, Chen Z, Tang G, Dou J, Sha S, Xu B, Liu C, Ma J, Zhang S, Li M, Fan D, Nie Y and Wu K. ACP5, a direct transcriptional target of FoxM1, promotes tumor metastasis and indicates poor prognosis in hepatocellular carcinoma. Oncogene 2014; 33: 1395-1406.
[18] Chao TY, Wu YY and Janckila AJ. Tartrate-resistant acid phosphatase isoform 5b (TRACP 5b) as a serum maker for cancer with bone metastasis. Clin Chim Acta 2010; 411: 1553-1564.
[19] Janckila AJ, Nakasato YR, Neustadt DH and Yam LT. Disease-specific expression of tartrate-resistant acid phosphatase isoforms. J Bone Miner Res 2003; 18: 1916-1919.
[20] Lakshminarasimhan R and Liang G. The role of DNA methylation in cancer. Adv Exp Med Biol 2016; 945: 151-172.
[21] Li J, Huang Q, Zeng F, Li W, He Z, Chen W, Zhu W and Zhang B. The prognostic value of global DNA hypomethylation in cancer: a meta-analysis. PLoS One 2014; 9: e106290.
[22] Zanini S, Renzi S, Limongi AR, Bellavite P, Giovinazzo F and Bermano G. A review of lifestyle and environment risk factors for pancreatic cancer. Eur J Cancer 2021; 145: 53-70.
[23] Ilic M and Ilic I. Epidemiology of pancreatic cancer. World J Gastroenterol 2016; 22: 9694-9705.
[24] Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol 2021; 18: 493-502.
[25] Jiang Y and Sohal DPS. Pancreatic adenocarcinoma management. JCO Oncol Pract 2023; 19: 19-32.
[26] Ansari D, Tingstedt B, Andersson B, Holmquist F, Sturesson C, Williamsson C, Sasor A, Borg D, Bauden M and Andersson R. Pancreatic cancer: yesterday, today and tomorrow. Future Oncol 2016; 12: 1929-1946.
[27] Orita T, Akimura T, Nishizaki T, Kamiryo T, Ikeyama Y, Aoki H and Ito H. Transferrin receptors in injured brain. Acta Neuropathol 1990; 79: 686-688.
[28] Krumpel M, Reithmeier A, Senge T, Baeumler TA, Frank M, Nyholm PG, Ek-Rylander B and Andersson G. The small chemical enzyme inhibitor 5-phenylnicotinic acid/CD13 inhibits cell migration and invasion of tartrate-resistant acid phosphatase/ACP5-overexpressing MDA-MB-231 breast cancer cells. Exp Cell Res 2015; 339: 154-162.
[29] Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, Coussens LM, Gabrilovich DI, Ostrand-Rosenberg S, Hedrick CC, Vonderheide RH, Pittet MJ, Jain RK, Zou W, Howcroft TK, Woodhouse EC, Weinberg RA and Krummel MF. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med 2018; 24: 541-550.
[30] Ho WJ, Jaffee EM and Zheng L. The tumour microenvironment in pancreatic cancer - clinical challenges and opportunities. Nat Rev Clin Oncol 2020; 17: 527-540.
[31] Feig C, Gopinathan A, Neesse A, Chan DS, Cook N and Tuveson DA. The pancreas cancer microenvironment. Clin Cancer Res 2012; 18: 4266-4276.
[32] Pothula SP, Xu Z, Goldstein D, Pirola RC, Wilson JS and Apte MV. Key role of pancreatic stellate cells in pancreatic cancer. Cancer Lett 2016; 381: 194-200.