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Original Article

Baicalein inhibits DDX60 to suppress pancreatic cancer growth and regulate the tumor microenvironment

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Abstract: Objective: To explore the effects of baicalein on immune cell infiltration and tumor progression in pancreatic cancer by modulating DDX60 expression. Methods: RNA-seq data of pancreatic cancer and normal tissues were obtained from the UCSC XENA database. DDX60 expression differences and their associations with patient prognosis and immune infiltration were analyzed. Panc02 pancreatic cancer cells were treated with baicalein (0, 20, 40, 60 μmol/L) for 24, 48, and 72 hours. Cell viability was assessed by MTT assay, while apoptosis and DDX60 expression were evaluated by flow cytometry and RT-qPCR, respectively. In vivo, tumor-bearing mice received baicalein, and tumor volume, immune cell infiltration, and DDX60 expression in tumor tissues were assessed. Results: DDX60 expression was significantly upregulated in pancreatic cancer tissues compared to normal tissues ($P < 0.05$). Patients with low DDX60 had better survival ($P < 0.05$). DDX60 levels correlated significantly with multiple immune cell types, including DCs, eosinophils, macrophages, neutrophils, T cell subsets, and NK cells ($P < 0.05$). Baicalein inhibited Panc02 cell proliferation and induced apoptosis in a dose- and time-dependent manner ($P < 0.05$), accompanied by downregulation of DDX60 ($P < 0.05$). In vivo, baicalein significantly suppressed tumor growth and increased $CD8^+$ T cells and macrophages in tumor tissues ($P < 0.05$). DDX60 expression decreased with increasing baicalein dosage ($P < 0.05$). Conclusion: Baicalein suppresses pancreatic cancer growth and promotes apoptosis, apparently through downregulation of DDX60 and modulation of immune responses in the tumor microenvironment.

Keywords: Baicalein, DDX60, pancreatic cancer, immune cell infiltration, cell proliferation, apoptosis

Introduction

Pancreatic cancer is one of the most lethal malignancies of the digestive system, with a steadily increasing incidence. According to the American Cancer Society, the 5-year survival rate for pancreatic cancer was only 11% in 2022, ranking it fourth in cancer-related mortality [1, 2]. Its pathogenesis is complex and often insidious, so early-stage disease typically presents without obvious clinical symptoms. As a result, most patients are diagnosed at advanced stages, missing the window for curative surgical intervention. It has been reported that only 15-20% of patients are eligible for radical resection [3]. Moreover, abnormal immune cell infiltration within the tumor microenvironment (TME) plays a critical role in resistance to immunotherapy, with KRAS mutation-driven immunosuppressive networks being particularly prominent. Therefore, identifying novel therapeutic targets that modulate the immune microenvironment - especially through natural compounds - has become a major research focus.

Baicalein, a flavonoid extracted from the dried roots of Scutellaria baicalensis (family Lamiaceae), has demonstrated anti-invasive and anti-metastatic effects in various cancers, including gastric, lung, and liver malignancies. However, its precise antitumor mechanisms remain unclear [4, 5]. To enhance therapeutic strategies for pancreatic cancer, this study investigated the inhibitory effects of baicalein on pancreatic cancer cells and preliminarily explored its underlying mechanisms. Using bioinformatic analysis, DDX60 was identified as a potential regulator of both cancer cell proliferation and immune infiltration. The study further assessed

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whether the antitumor activity of baicalein involved modulation of DDX60 expression.

Materials and methods

Database information

RNA-seq data in TPM format were obtained from UCSC XENA (https://xenabrowser.net/datapages/), uniformly processed using the Toil pipeline. The dataset included 167 GTEx normal tissue samples, 4 TCGA adjacent normal tissue samples, and 179 TCGA pancreatic tumor tissue samples.

Bioinformatic methods

Differential gene expression analysis was conducted using R packages: ggplot2 (v3.4.4), stats (v4.2.1), and car (v3.1-0). Statistical methods were selected based on data characteristics, and visualizations were generated using ggplot2.

Prognostic analysis was performed using the survival (v3.3.1), survminer (v0.4.9), and ggplot2 (v3.4.4) packages. The proportional hazards assumption was tested and Cox regression models were fitted. Results were visualized using survminer and ggplot2.

Immune infiltration analysis was conducted by correlating gene expression with immune cell infiltration matrix data. Results were visualized as lollipop plots and heatmaps using ggplot2.

Cell lines and animals

Panc02 mouse pancreatic cancer cells were obtained from the Committee for the Preservation of Typical Culture Collection, Chinese Academy of Sciences. SPF-grade male Kunming mice (18-22 g) were purchased from the Laboratory Animal Center of Chongqing Medical University.

Cell culture

Panc02 cells were cultured in DMEM high-glucose medium supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 incubator. Cells were passaged when confluence reached 70-80%.

MTT assay for cell growth inhibition

Panc02 cells in the logarithmic growth phase were digested with trypsin and resuspended into a single-cell suspension. Cells were seeded in 96-well plates at a density of 5×10^3 cells/200 μL per well. After attachment, cells were washed twice with PBS, and treated with 200 μL DMEM complete medium containing baicalein (CAS: 21967-41-9, Sigma, USA) at concentrations of 0, 5, 10, 20, 40, 60, 80, and 160 μmol/L. Each concentration was tested in five replicates. Cells were incubated for 24, 48, and 72 hours, and cell viability was assessed using an MTT Cell Proliferation and Cytotoxicity Assay Kit (Beyotime, Shanghai, China). After adding 20 μL of 5 g/L MTT solution to each well, plates were incubated for 4 hours. The supernatant was then removed, 150 μL of DMSO was added, and absorbance at 490 nm was measured using an automatic microplate reader. The growth inhibition rate was calculated as: Inhibition rate (%) = $(1\text{-}A_{\text{Medicated group}}/A_{\text{Blank group}}) \times 100\%$.

Flow cytometry for detection of apoptosis

Panc02 cells in the logarithmic growth phase were digested with trypsin and resuspended to form a single-cell suspension. Cells were seeded into 6-well plates at a density of 3×10^5 cells/well. After adherence, 2 mL of DMEM containing 0, 20, 40, or 80 μmol/L baicalein was added. After 48 hours of incubation, cells were harvested by trypsinization and kept at 4°C. Cells were centrifuged at 1500 rpm for 5 min, the supernatant was discarded, and the cells were washed twice with pre-chilled PBS. After another centrifugation, the pellet was resuspended in 200 μL of 1 × Binding Buffer. Annexin V-FITC (5 μL) was added, mixed, and incubated for 10 min at room temperature in the dark. PI (10 μL) was then added and incubated for an additional 5 min in the dark. Apoptosis was analyzed by flow cytometry. Each group was tested in duplicate.

Flow cytometry for cell proliferation analysis

Panc02 cells in the logarithmic phase were trypsinized to generate a single-cell suspension and seeded into 6-well plates at 3×10^5 cells/well. After adherence, cells were treated with 0, 20, 40, or 80 μmol/L baicalein in DMEM for 48 h. Cells were harvested, centrifuged at 1500 rpm for 5 min, and fixed in pre-chilled 75% ethanol overnight at 4°C. After washing with PBS and repeating centrifugation, 500 μL PI/RNase A staining solution (Kaiji Biotechnology, Jiangsu,

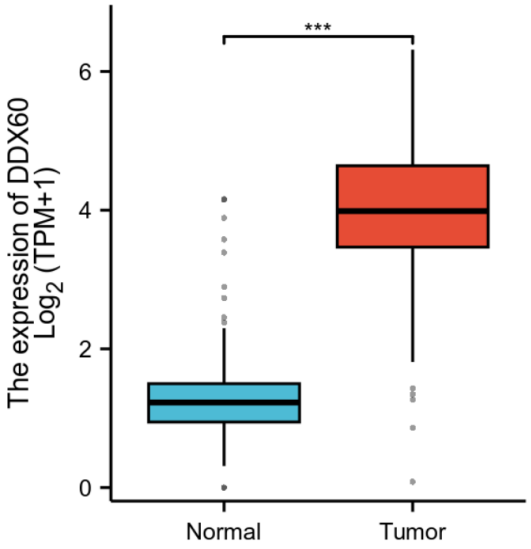

Figure 1. DDX60 expression in pancreatic cancer tissues and normal tissues (***$P < 0.001$).

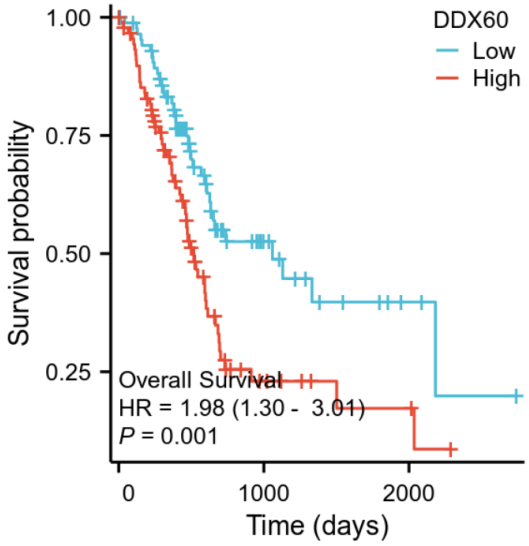

Figure 2. Analysis of DDX60 expression and patient survival prognosis.

China) was added. Cells were incubated for 30 min at room temperature in the dark. Cell cycle distribution was analyzed by flow cytometry using a 488 nm laser for red fluorescence detection. Each group included two replicates.

RT-qPCR for DDX60 mRNA expression

Panc02 cells were cultured as described above and then treated with 0, 20, 40, or 80 μmol/L baicalein for 48 h. Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions. RNA concentration and purity were assessed by UV spectrophotometry. cDNA was synthesized using a reverse transcription kit, and qPCR was performed using the following conditions: 95°C for 19 min; 40 cycles of 95°C for 15 s, 60°C for 30 s, and 65°C for 30 s. The following primers were used: DDX60: Forward: 5'-CGCAAGCCAGACAGTCCTACA-3'; Reverse: 5'-AAACATCGCCCTGTCTCACGGA-3'; GAPDH: Forward: 5'-CATCACTGCCACCCAGAAGACTG-3'; Reverse: 5'-ATGCCAGTGAGCTTCCCGTTCAG-3'.

Relative DDX60 expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the reference gene. All experiments were repeated three times.

Tumor inoculation and animal grouping

Forty SPF-grade Kunming mice were housed under controlled conditions (25°C, 55% humidity, 12 h light/dark cycle) and acclimated for 3 days. Panc02 cells were prepared at 1×10^7 cells/mL, and 0.2 mL of the suspension was injected subcutaneously under the right forelimb armpit of each mouse. Mice were monitored daily for tumor development and local reactions. Tumor dimensions (length, width, and height) were measured with calipers, and volume was calculated as: Tumor volume (V) = length × width × height; Tumor formation was defined as nodules exceeding 6 mm × 5 mm × 5 mm.

After 7 days, 34 mice developed tumors and were randomized into four groups: control (n = 8), low-dose (10 mg/kg, n = 8), medium-dose (20 mg/kg, n = 9), and high-dose (40 mg/kg, n = 9). Mice were intraperitoneally injected with 0.2 mL of either normal saline or baicalein solution once daily. Tumor volume was measured on days 7, 14, and 21. On day 21, mice were anesthetized with 4-5% isoflurane and euthanized by cervical dislocation. Tumors were excised, weighed, and the tumor inhibition rate was calculated: Inhibition rate (%) = (V control - V treatment group)/V control group × 100%.

Flow cytometry analysis of immune cells in mouse tumor tissues

Tumor tissues (~1 × 1 cm^3) from each mouse group were placed in 15 mL centrifuge tubes containing 3-5 mL digestion solution (5% DMEM, collagenase at 1 mg/mL, and DNase I at 200 μg/mL) and incubated at 37°C for 40 minutes. Digestion was stopped by adding 5

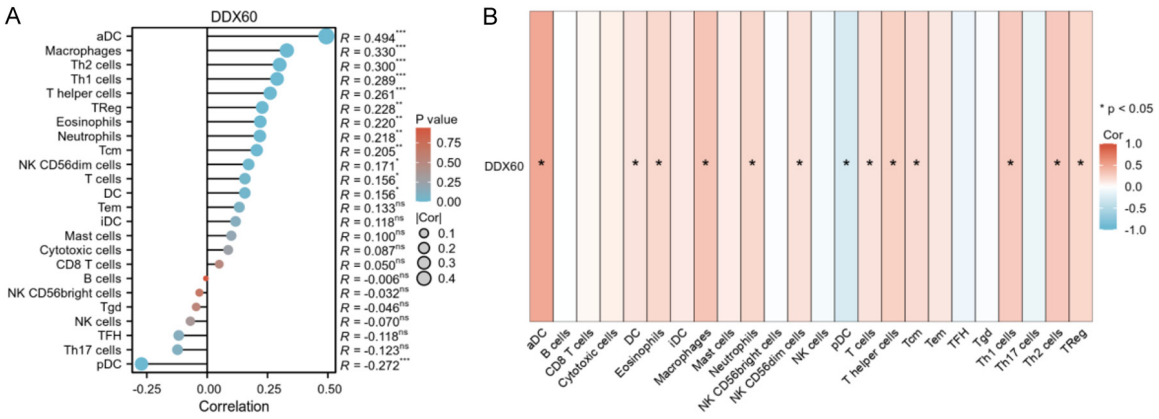

Figure 3. Relationship between DDX60 and immune infiltration in pancreatic cancer. A: Lollipop plot of immune infiltration, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. B: Heat map of immune infiltration correlation, *$P < 0.05$.

Table 1. Effects of baicalein on the growth inhibition rate of Panc02 cells (n = 5)

Concentration	24 h	48 h	96 h
0 μmol/L	0	0	0
5 μmol/L	3.72 ± 1.08*	6.72 ± 2.10*,#	8.46 ± 1.79*,#,Δ
10 μmol/L	5.82 ± 1.64*	11.23 ± 2.71*,#	13.75 ± 3.18*,#,Δ
20 μmol/L	15.23 ± 4.28*	19.83 ± 4.79*,#	30.21 ± 6.20*,#,Δ
40 μmol/L	37.41 ± 8.30*	45.62 ± 6.92*,#	57.49 ± 7.39*,#,Δ
80 μmol/L	49.54 ± 8.32*	61.95 ± 7.90*,#	65.62 ± 9.30*,#
160 μmol/L	67.32 ± 13.41*	69.49 ± 8.33*,#	71.32 ± 10.93*,#

Note: Compared to 0 μmol/L concentration at the same time, *$P < 0.05$; Compared to the same concentration for 24 h, #$P < 0.05$; Compared to the same concentration for 48 h, Δ$P < 0.05$.

mL DMEM high-glucose medium with 10% FBS. The cell suspension was filtered through a 70 μm strainer, and any remaining tissue fragments were further dissociated using a 2 mL syringe. Cells were centrifuged at 400 g for 5 minutes at 4°C and resuspended in PBS to adjust the concentration to 1×10^7 cells/mL.

Aliquots of 100 μL cell suspension were stained with viability dye (1:1000 dilution) for 10 minutes at room temperature in the dark. After washing with 1 mL PBS and centrifugation, cells were resuspended in 100 μL PBS and incubated with 5 μL surface antibodies (CD3-BUV395, CD4-PerCP-Cy5.5, CD8-Alexa Fluor 700, CD11b-FITC, F4/80-BV421) for 30 minutes at room temperature in the dark, following manufacturer's instructions. Cells were washed again and finally resuspended in 300 μL PBS for flow cytometry analysis.

Reagents used included Fixable Viability Dye eFluor™ 780 (eBioscience, USA), CD45-V500, CD3-BUV395, CD4-PerCP-Cy5.5 (BD Pharmingen, USA), CD8-Alexa Fluor 700 (eBioscience, USA), CD11b-FITC, and F4/80-BV421 (BioLegend, USA).

RT-qPCR for detection of DDX60 mRNA in tumor tissues

Tumor tissues from each group were collected, and total RNA was extracted using TRIzol reagent (Invitrogen, USA). RT-qPCR was performed to assess the relative expression of DDX60 mRNA. Experimental procedures followed the protocol described in section 1.8.

Statistical analysis

All data were analyzed using SPSS version 27.0. For measured data (mean ± SD) with a normal distribution, one-way ANOVA was performed followed by LSD-t post hoc testing. A *P*-value < 0.05 was considered significant.

Results

Comparison of DDX60 expression in pancreatic cancer and normal tissue

DDX60 expression was significantly higher in pancreatic cancer tissues compared to normal pancreatic tissues ($P < 0.05$), as shown in **Figure 1**.

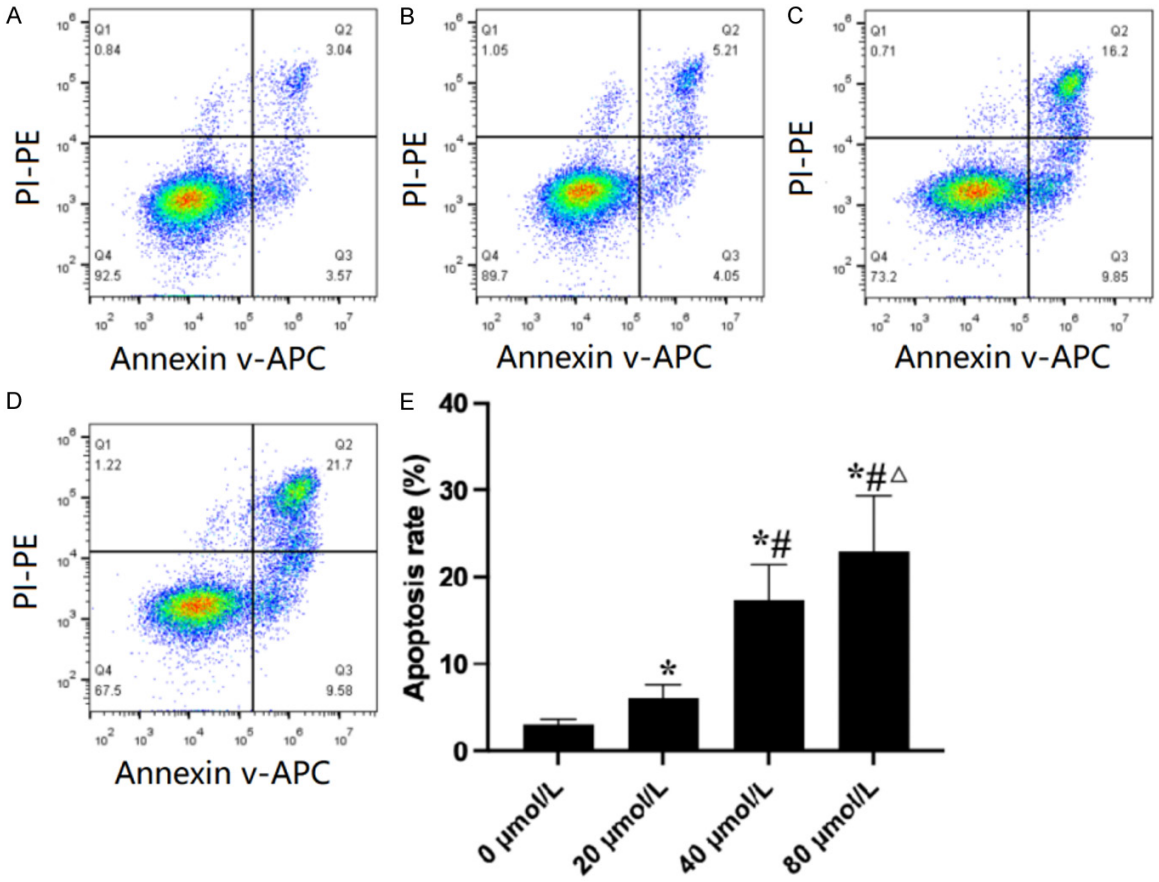

Figure 4. Effect of baicalein on the apoptosis rate of Panc02 cells. Notes: (A) 0 μmol/L baicalein; (B) 20 μmol/L baicalein; (C) 40 μmol/L baicalein; (D) 80 μmol/L baicalein; (E) Statistical plot of apoptosis rate of each concentration baicalein group (compared with 0 μmol/L group, $*P < 0.05$; Compared with 20 μmol/L group, $^{\#}P < 0.05$; Compared with 40 μmol/L, $^{\triangle}P < 0.05$).

Association between DDX60 expression and prognosis in pancreatic cancer

Survival analysis indicated that patients with low DDX60 expression had significantly better overall survival than those with high expression ($P < 0.05$), as shown in **Figure 2**.

Correlation between DDX60 expression and immune infiltration

Immune infiltration analysis revealed that DDX60 expression was significantly correlated with the infiltration of aDCs, DCs, eosinophils, macrophages, neutrophils, CD56^dim NK cells, pDCs, total T cells, T helper cells, Tcm, Th1, Th2, and Treg cells (all $P < 0.05$), as shown in **Figure 3**.

Effect of baicalein on Panc02 cell growth

Baicalein significantly inhibited the growth of Panc02 cells in a concentration- and time-dependent manner ($P < 0.05$), as presented in **Table 1**.

Effect of baicalein on apoptosis of Panc02 cells

The apoptosis rate of Panc02 cells increased significantly with rising baicalein concentrations ($P < 0.05$). Notably, the 80 μmol/L baicalein group showed a significantly higher apoptosis rate than all other groups, as shown in **Figure 4**.

Effect of baicalein on Panc02 cell proliferation

Baicalein treatment significantly reduced the proliferation of Panc02 cells in a dose-dependent manner ($P < 0.05$). As shown in **Figure 5**, the proportion of cells in the G0/G1 phase was markedly elevated in the 80 μmol/L group, while the proliferation rate was significantly lower than in other groups.

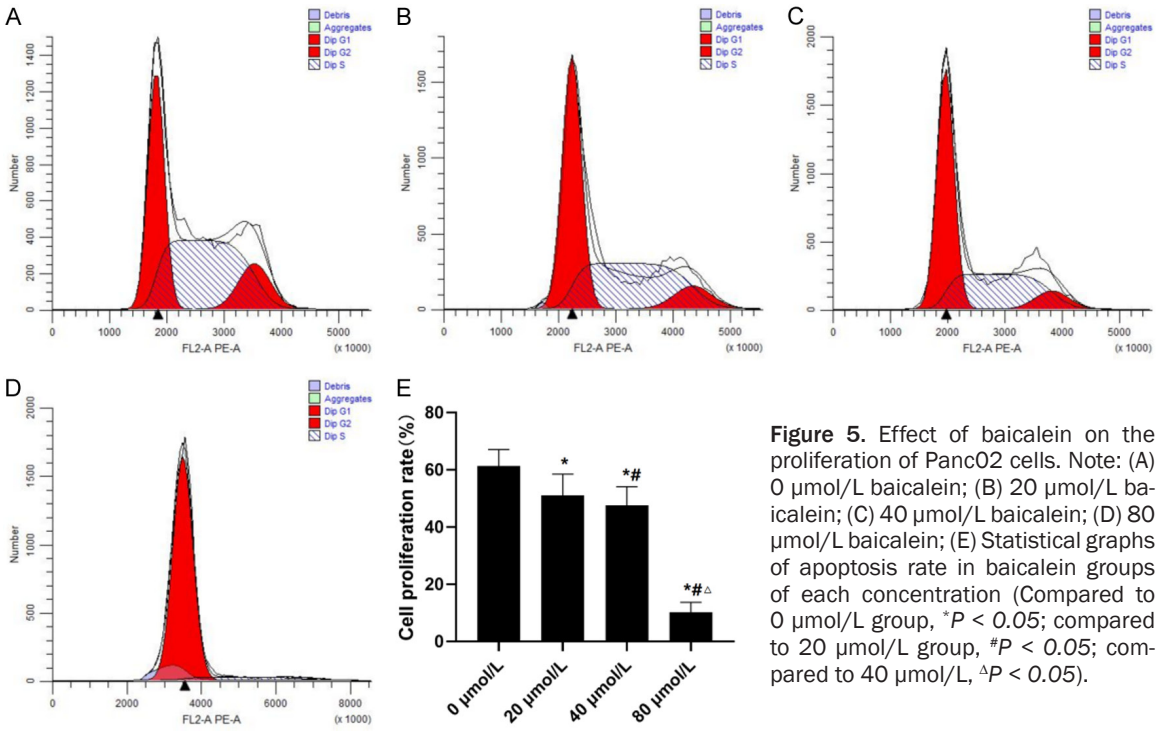

Figure 5. Effect of baicalein on the proliferation of Panc02 cells. Note: (A) 0 μmol/L baicalein; (B) 20 μmol/L baicalein; (C) 40 μmol/L baicalein; (D) 80 μmol/L baicalein; (E) Statistical graphs of apoptosis rate in baicalein groups of each concentration (Compared to 0 μmol/L group, $^{*}P < 0.05$; compared to 20 μmol/L group, $^{\#}P < 0.05$; compared to 40 μmol/L, $^{\triangle}P < 0.05$).

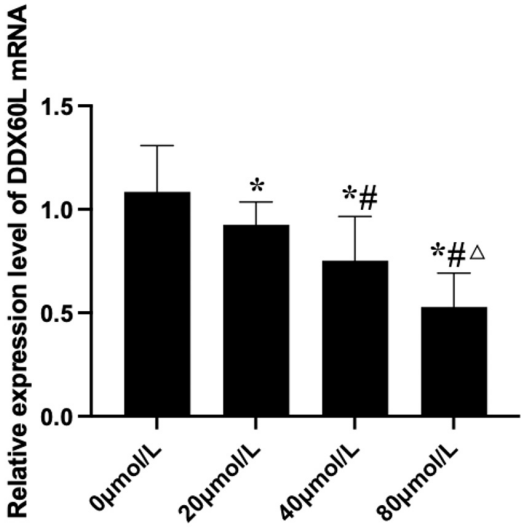

Figure 6. Effects of baicalein on the expression of DDX60 gene in Pan02 cells (Compared to 0 μmol/L group, $^{*}P < 0.05$; compared to 20 μmol/L group, $^{\#}P < 0.05$; compared to 40 μmol/L, $^{\triangle}P < 0.05$).

Effect of baicalein on DDX60 expression in Panc02 cells

DDX60 mRNA expression in Panc02 cells decreased significantly with increasing baicalein concentration ($P < 0.05$), as shown in **Figure 6**.

Effect of baicalein on tumor volume and inhibition in mice

In both the 14-day and 21-day experiments, tumor volumes in all baicalein-treated groups were significantly smaller than those of the control group ($P < 0.05$), as shown in **Tables 2**, **3**.

Effect of baicalein on tumor-infiltrating immune cells in mice

The proportions of $CD8^{+}$ T cells and macrophages in tumor tissues increased significantly with higher baicalein concentrations ($P < 0.05$), as shown in **Figures 7** and **8**.

Effect of baicalein on DDX60 expression in tumor tissues

DDX60 mRNA expression in mouse tumor tissues decreased significantly in a dose-dependent manner with baicalein treatment ($P < 0.05$), as shown in **Figure 9**.

Discussion

Pancreatic cancer is a highly aggressive malignancy of the gastrointestinal tract, characterized by an insidious onset, late clinical presentation, early metastasis, and poor prognosis. It ranks among the top ten malignancies in terms

Table 2. Changes in tumor volume of mice in each group (mm^3)

Group	7 d	14 d	21 d
Control group (n = 8)	185.23 ± 17.30	773.45 ± 60.39	1608.35 ± 249.58
Low dose group (n = 8)	191.78 ± 21.39	709.97 ± 49.32*	1329.48 ± 154.60*
Medium dose group (n = 9)	192.53 ± 18.66	598.63 ± 61.59*,#	1179.56 ± 169.80*,#
High-dose group (n = 9)	187.06 ± 20.93	476.98 ± 45.20*,#,Δ	1084.52 ± 210.93*,#,Δ

Note: Compared to the control group, $^{*}P < 0.05$; Compared to the low-dose group, $^{\#}P < 0.05$; Compared to the medium dose group, $^{\Delta}P < 0.05$.

Table 3. Comparison of tumor inhibition rate of mice in each group (%)

Group	Tumor inhibition rate
Control group (n = 8)	0
Low-dose group (n = 8)	17.84 ± 3.40*
Medium dose group (n = 9)	26.67 ± 6.94*,#
High-dose group (n = 9)	32.59 ± 7.03*,#,Δ

Note: Compared to the control group, $^{*}P < 0.05$; Compared to the low-dose group, $^{\#}P < 0.05$; Compared to the medium dose group, $^{\Delta}P < 0.05$.

of both incidence and mortality rates [6]. Despite extensive research, the pathogenesis of pancreatic cancer remains incompletely understood. Major risk factors include long-term smoking, excessive alcohol consumption, a family history of pancreatic cancer, obesity, chronic pancreatitis, and new-onset diabetes mellitus [7-9]. The etiology is multifactorial, involving complex interactions between genetic alterations, dysregulated signaling pathways, lifestyle behaviors, and environmental exposures, yet no unified conclusion has been reached. This lack of consensus is one of the primary barriers to advancing clinical research and treatment strategies for pancreatic cancer [10-12].

The TME of pancreatic cancer exhibits distinct features, including abundant extracellular matrix components, dense immune cell infiltration, extensive fibrosis, and profound immunosuppression, all of which contribute to its aggressiveness [13-15]. Immunosuppression is thought to result from the accumulation of bone marrow-derived cells - such as granulocytes, macrophages, and monocytes - within the tumor, which in turn impairs T-cell-mediated antitumor responses [16, 17]. Consequently, dysregulation of the immune microenvironment is considered a key contributor to tumor progression, and immune-based therapeutic strategies have been increasingly investigated across various malignancies [18-20].

Baicalein, a flavonoid compound extracted from the dried root of Scutellaria baicalensis, has been widely used in traditional Chinese medicine. It exhibits diverse pharmacologic activities, including antioxidant, antibacterial, anti-inflammatory, antitumor, cardioprotective, nephroprotective, and neuroprotective effects [21-23]. Baicalin is the glycosyloxyflavone that is the 7-O-glucuronide of bicalein. In the body, baicalin is hydrolyzed into baicaleinby intestinal flora, and baicalein is then subsequently reconverted into baicalinin the liver through a second metabolic transformation [24-26]. Previous studies have shown that baicaleinexerts significant antiproliferative effects in various malignancies at both the cellular and molecular levels [27-29]. Preliminary experiments in this study suggest that baicaleinexhibits notable antitumor effects against pancreatic cancer cells; however, the specific mechanisms underlying these effects remain largely unexplored.

In this study, we propose that baicalein inhibits tumor growth by modulating immune cell infiltration within the TME. Previous research has suggested that DDX60, an RNA-binding protein (RBP), maintains the stability of double-stranded RNA (dsRNA) and participates in tumor immune regulation. Modulation of DDX60 expression has been shown to affect T cell and $CD8^+$ T cell infiltration in tumor tissues of murine models [30, 31].

Based on this, we conducted a bioinformatic analysis using pancreatic cancer data from the UCSC XENA database. The results showed that DDX60 expression was significantly upregulated in pancreatic cancer tissues compared to normal tissues, and patients with low DDX60 expression exhibited significantly better survival outcomes than those with high expression. Furthermore, DDX60 expression was strongly correlated with the infiltration levels of multiple immune cell types, including aDCs, DCs, eosinophils, macrophages, neutrophils, CD56^dim

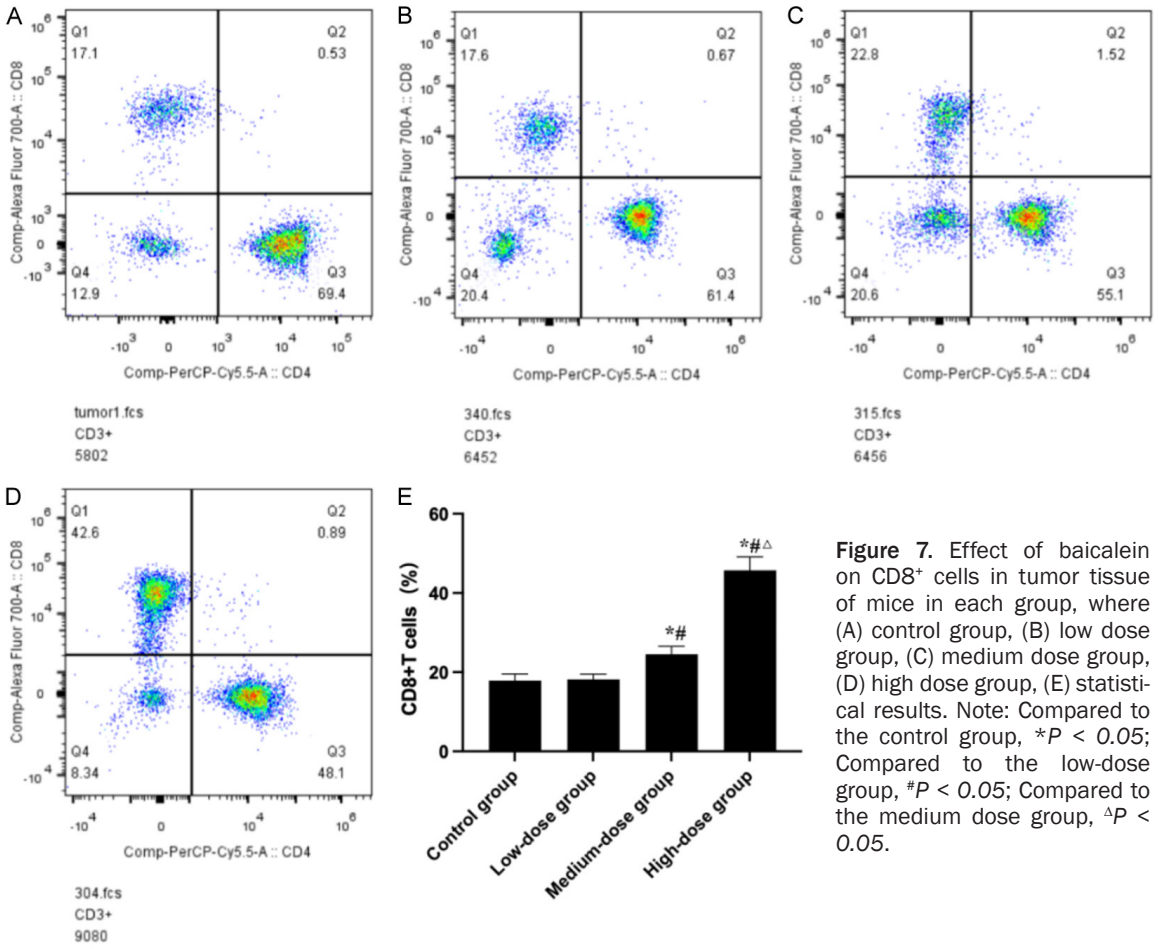

Figure 7. Effect of baicalein on CD8⁺ cells in tumor tissue of mice in each group, where (A) control group, (B) low dose group, (C) medium dose group, (D) high dose group, (E) statistical results. Note: Compared to the control group, $^{*}P < 0.05$; Compared to the low-dose group, $^{\#}P < 0.05$; Compared to the medium dose group, $^{\triangle}P < 0.05$.

NK cells, pDCs, total T cells, T helper cells, Tcm, Th1, Th2, and Treg cells. These findings suggest that DDX60 may act as an oncogene in pancreatic cancer and is closely associated with immune dysregulation.

We further investigated the antitumor effects of baicalein in cellular and animal models, and assessed its effect on immune infiltration. Preliminary findings revealed that baicalein significantly inhibited pancreatic cancer cell proliferation and subcutaneous tumor growth, which was accompanied by downregulation of DDX60 expression. Additionally, we observed a dose-dependent increase in CD8⁺ T cells and macrophages within the tumor tissues of treated mice. These results align with previous studies [32, 33], which reported that baicalin activated the proliferation of CD8⁺ T cells. These are key cytotoxic lymphocytes that eliminate malignant cells by immune activation [34, 35]. Baicalein may thus stimulate CD8⁺ T cell proliferation and recruitment by enhancing the host immune response. Similarly, macrophages - crucial innate immune cells - contribute to immune surveillance and inflammatory regulation, and their activation plays a vital role in reshaping the TME during antitumor therapy.

Based on these findings, we hypothesize that the antitumor effect of baicalein is mediated through the regulation of DDX60, enhancement of immune activation, and modulation of the tumor immune microenvironment.

However, several limitations must be acknowledged. First, this study only provided preliminary evidence that baicalein downregulates DDX60 to influence immune cell infiltration; the detailed molecular mechanism remains unclear. Second, the experimental system was limited to mouse models and the Panc02 cell line, lacking validation with clinical specimens and pharmacokinetic data. In future work, integrating single-cell RNA sequencing could help elucidate the immunoregulatory network involv-

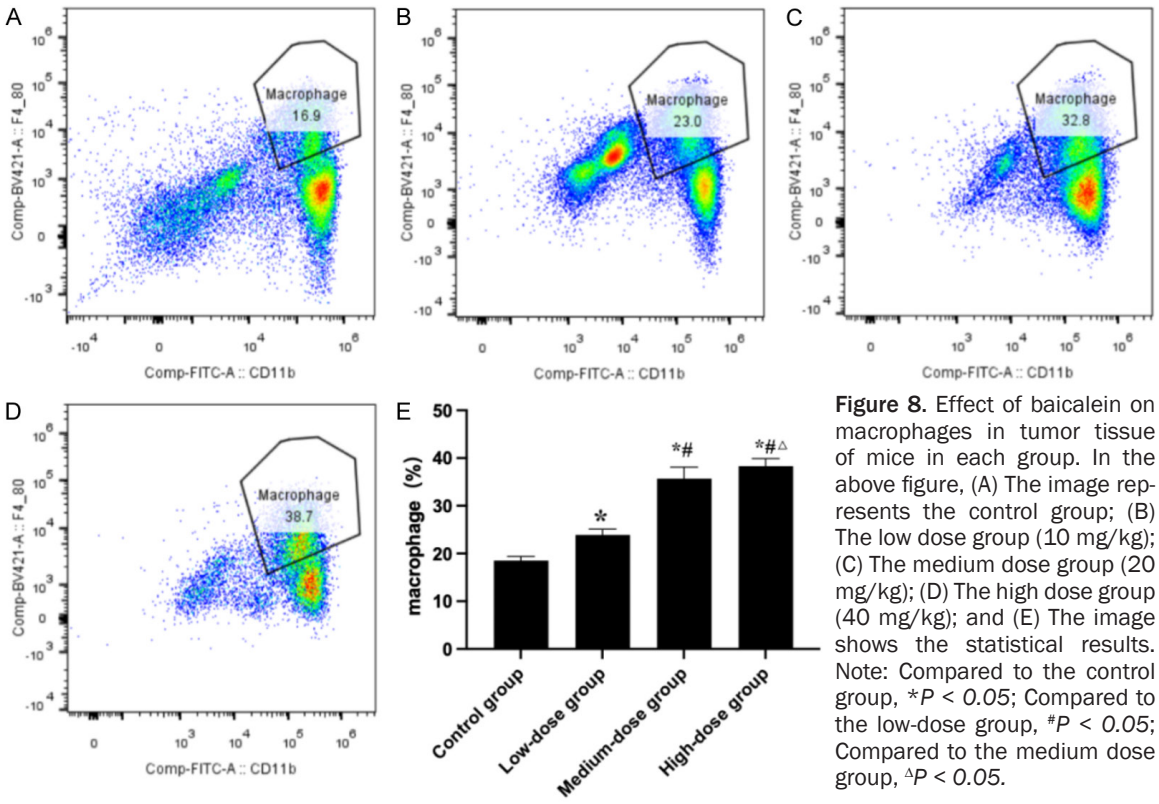

Figure 8. Effect of baicalein on macrophages in tumor tissue of mice in each group. In the above figure, (A) The image represents the control group; (B) The low dose group (10 mg/kg); (C) The medium dose group (20 mg/kg); (D) The high dose group (40 mg/kg); and (E) The image shows the statistical results. Note: Compared to the control group, $^{*}P < 0.05$; Compared to the low-dose group, $^{\#}P < 0.05$; Compared to the medium dose group, $^{\triangle}P < 0.05$.

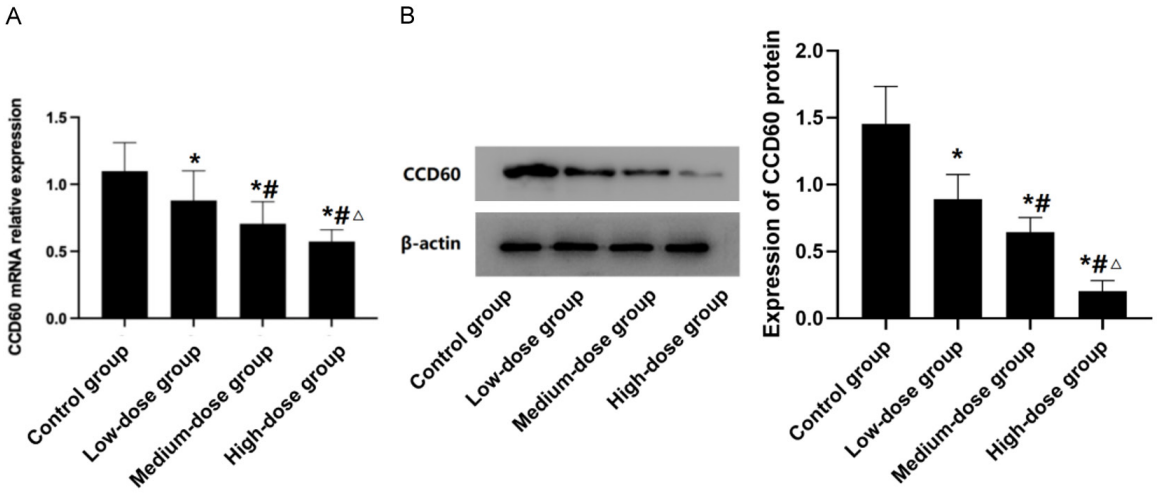

Figure 9. Effect of baicalein on macrophages in tumor tissues of different mouse groups. Note: Compared to the control group, $^{*}P < 0.05$; compared to the low-dose group, $^{\#}P < 0.05$; compared to the medium-dose group, $^{\triangle}P < 0.05$. In the figure, (A) The influence of DDX60 mRNA expression in each group of tumor tissues. (B) Statistical analysis of DDX60 protein expression and its gray value in each group of tumor tissues.

ing DDX60. Moreover, developing a baicalein-based nanodelivery system may enhance its tumor-targeting capabilities, offering a promising avenue for precision immunotherapy in pancreatic cancer.

In conclusion, baicalein effectively suppresses pancreatic cancer cell proliferation, induces apoptosis, and inhibits tumor growth *in vivo*. These effects may be mediated by downregulation of DDX60 and enhancement of immune

activation within the TME, warranting further in-depth investigation.

Disclosure of conflict of interest

None.

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References

[1] Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol 2021; 18: 493-502.
[2] Kolbeinsson HM, Chandana S, Wright GP and Chung M. Pancreatic cancer: a review of current treatment and novel therapies. J Invest Surg 2023; 36: 2129884.
[3] Stoffel EM, Brand RE and Goggins M. Pancreatic cancer: changing epidemiology and new approaches to risk assessment, early detection, and prevention. Gastroenterology 2023; 164: 752-765.
[4] Cai J, Chen H, Lu M, Zhang Y, Lu B, You L, Zhang T, Dai M and Zhao Y. Advances in the epidemiology of pancreatic cancer: trends, risk factors, screening, and prognosis. Cancer Lett 2021; 520: 1-11.
[5] Karunakaran M and Barreto SG. Surgery for pancreatic cancer: current controversies and challenges. Future Oncol 2021; 17: 5135-5162.
[6] Zhao Y, Tang J, Jiang K, Liu SY, Aicher A and Heeschen C. Liquid biopsy in pancreatic cancer - current perspective and future outlook. Biochim Biophys Acta Rev Cancer 2023; 1878: 188868.
[7] Jiang Z, Zhang W, Zhang Z, Sha G, Wang D and Tang D. Intratumoral microbiota: a new force in diagnosing and treating pancreatic cancer. Cancer Lett 2023; 554: 216031.
[8] Yoon AR, Hong J, Jung BK, Ahn HM, Zhang S and Yun CO. Oncolytic adenovirus as pancreatic cancer-targeted therapy: where do we go from here? Cancer Lett 2023; 579: 216456.
[9] Brozos-Vazquez E, Toledano-Fonseca M, Costa-Fraga N, Garcia-Ortiz MV, Diaz-Lagares A, Rodriguez-Ariza A, Aranda E and Lopez-Lopez R. Pancreatic cancer biomarkers: a pathway to advance in personalized treatment selection. Cancer Treat Rev 2024; 125: 102719.
[10] Dudley B and Brand RE. Pancreatic cancer surveillance and novel strategies for screening. Gastrointest Endosc Clin N Am 2022; 32: 13-25.
[11] Montalvo-Jave EE, Nuno-Lambarri N, Lopez-Sanchez GN, Ayala-Moreno EA, Gutierrez-Reyes G, Beane J and Pawlik TM. Pancreatic cancer: genetic conditions and epigenetic alterations. J Gastrointest Surg 2023; 27: 1001-1010.
[12] Mazer BL, Lee JW, Roberts NJ, Chu LC, Lennon AM, Klein AP, Eshleman JR, Fishman EK, Canto MI, Goggins MG and Hruban RH. Screening for pancreatic cancer has the potential to save lives, but is it practical? Expert Rev Gastroenterol Hepatol 2023; 17: 555-574.
[13] Tang R, Xu J, Wang W, Meng Q, Shao C, Zhang Y, Lei Y, Zhang Z, Liu Y, Du Q, Sun X, Wu D, Liang C, Hua J, Zhang B, Yu X and Shi S; Chinese Study Group for Pancreatic Cancer. Targeting neoadjuvant chemotherapy-induced metabolic reprogramming in pancreatic cancer promotes anti-tumor immunity and chemoresponse. Cell Rep Med 2023; 4: 101234.
[14] van Dam JL, Janssen QP, Besselink MG, Homs MYV, van Santvoort HC, van Tienhoven G, de Wilde RF, Wilmink JW, van Eijck CHJ and Groot Koerkamp B; Dutch Pancreatic Cancer Group. Neoadjuvant therapy or upfront surgery for resectable and borderline resectable pancreatic cancer: a meta-analysis of randomised controlled trials. Eur J Cancer 2022; 60: 140-149.
[15] Ulanja MB, Moody AE, Beutler BD, Antwi-Amoabeng D, Rahman GA and Alese OB. Early-onset pancreatic cancer: a review of molecular mechanisms, management, and survival. Oncotarget 2022; 13: 828-841.
[16] Barreto SG and D'Onise K. Pancreatic cancer in the Australian population: identifying opportunities for intervention. ANZ J Surg 2020; 90: 2219-2226.
[17] Yu B, Shao S and Ma W. Frontiers in pancreatic cancer on biomarkers, microenvironment, and immunotherapy. Cancer Lett 2025; 610: 217350.
[18] Attebury H and Daley D. The gut microbiome and pancreatic cancer development and treatment. Cancer J 2023; 29: 49-56.
[19] Chen L, Xing X, Zhu Y, Chen Y, Pei H, Song Q, Li J and Zhang P. Palmitoylation alters LDHA activity and pancreatic cancer response to chemotherapy. Cancer Lett 2024; 587: 216696.
[20] Hernandez D, Wagner F, Hernandez-Villafuerte K and Schlander M. Economic burden of pancreatic cancer in Europe: a literature review. J Gastrointest Cancer 2023; 54: 391-407.
[21] Sipra QUAR, Islam M, Riaz IB, Zhaohui J, Babiker HM, Bekaii-Saab TS and Sonbol MB. Contemporary management of pancreatic cancer from an internist perspective. Am J Med 2021; 134: 576-586.

[22] Jiang Z, Zheng X, Li M and Liu M. Improving the prognosis of pancreatic cancer: insights from epidemiology, genomic alterations, and therapeutic challenges. Front Med 2023; 17: 1135-1169.
[23] Guo X, Wang P, Li Y, Chang Y and Wang X. Microbiomes in pancreatic cancer can be an accomplice or a weapon. Crit Rev Oncol Hematol 2024; 194: 104262.
[24] Singhi AD and Wood LD. Early detection of pancreatic cancer using DNA-based molecular approaches. Nat Rev Gastroenterol Hepatol 2021; 18: 457-468.
[25] Park MN. Therapeutic strategies for pancreatic-cancer-related type 2 diabetes centered around natural products. Int J Mol Sci 2023; 24: 15906.
[26] Rosenzweig A, Berlin J, Chari S, Kindler H, Matrisian L, Mayoral A, Mills J, Nissen N, Picozzi V, Zelada-Arenas F and Fleming J. Management of patients with pancreatic cancer using the "right track" model. Oncologist 2023; 28: 584-595.
[27] Espona-Fiedler M, Patthey C, Lindblad S, Sarro I and Ohlund D. Overcoming therapy resistance in pancreatic cancer: new insights and future directions. Biochem Pharmacol 2024; 229: 116492.
[28] Papadakos SP, Dedes N, Pergaris A, Gazouli M and Theocharis S. Exosomes in the treatment of pancreatic cancer: a moonshot to PDAC treatment? Int J Mol Sci 2022; 23: 3620.
[29] Truong E, Pandol S and Jeon C. Uniting epidemiology and experimental models: pancreatic steatosis and pancreatic cancer. EBioMedicine 2022; 79: 103996.
[30] Tonini V and Zanni M. Early diagnosis of pancreatic cancer: what strategies to avoid a foretold catastrophe. World J Gastroenterol 2022; 28: 4235-4248.
[31] Madadjim R, An T and Cui J. MicroRNAs in pancreatic cancer: advances in biomarker discovery and therapeutic implications. Int J Mol Sci 2024; 25: 3914.
[32] Wu BU. Diabetes and pancreatic cancer: recent insights with implications for early diagnosis, treatment and prevention. Curr Opin Gastroenterol 2021; 37: 539-543.
[33] Xu J, Pham MD, Corbo V, Ponz-Sarvise M, Oni T, Ohlund D and Hwang CI. Advancing pancreatic cancer research and therapeutics: the transformative role of organoid technology. Exp Mol Med 2025; 57: 50-58.
[34] Chen Z, Wang Z, Bao H and Ma S. Gut microbiota and pancreatic cancer risk, and the mediating role of immune cells and inflammatory cytokines: a Mendelian randomization study. Front Immunol 2024; 15: 1408770.
[35] Wang S, You L, Dai M and Zhao Y. Mucins in pancreatic cancer: a well-established but promising family for diagnosis, prognosis and therapy. J Cell Mol Med 2020; 24: 10279-10289.