Prospective Cohort Study

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Clinical efficacy and chemoresistance analysis of precision neoadjuvant chemotherapy for borderline resectable pancreatic cancer: a prospective, single-arm pilot study

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Background: Neoadjuvant chemotherapy (NAC) can improve the survival outcomes of patients with pancreatic cancer, but for borderline resectable pancreatic cancer (BRPC) the proportion of conversion to surgery remains unsatisfactory. This single-arm pilot study aimed to assess the clinical efficacy and safety of NAC based on patient-derived organoids (PDOs) for BRPC.
Methods: Biopsy samples from BRPC patients were collected for generating PDOs. Gemcitabine plus nab-paclitaxel as NAC was initially administrated for one cycle, and then the treatment regimen was adjusted based on the PDO drug sensitivity testing. The primary endpoint was the objective response rate (ORR). Secondary endpoints included R0 resection rate, NAC-related adverse events (AEs), and postoperative complications. Exploratory objectives were to assess the chemoresistance to gemcitabine.
Results: Totally 19 of 25 patients were eligible for the study, among whom 16 achieved partial response and received surgical resection, with the ORR of 84.2% (16/19). The R0 resection rate was 81.3% (13/16). During NAC, 8 (42.1%, 8/19) patients experienced different grades of AEs, mainly including grade 2 myelosuppression (26.3%), cutaneous pruritus (5.3%), and diarrhea (5.3%). scRNA-seq analysis of duct cells showed that the transcriptome in aneuploid cells may affect gemcitabine resistance via multiple pathways, among which upregulation of drug-resistant genes (*OLFM4, AGR2, MUC5AC, MUC1, HMGA1, REG4, IL17RB, GCNT3, AKR1B10, ITGA6, HMGCS2*, and *SQLE*) and downregulation of sensitive genes (*SIK1, HEXIM1, SPINT2, GADD45*, and *TIMP2*) played crucial roles. Changes in the interactions between cancer cells and other cell groups may also involve in gemcitabine resistance.
Conclusion: PDO-based NAC shows a promising resectable rate in BRPC patients, with good tolerance. Potential drug-resistant and sensitive genes and cell–cell interaction changes may participate in the development of gemcitabine resistance.

Keywords: gemcitabine resistance, neoadjuvant chemotherapy, pancreatic cancer, patient-derived organoids, single-cell RNA sequencing

HIGHLIGHTS

- It is the first prospective, interventional study using patient-derived organoids (PDOs) for guiding neoadjuvant chemotherapy (NAC) regimens in pancreatic cancer.
- The resectable rate of patients is effectively improved through the PDO-based NAC.
- The transcriptome in aneuploid cells may affect gemcitabine resistance via multiple pathways.
- Changes in cell–cell interactions may participate in the development of gemcitabine resistance.

Pancreatic cancer is the seventh leading cause of cancer deaths in males and females worldwide[1]. As the most common type, pancreatic ductal adenocarcinoma (PDAC) only shows around 11% of 5-year survival rate[2]. For the preoperative assessment of nonmetastatic pancreatic cancers, three surgical stages have emerged, including resectable, borderline resectable, and unresectable. It is reported that approximately 15% of patients are assessed as resectable or borderline resectable[3]. Despite improved overall survival (OS) with adjuvant chemotherapy[4,5], there still exist around 50% of patients incapable of receiving adjuvant chemotherapy due to surgical complications, early recurrence, or clinical deterioration[6-8].

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Currently, neoadjuvant chemotherapy (NAC) has been extensively applied in clinic. Compared with upfront surgery, NAC may improve survival in resectable pancreatic cancer and borderline resectable pancreatic cancer (BRPC)[9]. Although the superiority of NAC in resectable pancreatic cancer remains conflicting, its importance in improving R0 resection rates and prognosis of BRPC patients has been confirmed. Several NAC regimens for pancreatic cancer have been recommended in clinical practice, in which GnP (gemcitabine plus nab-paclitaxel) and mFOLFIRINOX (calcium folinate, oxaliplatin, irinotecan and 5-fluorouracil) regimens are the most common. However, these empirically derived regimens may be effective for some patients but for others may be ineffective. How to choose the most effective regimen becomes one of the core concerns in the current NAC for pancreatic cancer.

Patient-derived organoids (PDOs), a kind of three-dimensional (3D) culture model, have been identified to highly preserve the histological, genetic, and molecular features of original tumors, with high potential for studying tumor biology and treatment response[10]. In previous studies, the role of PDOs in predicting response to conventional chemotherapy has been investigated, showing a high positive predictive value[11,12]. Grossman *et al* demonstrated a high correlation between the PDO drug sensitivity and clinical responses in pancreatic cancer, supporting the feasibility of PDOs in tailoring therapies to improve clinical outcomes at the patient-specific level[13]. Here, we conducted a prospective, single-arm pilot study to assess the clinical efficacy and safety of PDO-based NAC for BRPC and to evaluate the potential chemoresistance to gemcitabine commonly used in pancreatic cancer.

Methods

Study design and patients

This was a prospective, single-arm, interventional trial. Written informed consent was provided by patients before inclusion. The work has been reported in line with the strengthening the reporting of cohort, cross-sectional and case-control studies in surgery criteria[14].

Eligible patients were 18–75 years old, had histologically and/or cytologically confirmed BRPC, required for NAC based on clinical assessment, had sufficient fresh samples for organoid establishment through endoscopic ultrasonography (EUS) or EUS-guided fine needle biopsy and Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. Patients with T4 in cTNM staging, invasion of the portal vein or portal vein cancerous thrombosis were excluded. Other exclusion criteria included history of other malignancies within 5 years, severe heart diseases such as congestive heart failure, symptomatic coronary artery disease, and arrhythmia or myocardial infarction in the past 12 months[15], history of psychotropic substance abuse that cannot be abstained or psychiatric disorders. Meanwhile, 10 patients receiving NAC were selected to explore the underlying mechanism for the development of chemoresistance to gemcitabine.

Treatment

GnP (gemcitabine: 400–1000 mg/m^2, nab-paclitaxel: 100–160 mg/m^2) was initially administrated for one cycle, and then the treatment was adjusted based on the PDO drug sensitivity testing. After each cycle, the patient response was assessed according to imaging examinations and tumor markers. When there were no metastases or locally unresectable diseases using computerized tomography (CT), surgical exploration was scheduled for patients within 4–6 weeks after the last NAC. For the adenocarcinoma of the pancreatic head and uncinate process, pancreaticoduodenectomy (Whipple) should be performed[16]. For pancreatic body and tail tumors, radical antegrade modular pancreatosplenectomy was applied[17]. Additionally, standard pancreatectomy with D2 lymph node dissection was carried out in all patients after exploration of the abdominal cavity.

Postoperatively, responses to NAC were evaluated through CT scans according to Response Evaluation Criteria in Solid Tumors (version 1.1), including complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD)[18]. The objective response rate (ORR) was defined as the proportion of patients with CR and PR in all patients. Modified Ryan scoring system was used for assessing tumor regression grades (TRG), including TRG0 (no viable cancer cells), TRG1 (single cell or rare small groups of cancer cells), TRG2 (residual cancer cells with significant tumor regression but more than single cell or rare small groups of cancer cells), and TRG3 (extensive residual cancer cells without significant tumor regression)[19]. NAC-associated adverse events (AEs) were recorded based on the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0), and surgical complications were assessed according to clinical diagnosis[20].

Organoid culture

PDO establishment and drug sensitivity testing were performed strictly according to the protocols provided by Kingbio Medical (Chongqing), Co., Ltd[21]. Briefly, the tumor tissues from biopsies were first washed with precooled Jiabili® tissue cleaning fluid, and then minced and digested with advanced Dulbecco's Modified Eagle Medium (DMEM)/F12 (L330KJ, BasalMedia; Supplementary Table 1. http://links.lww.com/JS9/E37) supplemented with 10 μmol/mL of Y-27 632 (HY-10 071, MCE) and 1 mg/mL of Collagenase Type I (C0130, Sigma) at 37°C. Meanwhile, the tissues were blown 10–20 times using pipette tips until no obvious cell masses appeared. The organoid passaging medium with advanced (DMEM)/F12, 10 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) (15 630 080, Gibco), 100 U/mL penicillin-streptomycin-amphotericin B (P7630, Solarbio), and 1 × GlutaMAX (Gibco, 35 050 061) was used to terminate the digestion. Through a 100-μm sieve, the supernatant was filtered. Subsequently, the equal volume of advanced DMEM/F12 containing 2% fetal calf serum (FCS500, ExCell Bio) was added to the collected filtrate. Following centrifugation, the cell pellet was resuspended in Matrigel (354 234, Corning) and 40 μL of the suspension was inoculated in 24-well plates (662 160, Grenier bio-one). After Matrigel was solidified, Jiabili® organoid medium for pancreatic cancer was added to each well and replaced every 3 days. Notably, to improve the success rate of organoid establishment, dome-like cultures were used in our study[22].

Histopathological analysis

The organoids collected were put into a centrifuge tube, and then blown to dissolve Matrigel after adding Dispase II (17 105 041, Gibco). After the suspension was transferred to a 15-mL centrifuge tube, they were incubated in a thermostatic

oscillator (37°C, 200 rpm) for 10–15 min until no Matrigel residues. Subsequently, Accutase (A11105-01, Gibco) was added for organoid dissociation, and a series of operations including dehydration, transparency, waxdip, and embedding were performed. The sections were stained with hematoxylin and eosin (H&E), and the expression of CD68 and Ki-67 was detected using immunohistochemistry.

Drug sensitivity testing

Organoid suspension was centrifuged after placing into a constant temperature oscillator for digestion. The supernatant was discarded, Jiabili® organoid digestive juice was added until the cell masses were digested into single cells. Subsequently, 5 mL DMEM (C11995500BT, Gibco) for resuspension was determined according to the size of cell pellets. When Jiabili® organoid medium for pancreatic cancer and Matrigel were added, the suspension seeded onto 96-well plates was placed in a 37°C incubator. After 24 h, the preprepared drug solution was added, and then incubation was performed at a 37°C, CO_2 incubator. Finally, CelltiterGlo 2.0 (G9243, Promega) was added according to the manufacturer's instructions and the value was read by a microplate reader.

The concentrations for PDO-based drug sensitivity testing were established based on the blood drug concentrations of each drug, encompassing both the upper and lower limits of blood drug concentrations (Supplementary Table 2. http://links.lww.com/JS9/E37). Based on the quantification of the inhibitory effects of drugs on tumor organoid growth, a binary classification method was employed to assess the potential efficacy of the drugs. If the inhibitory rate was ≥50%, the drug was considered sensitive; if the inhibitory rate was <50%, the drug was considered resistant.

scRNA-seq analysis

The tumor sample from each patient was divided into three portions for organoid culture, histopathological, and scRNA-seq analysis, among which 10 samples were selected for scRNA-seq analysis based on the changes of tumor markers CA19-9 and tumor regression status after PDO-guided NAC, including five cases of sensitivity and five cases of resistance to gemcitabine used in NAC regimen. Totally 109 718 cells were extracted. Cells with unique molecular identifier (UMI) numbers <1000 or with detected genes <500 or with over 20% mitochondrial-derived UMI counts were considered low-quality and removed. Subsequently, the UMI count matrix was log normalized, and top 2000 variable genes were used to create potential Anchors with FindIntegrationAnchors function of Seurat. The main cell clusters were identified with the FindClusters function. Finally, cells were clustered into 13 major types, which were visualized with tSNE or UMAP plots. To identify the cell type for each cluster, we detected gene markers using the FindAllMarkers function in Seurat package (version 4.3.0), and annotated cell types using ScType tools[23].

Differentially expressed gene (DEG) and functional enrichment analysis

DEGs were determined with the FindMarkers/FindAllMarkers function. For computing DEGs, all genes were probed that the expression difference on a natural log scale was at least 0.5 and the adjusted P value was <0.05. To sort out functional categories of genes, Gene Ontology (GO) and KEGG pathways were identified using KOBAS 2.0[24]. The hypergeometric test and Benjamini-Hochberg false discovery rates were used to define the enrichment of each term.

Cell–cell communications

Cell–cell interactions based on the expression of known ligand–receptor pairs in different cell types were inferred using CellChat (version 2.1.1)[25]. To identify potential cell–cell communication networks in pancreatic cancer, we loaded the normalized counts into CellChat and selected the secreted signaling pathways, with the precompiled protein–protein interactions as the priori network information. For the main analyses, the core functions computeCommunProb, computeCommunProbPathway, and aggregateNet were applied using standard parameters and fixed randomization seeds. Additionally, Monocle2 (version 2.26.0) was performed on ductal aneuploid cells to uncover the pseudotime trajectory.

Endpoints

In this study, the primary endpoint was the ORR. Secondary endpoints included R0 resection rate, NAC-related AEs, and major postoperative complications. R0 resection referred to complete tumor resection without microscopic or macroscopic residual diseases. The exploratory endpoints included analysis of transcriptional differences in aneuploid cells and intercellular communication differences in cancer cells and other cell groups of patients with and without chemoresistance to gemcitabine.

Statistical analysis

Continuous variables were presented as the median and interquartile [M (Q1, Q3)] and compared using the Mann–Whitney U test. Categorical variables were shown as cases with percentages [n(%)] and compared by the Fisher's exact test. A two-sided $P < 0.05$ was statistically significant. Statistical analysis was performed using R package (version 4.0.2).

Results

Patient characteristics

Between June 2023 and 25 March 2024, patients were diagnosed with BRPC. Due to one case with tumors invading the portal vein, one case with myocardial infarction in the past 12 months, and four cases without performing drug sensitivity testing, finally 19 cases were included into the study. The study flow chart is described in Figure 1.

The patients enrolled in the study were all diagnosed with borderline resectable PDAC, with the median age of 59 years and median tumor diameter of 3.45 cm. Most patients were males (68.4%) and had TNM staging of grade III (73.7%). Eight patients (42.1%) suffered from arterial invasion. Except for two cases with type 2 diabetes mellitus, the remaining (89.5%) had no concomitant diseases. The baseline information of all patients included in our study are listed in Table 1.

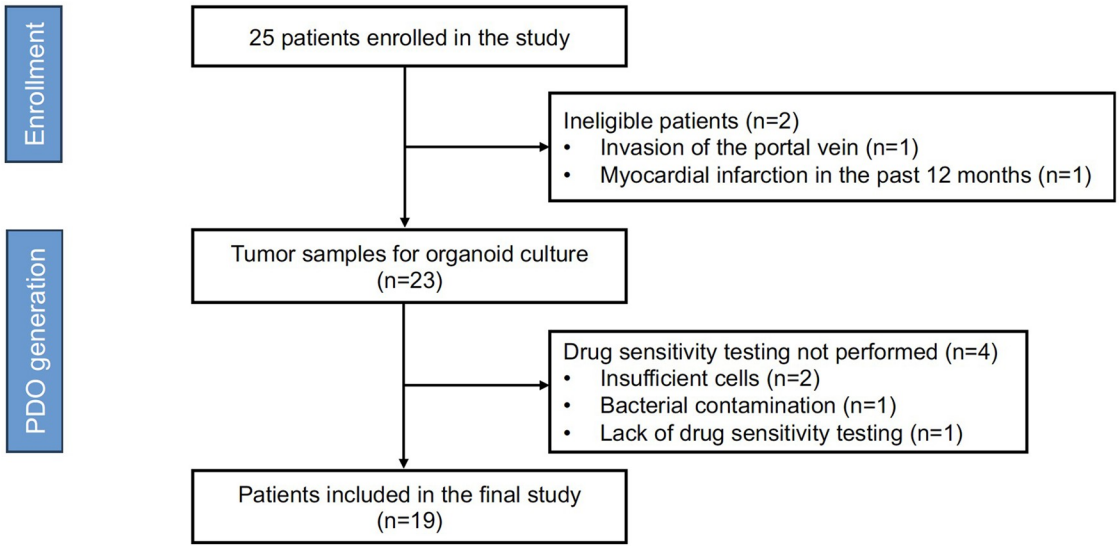

Figure 1. The flow chart of the patient selection process

PDO generation and characterization

As shown in Figure 1, a total of 23 samples were used for generating PDOs, among which 20 PDOs were cultured successfully (87.0%, 20/23) due to two samples with insufficient cells that were defined as one or fewer tumor cells in every 4X field on days 2 and 3 of plating, and one with uncontrollable bacterial contamination that was forced to terminate the culture. Notably, one PDO did not perform drug sensitivity testing owing to lack of expansion, thus it was also excluded from the study. Finally, 19 PDOs were used for drug sensitivity testing. The median culture time prior to testing was 20 days (range: 16–21 days), and the median time for performing the testing alone was 4 days. It could be observed that early organoids mainly presented as single cells or small cell clusters, but with organoid maturity, most of them showed thin-walled vesicles (Figure 2A). Additionally, a relatively homogeneous appearance was observed in the PDOs, consisting of duct cells, gland cells, multinuclear cells, glandular structures, as well as abundant vesicular/solid cell complexes. A relatively good accordance between the PDOs and original tumors was also shown regarding the expression of CD68 and Ki-67 (Figure 2B).

NAC outcomes and AEs

Totally 19 patients received NAC, with the median treatment course of 2 (range: 1–3). Notably, one died of infection of biliary tract after the initial treatment with GnP. Among the remaining cases, two did not underwent surgical resection due to PD, and 16 achieved PR and successfully received surgical resection. The ORR was 84.2% (16/19). Of the 16 patients receiving surgical resection, there were 13 cases with R0 resection and three with R1 resection, with the R0 resection rate of 81.3% (13/16) (Table 2). Importantly, the tumor diameter after NAC was 2.2 (1.9–2.8) cm, significantly lower than the 3.45 (3.0–4.5) cm before NAC (P<0.001).

During NAC, 8 (42.1%) of 19 patients experienced different grades of AEs, mainly including grade 2 myelosuppression (26.3%, 5/19), cutaneous pruritus (5.3%, 1/19) and diarrhea (5.3%, 1/19); only one patient (5.3%, 1/19) experienced grade 3 myelosuppression (Table 2). No postoperative complications occurred.

PDO drug sensitivity for tailoring NAC regimens

One of the major purposes of our study was to determine the special sensitive drugs for each patient at the individual level, thus improving the patient clinical response. Here, we presented three cases to demonstrate the importance of PDO-based NAC in pancreatic cancer.

Patient 1 was diagnosed with T2NXM0 PDAC. CT scan showed a neoplasm (3.3 × 3.1 cm) in the uncinate process of pancreas (**Figure S1A.** http://links.lww.com/JS9/E37). GnP (gemcitabine, 800 mg/m^2; nab-paclitaxel, 150 mg/m^2) was administrated for 1 cycle, during which the biopsy samples were used for PDO drug sensitivity testing. The results indicated sensitive to GnP (**Figure S1B.** http://links.lww.com/JS9/E37). Based on clinical practice and drug sensitivity results, GnP regimen continued to be used. The tumor significantly shrank after five cycles, and the level of CA19-9 was markedly decreased (**Figure S1C,** 1D. http://links.lww.com/JS9/E37). Through assessment, PR was achieved, and pancreatoduodenectomy plus radiofrequency ablation was performed.

Patient 2 experienced T2NXM0 PDAC. CT scan indicated a nodular shadow in the uncinate process of the pancreatic head and flake-like shadows close to lymph nodes and blood vessels (**Figure S2A.** http://links.lww.com/JS9/E37). During GnP (gemcitabine, 800 mg/m^2; nab-paclitaxel, 160 mg/m^2) treatment, the drug sensitivity testing was performed, indicating highly sensitive to mFOLFIRINOX regimen (**Figure S2B.** http://links.lww.com/JS9/E37). Therefore, the patient was switched to mFOLFIRINOX regimen. After treatment, the patient was assessed as PR (**Figure S2C,** 2D. http://links.lww.com/JS9/E37) and underwent R0 resection.

Patient 3 developed T2NXM0 PDAC. Through CT scan, a space-occupying lesion was found in the pancreatic body and

Table 1

Baseline information of all patients enrolled in our study

Patient No.	Age, years	Gender	Body height, cm	Body weight, kg	Preoperative radiological assessment	Vascular invasion	TNM staging	ECOG score	Concomitant diseases	CA19-9, U/mL	CA125, U/mL	CEA, ng/mL	Pre-treatment tumor diameter, cm
Patient 1	34	Male	168.0	55.0	BRPC	Veins	T2N2M0/III	0	No	120.98	24.8	1.93	3.3
Patient 2	76	Female	152.0	47.0	BRPC	Veins	T3NXM0/III	1	Type 2 diabetes	306.27	34.6	2.62	4.9
Patient 3	69	Female	164.0	51.0	BRPC	Veins	T3NXM0/III	1	Type 2 mellitus	2233.5	34.4	8.93	4.5
Patient 4	87	Male	159.0	51.4	BRPC	Arteria + veins	T2NXM0/III	0	No	883.82	29.8	5.16	3.0
Patient 5	52	Female	162.0	44.8	BRPC	Arteria + veins	T3NXM0/III	1	No	15.37	844.4	100.19	/
Patient 6	55	Male	171.0	68.9	BRPC	Veins	T2NXM0/III	0	No	9.99	10.5	1.7	3.8
Patient 7	63	Male	168.0	63.7	BRPC	Arteria	T2NXM0/III	0	No	9358.27	20	11.08	3.3
Patient 8	69	Male	160.0	54.0	BRPC	Arteria	T2NXM0/III	1	No	45.27	12.5	11.4	2.9
Patient 9	49	Male	156.5	52.0	BRPC	Veins	T2NXM0/II	0	No	186.09	12.2	1.92	2.73
Patient 10	53	Male	147.0	49.4	BRPC	Veins	T2NXM1/III	0	No	23.92	33.9	1.91	3.5
Patient 11	55	Male	181.0	71.5	BRPC	Arteria + veins	T3NXM0/III	0	No	12 000	5.7	5.28	4.8
Patient 12	59	Female	158.0	57.1	BRPC	Veins	T2NXM0/III	1	No	198.89	20.6	11.3	3.3
Patient 13	60	Male	165.0	63.0	BRPC	Veins	T2NXM0/II	1	No	30.07	15.8	13	2.3
Patient 14	53	Male	170.0	56.0	BRPC	Veins	T2NXM0/II	1	No	19.76	15.1	9.8	2.2
Patient 15	60	Male	165.0	54.0	BRPC	Veins	T2NXM0/II	0	No	1200	5.5	7.6	3.0
Patient 16	70	Female	167.0	60.5	BRPC	Veins	T2NXM0/II	1	No	978.27	84.5	7.3	3.8
Patient 17	58	Male	169.0	69.9	BRPC	Arteria	T2NXM0/III	1	No	27.21	20.8	10.28	3.4
Patient 18	72	Female	146.4	43.4	BRPC	Arteria + veins	T3NXM0/III	1	No	6.83	267.8	2.53	4.3
Patient 19	54	Male	174.5	61.9	BRPC	Arteria	T3NXM0/III	1	No	200.13	22.4	5.9	5.1

TNM staging is based on AJCC, 8th edition.

Abbreviations: BRPC, borderline-resectable pancreatic cancer; ECOG, Eastern Cooperative Oncology Group; CA, carbohydrate antigen; CEA, carcinoembryonic antigen.

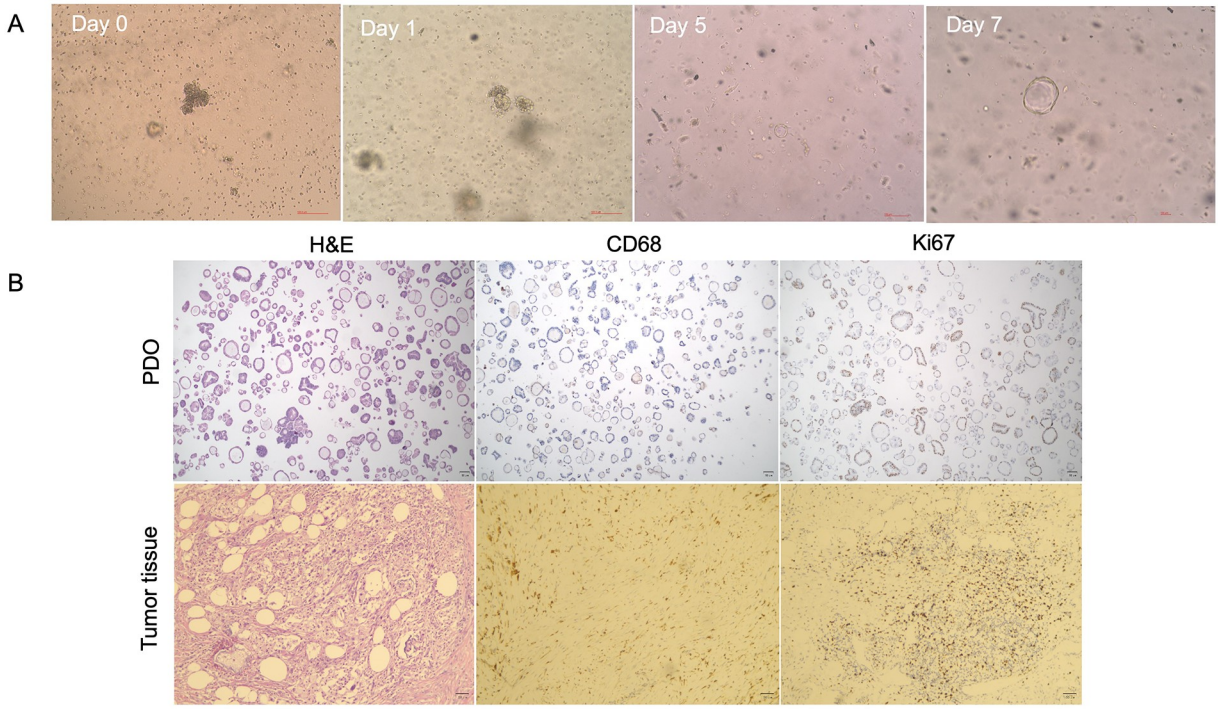

Figure 2. Changes in pancreatic cancer organoids days 0, 1, 5, and 7(A). H&E and immunohistochemical staining of CD68 and Ki-67 in PDOs and tumor tissues (B), with the magnification of × 100

tail, possibly accompanying by peripheral lymph node metastasis (**Figure S3A.** http://links.lww.com/JS9/E37). After initial treatment with GnP (gemcitabine, 400 mg/m^2; nab-paclitaxel, 100 mg/m^2), it was observed increased levels of CA 19-9, CA 15-3, and CA125, during which drug sensitivity results showed sensitive to GnP, and olaparib might be the most potential

Table 2

Clinical outcomes of all patients after treatment with PDO-based NAC

Patient No.	Treatment course	Tumor diameter after NAC, cm	Radiological assessment	Surgical types	TRG	Postoperative tumor staging	NAC-related adverse events
Patient 1	2	3.0	PR	R0	3	T2N0M0/Ib	No
Patient 2	2	3.7	PR	R1	2	T2N1M0/IIb	Grade 2 myelosuppression
Patient 3	1	4.0	PR	R0	3	T2N0M0/Ib	No
Patient 4	2	2.5	PR	R0	2	T2N1M0/IIb	Grade 2 cutaneous pruritus
Patient 5	1	/	/	No	/	/	/
Patient 6	1	2.0	PR	R0	1	T2N0M0/Ib	Grade 2 myelosuppression
Patient 7	3	2.3	PR	R0	2	T2N0M0/Ib	No
Patient 8	3	1.5	PR	R0	2	T1cN1M0/IIb	No
Patient 9	3	2.0	PR	R1	3	T2N1M0/IIb	No
Patient 10	3	1.7	PR	R0	1	T1cN1M0/IIb	No
Patient 11	3	2.8	PR	R0	2	T2N0M0/Ib	No
Patient 12	4	3.9	PD	No	/	/	Grade 2 myelosuppression
Patient 13	1	1.9	PR	R0	2	T1cN0M0/Ia	Grade 2 myelosuppression
Patient 14	1	1.6	PR	R0	2	T1cN0M0/Ia	No
Patient 15	2	2.2	PR	R0	3	T2N0M0/IIa	No
Patient 16	3	2.0	PR	R0	2	T2N0M0/Ib	No
Patient 17	1	1.0	PR	R0	1	T1cN0M0/Ia	Grade 2 myelosuppression
Patient 18	3	5.0	PD	No	/	/	Grade 3 myelosuppression
Patient 19	5	2.2	PR	R1	2	T2N0M0/IIa	Grade 2 diarrhea

Modified Ryan scoring system was used for tumor regression grades.

Abbreviations: NAC, neoadjuvant chemotherapy; PR, partial response; PD, progressive disease; TRG, tumor regression grade.

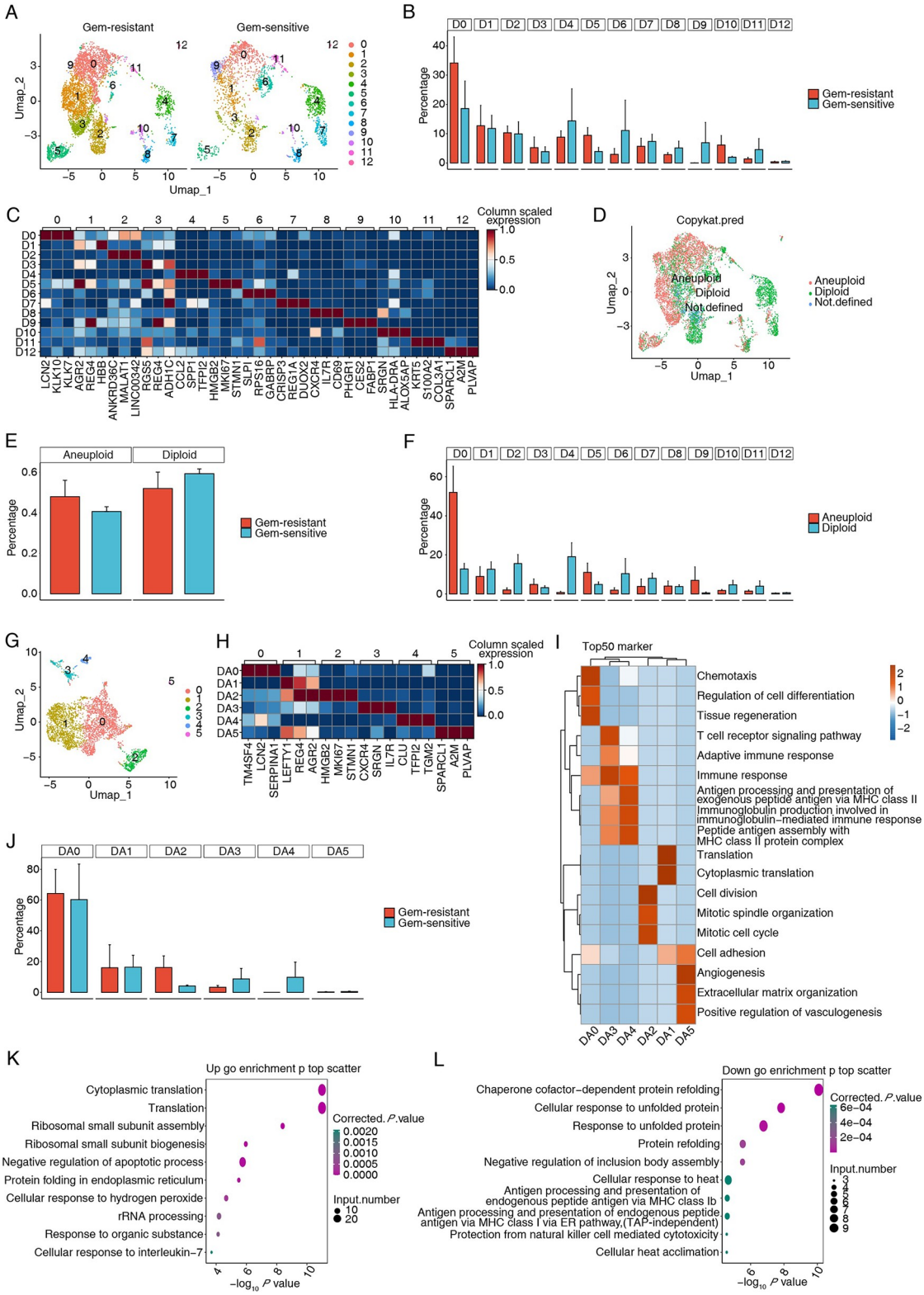

Figure 3. Unsupervised clustering analysis of duct cells and a UMAP plot of 13 duct cell clusters (A). The proportion of cells in different duct cell clusters between resistant and sensitive groups (B). A heat map showing the top three marker genes of each duct cell cluster (C). A UMAP plot showing aneuploids, diploids, and undefined ductal cells between resistant and sensitive groups (D). Proportions of aneuploids and diploids between resistant and sensitive groups (E). Proportions of aneuploids and diploids in duct cell clusters (F). Re-clustering of duct malignant cells and a UMAP plot of six aneuploid cell clusters (G). A heat map showing the top three marker genes of each aneuploid cell cluster (H). GO analysis of top 50 marker genes in each aneuploid cell cluster (I). Proportions of different aneuploid cell clusters between resistant and sensitive groups (J). GO pathways of upregulated genes (K) and downregulated genes in aneuploid cells of gemcitabine-resistant samples (L)

candidate (**Figure S3B.** http://links.lww.com/JS9/E37). Thus, olaparib was added to GnP regimen. Significant reductions in tumors and tumor markers were both observed after treatment (**Figure S3C,** 3D. http://links.lww.com/JS9/E37), and R0 resection was performed.

Transcriptional differences in aneuploid cells

Considering pancreatic duct cells in PDAC were the primary cells for cancer transformation, we extracted 8104 duct cells. Through clustering analysis, 13 duct cell clusters were obtained (Figure 3A). It could be observed that D0 cluster was significantly enriched in the gemcitabine-resistant group (Figure 3B), and top three markers for each cluster were presented in Figure 3C. Subsequently, we used copyKAT to classify duct cells into aneuploids, diploids, and undefined ductal cells (Figure 3D), and found a higher proportion of aneuploids in the resistant group, suggesting a stronger cancer cell enrichment (Figure 3E). Additionally, the proportion of aneuploids in D0 cluster was also extremely high (Figure 3F).

To explore the mechanism of gemcitabine resistance in cancer cells, we extracted all aneuploids from duct cells and obtained six clusters (Figure 3G), and most of the marker genes were specifically expressed in the corresponding duct aneuploid clusters (Figure 3H). GO analysis of the top 50 marker genes showed DA0 cluster was mainly enriched in the chemotaxis, regulation of cell differentiation and tissue regeneration pathways; DA3 and DA4 clusters were enriched in various immune-related pathways; DA2 cluster was enriched in the mitotic cell cycle pathway; DA1 cluster was enriched in translation and cytoplasmic translation, and DA5 cluster was enriched in the pathways including cell adhesion, angiogenesis, extracellular matrix organization, and positive regulation of angiogenesis (Figure 3I). Moreover, there were some differences between resistant and sensitive groups regarding the proportions of DA2 cluster and DA3, DA4 clusters, unveiling that variations in the proportion of these clusters may be associated with gemcitabine resistance (Figure 3J). Subsequently, we analyzed DEGs from aneuploid cells in the two groups. GO analysis revealed that upregulated genes were mainly enriched in the pathways including negative regulation of apoptosis, protein folding in endoplasmic reticulum, and cellular response to interleukin-7 (Figure 3K); downregulated genes were enriched in the pathways like protein refolding, antigen processing, and presentation of endogenous peptide antigens via MHC class Ib and via MHI class I via ER pathway (Figure 3K). By screening these DEGs, we found that the expressions of *OLFM4, AGR2, MUC5AC, MUC1, HMGA1, REG4, IL17RB, GCNT3, AKR1B10, ITGA6, HMGCS2*, and *SQLE* were upregulated (**Figure S4A.** http://links.lww.com/JS9/E37), while those of *SIK1, HEXIM1, SPINT2, GADD45*, and *TIMP2* were downregulated in gemcitabine-resistant duct aneuploid cells (**Figure S4B.** http://links.lww.com/JS9/E37). These findings suggest that D0 cluster may be the major gemcitabine-resistant duct aneuploid cluster in pancreatic cancer, and the transcriptome in aneuploid cells may affect gemcitabine resistance via multiple pathways, among which upregulation of drug-resistant genes and downregulation of sensitive genes may play important roles.

Intercellular communications between cancer cells and other cell groups

By defining all duct aneuploid cells as cancer cells, we constructed the potential interaction between cancer cells and other cell groups of the resistant and sensitive samples using CellChat and observed the changes of the interaction in the development of resistance to gemcitabine. Compared with the sensitive group, the number and intensity of cell–cell interaction signals were significantly increased in the resistant group (Figure 4A, B), and cancer cells interacted with various other cell subgroups (Figure 4C). Regarding information flow in the important signaling pathways of cell–cell interactions, we found that the intensity of SELE, ANGPLT, CLDN, and PLAU signaling pathways was significantly upregulated, while that of CypA, CD45, and ICAM pathways was downregulated in the resistant group (Figure 4D). Meanwhile, the resistant group showed increased number and intensity of the interaction between cancer cells and other cells (Figure 4E). Subsequently, through extraction of the ligand-receptor pathways of cancer cells as ligand cells, we observed that during the interaction between cancer cells and various other cells in the resistant group, the ligand-receptor pathways like PPIA-BSG, ANXA2-FPR2, and KITLG-KIT were upregulated, while those including HLA-A/HLA-B/HLA-C-CD8A were downregulated (Figure 4F, G). When the ligand-receptor pathways of cancer cells as recipient cells were extracted, we found in the interaction between cancer cells and various other cells in the resistant group, the ligand-receptor pathways such as APP-CD74, LAMB1-ITGA6_ITGB1, and LAMB1-CD44 were upregulated, but those with SDC1 as the receptor like COL1A1-SDC1, FN1-SDC1, and THBS2-SDC1 were downregulated (Figure 4H-J). Overall, the interactions between cancer cells and other cells may be involved in gemcitabine resistance.

To understand the changes in cancer cells from sensitivity to resistance, we performed a pseudotime analysis on all duct aneuploid cells, which showed significant differences between resistant and sensitive groups (**Figure S5A.** http://links.lww.com/JS9/E37). Duct aneuploid cells were classified into three branches (S1-S3; **Figure 52B**). For each branch, top three marker genes in cells were shown in **Figure S5C** (http://links.lww.com/JS9/E37). S2 branch was mainly enriched in the resistant group, while S3 was in the sensitive group (**Figure S5D.** http://links.lww.com/JS9/E37). GO analysis of the top 50 marker genes in the S1–S3 branches exhibited that S3 branch was primarily enriched in the pathways like tight junctions, fat digestion and absorption, and leukocyte transendothelial migration; S1 branch was enriched in the antigen processing and presentation, and IL17 signaling pathways; S2 branch was primarily enriched in the ribosome pathway, suggesting that the transformation of resistance to gemcitabine in cancer cells may be related to immunity (**Figure S5E.** http://links.lww.com/JS9/E37). Additionally, we analyzed the dynamic expression of the branch-related regulatory genes based on the branch expression analysis model (**Figure S5F.** http://links.lww.com/JS9/E37). Among the genes with expression changes in the process of branch differentiation, *AGR2, MMP7, OLFM4, NR4A1*, and *CDKN1A* might be related to the differentiation of S1 into the drug-resistant S2 branch or sensitive S3 branch (**Figure S5G.** http://links.lww.com/JS9/E37).

Discussion

To the best of our knowledge, this was the first prospective, single-arm pilot study to assess the efficacy and safety of PDO-based NAC for BRPC, and the results demonstrated improved resectable

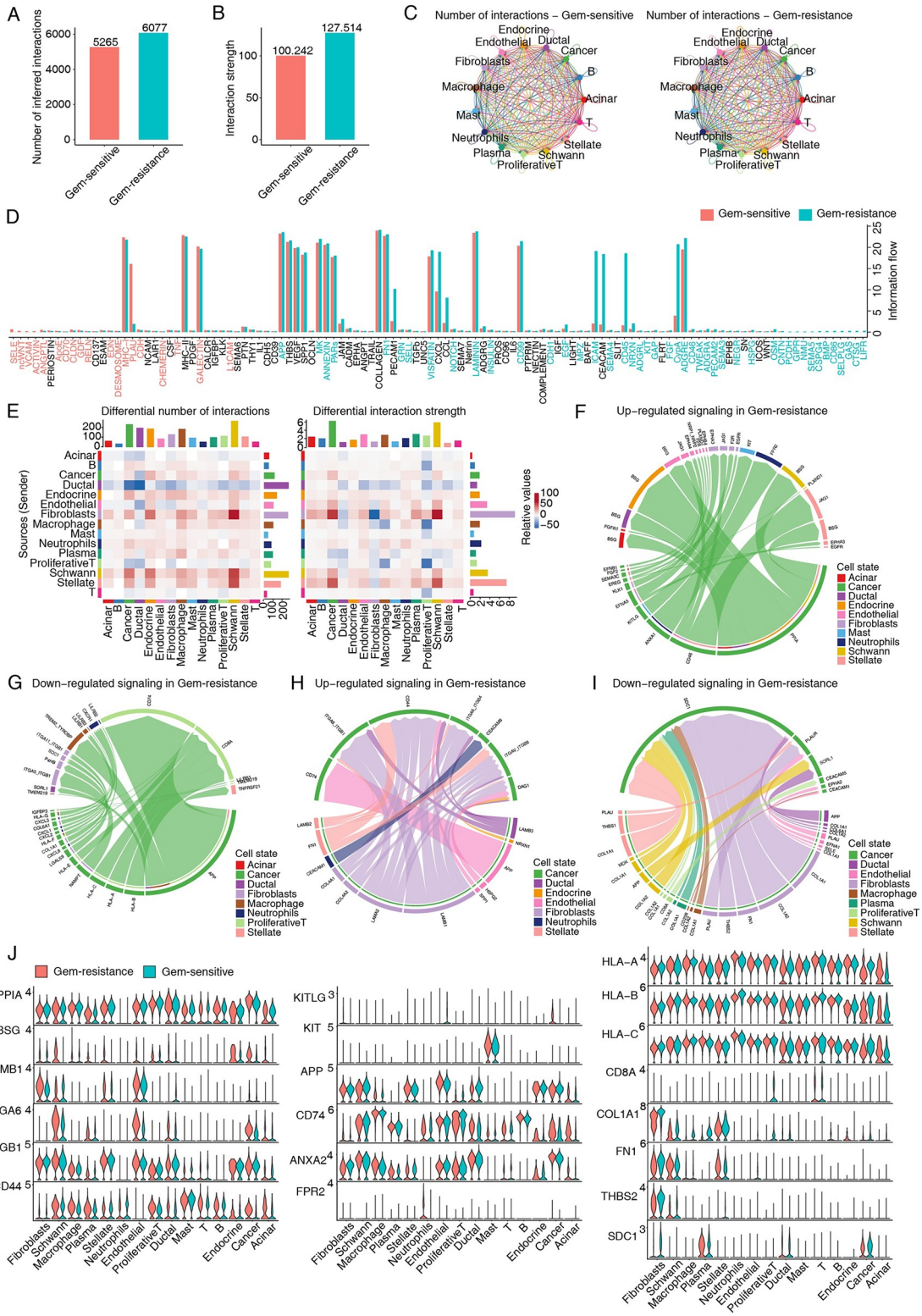

Figure 4. The total number and intensity of cell–cell interaction signals between cancer cells and other cell populations in the resistant and sensitive groups (A and B). (C) Communications between cancer cells and other cell populations using CellChat (C). Proportion of the overall information flow in the important signaling pathways of cell–cell interactions (D). The overall information flow for a signal network is calculated by summarizing all the communication probabilities in that network. The number and intensity of interactions between cancer cells and other cell groups based on a heat map (E). The upregulated and downregulated cancer cells as the ligand-receptor pathways of ligand cells (F and G) and as the ligand-receptor pathways of receptor cells (H and I) in the resistant group via chord diagrams. The expression of differential ligand-receptor pathway genes based on violin diagrams, including *PPIA-BSG, ANXA2-FPR2, KITLG-KIT, HLA-A/HLA-B/HLA-C-CD8A, APP-CD74, LAMB1-ITGA6_ITGB1, LAMB1-CD44, COL1A1-SDC1, FN1-SDC1*, and *THBS2-SDC1*

rates in BRPC, with good tolerance to AEs. Through scRNA-seq analysis, we identified potential genes resistant and sensitive to gemcitabine, as well as the possible cell–cell interactions in the development of chemoresistance to gemcitabine.

In our study, the ORR as the primary endpoint was up to 84.2%. Meanwhile, the R0 resection rate reached 81.3%, significantly higher than 64.7% reported in a previous study that used neoadjuvant GnP or FOLFIRINOX[15]. Currently, there are various NAC regimens for pancreatic cancer[26-30], among which FOLFIRINOX/mFOLFIRINOX and GnP regimens present the most clinical evidence and are also the first-line treatment strategies recommended by the NCCN guidelines for pancreatic cancer[31]. The NAC with these two regimens has been demonstrated to be feasible and well-tolerated in pancreatic cancer, showing favorable survival outcomes[32,33]. However, there are lack of the randomized controlled trials comparing the neoadjuvant efficacy of GnP with FOLFIRINOX/mFOLFIRINOX. In a real-world study, FOLFIRINOX regimen was superior to GnP in clinical efficacy, but with higher standards for patients' physical conditions, which was more likely to cause the adjustment of treatment regimens due to intolerance to chemotherapy[34]. Based on the clinical practice, in our study GnP was routinely selected as the initial NAC for pancreatic cancer. Despite the presence of potentially increased efficacy, FOLFIRINOX is associated with elevated toxicities[35].

Over the past decade, PDOs have been identified to be a superior pre-clinical model for drug screening and prediction of cancer treatment responses[36-38]. They allow for biomarker discovery and drug screening to identify potential drugs at the patient-specific level, thus offering personalized treatment for patients[39,40]. Several studies have retrospectively tested the feasibility of PDOs in predicting the response to conventional chemotherapy[11-13]. Based on the organoid gene expression signatures of chemosensitivity, Tiriac *et al.* classified the pancreatic cancer patients into gemcitabine-sensitive and -resistant groups, and found that the patients sensitive to gemcitabine had a significantly better progression free survival[12]. To better predict the clinical outcomes of pancreatic cancer patients, Grossman *et al.* developed a method for classifying PDOs as sensitive or resistant to chemotherapy regimens, and PDOs from different patients showed a spectrum of sensitives to the drugs tested, including gemcitabine, paclitaxel, 5-Fu, etc[13]. These findings highlight the association of PDO drug sensitivity testing and clinical response at the individual level, and the promise of using PDO drug sensitivity data to improve response rates and minimize toxicity by avoiding noneffective drugs. In this study, we first utilized the PDO drug sensitivity to tailor the NAC options in patients with BRPC and demonstrated promising resectable rates. Importantly, no serious AEs occurred during NAC. Additionally, our results also exhibited that the PDO-based NAC could also increase the use of targeted drugs to ameliorate the treatment response. In a recently published consensus, the PDO-based drug sensitivity testing has been recommended to guide targeted therapy in cancer[41].

Through clustering of duct cells and classification of aneuploids and diploids using CopyKAT, we identified the genes resistant to gemcitabine in aneuploid cells of pancreatic cancer, including *OLFM4, AGR2, MUC5AC, MUC1, HMGA1, REG4, IL17RB, GCNT3, AKR1B10, ITGA6, HMGCS2*, and *SQLE*. HMGA1 can promote chemoresistance to gemcitabine in pancreatic adenocarcinoma via the Akt-dependent mechanism[42]. The high expression of OLFM4 participated in chemoresistance in pancreatic cancer and was associated with poor survival outcomes[43]. AGR2 can promote the dissemination of pancreatic cancer cells, and its knockdown can induce cell apoptosis and decrease chemoresistance[44,45]. Other genes like *MUC1, IL17RB*, and *HMGCS2* have also been reported to induce drug resistance in pancreatic cancer via diverse mechanisms[46-48]. Moreover, the expression of *SIK1, HEXIM1, SPINT2, GADD45*, and *TIMP2* was downregulated in aneuploid cells of pancreatic cancer, which may play inhibitory effects in the progression of pancreatic cancer[49-52] and can be sensitive genes to gemcitabine. These findings further suggest that both the drug-resistant genes and drug-sensitive genes identified in our study may be useful markers for evaluating pancreatic cancer progression and may serve as promising candidates for targeted therapies in pancreatic cancer. By exploring the potential of cell–cell interactions in the development of chemoresistance to gemcitabine, we showed that the ligand-receptor pathways that interacted between cancer cells whether as ligand or receptor cells and other cells were significantly different between gemcitabine-resistant and gemcitabine-sensitive patients, such as APP-CD74, LAMB1-ITGA6_ITGB1, LAMB1-CD44 and more, suggesting these cell–cell interactions may involve in the development of chemoresistance to gemcitabine. In pancreatic cancer cells, suppression of APP processing is conductive to reinforcing gemcitabine-mediated cytotoxicity, and meanwhile CD74 could facilitate a pro-inflammatory tumor microenvironment through induction of S100A8 and S100A9 secretion[53,54]. ITGA6 and RPSA can promote the invasion and metastasis of pancreatic cancer synergistically through PI3K and MAPK signaling pathways[55]. Furthermore, ITGB1 is also found to be a potential drug target for pancreatic cancer[56].

One of the limitations in our study is a single-arm design, lacking randomized comparison with the control arm, which may influence the superiority of PDO-guided NAC over standard chemotherapy. Despite being a prospective establishment of the PDOs as a potential platform for tailoring NAC, the number of evaluable patients was limited, possibly affecting the statistical testing power. In the future, we will further conduct large-scale randomized controlled studies to verify our findings. Notably, due to presence of hysteretic nature in the PDO drug sensitivity testing report, the empirically derived NAC with GnP was initially used in clinic practice, which may affect our results to some extent. The prognostic data utilized to evaluate the effect of PDO-guided NAC on the patient survival outcomes, such as overall survival or disease-free survival, were also missing, which would be further provided in our subsequent studies if the research continues as planned.

Conclusion

PDO-based NAC shows a promising resectable rate in BRPC patients, with good tolerance, which may provide some novel sights into the selection of NAC options in pancreatic cancer. Potential drug-resistant and sensitive genes and cell–cell interaction changes may involve in the development of chemoresistance to gemcitabine.

Ethical approval

The protocol was approved by the Institutional Review Board of The Second Affiliated Hospital of Army Medical University

(No.: 2023-076-01). The trial was conducted in accordance with the principles of Declaration of Helsinki and Good Clinical Practice guidelines.

Consent

All patients gave written informed consent. All authors have read and approved of its submission to this journal.

Author contributions

Y.G.H., Y.N.Z., W.W.W., X.B.H. and L.Z. participated in study conception and design. Y.Y.Y. and Z.W. were responsible for data acquisition, data analysis, and interpretation. C.Y.Z. and J.L. provided technical and material support. Y.G.H., Y.N.Z., and W.W.W. drafted the manuscript. L.Z. and X.B.H. critically revised the manuscript. All authors reviewed the manuscript and agreed to submit it for publication.

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Conflicts of interest disclosure

The authors declare that they have no conflict of interests.

Data availability statement

The datasets used during this study are available from corresponding authors upon reasonable request.

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