Abstract

Pancreatic cancer is the third-leading cause of death among all cancer cases in the United States according to the data from National Cancer Institute. Prognosis of pancreatic cancer is generally poor due to diagnostic challenges and limited treatment options. We carefully studied the growth behavior of PANC-1 pancreatic cancer cell lines under various oxygen levels and tested the efficacy of FDA approved chemotherapy drugs under the physiologically realistic oxygen condition, known as hypoxia, to determine the optimized in-vitro treatment strategy. Two of the most common pancreatic cancer drugs are Cisplatin and 5-Fluorouracil. Cisplatin is a platinum base drug, and its mode of action is apoptosis by causing mispairing of nucleotides. The mechanism of 5-Fluorouracil is forming a complex to inhibit DNA replication and repair. A hypoxic chamber is used in this experiment to mimic the pancreas in the human body. In this experiment we are testing PANC-1 cells in a hypoxic environment and in normal conditions to see the efficacy of the drug action under varying oxygen levels. The cell images were imported into an automatic cell counting software called Fiji. The data was analyzed and pointed as individual growth curves. Based on our time course data, 5-Fluorouracil inhibited PANC-1 more effectively under the hypoxia environment compared to the normoxia environment. Additionally, Cisplatin inhibited cell growth more effectively in the hypoxia environment compared to the normoxia environment.

Materials and Methods

Culture of PANC-1 Cells

Cells were cultured at 37 degrees Celsius under normal conditions (5% CO₂). The experiment requires a total of 4.2x10⁴ viable PANC-1 cells, which will be centrifuged into pellet(s) and subsequently resuspended into a total cell culture volume of 24 mL. This will then be distributed into the 96-well plates in aliquots of 180 uL for culture, treatment, and analysis.

Drugs, Drug Dilutions, and Cell Treatments

A dilution series will be performed to obtain different testing concentrations of the chemotherapeutic drugs Cisplatin and 5-Fluorouracil. The concentrations will be 1mM, 100uM, 10uM, 1uM, 100nM, and 10nM, and the amounts used for the dilutions is diagrammed in Figure 2. These will then be diluted by another factor of ten when they are added to the sample, giving final treatment concentrations of 100uM, 10uM, 1uM, 100nM, 10nM, and 1nM.

O₂ Treatments, Apoptosis Assay, and Cellular Energetics

Different plates of samples under treatment will be placed into conditions of normoxia, ~5% oxygen, and ~1% oxygen (hypoxia). These environmental goals will be achieved and validated using the AnaeroPack System from MGC and dry anaerobic testing strips. After allowing the cells the opportunity to proliferate, the cells were analyzed using a Fiji program to count the cells.

Conclusion

- The growth rate of PANC-1 was faster under Normoxia conditions (without drug treatment).
- The greater dosage of both (cisplatin and 5-Fluorouracil) chemotherapy drugs killed cells at a higher rate. Demonstrating robust dosage response of the PANC-1 cells.
- Based on our time course data, 5-Fluorouracil inhibited PANC-1 more effectively under the hypoxia environment.
- Additional, Cisplatin inhibited cell growth more effectively in the hypoxia environment compared to the normoxia environment.

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