Predicting the chemosensitivity of pancreatic cancer cells as a personalized therapy

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Abstract

Background. Annually, approx. 4000 patients are diagnosed with pancreatic cancer in Poland, and the number of deaths is close to the number of diagnoses. Such a high morbidity/mortality ratio is caused by a high percentage of unresectable lesions (about 80%) and chemoresistance, which, among other things, is due to the specific desmoplastic environment. Currently, there are 2 main systemic treatment regimens for pancreatic cancer: FOLFIRINOX (which is a combination of folic acid, fluorouracil (5-FU), irinotecan, and oxaliplatin) and combined treatment with nab-paclitaxel plus gemcitabine (NPXL+GMC).

Objectives. In order to increase the effectiveness of systemic treatments for individual patients, cell lines derived from resected pancreatic tumors were developed and their chemosensitivity to various agents was examined. The hypothesis was that patients may benefit from individualization of chemotherapy.

Materials and methods. Patients with histopathologically confirmed pancreatic cancer were operated on using irreversible electroporation (IRE) procedure. After isolating and establishing individual cell lines, chemosensitivity to 5-FU, GMC and NPXL was determined using MTT assay in primary and metastatic cell cultures.

Results. Three primary cell lines were isolated for the prediction of chemosensitivity. Gemcitabine was shown to be more effective at lower doses compared to 5-FU, while NPXL was more effective than 5-FU, and both of these were less effective in metastatic cells. Pancreatic cancer cell chemoresistance was confirmed in stage IV.

Conclusions. Determination of chemosensitivity profiles using cell lines may help in the selection of systemic treatments for individual patients. This method can be the basis for a personalized planned chemotherapeutic protocol.

Key words: pancreatic cancer, personalized therapy, chemosensitivity of pancreatic cancer cells

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Background

Chemosensitivity of pancreatic cancer cells is a part of the "Personalization of pancreatic cancer treatment" program. The aim of this program is to match the most effective chemotherapy to an individual case to improve outcome for the patients. Patients were operated in the 2nd Department of General and Oncological Surgery of the University Clinical Hospital in Wrocław, Poland.

Objectives

The aim of the study is the personalization of pancreatic cancer treatment. According to treatment guidelines, pancreatic cancer patients receive FOLFIRINOX (a combination of folic acid, fluorouracil (5-FU), irinotecan, and oxaliplatin) or nab-paclitaxel plus gemcitabine (NPXL+GMC), without confirmation of sensitivity. There is a possibility of inadequate chemotherapy. However, matching proper systemic treatment for an individual patient can result in longer overall survival (OS) and progression-free survival (PFS). The question was whether examination of chemosensitivity can influence patient outcome and whether this examination may be mandatory before treatment in future.

Patients were operated on under general and epidural anesthesia. Samples were collected during the operation and delivered immediately to the Department of Molecular and Cellular Biology at the Wroclaw Medical University, Poland, where individual cell lines were isolated.

The patients gave informed consent prior to participation in the study. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethical Committee at Wroclaw Medical University (approval No. KB-330/2018).

Materials and methods

Cell culture

In this study, a primary cell culture technique was used. Fresh tumor samples were acquired from 2 patients during biopsy and the tissue was processed directly after surgery. From the 1st patient, biopsy from a primary pancreatic cancer (PPCC1) was taken, and from the 2nd patient, 2 biopsies were taken: the 1st from the primary tumor (PPCC2) and the 2nd from metastatic tissue (MPCC2). Cells were isolated from tissue samples according to a previously described procedure.[1,2] Briefly, the tissue was gently rinsed with sterile phosphate-buffered saline (PBS; BioShop, Burlington, Canada). Next, samples were minced in Petri dishes (Sigma-Aldrich, St. Louis, USA) using a scalpel and suspended in dedicated culture medium (L-15; Gibco, Life Technologies, Carlsbad, USA). Part of the suspended material was immediately transferred to 25 cm^3 culture flasks. For the first 3 days, the medium was replaced daily and care was taken not to discard any non-attached tissue fragments. Following this, the medium was fully replaced twice a week. The average time to obtain confluence in both the Petri dishes and the culture flasks was 14 days. The cells were cultivated in a modified high-glucose Leibovitz's L-15 medium (Gibco) supplemented with 10% fetal bovine serum (FBS), 1% antibiotics (penicillin and streptomycin), 1.5% sodium bicarbonate (7.5%; Gibco), 1% MEM vitamin solution (Sigma-Aldrich), 0.5% UltraGlutamine™ 1 (Lonza, Basel, Switzerland), 0.1% glucose (45%; Sigma-Aldrich), and 0.7% aprotinin (BioShop). The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

MTT assay

Cytotoxicity of the chemotherapeutics was determined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma-Aldrich) assay. This is a colorimetric assay that measures the metabolic rate in viable cells. Cells were seeded at 4×10^3 cells per well in 96-well plates (Greiner, Pleidelsheim, Germany) and left to adhere for 24 h. The cells were then exposed to the chemotherapeutic drugs (GMC, 5-FU, NPXL) for 24 h and 48 h to evaluate short-term and long-term effects on cell viability. The drugs were dissolved in sterile PBS and then with diluted in culture medium at concentrations of 0–1000 µg/mL. Absorbance of the wells was measured using a multi-well scanning spectrophotometer at 570 nm (Glomax; Promega Polska, Pisz, Poland). Absorbance values were expressed as a percentage of treated cells compared to control cells. All experiments were performed in triplicate.

Patient data

Before surgery, a 62-year-old male with diabetes and an ECOG score of 0 received 6 courses of NPXL+GMC. The patient's histopathological results showed a regression of the neoplastic lesions, which was confirmed by a decrease in serum carbohydrate antigen 19-9 (CA 19-9) from 1280.98 U/mL to 18.98 U/mL and radiological regression according to the Response Evaluation Criteria in Solid Tumors (RECIST). This also correlated with the results of the chemosensitivity testing (see below). After the procedure, the patient was treated with 7 courses of FOLFIRINOX according to drug protocols approved in Poland. During chemotherapy, progression of the disease in the retroperitoneal space occurred. Therefore, in line with the results obtained from the chemosensitivity testing, we returned to treatment with NPXL+GMC (8 courses). Unfortunately, this treatment regimen was not eligible for reimbursement because drug programs in Poland do not provide for a return to previous regimens.

Another patient, a 68-year-old female with hypertension and a ECOG score of 1, received 8 cycles of GMC alone and

was qualified for the IRE procedure. Two cell lines were isolated from this patient from the primary and metastatic lesions. In both lines, chemoresistance was viable. Therefore, patient died 2 months after the IRE procedure.

Statistical analyses

As the number of patients was too small to generalize the results of the analysis for the population, our analysis aimed to prove the studied effects. The survival of 3 line cells (PCC1, PCC2 and MPCC2) was analysed for 3 drugs: fluorouracil, gemcitabine and nab-paclitaxel, in addition to exposition time and dose (Fig. 1). Analysis was performed using R-package "nparLD" function (R Foundation for Statistical Computing, Vienna, Austria).[3] This package is a nonparametric rank-based tool for the analysis of variance in repeated measures design, and does not require any assumptions on data distribution.[4] Due to these features it can be used for a small number of replications (n = 3 in this study). Overall effect was tested with the use of the "nparLD" function, according to a two-way analysis of variance (ANOVA) design. Values of $p \leq 0.05$ were considered statistically significant.

Results

Results indicated higher efficacy of GMC, even at low concentrations, in a cell line derived from a patient diagnosed with pancreatic cancer (PPCC1). This was highlighted by a pronounced effect of time (much lower survival rate after 72 h when compared to 24 h, $p < 0.001$) and a small but marginally statistically significant effect of the dose ($p = 0.041$). The efficacy of the 5-FU in the cell line taken from the 1st patient (PPCC1) increased with both the dose ($p < 0.001$) and time ($p < 0.001$). In the cell line taken from the 2nd patient (PPCC2), cell viability was higher after 48 h for both NPXL and 5-FU. However, there

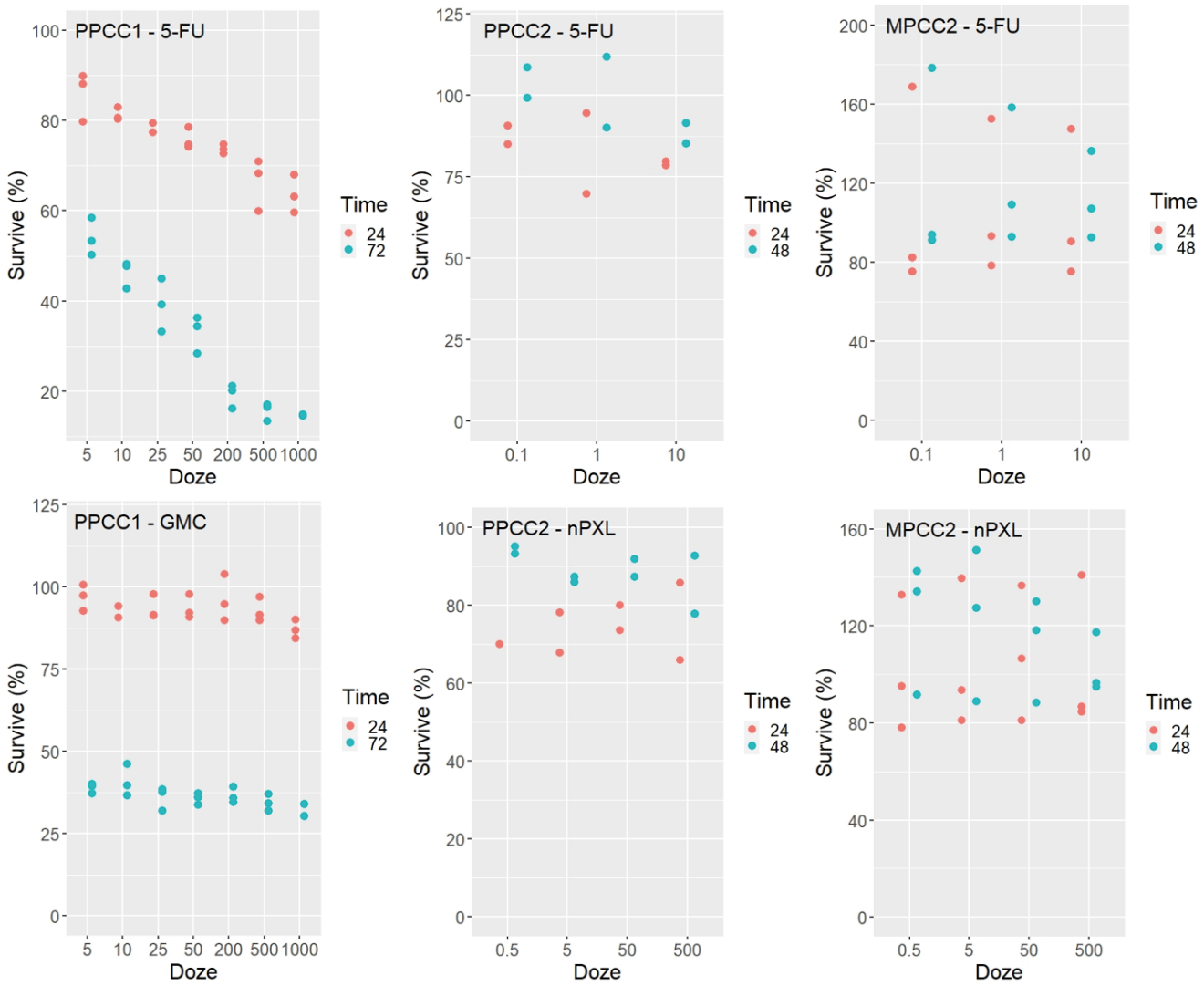

Fig. 1. The effect of the dose and the duration of administering fluorouracil (5-FU), gemcitabine (GMC) and nab-paclitaxel (NPXL) on the survival of cells from the primary pancreatic cancer lesion (biopsy from the 1st patient (PPCC1) and primary tumor biopsy from the 2nd patient (PPCC2)), and from the metastatic lesion (biopsy from the metastatic tissue from the 2nd patient (MPCC2)) after 24 h, 48 h and 72 h

Table 1. The effect of drug dose (dose) and exposition time (time) on cell survival (results of nonparametric analysis of variance (ANOVA))

Drug	Cell origin	Predictor	F	df	p-value
GMC	PPCC1	dose	2.41	4.41	0.0412
		time	448.37	1.00	0.0000
		dose:time	2.25	4.27	0.0564
5-FU	PPCC1	dose	58.62	2.96	0.0000
		time	1404.19	1.00	0.0000
		dose:time	0.38	3.95	0.8185
	PPCC2	dose	0.72	1.15	0.4133
		time	28.17	1.00	0.0000
		dose:time	0.17	1.60	0.7980
	MPCC2	dose	0.08	1.85	0.9092
		time	8.58	1.00	0.0034
		dose:time	0.06	1.77	0.9218
nPXL	PPCC2	dose	0.28	1.36	0.6709
		time	160.19	1.00	0.0000
		dose:time	7.81	1.51	0.0014
	MPCC2	dose	0.05	2.82	0.9812
		time	5.71	1.00	0.0169
		dose:time	0.92	1.66	0.3835

5-FU – fluorouracil; GMC – gemcitabine; NPXL – nab-paclitaxel; PPCC1 – cell line obtained via biopsy from the 1st patient; PPCC2 – cell line obtained via primary tumor biopsy from the 2nd patient; MPCC2 – cell line obtained via biopsy from the metastatic lesion tissue from the 2nd patient.

was no statistically significant effect of the dose (p = 0.671 and p = 0.413, respectively), which was an evidence of chemoresistance (PPCC2).

The same situation occurred in the metastatic cell line (MPCC2). No effect of dose was observed (p = 0.909 and p = 0.981 in 5-FU and nPXL, respectively), though the viability of the cells increased significantly (p = 0.003 and p = 0.017, respectively) after 48 h (Table 1).

Discussion

At present, pancreatic cancer is the 4th most common cause of cancer-related death worldwide, and it is estimated that by 2030, it will be the 2nd most common cause of death after lung cancer.[5] The incidence of pancreatic cancer continues to rise both in Poland and worldwide, and the mortality rate remains at a level almost equal to the incidence rate. Globally, the number of cases in 2018 was 458,918 and the number of deaths was 432,242.[6] The reasons for the high mortality/morbidity ratio are late detection, a lack of sensitive and specific markers for the disease, a lack of novel therapies, and chemoresistance.

The primary treatment modalities for pancreatic cancer are surgical resection and adjuvant chemotherapy. Unfortunately, resection is only possible in 15–20% of cases, and 80% of patients who undergo resection will have a recurrence within a year.[7] Regarding adjuvant treatment, there are 2 treatment regimens available: NPXL+GMC and FOLFIRINOX. Although the differences between OS and PFS for both regimens are statistically insignificant, NPXL+GMC has been shown to be more effective, with indications of less toxicity.[8–10]

Unfortunately, pancreatic cancer is a chemoresistant disease. This is due to acquired abnormalities in the tumor cell membrane transport protein hENT1,[11] nucleoside enzymes[12,13] and changes in the epithelial–mesenchymal transition.[14,15] One of the key mechanisms of pancreatic cancer chemoresistance is stellate cell activation, which induces a change in tumor bedding to the desmoplastic environment.[16–18] Chemoresistance was confirmed in our study in the 2nd patient with metastatic disease.

Due to the poor prognosis, personalized therapy is being explored for pancreatic cancer patients. Research has been carried out on the benefits of establishing tumor gene profiles[18] and immunotherapy.[19,20] Unfortunately, neither therapy has been shown to be effective to date. Determining the chemosensitivity of tumor cell lines derived from a given patient seems to be a good direction for the personalization of treatment. By comparing the relative effectiveness of 2 lines of treatment with proven efficacies (NPXL+GMC and FOLFIRINOX), we can choose the more effective treatment for an individual patient. The results of one of the patients treated at the University Clinical Hospital indicated a higher chemosensitivity to GMC, which correlated with the clinical course. Clinical and radiographic regression occurred during NPXL+GMC therapy. However, after the conversion into FOLFIRINOX, which was stipulated by the current oncological treatment regimens, tumor progression occurred.

In 2 of the cell lines, we confirmed chemoresistance in metastatic and progressive disease, so the prognosis for the patient was poor. Thus, the determination of chemosensitivity may be important for patient prognosis and requires further studies with a larger number of patients.

Limitations

The main limitation of this study was the low number of examined tissue specimens. Further studies with larger samples and more comprehensive evaluations are necessary. The study was also limited by the determination of chemosensitivity for only a few chemotherapeutics (GMC 5-FU, NPXL). It would be advisable to determine the chemosensitivity for each chemotherapeutic, or for the full NPXL+GMC and FOLFIRINOX regimens, to gather complete data.

Conclusions

Examined cell lines have different biology and different chemosensitivity in every patient, and cancer progression is related to chemoresistance of cancer cells. The determination of chemosensitivity in pancreatic cancer cell lines derived from a given patient may be useful in the selection of a systemic treatment. At present, this is not a standard technique and it is not included in treatment protocols. However, in the future, this procedure may be used as a guideline to select the best systemic treatment for an individual patient and individual stage.

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