Original Article

Bioinformatics-based screening of key genes associated with gemcitabine resistance in advanced pancreatic ductal adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) ranks among the deadliest cancers globally. Despite gemcitabine being a primary chemotherapeutic agent, many patients with PDAC develop resistance, significantly limiting treatment efficacy. This study aims to screen and validate key genes associated with gemcitabine resistance in advanced PDAC using bioinformatics analysis and clinical sample validation, thereby providing potential noninvasive biomarkers and therapeutic targets for overcoming chemoresistance.

Methods: This study used bioinformatics approaches to analyze gene expression data from the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases, identifying differentially expressed genes (DEGs) associated with gemcitabine resistance in advanced PDAC. A total of 122 patients with advanced PDAC were selected for the study and divided into gemcitabine-sensitive and gemcitabine-resistant groups post-treatment. The expression levels of key genes in patients' serum were measured using enzyme-linked immunosorbent assay, and both univariate and multivariate analyses were performed to assess their potential as noninvasive biomarkers for predicting resistance.

Results: Ten upregulated DEGs related to gemcitabine resistance were identified. Among these genes, cathepsin E (*CTSE*) was significantly negatively correlated with overall survival, disease-specific survival, and progression-free interval in patients with PDAC and was thus identified as a significant key gene. Further clinical sample validation confirmed that *CTSE* expression level was significantly higher in the resistant group of patients with advanced PDAC compared to the sensitive group, establishing *CTSE* as an independent predictor of gemcitabine resistance.

Conclusions: *CTSE* is a key gene associated with gemcitabine resistance in advanced PDAC and shows promise as a target for enhancing responsiveness to gemcitabine treatment.

Keywords: Advanced pancreatic ductal adenocarcinoma (advanced PDAC); gemcitabine; resistance; cathepsin E (*CTSE*)

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Introduction

Pancreatic cancer is the 14th most prevalent cancer worldwide and ranks as the 5th leading cause of cancer-related mortality (1,2), with approximately 95% of pancreatic cancer cases being pancreatic ductal adenocarcinoma (PDAC). The worldwide 5-year survival rate for patients with pancreatic cancer continues to be under 10% (3,4), with its high mortality rate chiefly being attributed to delayed diagnosis and limited responsiveness to chemotherapy. Gemcitabine, as the standard first-line chemotherapeutic agent, is frequently selected as the treatment of choice to prolong life in many patients with advanced PDAC (5). However, resistance to gemcitabine greatly limits its therapeutic effect and often occurs within a few months of treatment initiation, resulting in progressive disease (PD) and decreased patient survival (6-8). Gemcitabine resistance in PDAC is a complex process involving various factors such as metabolic reprogramming (9), DNA repair pathways (10), epigenetics (11), and the tumor microenvironment (12,13). Identifying key genes that can predict resistance remains a significant challenge.

The application of bioinformatics has become an indispensable part of modern cancer research and involves using high-throughput genomic data to uncover the molecular mechanisms of cancer. By analyzing data from public databases including the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), researchers can identify gene expression patterns associated with differences in drug responsiveness (14,15). This approach holds promise for revealing new targets to overcome gemcitabine resistance in PDAC.

This study aimed to leverage the GEO and TCGA databases to identify differentially expressed genes (DEGs) related to gemcitabine resistance in PDAC through bioinformatics methods and to further validate their clinical application potential. Our findings differ from previous study by identifying *CTSE* as a novel (16), independent predictor of gemcitabine resistance in advanced PDAC. This discovery highlights the potential of *CTSE* as a target for overcoming gemcitabine resistance, a crucial step toward improving therapeutic outcomes in PDAC patients. Additionally, the results of this study may open avenues for personalized treatment strategies based on the molecular profile of patients' tumors. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2374/rc).

Highlight box

Key findings

- Ten upregulated differentially expressed genes (DEGs) related to gemcitabine resistance were identified in patients with advanced pancreatic ductal adenocarcinoma (PDAC).
- Cathepsin E (*CTSE*) was identified as a significant key gene and was negatively correlated with overall survival, disease-specific survival, and progression-free interval.
- High expression levels of CTSE in serum were associated with gemcitabine resistance as validated through clinical samples.

What is known and what is new?

- Gemcitabine resistance is a major challenge in treating PDAC, necessitating the identification of reliable biomarkers for predicting treatment outcomes.
- This study highlights CTSE as a novel, significant biomarker for gemcitabine resistance in PDAC, providing a potential target to enhance treatment responsiveness.

What is the implication, and what should change now?

- The identification of CTSE as a predictive biomarker for gemcitabine resistance implies a pivotal shift toward more personalized treatment strategies in PDAC.
- Clinical protocols should consider integrating CTSE level assessments to tailor gemcitabine use, potentially enhancing therapeutic efficacy and patient outcomes in advanced PDAC.

Methods

Data sources

Data containing information on patients with advanced PDAC were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The GSE62165 dataset includes 13 normal tissues and 18 tumor tissues from advanced PDAC, while the GSE140077 dataset includes 6 pairs of PDAC tissues that are sensitive and resistant to gemcitabine. The dataset inclusion criteria were as follows: (I) full-genome messenger RNA (mRNA) expression microarray data; (II) either standardized or original datasets; and (III) more than three samples in the dataset.

Data processing and DEGs screening

Principal component analysis (PCA) with R language (The R Foundation for Statistical Computing) was performed on the samples from the two datasets mentioned above, separately for different microarrays to observe the distribution between groups. The GEO2R online tool

(https://www.ncbi.nlm.nih.gov/geo/geo2r/) was used to analyze the DEGs in each dataset, with the selection criteria set at |log fold change| ≥2 and an adjusted P value <0.05 for screening DEGs. DEGs common to both microarrays could contain genes with inconsistent up- or downregulation, sometimes leading to false-positive results due to different experimental conditions or technical differences. To eliminate this confounding factor and to select potential clinical diagnostic and prognostic prediction targets, only upregulated genes in the common DEGs were analyzed, as their expression upregulation might indicate a key role in the resistance process. Heatmaps and volcano plots for the DEGs derived from the two datasets were created using CHDTEPDB (17). A Venn diagram was employed to determine the intersection of upregulated DEGs from both datasets, with genes that consistently showed upregulated expression associated with gemcitabine resistance in advanced PDAC being obtained.

Data analysis of TCGA database

The expression of candidate upregulated DEGs was investigated in tumor and normal tissues of pancreatic cancer within TCGA database. The clinical parameters of patients with pancreatic cancer were also downloaded from the database. Subsequently, a comparative analysis was conducted to clarify the correlation between these DEGs and the prognosis of patients with pancreatic cancer.

Clinical data

A total of 122 patients with advanced PDAC were selected from those admitted to the Sir Run Run Hospital, Nanjing Medical University between May 2021 and May 2023. The inclusion criteria for patients were as follows: (I) diagnosed with advanced PDAC through pathological or imaging examinations; (II) an expected survival period greater than 3 months; (III) no history of chemotherapy or radiotherapy before enrollment. The exclusion criteria were as follows: (I) incomplete medical records; (II) presence of other malignant tumors; (III) severe liver or kidney dysfunction; and (IV) contraindications to the drugs used in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study received approval from the Ethics Committee of Sir Run Run Hospital Nanjing Medical University (No. 2023-SR-007) and informed consent was taken from all the patients.

Efficacy evaluation and grouping

Gemcitabine was administered intravenously at a dose of 1,000 mg/m^2 over 30 minutes once a week (on days 1, 8, and 15) and followed by a week off after 3 weeks of continuous treatment. Each cycle lasted 28 days, and cycles were repeated until PD or patient intolerance. Clinical efficacy was assessed based on solid tumor response criteria, with patients achieving complete response (CR) or partial response (PR) categorized into the sensitive group, and those with stable disease (SD), PD, or relapse within 6 months after achieving CR or PR categorized into the resistant group.

Enzyme-linked immunosorbent assay (ELISA) detection of key gene cathepsin E (CTSE) expression in the serum of patients with advanced PDAC

CTSE protein levels in serum were determined using an ELISA kit provided by Wenzhou Kemao Biotechnology Co., Ltd. (Wenzhou, China). Initially, 100 µL of patient serum samples were added to microplates precoated with anti-*CTSE* antibodies and incubated at 37 ℃ for 2 hours. Subsequently, primary antibodies were added and incubated at the same temperature for another hour. The microplates were washed with a washing buffer to remove unbound substances, followed by the addition of enzyme-labeled secondary antibodies and a 15-minute incubation. The reaction was stopped with a stop solution and followed by the addition of a substrate for color development. The absorbance was read at a wavelength of 450 nm using an enzyme reader, and the concentration of *CTSE* protein in the samples was calculated based on the standard curve. All samples were tested in triplicate (18).

Statistical analysis

Statistical evaluations and graph construction were performed using SPSS 26 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA). For the analysis of DEGs, P values and their adjustments were calculated using the *t*-test, with the false-discovery rate method applied for corrections. Patient survival was analyzed using the Kaplan-Meier method, with significance assessed via the log-rank test. Normally distributed quantitative data are expressed as the mean ± standard deviation, and group comparisons were conducted

Figure 1 Venn diagram of upregulated genes from the GSE62165 and GSE140077 datasets in the Gene Expression Omnibus database.

with the *t*-test. Count data (n), were analyzed using the chi-square test. Logistic regression was employed to pinpoint independent risk factors that influence gemcitabine resistance in patients with advanced PDAC, with statistical significance set at a P value <0.05.

Results

Screening of key genes associated with gemcitabine resistance in advanced PDAC

By intersecting the upregulated DEGs from the two datasets using a Venn Diagram, 10 genes associated with gemcitabine resistance in advanced PDAC were identified. These genes were *CCL20*, *CP*, *PTGS2*, *MUC4*, *KIF26B*, *FBXO32*, *CTSE*, *ZFPM2*, *CXCL14*, and *DIO2* (*Figure 1*).

Expression of DEGs in the pancreatic cancer data from TCGA database

In the analysis of the aforementioned 10 candidate upregulated DEGS in TCGA database, only *MUC4* and *CTSE* exhibited significant differences in expression between pancreatic cancer tumor tissues and adjacent normal tissues, with both acting as oncogenes (P<0.05) (*Figure 2A*). Construction of receiver operating characteristic (ROC) curves showed that the area under the curve (AUC) for both genes was greater than 0.7, indicating good predictive distinction value for these genes (*Figure 2B*). Analyzing the clinical data of patients with pancreatic cancer to observe the correlation between different expressions of these two genes and patient prognosis revealed that only patients with low *CTSE* expression had better outcomes [overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI)], with statistically significant differences (P<0.05) (*Figure 2C,2D*). *MUC4* mainly promotes tumor progression by affecting cell surface signaling (19,20) and has limited impact on resistance and the microenvironment. In contrast, *CTSE* promotes resistance by degrading the extracellular matrix (21) and remodeling the tumor microenvironment (22), thereby limiting the penetration of chemotherapeutic drugs. Therefore, we identified *CTSE* as the key gene associated with gemcitabine resistance in advanced PDAC in this study.

Serum CTSE levels

After treatment with gemcitabine, 87 patients were categorized into the sensitive group and 35 into the resistant group. In the comparison of the serum *CTSE* levels between the two groups, the resistant group had significantly higher serum *CTSE* levels at 4.17±0.78 ng/mL compared to the sensitive group at 3.67±0.76 ng/mL (P=0.001; *Figure 3*).

Univariate analysis of factors affecting gemcitabine resistance in patients with advanced PDAC

Univariate analysis showed no significant differences in age, gender, tumor location, history of hypertension, or history of hyperglycemia between the sensitive and resistant groups (all P values >0.05). However, significant differences were observed in tumor diameter, lymph node metastasis, vascular invasion, and serum *CTSE* levels (all P values <0.05), suggesting that these factors, particularly tumor diameter, lymph node metastasis, vascular invasion, and serum *CTSE* levels, may influence gemcitabine resistance in patients with advanced PDAC (*Table 1*).

Logistic multivariate regression analysis

Clinical characteristics that were significantly different in the univariate analysis were incorporated into a logistic multivariate regression analysis. The findings revealed that tumor diameter, lymph node metastasis, vascular invasion, and serum *CTSE* levels are independent risk factors of gemcitabine resistance in patients with advanced PDAC (all P values <0.05) (*Table 2*).

Discussion

Initially, through the analysis of data from the GEO database, we identified 10 upregulated genes associated with

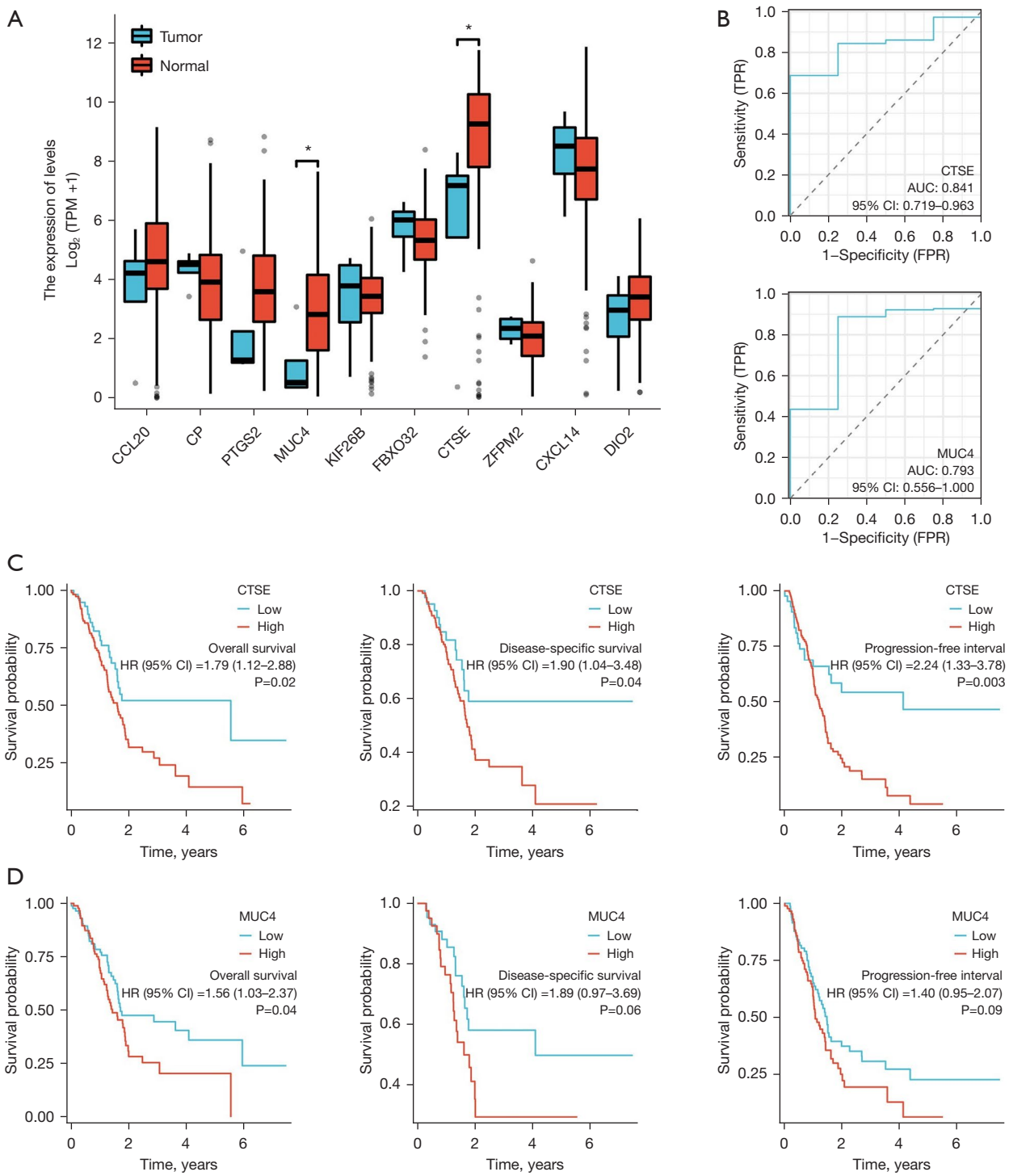

Figure 2 Expression of differentially expressed genes in the pancreatic cancer data from TCGA database. (A) Expression of 10 candidate upregulated differentially expressed genes in unmatched pancreatic cancer tissues (tumor =179 cases; normal =4 cases). (B) Receiver operating characteristic curve analysis demonstrating good discrimination ability between tumor and normal tissues for *MUC4* and *CTSE*. (C,D) Correlation of *CTSE* and *MUC4* expression with prognosis, including the overall survival, disease-specific survival, and progression-free interval, of patients with pancreatic cancer in TCGA database. *, P<0.05. TPM, transcripts per million; *CTSE*, cathepsin E; AUC, area under the curve; CI, confidence interval; FPR, false positive rate; TPR, true positive rate; HR, hazard ratio; TCGA, The Cancer Genome Atlas.

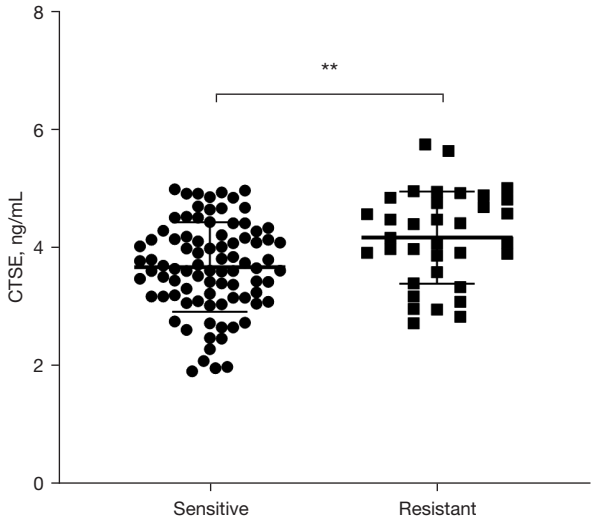

Figure 3 Serum CTSE levels. **, P<0.01. CTSE, cathepsin E.

gemcitabine resistance in patients with advanced PDAC.

Further validation with TCGA database showed that the expression levels of the *CTSE* and *MUC4* genes differed significantly between pancreatic cancer tissues and adjacent normal tissues, with *CTSE*'s expression being more closely associated with patient prognosis. Specifically, high expression of *CTSE* correlates with poorer OS, DSS, and PFI, indicating its oncogenic role in pancreatic cancer. Compared to *MUC4*, *CTSE* has greater potential for predicting gemcitabine resistance.

CTSE is an aspartic protease expressed in immune cells, gastrointestinal mucosal cells, and lymphoid tissues (23); is involved in protein degradation and extracellular matrix remodeling (21,24); and is closely associated with changes in the tumor microenvironment. In various cancers, *CTSE* has an oncogenic role, particularly in digestive system tumors such as pancreatic cancer, gastric cancer, and colorectal cancer, in which its high expression is closely related to tumor aggressiveness and malignancy (25-27). Research by Li *et al.* indicated that *CTSE* expression is elevated in pancreatic cancer and its precursors (such as pancreatic intraepithelial neoplasias) and that its expression levels increase with PD (28). Notably, besides its role in cancer promotion, *CTSE* is also potentially involved in cancer resistance, especially in chemotherapy-resistant tumors, such as those of patients in rectal cancer undergoing radiochemotherapy, in whom high *CTSE* expression is associated with tumor recurrence and poor prognosis. This may be due to *CTSE* forming a defensive mucous barrier that prevents drug penetration, thereby enhancing tumor resistance (25). Another study showed that *CTSE* and other lysosomal proteases can promote cancer progression and resistance by degrading the extracellular matrix, participating in apoptosis, and affecting the mechanisms of response in cancer therapy (29). Combined with the results from the GEO database in this study, the high expression of *CTSE* in PDAC suggests that it is closely related to gemcitabine resistance, further supporting its role in the mechanism of chemotherapy resistance. *CTSE* promotes the remodeling of the PDAC microenvironment by degrading the extracellular matrix, thereby affecting PDAC aggressiveness and chemotherapy sensitivity. This mechanism suggests that inhibiting *CTSE* activity may enhance the efficacy of chemotherapy drugs, offering a potential targeted treatment strategy for patients with PDAC.

Furthermore, in our analysis of 122 patients with advanced PDAC, the measurement of serum *CTSE* levels further supports the potential of *CTSE* as a predictive factor for resistance. Patients with gemcitabine resistance had significantly higher *CTSE* levels compared to the sensitive group, and thus serum *CTSE* levels were an independent predictor of gemcitabine resistance. This suggests that *CTSE* could serve as a noninvasive biomarker for predicting resistance to gemcitabine in patients.

The discovery of genes such as *CTSE*, which play a pivotal role in gemcitabine resistance, may open new avenues for targeted therapies. Inhibiting the activity of genes involved in resistance mechanisms, such as those regulating extracellular matrix remodeling, autophagy, or drug efflux, could enhance the efficacy of gemcitabine and overcome resistance. Future clinical studies may focus on developing small molecule inhibitors or biologics to target these key genes directly.

Certain limitations to our study should be mentioned. First, although we validated our findings through two datasets and TCGA database, the role of *CTSE* in gemcitabine resistance in PDAC still needs further confirmation through larger-scale independent cohort studies. Additionally, while our data indicate that *CTSE* is a potential marker of resistance, its exact mechanisms remain unclear, and future in-depth molecular experiments are needed to clarify the functional role of *CTSE* in gemcitabine resistance (30). Moreover, the primary focus of this study was the relationship between *CTSE* and gemcitabine resistance, and it did not thoroughly explore other potential

Table 1 Univariate analysis of factors influencing gemcitabine resistance in patients with advanced pancreatic ductal adenocarcinoma

Clinical characteristics	Sensitive group (n=87)	Resistant group (n=35)	t/χ^2	P
Age (years)	59.67±8.11	60.39±8.71	0.4599	0.65
Sex			1.128	0.29
Male	49	16		
Female	38	19		
Tumor diameter (cm)			5.919	0.02
<5	51	12		
≥5	36	23		
Tumor location			1.168	0.28
Pancreatic head	54	18		
Pancreatic body/tail	33	17		
Lymph node metastasis			6.060	0.01
Yes	31	21		
No	56	14		
Hypertension			0.6900	0.41
Yes	40	19		
No	47	16		
Hyperglycemia			2.663	0.10
Yes	38	21		
No	49	14		
Vascular invasion			5.682	0.02
Yes	34	22		
No	53	13		
Serum *CTSE* (ng/mL)	3.67±0.76	4.17±0.78	3.271	0.001

Data are presented as mean ± standard deviation or number. *CTSE*, cathepsin E.

Table 2 Logistic multivariate regression analysis

Clinical characteristics	Exp (B) (95% CI)	P
Tumor diameter	3.354 (1.324–8.495)	0.01
Lymph node metastasis	3.221 (1.290–8.041)	0.01
Vascular invasion	2.517 (1.024–6.186)	0.044
Serum *CTSE*	2.153 (1.200–3.863)	0.01

CI, confidence interval; *CTSE*, cathepsin E.

mechanisms by which high *CTSE* expression might impact patient prognosis. Future research should conduct a more comprehensive analysis of the specific roles of *CTSE* in the tumor microenvironment, inflammatory pathways, and other related mechanisms.

Conclusions

Overall, our study through bioinformatics screening and preliminary clinical validation suggests that *CTSE* may be an important predictive factor for gemcitabine resistance in patients with advanced PDAC. These findings emphasize the inhibition of *CTSE* activity as a potential strategy, which could enhance responsiveness to gemcitabine treatment and thus open new directions for research on the resistance mechanisms in pancreatic cancer.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2374/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2374/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2374/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2024-2374/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Sir Run Run Hospital Nanjing Medical University (No. 2023-SR-007) and informed consent was taken from all the patients.

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin 2024;74:12-49.
2. Global Burden of Disease 2019 Cancer Collaboration; Kocarnik JM, Compton K, et al. Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. JAMA Oncol 2022;8:420-44.
3. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol 2021;18:493-502.
4. Wang Y, Cui C, Yu Q, et al. Development and validation of a nomogram for predicting survival in patients with pancreatic ductal adenocarcinoma after radical pancreatoduodenectomy. Oncologie 2023;25:51-9.
5. Piquemal D, Bruno R, Bournet B, et al. Performance of a blood-based RNA signature for gemcitabine-based treatment in metastatic pancreatic adenocarcinoma. J Gastrointest Oncol 2023;14:997-1007.
6. Qi R, Bai Y, Li K, et al. Cancer-associated fibroblasts suppress ferroptosis and induce gemcitabine resistance in pancreatic cancer cells by secreting exosome-derived ACSL4-targeting miRNAs. Drug Resist Updat 2023;68:100960.
7. Zhou T, Xie Y, Hou X, et al. Irbesartan overcomes gemcitabine resistance in pancreatic cancer by suppressing stemness and iron metabolism via inhibition of the Hippo/YAP1/c-Jun axis. J Exp Clin Cancer Res 2023;42:111.
8. Teh YH, Jing R, Ramasamy R, et al. More than just a KRAS inhibitor: DCAI abrogates the self-renewal of pancreatic cancer stem cells in vitro. Oncologie 2023;25:671-80.
9. Ma MJ, Shi YH, Liu ZD, et al. N6-methyladenosine modified TGFB2 triggers lipid metabolism reprogramming to confer pancreatic ductal adenocarcinoma gemcitabine resistance. Oncogene 2024;43:2405-20.
10. Xu L, Ma X, Zhang X, et al. hsa_circ_0007919 induces LIG1 transcription by binding to FOXA1/TET1 to enhance the DNA damage response and promote gemcitabine resistance in pancreatic ductal adenocarcinoma. Mol Cancer 2023;22:195.
11. Nguyen CDK, Colón-Emeric BA, Murakami S, et al.

PRMT1 promotes epigenetic reprogramming associated with acquired chemoresistance in pancreatic cancer. Cell Rep 2024;43:114176.
12. Lee YS, Kim HS, Kim HJ, et al. The role of LOXL2 induced by glucose metabolism-activated NF-κB in maintaining drug resistance through EMT and cancer stemness in gemcitabine-resistant PDAC. J Mol Med (Berl) 2023;101:1449-64.
13. Natu J, Nagaraju GP. Gemcitabine effects on tumor microenvironment of pancreatic ductal adenocarcinoma: Special focus on resistance mechanisms and metronomic therapies. Cancer Lett 2023;573:216382.
14. Athar M, Manhas A, Rana N, et al. Computational and bioinformatics tools for understanding disease mechanisms. Biocell 2024;48:935-44.
15. Shahrajabian MH, Sun W. Survey on Multi-omics, and Multi-omics Data Analysis, Integration and Application. Current Pharmaceutical Analysis 2023;19:267-81.
16. Ye H, Li T, Wang H, et al. TSPAN1, TMPRSS4, SDR16C5, and CTSE as Novel Panel for Pancreatic Cancer: A Bioinformatics Analysis and Experiments Validation. Front Immunol 2021;12:649551.
17. Song Z, Yu J, Wang M, et al. CHDTEPDB: Transcriptome Expression Profile Database and Interactive Analysis Platform for Congenital Heart Disease. Congenital Heart Disease 2024;18:693-701.
18. Tian X, He X, Qian S, et al. Immunoregulatory effects of human amniotic mesenchymal stem cells and their exosomes on human peripheral blood mononuclear cells. Biocell 2023;47:1085-93.
19. Liu J, Xing R, Shao J, et al. Relationship Between MUC4 Variants and Metastatic Recurrence in Colorectal Cancer. Int J Gen Med 2023;16:5077-87.
20. Hu CM, Huang CC, Hsu MF, et al. Oncogenic KRAS, Mucin 4, and Activin A-Mediated Fibroblast Activation Cooperate for PanIN Initiation. Adv Sci (Weinh) 2023;10:e2301240.
21. Fonović M, Turk B. Cysteine cathepsins and extracellular matrix degradation. Biochim Biophys Acta 2014;1840:2560-70.
22. Li Y, Xu C, Wang B, et al. Proteomic characterization of gastric cancer response to chemotherapy and targeted therapy reveals new therapeutic strategies. Nat Commun 2022;13:5723.
23. Mengwasser J, Babes L, Cordes S, et al. Cathepsin E Deficiency Ameliorates Graft-versus-Host Disease and Modifies Dendritic Cell Motility. Front Immunol 2017;8:203.
24. Vidak E, Javoršek U, Vizovišek M, et al. Cysteine Cathepsins and their Extracellular Roles: Shaping the Microenvironment. Cells 2019;8:264.
25. Chou CL, Chen TJ, Tian YF, et al. CTSE Overexpression Is an Adverse Prognostic Factor for Survival among Rectal Cancer Patients Receiving CCRT. Life (Basel) 2021;11:646.
26. Pontious C, Kaul S, Hong M, et al. Cathepsin E expression and activity: Role in the detection and treatment of pancreatic cancer. Pancreatology 2019;19:951-6.
27. Konno-Shimizu M, Yamamichi N, Inada K, et al. Cathepsin E is a marker of gastric differentiation and signet-ring cell carcinoma of stomach: a novel suggestion on gastric tumorigenesis. PLoS One 2013;8:e56766.
28. Li H, Li Y, Cui L, et al. Monitoring pancreatic carcinogenesis by the molecular imaging of cathepsin E in vivo using confocal laser endomicroscopy. PLoS One 2014;9:e106566.
29. Rudzińska M, Parodi A, Soond SM, et al. The Role of Cysteine Cathepsins in Cancer Progression and Drug Resistance. Int J Mol Sci 2019;20:3602.
30. Seyfinejad B, Jouyban A. Importance of Method Validation in the Analysis of Biomarker. Current Pharmaceutical Analysis 2022;18:567-9.

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