3D reconstructed pancreas: A model capturing the unique tumor microenvironment and stromal architecture of pancreatic cancer

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Introduction

- The prognosis for people with pancreatic cancer is poor, with an average 5-year survival rate of 10% and only 3% for those with metastatic illness. Pancreatic cancer patients have few therapeutic options, and innovative therapies are sorely needed to improve treatment of the disease. This is mainly due to the late diagnosis and partially due to the biology of the disease.
- It has been shown that human pancreatic tumors have an outer layer of stiff extracellular matrix (ECM) that functions as a physical barrier, preventing drugs from penetrating the tumor (Persman, 2020).1
- To address this with our model, we have incorporated a collagen capsule and have co-cultured pancreatic tumor cell lines with primary activated pancreatic stromal cells.

We have created a 3D model of the pancreatic tumor microenvironment, the Reconstructed Pancreas (r-Pancreas), which is formulated to recapitulate the typical ECM of pancreatic tumors. We tested the response of BxPC-3, PAN-1 and Mia PaCa-2 pancreatic tumor cell lines embedded in this ECM to standard of care therapies. We demonstrated that gemcitabine was effective against pancreatic tumor cells cultured in r-Pancreas (IC50 = 0.4-0.8 µM), while 5-fluorouracil (5FU) was ineffective (IC50 not reached).

In nude mice, subcutaneous PANC-1 tumors exhibited similar responses to gemcitabine and 5FU. To address this with our model, we have incorporated a collagen capsule and have co-cultured the tumor cells with activated pancreatic fibroblasts.

Methods

- PAN-1 and MIA PaCa-2 were mixed with ECM and plated in a 96-well plate. For the 2D setup, ECM was not added and the cells were plated at the bottom of the wells. Pancreatic cancer-specific supplement was added into the media for both the r-Pancreas and 2D setup. Gemcitabine and 5FU were administered into the medium 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured (Kirshner, 2022).
- Collagen-1 (Corning; Durham, NC) was added into the transwell insert and allowed to polymerize. BxPC-3, PAN-1 and MIA PaCa-2 were mixed with ECM and plated in a 96-well plate. Pancreatic cancer-specific supplement was added into the medium. Gemcitabine was administered into the medium 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured accordingly.
- BxPC-3, PAN-1 and MIA PaCa-2, along with human pancreatic fibroblasts, were mixed with ECM and plated in a 96-well plate. Pancreatic cancer-specific supplement was added into the medium. Gemcitabine was administered into the medium 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured.
- Female NOD:scid (NSG) mice were implanted with 1.0E+07 human PAN-1 cells (SC = high axilla) in 50% Matrigel. After staking, tam mice per group were treated with either vehicle, intraperitoneal gemcitabine at 100 mg/kg every 3 days for 4 cycles (IP, 100 mg/kg, Q3Dx4), or intraperitoneal 5FU at 100 mg/kg every 7 days for 3 cycles (IP, 100 mg/kg, Q7Dx3). Tumors were measured every 2 days from 18 to 81 days post-tumor implant.

All animal work was performed in an AALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane endpoints criteria on all studies.

Results and Conclusions

- Capturing the components of the tumor microenvironment, which serve as both a physical barrier and a source of stromal-driven resistance to therapeutics, is one of the issues faced by drug developers searching for agents to combat pancreatic cancer.
- The addition of a collagen layer separating the r-Pancreas + BxPC-3, PAN-1 or MIA PaCa-2 mixture from the medium containing gemcitabine reduced the sensitivity of the cancer cells to gemcitabine mimicking the ECM around pancreatic tumors and preventing drug penetration. Incorporating a collagen-rich capsule increased physiological relevance of the r-Pancreas model.
- The addition of pancreatic fibroblasts to the r-Pancreas + BxPC-3, PAN-1 or MIA PaCa-2 mixture reduced the sensitivity of the cancer cells to gemcitabine demonstrating the protective effect of fibroblasts against drug treatment in pancreatic tumor models. The IC50 values for the pancreatic tumor cell lines treated with gemcitabine increased by two-fold when co-cultured with activated pancreatic fibroblasts, compared to control sets of tumor cells alone (IC50 = 1-3 µM). Creating a co-culture of primary activated pancreatic stromal cells and pancreatic tumor cell lines produced a more complete 3D model that permits the testing of potential therapeutic agents inside the pancreatic tumor microenvironment.
- The response of the human pancreatic cancer cell line, PAN-1, to gemcitabine and 5FU in 3D r-Pancreas ECM mirrored the in vivo response of subcutaneously implanted PAN-1 cells in female nude mice.
- Our data suggests, that to be clinically relevant, in vitro models of pancreatic cancer must integrate tumor-specific components of the microenvironment, including collagen cap and activated fibroblasts.

References