FOLFIRINOX Versus Gemcitabine-based Therapy for Pancreatic Ductal Adenocarcinoma: Lessons from Patient-derived Cell Lines

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Abstract. *Background/Aim:* FOLFIRINOX [fluorouracil (5-FU), irinotecan, oxaliplatin] and gemcitabine plus nab-paclitaxel are standard treatments for patients with pancreatic ductal adenocarcinoma (PDAC). Despite efficacy rates of less than 32%, evidence is lacking to guide the use of one drug over the other. Herein, we compared the sensitivity of patient-derived PDAC cell lines to each of these regimens. *Materials and Methods:* Changes in the growth of 19 low-passage patient-derived PDAC cell lines were evaluated in response to treatment with FOLFIRINOX and gemcitabine plus paclitaxel (Gem-Pac). *Results:* Six cell lines exhibited optimal sensitivity (high E_{Max} and low GI_{50}) to FOLFIRINOX and three cell lines exhibited optimal sensitivity to Gem-Pac. Several cell lines that were optimally sensitive to one drug regimen exhibited very poor response to the other. *Conclusion:* Further characterization of cancer cells exhibiting preferential sensitivity to each of these regimens may allow the identification of biomarkers to guide the selection of appropriate chemotherapy for a given patient.

Over the last decade, significant progress has been made in the treatment of pancreatic ductal adenocarcinoma (PDAC). While complete surgical resection remains the only curative treatment, advances in chemotherapy have helped improve patient outcomes and median overall survival. Currently, the two most commonly employed regimens are FOLFIRINOX and gemcitabine plus nanoparticle albumin-bound (nab) paclitaxel (Abraxane); 31.6% and 23% of patients who undergo neoadjuvant treatment with these agents, respectively, demonstrate a response to therapy (1, 2).

FOLFIRINOX is a combination therapy consisting of fluorouracil (5-FU), irinotecan, oxaliplatin, and a drug that enhances the efficacy of 5-FU, leucovorin. FOLFIRINOX targets DNA replication, at least in part, by cross-linking DNA (oxaliplatin) and inhibiting the activities of thymidylate synthase (5-FU) and topoisomerase 1 (irinotecan) (3). In contrast, gemcitabine and paclitaxel target unique pathways: gemcitabine is a self-potentiating nucleoside analog that functions to inhibit DNA synthesis, and paclitaxel stabilizes microtubules leading to a defective mitotic process and cell death (4, 5). While paclitaxel was initially modified with albumin to eliminate the need for an allergy-inducing solvent required for taxol solubility, albumin may also enhance delivery to stroma-rich tumors and increase gemcitabine concentrations inside cancer cells (6).

Both combination therapies have become standard treatments in patients with advanced pancreatic cancer. In the neoadjuvant setting, they are associated with improved tumor down-staging, converting initially unresectable tumors into successful resections with R0 margins (1, 2, 7). However, no clear evidence exists to support the use of one drug regimen over the other in any given patient (8-12). Since less than a third of patients exhibit a response to these treatments, the

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ability to identify a preferential response to one drug regimen versus another for a given patient would be highly desirable. The aim of this study was to evaluate and stratify the sensitivity of low-passage PDAC cell lines derived from primary and metastatic tumors to these regimens and investigate the role of the tumor microenvironment in cell line sensitivity.

Materials and Methods

Chemotherapeutic preparation. Irinotecan, 5-FU, oxaliplatin, leucovorin, gemcitabine, and paclitaxel were obtained from Selleckchem (Houston, TX, USA). The drugs were dissolved in dimethyl sulfoxide (DMSO) or nuclease-free water and stored at -80°C . Drugs constituting FOLFIRINOX and gemcitabine plus paclitaxel (Gem-Pac) were combined in the following molar ratios analogous to those used in patients; FOLFIRINOX: 1.00 irinotecan, 80.95 5-FU, 0.80 oxaliplatin, 1.07 leucovorin; Gem-Pac: 1.00 gemcitabine, 0.04 paclitaxel (2, 13).

Patient samples and clinical data. Cell lines were established from xenograft tumors derived from surgically resected primary and metastatic PDAC tumors. The chemosensitivities of 19 patient-derived low-passage PDAC cell lines were tested. Patient clinical data corresponding to each PDAC cell line was derived from a prospective clinical database. Patients gave written, informed consent and the study was approved by our institutional review committee.

Cell culture. Cells were maintained in a humidified incubator at 37°C with 5% CO_2 and grown in DMEM/F-12 media containing 10% fetal bovine serum and 1% antibiotic-antimycotic. Conditioned media was prepared by plating cells at 3.0×10^6 cells per 10-centimeter cell culture dish. Media was changed 24 h after plating and conditioned media were collected after an additional 24 h of incubation. Conditioned media was cleared by centrifugation at 1,000 RPM for five minutes at 4°C prior to storage at -80°C .

Cellular growth evaluation. Cell lines were plated in triplicate on 96-well plates at a density of 2,000 cells per well. After 24 h of incubation, serial dilutions of FOLFIRINOX (ranging from 6×10^{-5} μMol to 0.4 μMol for irinotecan, 5×10^{-3} μMol to 32.3 μMol for 5-FU, 5×10^{-5} μMol to 0.32 μMol for oxaliplatin, and 6×10^{-5} μMol to 0.42 μMol for leucovorin), Gem-Pac (ranging from 5×10^{-6} μMol to 0.31 μMol for gemcitabine and 2×10^{-7} μMol to 0.014 μMol for paclitaxel), gemcitabine alone (ranging from 4×10^{-3} μMol to 1.25 μMol), and paclitaxel alone (ranging from 2×10^{-7} μMol to 0.014 μMol) were added to the cells. These concentrations were selected based on the prior irinotecan reference concentration used to treat human colon cancer cell lines, LoVo (14). In a clinical setting, the 5-FU component of FOLFIRINOX is administered in an initial bolus followed by a continuous IV infusion over 46 h, whereas, here, the chemotherapeutics were consolidated into a single administration. After five days of incubation, cell viability was assessed with resazurin-based assays using a SpectraMax M5 plate reader. At least three independent experiments were conducted for each cell line.

Data analysis. Statistical analysis was performed using the GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, CA, USA). The maximum growth inhibition (E_{Max}) achieved at the

highest concentration of drug tested and the concentration of drug needed to achieve 50% cellular growth inhibition (GI_{50}) of each cell line in response to chemotherapeutic treatment was determined from the dose-response data of at least three separate experiments performed in triplicate. Statistical significance was determined using Student's t-test, log-rank test, and Pearson's correlation using GraphPad Prism 7.0 software. Significance was defined as $p < 0.05$.

Evaluation of drug interactions. The interaction between gemcitabine and paclitaxel was evaluated by isobolographic analysis, a dose-oriented geometric method of assessing drug interactions (15). The combination index (CI) values were calculated based on the following equation:

$$CI = [(GI_{50} \text{ of Gem-Pac relative to Gemcitabine}) / (GI_{50} \text{ of Gemcitabine Alone})] + [(GI_{50} \text{ of Gem-Pac relative to Paclitaxel}) / (GI_{50} \text{ of Paclitaxel Alone})]$$

By this calculation, $CI > 1.3$ indicates antagonism, $CI = 1.1$ -1.3 indicates moderate antagonism, $CI = 0.9$ to 1.1 indicates an additive effect, $CI = 0.8$ -0.9 indicates slight synergism, $CI = 0.6$ -0.8 indicates moderate synergism, $CI = 0.4$ -0.6 indicates synergism, and $CI = 0.2$ -0.4 indicates strong synergism. The interaction between 5-FU, irinotecan, oxaliplatin, and leucovorin was not evaluated, since monotherapy with each of these agents is not of clinical interest.

Results

Evaluation of PDAC cell line chemosensitivity. We exposed 19 low-passage patient-derived PDAC cell lines (Table I) to increasing doses of FOLFIRINOX and gemcitabine plus paclitaxel (Gem-Pac) to investigate the inherent sensitivities of the cell lines. Representative dose-response curves for six cell lines are shown in Figure 1A. The maximum growth inhibition (E_{Max}) achieved at the highest concentration of drug tested was calculated and the cell lines were defined as having high ($>75\%$), moderate (50-75%), or low ($<50\%$) E_{Max} . An E_{Max} of $>50\%$ was observed in each cell line in response to at least one of the two drug combinations (Figure 1B). All PDAC cell lines exhibited a high ($n=15$, 78.9%) or moderate ($n=4$, 21.1%) E_{Max} in response to FOLFIRINOX. In contrast, cell lines exhibited a broad distribution of E_{Max} in response to Gem-Pac; six cell lines (31.6%) exhibited high E_{Max} , seven (36.8%) exhibited moderate E_{Max} , and six (31.6%) exhibited low E_{Max} . Eleven cell lines (57.9%) exhibited preferential maximum growth inhibition to FOLFIRINOX over Gem-Pac, while only one cell line (MGH1415) demonstrated a preferential response to Gem-Pac over FOLFIRINOX.

In addition to E_{Max} , the concentration of drug needed to achieve 50% cellular growth inhibition (GI_{50}) of each cell line in response to FOLFIRINOX and Gem-Pac was calculated relative to the concentrations of irinotecan and gemcitabine in each combination chemotherapy, respectively. Based on the GI_{50} values, cell lines were defined as having high (<11 nMol FOLFIRINOX, <20 nMol Gem-Pac), moderate (14-400 nMol FOLFIRINOX, 30-314 nMol Gem-

Table I. Histopathological features of PDAC cell lines.

Cell line	Gender	Age	Primary/Met	Grade	Therapy	
					Neoadjuvant	Adjuvant
MGH617	Male	57	Primary	III	None	None
MGH1108	Female	73	Liver Met	N/A	None	None
MGH1152	Female	56	Primary	II	None	None
MGH1157	Female	62	Primary	II	None	None
MGH1222	Female	78	Primary	II	None	None
MGH1275	Male	70	Liver Met	N/A	None	None
MGH1289	Male	56	Primary	I	None	Gem + Cap
MGH1294	Female	37	Primary	II	None	None
MGH1300	Female	87	Primary	N/A	None	None
MGH1309	Male	62	Primary	III	None	None
MGH1312	Male	51	Liver Met	III	None	None
MGH1319	Male	66	Primary	III	None	None
MGH1326	Female	82	Primary	II	None	None
MGH1415	Male	64	Liver Met	N/A	None	None
MGH1473	Male	61	Lung Met	N/A	None	None
MGH1498	Female	68	Primary	III	None	None
MGH1513	Male	64	Primary	II	Cap + R.T.	None
MGH1925	Female	75	Peritoneal Met	II	None	FX + LDE225
MGH1968	Male	79	Primary	II	None	None

Gem: Gemcitabine; Cap: capecitabine; R.T.: radiation therapy; FX: FOLFIRINOX.

Pac), or low (>400 nMol FOLFIRINOX, >314 nMol Gem-Pac) sensitivity to these chemotherapeutics (Figure 1C). In general, the growth of PDAC cell lines was more broadly sensitive to FOLFIRINOX than to Gem-Pac. Only one cell line (5.3%) exhibited low sensitivity to FOLFIRINOX compared to the six cell lines (31.6%) with low sensitivity to Gem-Pac. The cell line (MGH1498) that exhibited low sensitivity to FOLFIRINOX also exhibited low sensitivity to Gem-Pac. Two cell lines (MGH1289 and MGH1294) exhibited both high sensitivity to FOLFIRINOX and low sensitivity to Gem-Pac.

While E_{Max} and GI_{50} individually provided valuable insight into the response of cells to FOLFIRINOX and Gem-Pac, the evaluation of both metrics in combination allowed us to identify cell lines that we classified as optimally sensitive to these chemotherapeutic combinations. Optimal sensitivity was defined as a combination of high E_{Max} (>75%) and low GI_{50} (<11 nMol FOLFIRINOX, <20 nMol Gem-Pac). Six cell lines (MGH1108, MGH1222, MGH1289, MGH1294, MGH1300, and MGH1312) exhibited optimal sensitivity to FOLFIRINOX (31.6%) while three cell lines (MGH1108, MGH1312, and MGH1415) exhibited optimal sensitivity to Gem-Pac (15.8%) (Figure 2). Two cell lines (MGH1108 and MGH1312) had similar sensitivities to these two regimens.

Characterization of gemcitabine and paclitaxel interaction. Gemcitabine had historically been used as a first-line therapy in the treatment of PDAC (2). While gemcitabine plus

nanoparticle albumin-bound paclitaxel has replaced gemcitabine alone as a standard neoadjuvant chemotherapy option for patients with PDAC, limited research has been conducted regarding the efficacy of paclitaxel monotherapy (6, 16). To better understand the role of gemcitabine and paclitaxel in combination, the effect of the individual drugs was evaluated in six cell lines that exhibited high GI_{50} in response to Gem-Pac. Given the different dilution ranges used to treat cells with Gem-Pac and gemcitabine alone, a reference dose of 0.31 μMol of gemcitabine was used to calculate E_{Max} for cell line response to both drugs. Dose-response curves generated for each cell line indicated that paclitaxel was the primary mediator of Gem-Pac sensitivity in terms of both E_{Max} and GI_{50} (Figure 3A and B). In regard to E_{Max} , paclitaxel drove the inhibitory effects of Gem-Pac in two cell lines (MGH1108 and MGH1473), gemcitabine drove the inhibitory effects of Gem-Pac in one cell line (MGH1415), and the remaining three cell lines (MGH1275, MGH1309, and MGH1312) exhibited similar E_{Max} in response to Gem-Pac, gemcitabine alone, and paclitaxel alone (Figure 3C). In regard to GI_{50} , all six cell lines exhibited a dramatically higher sensitivity to paclitaxel relative to Gem-Pac than to gemcitabine relative to Gem-Pac (Figure 3D). To better define the relationship between gemcitabine and paclitaxel in combination, the interaction between these drugs was evaluated by isobolographic analysis (15). The combination index (CI) values for the six treated cell lines indicated a synergistic interaction between gemcitabine and paclitaxel in

Figure 1. The response of PDAC cell lines to chemotherapeutic treatment. (A) Representative dose-response curves for six PDAC cell lines (MGH617, MGH1152, MGH1275, MGH1294, MGH1309, MGH1319) treated with FOLFIRINOX (left) and Gem-Pac (right). Cellular growth was evaluated by resazurin assay after incubation for five days with treatment. (B) The maximum growth inhibition (E_{Max}) achieved at the highest concentration of drug tested for all 19 PDAC cell lines in response to treatment with FOLFIRINOX (red) and Gem-Pac (blue). (C) The concentration of drug needed to achieve 50% cellular growth inhibition (GI_{50}) for all 19 PDAC cell lines in response to treatment with FOLFIRINOX (left) and Gem-Pac (right). All results are presented as the average $\pm$ standard error of the mean from at least three experiments performed in triplicate.

three cell lines (MGH1108, MGH1275, and MGH1473; $CI=0.32-0.52$) and an additive interaction between gemcitabine and paclitaxel in the remaining three cell lines (MGH1309, MGH1312, MGH1415; $CI=0.99-1.09$).

The effect of the tumor microenvironment on chemosensitivity. PDAC is characterized by a dense stromal component in which cancer-associated fibroblasts (CAFs) comprise the majority of the non-neoplastic cells of the tumor (17). CAFs have been implicated as a key barrier to chemotherapeutic treatment of PDAC, in part by enhancing the chemoresistance of PDAC

cells (17). To assess whether the chemosensitivity of PDAC cell lines to FOLFIRINOX and Gem-Pac is affected by CAFs, we employed two CAF lines derived from PDAC (CAF-1 and CAF-2) and one immortalized hepatic stellate cell line (LX-2). The hepatic-derived cell line was used to interrogate whether liver-derived fibroblasts likely found in liver metastases impart a differential effect on the chemosensitivity of PDAC cells.

Cell lines that exhibited optimal sensitivity to both FOLFIRINOX and Gem-Pac (MGH1108) or were exclusively optimally sensitive to FOLFIRINOX (MGH1222) or Gem-Pac (MGH1415) were treated with chemotherapeutics in the

Figure 2. Identification of PDAC cell lines exhibiting optimal sensitivity to chemotherapeutic treatment. The E_{Max} and GI_{50} of each cell line were plotted. Cell lines exhibiting optimal sensitivity ($E_{Max} < 75\%$ and $GI_{50} < 11$ nMol FOLFIRINOX; < 20 nMol Gem-Pac) are indicated by the blue box. Cell lines with unique optimal sensitivity to FOLFIRINOX are indicated by red circles (MGH1222, MGH1289, MGH1294, and MGH1300), and the cell line with unique optimal sensitivity to Gem-Pac is indicated by a white circle (MGH1415). Cell lines optimally sensitive to both FOLFIRINOX and Gem-Pac are indicated by red and white circles (MGH1108 and MGH1312).

presence of conditioned media from each of the three fibroblast lines: CAF-1, CAF-2, and LX-2. While the E_{Max} of cell lines treated with FOLFIRINOX was not altered by the presence of fibroblast-conditioned media, the sensitivity of MGH1108 cells ($GI_{50}=2.38$ nMol) was dramatically decreased in the presence of conditioned media from one fibroblast line (CAF-2), with a ten-fold increase in GI_{50} observed ($GI_{50}=22.26$ nMol; $p=0.014$) (Figure 4A). The sensitivity of MGH1222 cells to FOLFIRINOX was not affected by fibroblast conditioned media. The sensitivity of cell line MGH1108 to Gem-Pac ($GI_{50}=4.28$ nMol) was decreased at least three-fold by conditioned media from CAF-1 ($GI_{50}=13.44$ nMol), CAF-2 ($GI_{50}=24.80$ nMol), and LX-2 ($GI_{50}=16.31$ nMol; $p<0.001$) (Figure 4C). A similar three-fold decrease in sensitivity to Gem-Pac was also observed in cell line MGH1415 ($GI_{50}=22.40$ nMol) when treated in LX-2 conditioned media ($GI_{50}=70.07$; $p>0.001$ nMol) (Figure 4D). However, CAF-1 and CAF-2 conditioned media did not significantly alter the GI_{50} of MGH1415 cells in response to Gem-Pac.

FOLFIRINOX and Gem-Pac are non-selective chemotherapeutics that may also impact the tumor microenvironment. Given the importance of the stroma in affecting the chemosensitivity of PDAC cells, the ability of these drugs to reduce the viability of CAFs may have profound effects on the chemotherapeutic response of the tumor. To evaluate the sensitivity of fibroblasts to FOLFIRINOX and Gem-Pac and assess whether their chemosensitivity is affected by the presence of PDAC,

fibroblast lines CAF-1, CAF-2, and LX-2 were treated with FOLFIRINOX and Gem-Pac in PDAC cell line conditioned media. Fibroblast lines exhibited a range of E_{Max} (68.2%-85.6%) and GI_{50} (FOLFIRINOX: 32.82 nMol-95.44 nMol; Gem-Pac: 4.387 nMol-10.98 nMol) in response to both drug combinations in unconditioned media that was comparable to the chemosensitivity of PDAC cells. When treated in PDAC cell line conditioned media, all fibroblast lines exhibited a decrease in sensitivity to both chemotherapeutics (Figure 5). A key difference in the ability of PDAC conditioned media to alter the chemotherapeutic response of fibroblasts was observed with E_{Max} ; all three fibroblast lines exhibited a decrease in E_{Max} to FOLFIRINOX when treated with PDAC cell line conditioned media ($p<0.05$), while the response of fibroblast lines to Gem-Pac was not affected under similar media conditions.

Discussion

FOLFIRINOX and gemcitabine plus Abraxane have become the two standard chemotherapeutic treatments in patients with metastatic PDAC. However, no evidence exists to guide the selection of one chemotherapy over the other for any given patient (8-12). This study found that patient-derived PDAC cell lines exhibit differential sensitivity to FOLFIRINOX and gemcitabine plus paclitaxel (Gem-Pac), reflecting the heterogeneity of patient response to these drug regimens; six of the 19 cell lines exhibited optimal sensitivity (high E_{Max}

Figure 3. The response of PDAC cell lines to gemcitabine and paclitaxel monotherapy. (A) Representative dose-response curves for three PDAC cell lines in response to treatment with Gem-Pac (dark blue) and paclitaxel alone (light blue). (B) Representative dose-response curves for three PDAC cell lines in response to treatment with Gem-Pac (dark blue) and gemcitabine alone (orange). (C) The E_{Max} of six PDAC cell lines in response to treatment with Gem-Pac (dark blue), paclitaxel alone (light blue), and gemcitabine alone (orange). A reference dose of 0.31 μMol of gemcitabine and 0.014 μMol of paclitaxel was used to calculate E_{Max} for cell line response to Gem-Pac; a reference dose of 0.31 μMol of gemcitabine was used to calculate E_{Max} for cell line response to gemcitabine alone; and a reference dose of 0.014 μMol of paclitaxel was used to calculate E_{Max} for cell line response to paclitaxel alone. (D) The GI_{50} of six PDAC cell lines in response to treatment with Gem-Pac, gemcitabine alone, and paclitaxel alone. Isobolographic analysis of these results yielded combination index (CI) values for the six treated cell lines.

and low GI_{50}) in response to treatment with FOLFIRINOX, while three of 19 exhibited optimal sensitivity to Gem-Pac. Two cell lines had similar sensitivities to the two regimens, while the other cell lines were not responsive to either chemotherapy regimen. Several cell lines that were optimally sensitive to one drug regimen exhibited very poor response to the other, indicating that one chemotherapy was clearly superior. Additionally, the higher overall proportion of FOLFIRINOX-susceptible cell lines compared to Gem-Pac-susceptible cell lines is consistent with analysis of real-world clinical data suggesting that FOLFIRINOX may be overall more effective than gemcitabine and Abraxane chemotherapy for metastatic pancreatic cancer, although this has not yet been confirmed in a randomized study (18).

These findings warrant further investigation to reveal whether characteristics of each cell line are related to chemosensitivity or chemoresistance to one or the other chemotherapy regimen. It is unlikely that these effects are linked to simple genetic

alterations; the four most commonly mutated genes in PDAC (KRAS, TP53, CDKN2A, and SMAD4) occur at higher rates than would account for these results, and additional genes are generally altered in less than 10% of tumors (19). However, a formal genetic analysis of these cell lines remains worthwhile. If the characteristics that define chemosensitivity or chemoresistance to one regimen or the other can be elucidated, the findings would have potential clinical implications. For example, knowing which chemotherapy is most effective for a given patient from the beginning of treatment could save precious time, potentially significantly improving the patient's clinical outcome. Likewise, knowing that two drug regimens are equally effective for a given patient could help direct the choice of a more tolerable chemotherapy and thereby improve the patient's quality of life. Finally, knowing which patients are unresponsive to chemotherapy altogether could spare the burden of a futile course of chemotherapy that would unnecessarily debilitate the patients' final stages of life. While these findings

Figure 4. Effect of fibroblast-conditioned media on PDAC cell line chemosensitivity. (A) Dose-response curves for PDAC cell line MGH1108 treated with FOLFIRINOX in the presence of conditioned media from MGH1108 and conditioned media from two PDAC-derived CAF lines, CAF-1 and CAF-2, and the hepatic-derived fibroblast line LX-2. (B) Dose-response curves for PDAC cell line MGH1222 treated with FOLFIRINOX in the presence of conditioned media from MGH1222, CAF-1, CAF-2, and LX-2. (C) Dose-response curves for MGH1108 treated with gemcitabine plus paclitaxel (Gem-Pac) in the presence of conditioned media from MGH1108, CAF-1, CAF-2, and LX-2. (D) Dose-response curves for PDAC cell line MGH1415 treated with Gem-Pac in the presence of conditioned media from MGH1415, CAF-1, CAF-2, and LX-2.

constitute exciting preliminary results, it is based on a limited number of cell lines and additional studies are needed to extend this and define markers of chemosensitivity and resistance.

The examination of previously uninvestigated comparative effects of gemcitabine and paclitaxel monotherapy on patient derived cell lines revealed that paclitaxel drove the growth inhibitory effects in five of the six most Gem-Pac-susceptible cell lines. Interestingly, one of these cell lines was dramatically more sensitive to Gem-Pac than to gemcitabine and paclitaxel alone, suggesting that this combination exerts a synergistic effect on a subset of PDAC cells. These findings begin to elucidate the potential interactions of gemcitabine and taxanes in combination chemotherapy regimens.

It is important to acknowledge and investigate the role of the characteristic dense, stroma-rich microenvironment in which PDAC cells grow. Cancer-associated fibroblasts (CAFs), cells that define the PDAC stroma, have been shown to play a role in decreasing patient sensitivity to chemotherapeutic treatment

in breast, lung, esophageal, and colorectal cancer (20-22). While PDAC cell lines overall exhibited a more dramatic reduction in chemosensitivity to Gem-Pac than to FOLFIRINOX when cultured with fibroblast-conditioned media, different cell lines exhibited a heterogeneous response to therapy, with several lines remaining unaffected by the presence of fibroblast-conditioned media. The diversity of CAF subtypes may, therefore, play a role in eliciting this heterogeneous response.

Understanding that chemotherapy impacts the growth and proliferation of both tumor and stromal cells, we also evaluated the impact of PDAC cell line conditioned media on fibroblast response to therapy. When treated in PDAC cell line conditioned media, all fibroblast lines exhibited a decrease in sensitivity to both drug regimens. Developing a better understanding of how the PDAC tumor microenvironment impacts the efficacy of different chemotherapeutics could help inform the nuances of PDAC tumor biology, as well as tailor treatment decisions for patients.

Figure 5. Effect of PDAC-conditioned media on fibroblast chemosensitivity. (A) Dose-response curves for PDAC-derived CAF-1 treated with FOLFIRINOX in the presence of conditioned media from CAF-1 and PDAC-derived cell lines MGH1108 and MGH1222. (B) Dose-response curves for PDAC-derived CAF-2 treated with FOLFIRINOX in the presence of conditioned media from CAF-2, MGH1108, and MGH1222. (C) Dose-response curves for hepatic derived LX-2 treated with FOLFIRINOX in the presence of conditioned media from LX-2, MGH1108, and MGH1222. (D) Dose-response curves for CAF-1 treated with gemcitabine plus paclitaxel (Gem-Pac) in the presence of conditioned media from CAF-1, MGH1108, and PDAC-derived cell line MGH1415. (E) Dose-response curves for CAF-2 treated with Gem-Pac in the presence of conditioned media from CAF-2, MGH1108, and MGH1415. (F) Dose-response curves for LX-2 treated with Gem-Pac in the presence of conditioned media from LX-2, MGH1108, and MGH1415.

With improved understanding of the intrinsic heterogeneity of pancreatic cancer in different patients, these findings support the need for future research towards customizing treatments to fit the diverse needs of these individuals. Cell lines may represent a unique tool for stratifying patient chemosensitivity; further proteomic and transcriptomic analyses of the cell lines used in our study could help identify biomarkers that ultimately guide the use of one drug regimen over the other for a given patient. These lessons learned from patient-derived PDAC cell lines could additionally help evaluate the validity of other recently published biomarkers that purport to predict chemotherapeutic response.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

ASL and CFC conceived the study. SKSB, DJB, CFC, MMM, and ASL participated in the design of the study. SKSB and DJB

performed the experiments. SKSB, DJB, JWC, KDL, ALW, CFC, and ASL analyzed and/or interpreted the data. UFW and OS generated CAF-2 cells used in this study. SKSB and DJB wrote the manuscript under the supervision of ASL with input from all authors.

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