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Research article

Identification of PSMD2 as a promising biomarker for pancreatic cancer patients based on comprehensive bioinformatics and *in vitro* studies

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ABSTRACT

Background: Pancreatic cancer patients have limited treatment options and extremely poor prognosis. Dysregulations of proteasome 26S subunit, non-ATPases (PSMDs) contribute to the development of various cancers, whereas the significance of PSMDs in pancreatic cancer is poorly understood. In the present study, we intended to explore the therapeutic potential of PSMDs in pancreatic cancer.

Methods: Based on TCGA database, the expression of PSMDs was analyzed in pancreatic cancer patients. Multivariate Cox regression and Kaplan–Meier survival analyses were conducted to investigate the prognostic value of PSMDs. The correlations between the expression of PSMD2/14 and immune cell infiltration, immune checkpoint genes' expression, enrichment of signaling pathways, and the sensitivity of chemotherapies were also evaluated. Knockdown and overexpression experiments were performed to explore the biological function of PSMD2/14. Immunoblotting was conducted to detect the downstream signaling pathway of PSMD2.

Results: Most of the PSMDs, except for PSMD5 and PSMD6, were significantly upregulated in pancreatic cancer tissues. Patients with higher grade tumor had increased mRNA levels of PSMD1/2/5/7/8/11/12/14. Survival and multivariate Cox regression analyses indicated that PSMD2 and PSMD14 were biomarkers of worse prognosis. High expression of PSMD2 and PSMD14 was positively correlated with the levels immune checkpoint genes but not with the infiltration of specific immune cell types. *In vitro* knockdown of PSMD2, but not PSMD14, increased the apoptosis, gemcitabine's toxicity and inhibited the growth capacity of MIA cells. Conversely, decreased apoptosis and gemcitabine sensitivity along with accelerated cell proliferation ability were observed in PSMD2-overexpressing PANC-1 cells. Mechanistically, PSMD2 activated the AKT/mTOR signaling pathway, consistent with the findings from KEGG and GSEA analysis. The AKT specific inhibitor MK-2206 exhibited higher cytotoxicity in MIA and PANC-1 cells with high PSMD2 expression. Importantly, MK-2206 largely reversed the oncogenic functions of PSMD2 on the growth and proliferation of PANC-1 cells.

Conclusion: In summary, our study provided a comprehensive bioinformatics analysis of PSMDs in pancreatic cancer. We identified that PSMD2 acted as a tumor-promoting protein in pancreatic

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cancer through the activation of the AKT/mTOR pathway. The overexpression of PSMD2 may be a potential biomarker that predicts the response of pancreatic cancer patients to AKT inhibitor treatments.

1. Background

Pancreatic ductal adenocarcinoma (PDAC) is the dominant type of primary pancreatic cancers, accounting for approximately 95 % of all cases [1]. The annual number of newly diagnosed PDAC patients has nearly tripled during the past three decades, with 196,000 diagnosed cases in 1990 and 495,773 new cases in 2020 [2,3]. Pancreatic cancer is a relatively uncommon malignancy; however, its high mortality, due to challenging diagnosis and therapy, makes it one of the deadliest cancers [4]. Despite great efforts have been performed to understand the risk factors, the overall five-year survival rate of pancreatic cancer remains almost less than 10 % [5]. Comprehensive analyses of the driver genes for pancreatic cancer are needed for us to develop potential biomarker or therapeutic targets for this deadly disease.

Ubiquitination and de-ubiquitination of the proteins are controlled by ubiquitin–proteasome system (UPS) in mammals. Dysregulation of UPS causes the degradation or stabilization of substrates, playing an important role in cellular proliferation, growth, survival, metabolism, inflammation and cancer development [6]. The most well-studied UPS factors include the members of ubiquitin-specific peptidase [7], OTU deubiquitinase [8], and E3 ubiquitin ligase families [9]. Their functions in carcinogenesis have been widely reported [9–12].

Proteasome 26S subunit, non-ATPase (PSMD) family members also play essential role in regulating proteins' ubiquitination and stability. Recent evidence has shown that abnormal expression of PSMDs promotes the growth and invasion of cancer cells by repressing the degradation of tumor suppressors or oncogenic proteins. For example, PSMD1 high expression confers the poor survival of oropharyngeal squamous cell carcinoma patients [13]. In lung cancer, overexpression of PSMD1 facilitates cancer cell growth through stabilization of PTEN-induced kinase 1 (PINK1). Besides PSMD1, the involvement of other PSMDs was investigated in numbers of cancer types. Overexpression of PSMD2 is closely correlated with tumor immune microenvironment (TIME) and tumor progression of bladder cancer [14]. PSMD3 acts as an oncogene in chronic myeloid leukemia via activating nuclear factor-kappa B signaling [15]. Upregulation of PSMD3 contributes to the progression of breast cancer via increasing the expression of HER2 [16]. PSMD4 triggers the development of HCC by modulating Akt/GSK-3β/cyclooxygenase2 (COX2) and P53 pathway [17]. PSMD7 potentiates the malignant progression and chemotherapy resistance in gastric cancer by stabilizing RAD23B protein [18]. PSMD7 also strengthens the stability of RAB1A to promote the development of bladder cancer [19]. Although numerous evidence has addressed the importance of PSMDs in the development of solid tumors, the significance PSMDs in pancreatic cancer remains to be determined.

Herein, we comprehensively analyzed the involvement of PSMDs in pancreatic cancer, focusing on their expression, prognostic value, and their correlation with TIME, chemotherapy sensitivity and pathway enrichment. We identified that PSMD2 acts as a tumor-promoting factor in pancreatic cancer by activating AKT/mTOR signaling pathway.

2. Methods

2.1. Bioinformatics analysis

The expression levels of PSMDs in the tumor tissues of pancreatic cancer patients and in the normal pancreatic tissues were analyzed from GEPIA database (http://gepia.cancer-pku.cn/index.html), an interactive web server to analyze the RNA sequencing expression data from the TCGA and the GTEx projects [20]. The expression levels of PSMDs in the tumor tissues of PAAD patients with different grades were analyzed from UALCAN (https://ualcan.path.uab.edu/index.html) [21]. The clinicopathologic prognostic information was downloaded from the literature [22].

We screened target genes using the following criteria: 1) significant differential expression in tumor tissues; 2) significant differential expression in tumor tissues of different grades; 3) significance in the Log-rank test; 4) significance in multivariate Cox regression analysis.

The transcriptome sequencing data of pancreatic cancer was downloaded from the TCGA website (https://portal.gdc.cancer.gov/). Clinical data were obtained from the literature by Liu et al. [22]. Based on the database, we conducted multivariate Cox regression analysis of PSMDs and the patients' clinicopathological index.

CIBERSORT immunoinfiltration analysis tool was used to analyze the selected PSMD genes. Differences of immune cell infiltration between high and low expression groups were mapped using R software. We also analyzed the expression differences of immune checkpoint genes (ICB) in the high and low expression groups.

We performed single-gene correlation analyses of PSMD2 and PSMD14, selecting the 1000 genes most positively correlated with PSMD2 and PSMD14 expression, respectively. Based on these 1000 genes, we performed GO and KEGG enrichment analyses using R software and HALLMARK GSEA enrichment analysis using GSEA 4.0.3 software [23].

2.2. Cell culture

Pancreatic cancer cell lines MIA PaCa-2 (MIA), SW1990 and PANC-1 were obtained from Procell (Wuhan, Hubei, China). MIA and

PANC-1 cells were cultured in Dulbecco modified Eagle's medium (Hyclone, Logan, UT, USA), and SW1990 cells were maintained in Leibovitz's L-15 (Procell). Both media contained 10 % fetal bovine serum (Gibco, Grand Island, NY, USA) and 1 % penicillin/streptomycin (P/S, Procell). An additional 2.5 % horse serum (Procell) was added to the culture medium of MIA cells. Both MIA and PANC-1 cells were maintained in a 37°C cell incubator with 5 % CO_2.

2.3. PSMD2 and PSMD14 knockdown

PSMD2 and PSMD14 were silenced using siRNAs targeting a negative control or PSMD2/14. RNAiMAX (Invitrogen, Carlsbad, CA, USA) was applied for the transfection of siRNAs. The siRNAs are listed as follows: siCtrl, 5′-UUCUCCGAACGUGUCACGU-3'; siPSMD2#1, 5′-GGUGUGUUCCGAAAGUUUA-3'; siPSMD2#2, 5′-GGAUGUGGUAGUACAGAAA-3'; siPSMD14#1, 5′-AUCCAGUGUUCCAAGCUAA-3'; siPSMD14#2, 5′-GAUCCAGUGUUCCAAGCUA-3'.

2.4. Overexpression of PSMD2

The overexpression lentivirus of PSMD2 was packaged by transfecting the 293FT cells with pCDH vectors containing the coding sequence of PSMD2, as well as the packaging vectors psPAX2 and pMD2.G. Seventy-two hours later, lentivirus was harvested from the culture supernatant and centrifuged at 80,000×*g* for 2–3 h. PANC-1 cells were then infected with the lentivirus. Polybrene was added to promote the infection efficiency.

2.5. Western blotting

RIPA (Beyotime, Shanghai, China) was used to lyse protein samples from the pancreatic cancer cells. After quantifying the protein concentration, 40–70 μg of proteins were run on SDS-PAGE gels. After 2 h of separation, the proteins were transferred onto the PVDF membranes. The membranes were then incubated with 5 % skim milk followed by incubation with the antibodies. The information of the antibodies is listed as below: PSMD2 (1:1000, cat no. 14748-1-AP, Proteintech, Chicago, IL, USA), p-AKT (1:2000, cat no. 4060, Cell Signaling, Danvers, MA, USA), AKT (1:2000, cat no. 2938, Cell Signaling), p-S6 (1:2000, cat no. 2211, Cell Signaling), S6 (1:2000, cat no. 2217, Cell Signaling) and β-actin (1:5000, cat no. 81115-1-RR, Proteintech), and secondary antibodies (1:10000, cat no. SA00001-2 Proteintech). Finally, the abundance of the proteins was examined using SuperSignal West Pico PLUS (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Reverse-transcription quantitative real-time PCR (RT-qPCR)

Total RNA was isolated using Trizol (cat no. 15596026CN, Invitrogen). Reverse transcription of the RNA was conducted by M-MLV reverse transcriptase (cat no. M1701, Promega, Madison, WI, USA). The qPCR assay was performed on a Bio-Rad system with qPCR mix (cat no. G3325-01, Servicebio Biotech, Wuhan, Hubei, China). PSMD2-F, 5′-ATCTGGCAGGAGAAGTGGCTA-3′, and PSMD2-R, 5′-GGGCCATGTTATAGGGGACG-3'. PSMD14-F, 5′-GATGGTTGTTGGTTGGTATCACA-3′, and PSMD14-R, 5′-GATGGTTGTTGGTTGGTATCACA-3'. PD-L1-F, 5′-TGGCATTTGCTGAACGCATTT-3′, and PD-L1-R, 5′-TGCAGCCAGGTCTAATTGTTTT-3'. PD-L2-F, 5′-ACCCTGGAATGCAACTTTGAC-3′, and PD-L2-R, 5′-ACCCTGGAATGCAACTTTGAC-3'. IDO1-F, 5′-GCCAGCTTCGAGAAAGAGTTG-3′, and IDO1-R, 5′-ATCCCAGAACTAGACGTGCAA-3'. GAPDH-F 5′-TGACTTCAACAGCGACACCCA-3′, and GAPDH-R, 5′-CACCCTGTTGCTGTAGCCAAA-3'.

2.7. Cell counting kit-8

A total of 3000 MIA or PANC-1 cells with knockdown or overexpression of PSMD2/14 were seeded in triplicate into each well of 96-well plates. Cell viability was measured by adding 10 μL of Cell Counting Kit-8 (cat no. C0039, CCK-8, Beyotime) into each well, followed by incubation at 37°C in a cell incubator for 3 h, and measuring the OD value at 450 nm.

2.8. Colony formation

A total of 1000 siCtrl, siPSMD2#1, and siPSMD2#2 MIA cells, and a total of 500 control and PSMD2 overexpressing PANC-1 cells were seeded into 6-well plates, supplied with 3 mL of complete culture medium. Five to seven days later, the colonies were stained with crystal violet.

2.9. Apoptosis

Apoptosis was examined using the PI + Annexin V staining kit. After trypsinizing the cells with EDTA-free trypsin and staining the cells with PI and annexin V reagent (cat no. C1062L, Beyotime), apoptosis was measured using a flow cytometer system (Beckman, Brea, CA, USA).

2.10. Statistical analysis

The experiments were repeated at least three times. Statistical analysis of the experimental data (mean ± standard deviation) was conducted using GraphPad prism 8.0 software. Differences between two groups were analyzed using Student's t-tests. One-way ANOVA followed by a Tukey's post hoc test was applied to analyze the difference among more than two groups. $p < 0.05$ indicated a significant difference.

3. Results

3.1. Transcript abundance of PSMDs in pancreatic cancer

To investigate the clinical relevance of PSMDs, we analyzed the transcript abundance of PSMD1-14 in pancreatic cancer patients. According to the database (cancer tissues, n = 179; normal tissues, n = 171), the transcript levels of PSMD1/2/3/4/7/8/9/10/11/12/13/14 were significantly enhanced, while PSMD6 mRNA levels were reduced in pancreatic cancer compared to normal samples (Fig. 1A). Then the transcript levels of PSMDs were analyzed in pancreatic cancer patients with different grades based on the UALCAN database. We found that PSMD1/2/5/7/8/11/12/14 were significantly upregulated in the patients of grades 2 and 3 as compared with those of grade 1 (Fig. 1B).

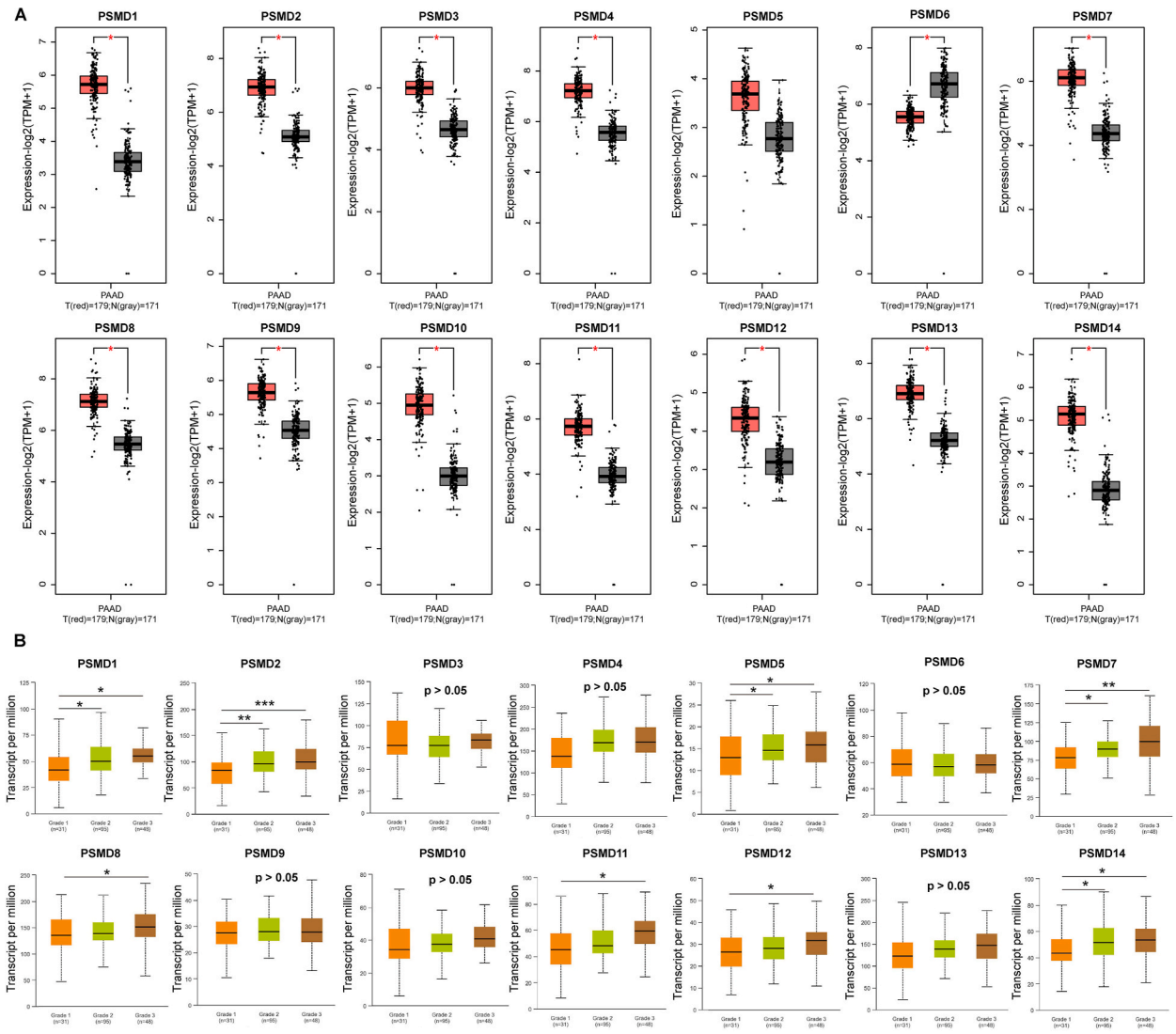

Fig. 1. mRNA abundance of PSMDs in pancreatic cancer patients. (A) The mRNA levels of PSMD1-14 in pancreatic cancer and normal samples from GEPIA2. PAAD, pancreatic adenocarcinoma; T, tumor samples; N, normal samples. (B) The mRNA levels of PSMD1-14 in pancreatic cancer patients with different grades from UALCAN. *$p < 0.05$. **$p < 0.01$.

3.2. Prognostic significance of PSMDs in pancreatic cancer patients

Next, we analyzed the prognostic significance of PSMDs in pancreatic cancer patients using the GEPIA database. Overall and disease-free survival were analyzed in patients with high and low expression of PSMD1-14. The results showed that the transcript abundance of PSMD1/3/4/5/8/9/10 was not associated with the survival of pancreatic cancer patients (Fig. 2A). Although high expression of PSMD6 was inversely correlated with patient survival, its mRNA expression was reduced in cancer tissues (Fig. 1A). High expression of PSMD2/7/11/13/14 predicted poorer disease-free survival in patients (Fig. 2A). To further explore the prognostic significance of PSMDs, multivariate Cox regression analysis was conducted. PSMD2, PSMD13, and PSMD14 were identified as the independent prognostic biomarkers, while other PSMDs were not (Fig. 2B).

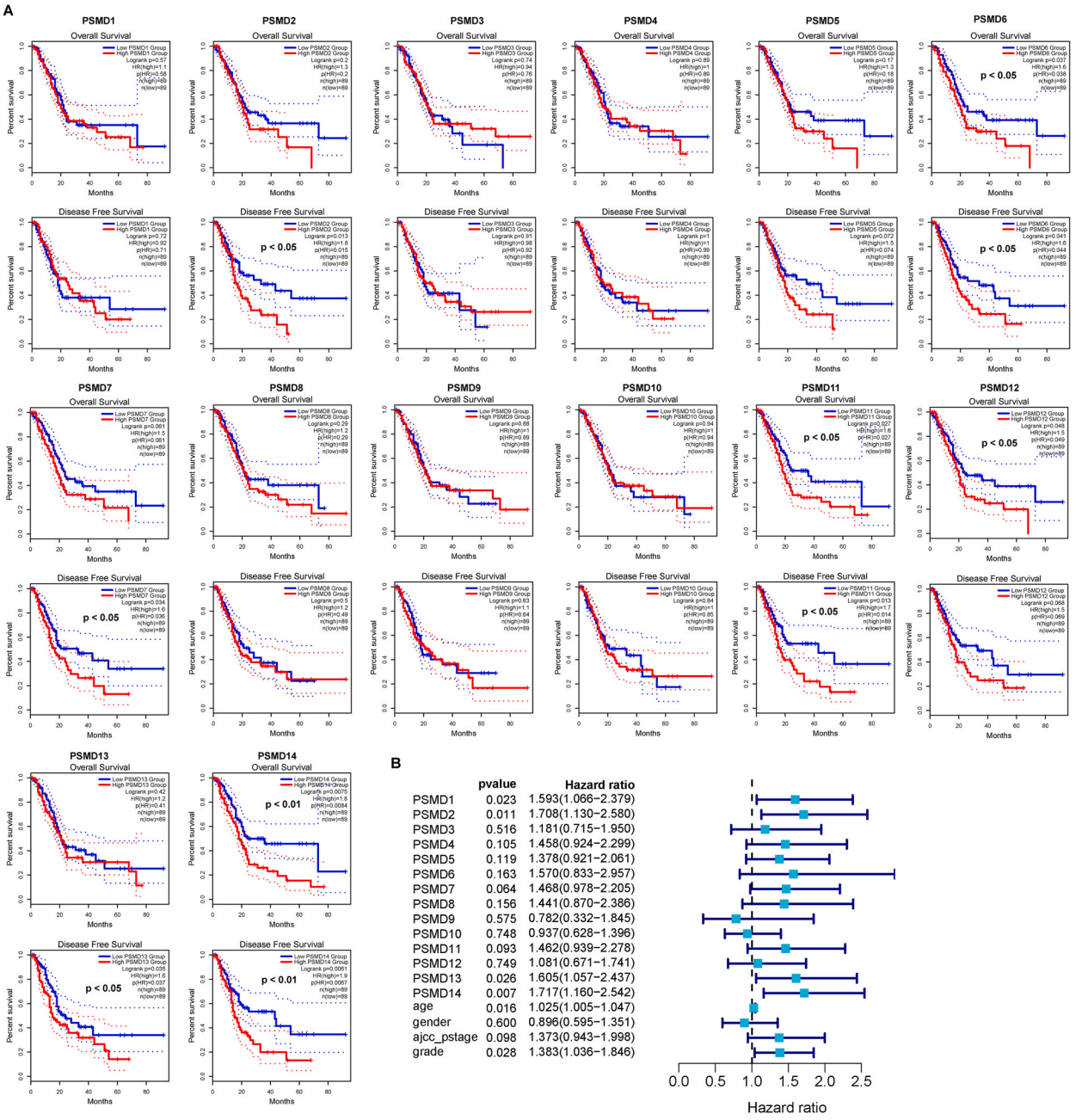

Fig. 2. The predictive significance of PSMDs on the prognosis of pancreatic cancer patients. (A) The relationship between the mRNA levels of PSMD1-14 and the overall and disease-free survival of pancreatic cancer patients. n = 89 per group. The KM analysis of PSMD1-14 is based on the median cutoff value. (B) Multivariate Cox analysis of PSMD1-14 in pancreatic cancer patients. p values are presented in the figure.

3.3. Relationship between PSMDs and TIME in pancreatic cancer

The tumor immune microenvironment (TIME) is closely associated with the progression of pancreatic cancer [24,25]. We then studied the correlation between PSMD2/14 and TIME based on TIMER2 database. The characteristics of immune cell infiltrations in pancreatic cancer patients were presented in Fig. 3A and B. Despite the mRNA abundances of PSMD2 and PSMD14 not being correlated with immune cells' infiltration (Fig. 3C and D), they were significantly associated with the expression levels of immune checkpoints. For example, PSMD2 abundance was positively correlated with the mRNA levels of CD274 (PD-L1), CTLA4, PDCD1LG2 (PD-L2), IDO1, CD276, TIGHT and HAVCR2 (TIM3), which are well-known immunotherapy targets (Fig. 3C). Based on RT-qPCR results, the mRNA levels of PD-L1, PD-L2 and IDO1 were positively regulated by PSMD2 (Figs. S1A and B). PSMD14 overexpression exhibited a positive correlation with the mRNA levels of CD274 (PD-L1), PDCD1LG2 (PD-L2) and CD276 (Fig. 3D). These results suggest that PSMD2 and PSMD14 may be associated with the activity of immune cells in pancreatic cancer.

3.4. Correlated signaling pathways of PSMD2 and PSMD14 based on GO, KEGG and GSEA analysis

To analyze the correlated signaling pathways of PSMD2 and PSMD14, we performed gene ontology (GO) functional annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) in patients with high expression of PSMD2 and PSMD14. According to GO analysis, high expression of PSMD2 correlated with chromosome segregation and DNA replication, while high expression of PSMD14 was associated with chromosome segregation and organelle fission (Figs. S2A and B). According to KEGG analysis, PSMD2 and PSMD14 were associated with pathways of amyotrophic lateral sclerosis, neurodegeneration, Alzheimer 's disease and cell cycle. Interestingly, PSMD2 was positively correlated with mTOR signaling (Fig. S3A). Based on GSEA analysis, both PSMD2 and PSMD14 showed a positive relationship with the PI3K/AKT/mTOR, mTORC1, hypoxia and glycolysis pathways (Fig. 4A and B). While high expression of PSMD2 correlated with the KRAS pathway, PSMD14 was positively correlated with MYC signaling (Fig. 4A and B).

3.5. Predictive value of PSMD2 and PSMD14 on chemotherapy

Chemotherapy is a widely used treatment option for pancreatic cancer patients. The response of the patients to chemotherapy is dramatically varied, we therefore analyzed whether the abundance of PSMD2/14 was associated with the sensitivity of chemotherapeutic drugs based on TCGA database. The results showed that the IC50 values of gemcitabine, oxaliplatin, fludarabine, paclitaxel, and cisplatin were significantly increased in patients with high expression of PSMD2 (Fig. 5A). Similarly, high expression of PSMD14 was associated with enhanced IC50 values of gemcitabine, oxaliplatin, fludarabine, and paclitaxel (Fig. 5B). These results suggest that high expression of PSMD2/14 predicts a lower response to chemotherapy in pancreatic cancer patients.

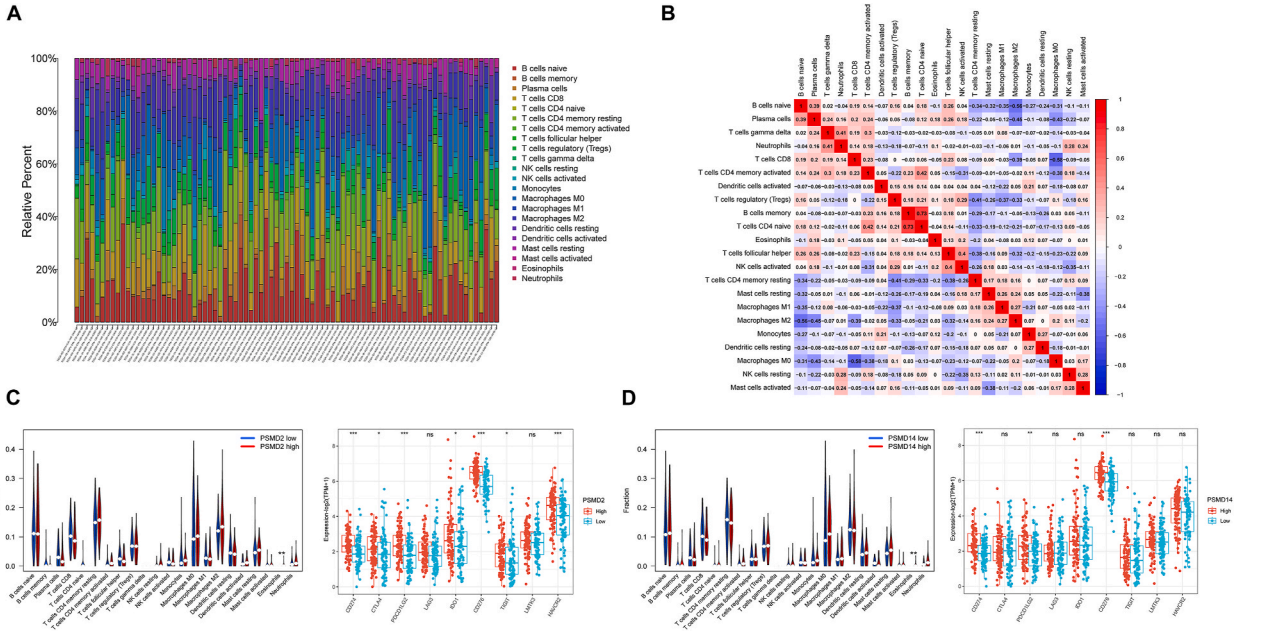

Fig. 3. The relationship between PSMDs' expression and TIME in pancreatic cancer. (A and B) Immune cell infiltration characteristics of pancreatic cancer patients from TCGA data. The abbreviations on the x-axis of A, such as TCGA-HZ-8519-01A-11R-2404-07, represent the sample ID in TCGA database. (C and D) The relationship between high/low expression of PSMD2 (C) or PSMD14 (D) and immune cell infiltration or ICB genes expression in pancreatic cancer patients. *p < 0.05. **p < 0.01. ***p < 0.001. ns, not significant.

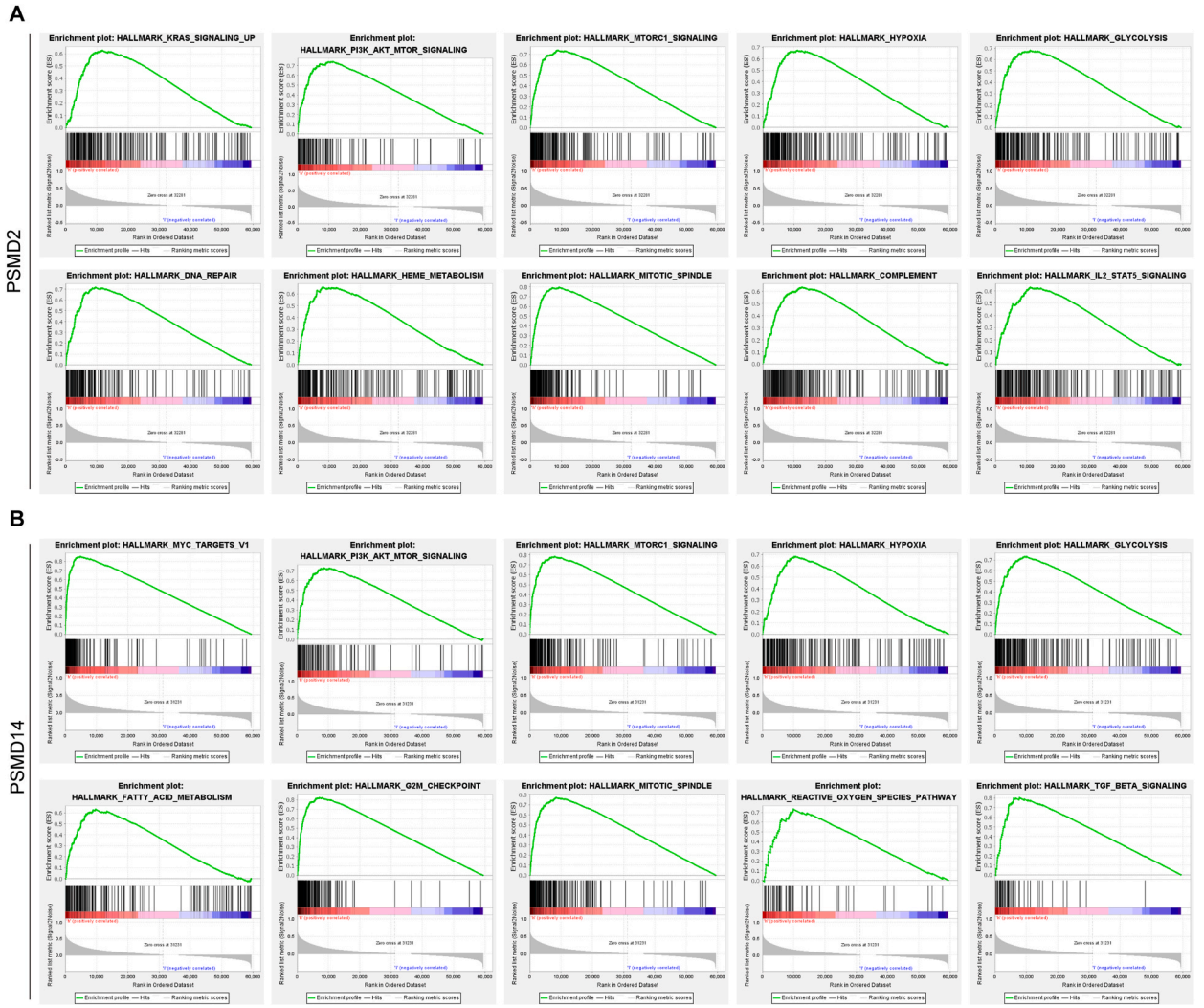

Fig. 4. Signaling pathways correlated with PSMD2 and PSMD14 gene based on GSEA analysis. (A and B) GSEA analysis of correlated signaling pathways in patients with high and low expression of PSMD2 (A) and PSMD14 (B). $p < 0.001$.

3.6. PSMD2 exhibits tumor-promoting function and influences sensitivity to gemcitabine

To study the biological role of PSMD2, we examined the mRNA expression of PSMD2 in pancreatic cancer cells PANC-1, MIA, and SW1990. PANC-1 cells had the lowest, while MIA cells had the highest mRNA level of PSMD2 (Fig. 6A). Therefore, we overexpressed PSMD2 in PANC-1 cells using lentivirus and knocked down PSMD2 in MIA cells using siRNAs (Fig. 6B). Overexpression of PSMD2 resulted in increased proliferation and colony growth ability of PANC-1 cells (Fig. 6C and D). By contrast, downregulation of PSMD2 suppressed the growth capacity of MIA cells (Fig. 6E–G). In addition, PSMD2 inhibited the apoptosis of MIA and PANC-1 cells (Fig. 6H and I). Gemcitabine is a promising chemotherapeutic drug for the treatment of pancreatic cancer patients. We demonstrated that PSMD2 overexpression reduced, while its knockdown increased the sensitivity of pancreatic cancer cells to the treatment of gemcitabine (Fig. 6J and K).

Then, we explored the function of PSMD14 by reducing the expression of PSMD14 in pancreatic cancer cells. We found that PSMD14 knockdown had a minimal effect on the proliferation of MIA cells (Figs. S4A and B). Knockdown of PSMD14 did not significantly influence the toxicity of gemcitabine (Fig. S4C).

Collectively, PSMD2, but not PSMD14, has a tumor-promoting function in pancreatic cancer. The expression level of PSMD2 affects the sensitivity of pancreatic cancer cells to gemcitabine.

3.7. PSMD2 activates AKT/mTOR signaling pathway

Lastly, we investigated the underlying mechanism of PSMD2. Overexpression of PSMD2 resulted in enhanced phosphorylation of

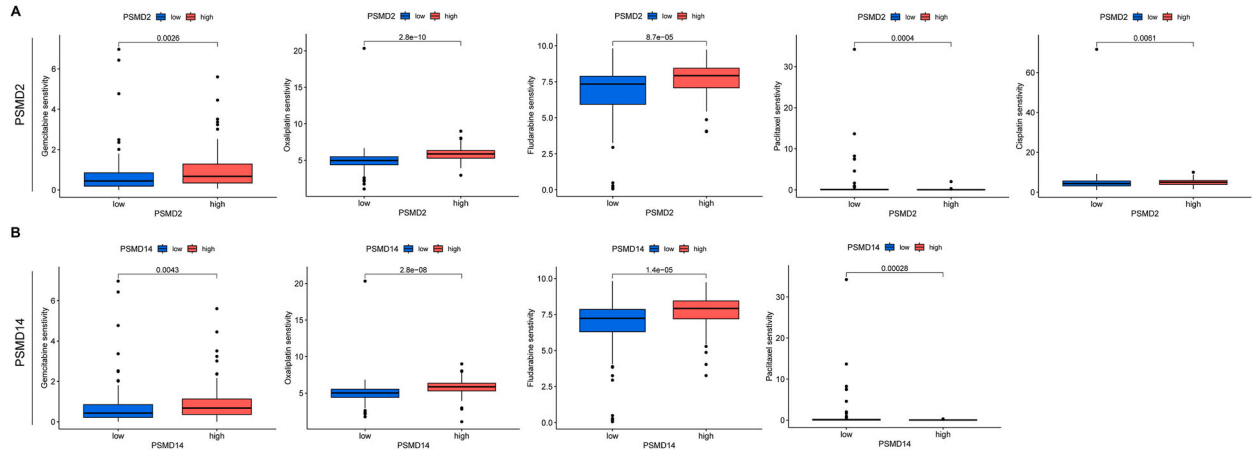

Fig. 5. PSMDs' expression predicts chemotherapeutic drugs' sensitivity in pancreatic cancer. (A and B) The relative sensitivity (Y axis represents IC50 of the drug) of gemcitabine, oxaliplatin, fludarabine, paclitaxel, and cisplatin in patients with high and low levels of PSMD2 (A) and PSMD14 (B). $p < 0.05$.

AKT and S6 protein (Fig. 7A). By contrast, downregulation of PSMD2 inactivated AKT and S6 in MIA cells (Fig. 7A). To validate the contribution of AKT/mTOR signaling, we treated cells overexpressing or knocked down for PSMD2 with different concentrations of the AKT-specific inhibitor MK-2206 for 48 h. The results showed that PANC-1 cells with overexpression of PSMD2 exhibited higher sensitivity to MK-2206, while downregulation of PSMD2 reduced the cytotoxicity of MK-2206 (Fig. 7B). Finally, PANC-1 cells with PSMD2 overexpression were treated with 10 μM MK-2206 for different durations. The results showed that 10 μM of MK-2206 significantly repressed the proliferation and colony formation capacity of PANC-1 cells overexpressing PSMD2 (Fig. 7C and D). Collectively, PSMD2 activation of AKT/mTOR promotes the growth of pancreatic cancer cells.

4. Discussion

Although great efforts are made to explore the underlying mechanisms triggering the development, drug resistance and immune escape of pancreatic cancers, little success has been achieved on clinical treatment. Compared to other malignant tumors, such as melanoma, lung cancer or gastric cancer, pancreatic cancer seems to be resistant to most of the anti-tumor drugs. Therefore, it is critical to perform a comprehensive bioinformatics analysis which can help us identify the therapeutic or diagnostic biomarkers for pancreatic cancer.

Dysregulation of ubiquitin–proteasome system (UPS) plays an essential role in the carcinogenesis of different malignancies. Besides the ubiquitin-specific peptidase, OTU de-ubiquitinase, and E3 ubiquitin ligase families, proteasome 26S subunit, non-ATPases (PSMDs) are also essential UPS members regulating the stability of substrates. In the present study, we comprehensively analyzed the clinical significance of PSMDs in pancreatic cancer. The mRNA expression of PSMD1/2/3/4/7/8/9/10/11/12/13/14 was enhanced in pancreatic cancer tissues. Overexpression of PSMD2/14 predicted the poorer disease-free survival of pancreatic cancer patients. Multivariate Cox regression analysis showed that PSMD2, PSMD13, and PSMD14 were the independent prognostic factors. In addition, PSMD2 and PSMD14 were highly expressed in high-grade tumor tissues. These results suggested that PSMD2 or PSMD14 might be a valuable diagnostic and therapeutic target for pancreatic cancer patients.

Increasing studies have suggested that PSMD2 and PSMD14 act as an oncogene in kinds of malignant tumors. Overexpression of PSMD2 is correlated with the progression of LUAD [26]. Upregulation of PSMD2 contributes to the growth of breast cancer cells through regulation of p21/p27-mediated cell cycle process [27]. PSMD2 regulation of MYH9 stability participates in the DNAJA4-suppressed migration and invasion of nasopharyngeal carcinoma [28]. PSMD2 competes with RACK1 to interact with β-catenin, which facilitates the progress of breast cancer [29]. These reports indicate the oncogenic function of PSMD2 in cancer development. Besides PSMD2, PSMD14 overexpression also promotes ovarian cancer development via regulating the glycolytic enzyme PKM2 or inhibiting autophagy [30,31]. PSMD14 stabilization of GRB2 contributes to the progression of hepatocellular carcinoma [32]. PSMD14 triggers the growth of gastric cancer via enhancing the stability of PTBP1 [33]. However, the function of PSMD2 or PSMD14 in pancreatic cancer remains to be determined. Here, we demonstrated that depletion of PSMD2, but not PSMD14, suppressed the growth ability of MIA cells. Ectopic expression of PSMD2 accelerated the growth rate of PANC-1 cells. The apoptosis of pancreatic cancer cells was suppressed by the overexpression of PSMD2. Our results indicate the oncogenic function of PSMD2 in pancreatic cancer.

Clinical studies suggest that some pancreatic cancer patients can benefit from treatments involving chemotherapeutics drugs such as gemcitabine, oxaliplatin, fludarabine, paclitaxel, and cisplatin [34–37]. However, a large number of patients exhibit little response to chemotherapy. Thus, it is urgent to identify biomarkers to predict the effectiveness of chemotherapy. Based on bioinformatics analysis from the TCGA database, overexpression of PSMD2 was obvious correlation with reduced response to gemcitabine, oxaliplatin, fludarabine, paclitaxel, and cisplatin treatment among pancreatic cancer patients, suggesting that high PSMD2 expression may

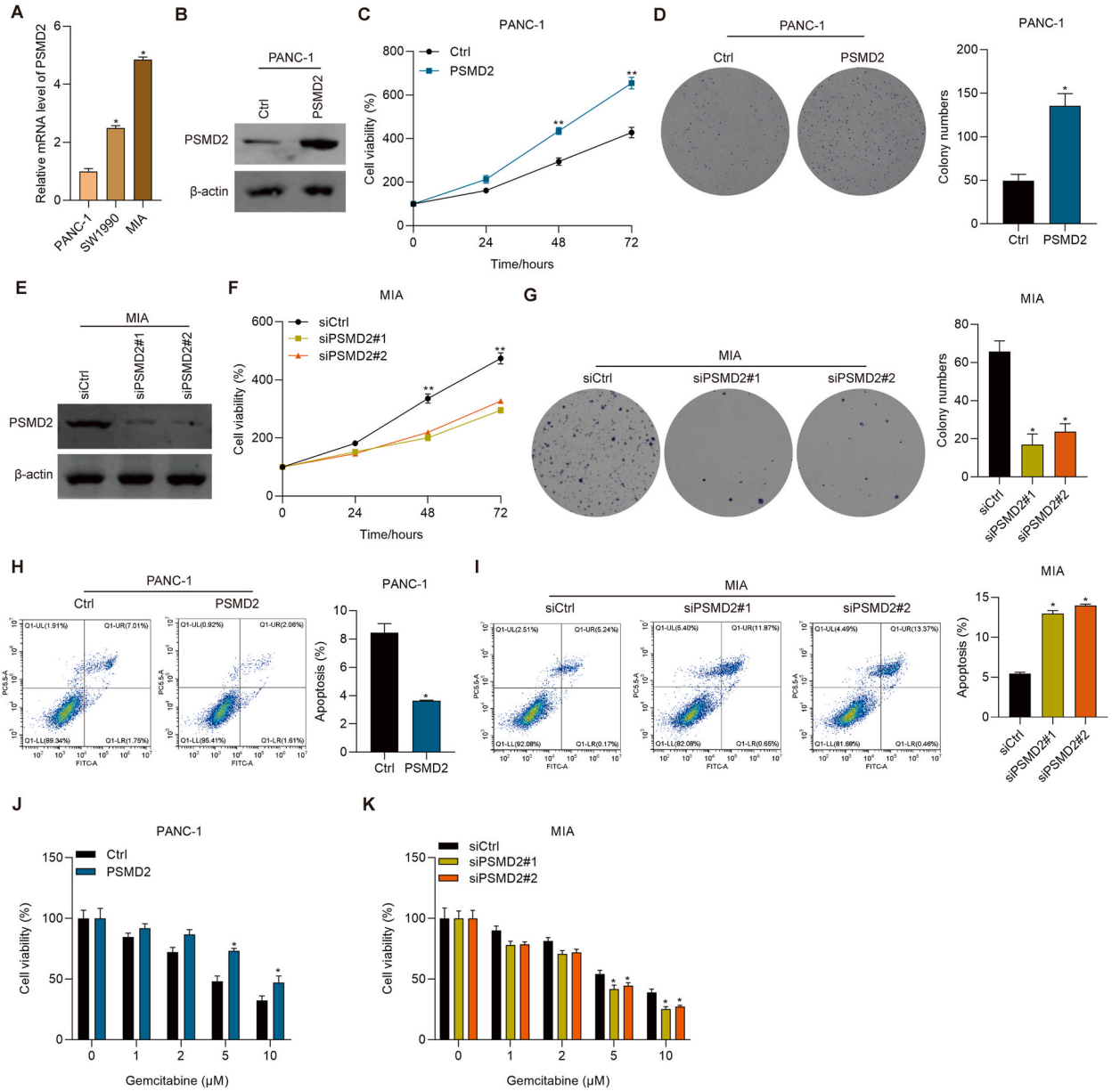

Fig. 6. PSMD2 exhibits tumor promoting function in pancreatic cancer. (A) RT-qPCR detection of PSMD2 in PANC-1, SW1990 and MIA cells. The expression of PSMD2 was normalized to GAPDH. (B) Immunoblotting detection of PSMD2 and β-actin in control (Ctrl) and PSMD2 overexpressed PANC-1 cells. The blots were cut before hybridization with antibodies. The original uncropped Western Blot images can be found in Fig. S5. (C) Cell growth rate was assessed by CCK-8 in PANC-1 cells. (D) Colony formation was assessed in PANC-1 cells. (E) Immunoblotting detection of PSMD2 and β-actin in MIA cells. The blots were cut before hybridization with antibodies. The original uncropped Western Blot images can be found in Fig. S5. (F) Cell growth rate was assessed by CCK-8 in MIA cells. (G) Colony formation was assessed in MIA cells. (H and I) Apoptosis was detected by PI/Annexin V staining in cells as described in B and E. (J and K) Ctrl and PSMD2 overexpressed PANC-1 cells, siCtrl, siPSMD2#1 and siPSMD2#2 MIA cells were treated with different concentrations of gemcitabine for 72 h and cell viability was detected by CCK-8 assay. *$p < 0.05$. **$p < 0.01$.

contribute to chemotherapy resistance. *In vitro*, we found that downregulation of PSMD2 enhanced the sensitivity of MIA cells to gemcitabine, while opposite results were observed in PANC-1 cell with overexpression of PSMD2. These results implied that PSMD2 abundance influenced the response of pancreatic cancers to gemcitabine. Nevertheless, follow-up studies should be conducted to further confirm the role of PSMD2 in the sensitivity to other chemotherapeutic drugs, such as oxaliplatin, fludarabine, paclitaxel, and cisplatin.

Hyperactivation of PI3K/AKT/mTOR signaling promotes the malignant progression of various cancers, including pancreatic cancer [38]. Targeting this signaling pathway is a potential therapeutic strategy for this deadly disease. However, the clinical evaluation of

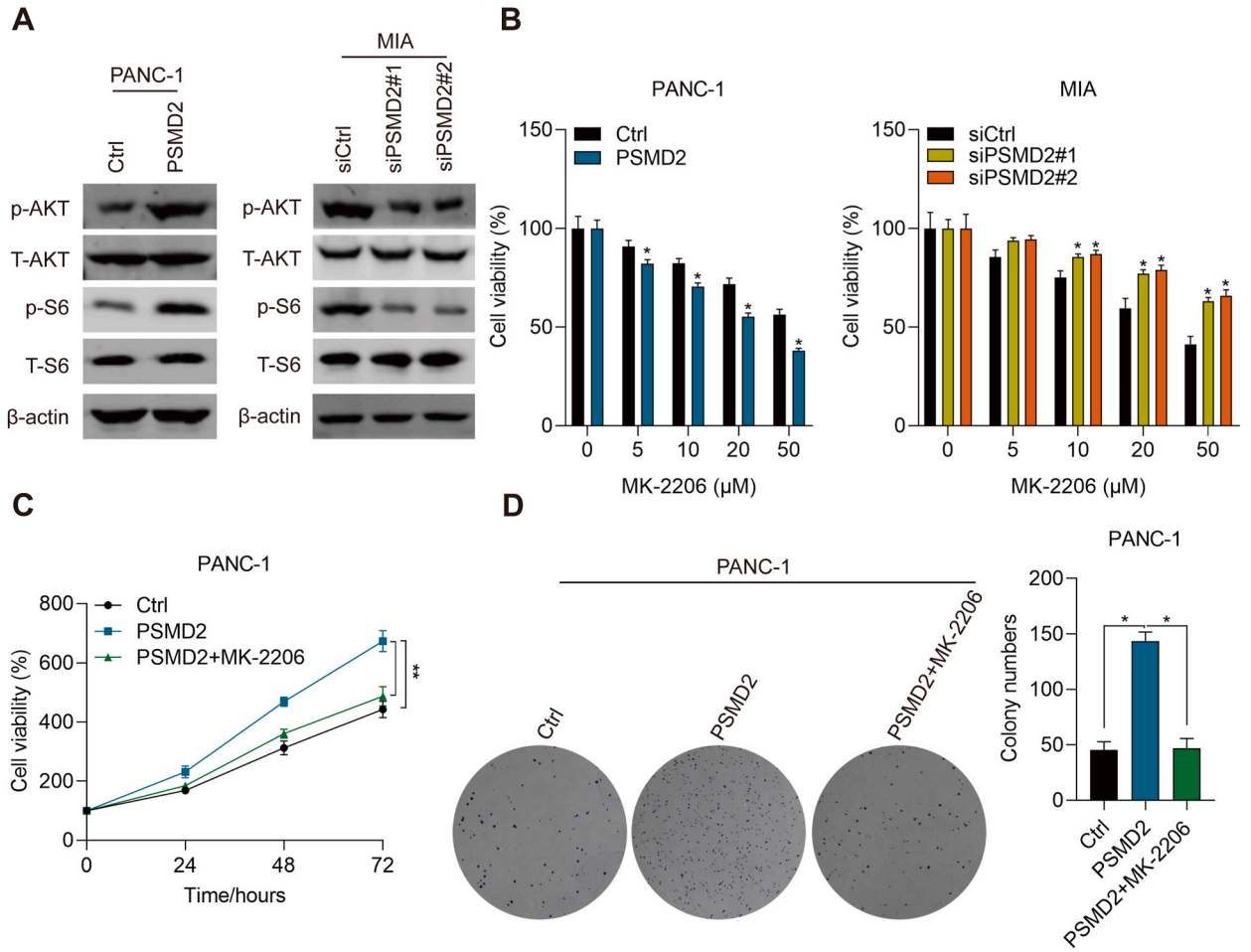

Fig. 7. PSMD2 potentiates AKT/mTOR signaling activity in pancreatic cancer. (A) Immunoblotting detection of AKT/mTOR signaling activity in PANC-1 and MIA cells. The blots were cut before hybridization with antibodies. The original uncropped Western Blot images can be found in Fig. S6. (B) Cells described in A were treated with different concentrations of MK-2206 and the cell viability was detected by CCK-8 assay. (C and D) PSMD2 overexpressed PANC-1 cells were treated with 10 μM MK-2206 for different time. Cell viability was detected by CCK-8 assay, and colonies were detected by crystal violet staining. *p < 0.05. **p < 0.01.

AKT specific inhibitor MK-2206 on advanced/metastatic pancreatic cancer patients has not yielded positive results, even when combined with other drugs, such as selumetinib, oxaliplatin, fluorouracil or dinaciclib [39,40]. We identified that PSMD2 potentiated the activity of AKT/mTOR signaling, which was consistent with KEGG and GSEA analysis of PSMD2-associated signaling pathway based on TCGA data. We also demonstrated that overexpression of PSMD2 significantly enhanced the cytotoxicity of MK-2206 in pancreatic cancer cells. Opposite results were observed when knocking down PSMD2. These results suggest that the expression PSMD2 is correlated with the sensitivity of MK-2206. Our findings are helpful to the clinical study of MK-2206 for the treatment of pancreatic cancer.

Dysregulation of PSMD2 plays a pivotal role in immune escape. For instance, high expression of PSMD2 is associated with the immune infiltration or anti-tumor immunity characteristics of bladder cancer, hepatocellular carcinoma, lung adenocarcinoma, and breast cancer [14,26,41,42]. Here, we showed that PSMD2 overexpression was positively associated with the mRNA abundance of CD274 (PD-L1), CTLA4, PDCD1LG2 (PD-L2), IDO1, CD276, TIGHT and HAVCR2 (TIM3), which are well-known immunotherapy targets. These results suggest that PSMD2 overexpression might be correlated with the immune escape of pancreatic cancer. Thus, the role of PSMD2 in regulating immune escape in pancreatic cancer should be studied in the follow-up study.

5. Conclusion

In conclusion, we have presented a comprehensive analysis of PSMDs in pancreatic cancer, focusing on their expression level, prognostic value, correlation with chemotherapy sensitivity, immune escape, and enriched signaling pathways. Based on the bioinformatics analysis and *in vitro* experiments, we not only comprehensively analyzed the clinical significance of PSMDs in pancreatic cancer patients, but also identified PSMD2, but not PSMD14, as an oncogenic protein in pancreatic cancer. Importantly, PSMD2 is a

potential biomarker to predict the sensitivity of the AKT inhibitor MK-2206 and chemotherapeutic drugs, such as gemcitabine, in pancreatic cancer. MK-2206 or other AKT inhibitors could be effective for pancreatic cancer patients with high expression of PSMD2. By contrast, patients with low expression of PSMD2 might be sensitive to gemcitabine treatment. If a targeted drug against PSMD2 is developed in the future, the combination of a PSMD2 inhibitor and gemcitabine could be a promising strategy for the treatment of pancreatic cancer. Therefore, our findings provided a clue that PSMD2 is a prospective therapeutic target and will be helpful for preclinical and clinical studies evaluating the effectiveness of chemotherapy, targeted therapy and immunotherapy in pancreatic cancers.

CRediT authorship contribution statement

Xiuxue Feng: Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Qiang Liu:** Writing – original draft, Validation, Resources, Methodology, Formal analysis, Data curation. **Huikai Li:** Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Jing Yang:** Writing – original draft, Formal analysis, Data curation. **Enqiang Linghu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the First Medical Center of Chinese PLA General Hospital.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40117.

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