Evaluation of New Targeted Therapies in 3D Culture Models for Pancreatic Cancer

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Abstract

Pancreatic Ductal Adenocarcinoma (PDAC) is a deadly cancer that displays a highly heterogeneous and complex tumor microenvironment. Novel therapeutic agents that target DNA regulatory networks known as super-enhancers (SEs) have recently gained attention in the potential treatment of PDAC. In order to accurately study the effects of super-enhancer inhibitors, cell culture models that accurately mimic the tumor microenvironment are necessary. In this experiment, two PDAC cell lines (B011 and B028) are grown under both 3D and 2D culture conditions and the growth inhibitory effects of three super-enhancer inhibitors (triptolide, THZ1, and THZ2) are determined. The 3D PDAC cell cultures of the B011 cell line demonstrate greater resistance to all three drugs tested from the class of super-enhancer inhibitors. Based on these findings, 3D PDAC culture models may provide more accurate means to study novel chemotherapy agents than traditional 2D culture methods. The implications of this are significant as it may pave the way for quick and affordable precision medicine modalities in the future for the treatment of PDAC.

Introduction

Pancreatic cancer is the third leading cause of cancer-related deaths in the United States and has a 5-year relative survival rate of just 9.3% (1). Pancreatic Ductal Adenocarcinoma (PDAC) accounts for >90% of pancreatic cancer cases and has a tendency for rapid invasion and metastasis (2). Super-enhancers (SEs) were defined as large clusters of transcriptional enhancers that control the expression of genes that define cell identity (3). Current literature suggests that the development of PDAC may be dependent on SE transcription activity, and there has been a growing body of research demonstrating the use of super-enhancer inhibitors as novel therapeutics for PDAC (4, 5).

In order to accurately study the effects of novel PDAC therapeutic agents, such as super-enhancer inhibitors, cell culture models that closely recapitulate the microenvironment and dynamics of the primary malignancy are needed. Traditional two-dimensional adherent cultures grown in a monolayer oversimplify cell-to-cell contact dynamics and lack three-dimensional organization. Three-dimensional cultures that are suspended in non-adherent plates more accurately mimic the physical and biochemical features of the primary tumor (6). Similar to tumors in patients (and unlike the 2D culture), therapeutics need to penetrate the multilayer cellular structure to reach tumor cells inside 3D spheroids. Thus, we hypothesize that PDAC cell cultures grown in 3D architecture will demonstrate greater resistance to treatment with super-enhancer inhibitors than their 2D counterparts. The super-enhancer inhibitor, triptolide, which is a natural compound isolated from the Chinese herb *Tripterygium wilfordii,* has shown to induce apoptosis in pancreatic cancer cells (7) and cause transcriptional downregulation of super-enhancer networks in pancreatic cancer cells (4). THZ1 and THZ2 are CDK7 inhibitors that have recently shown to induce gene expression changes in PDAC cell lines that are similar to those of triptolide (4).

Materials and methods

Cell culture maintenance and conditions

Low passage tumor cells were isolated from the tumor samples of two PDAC patients (B011 and B028). Cells were obtained from passage 1 for B011 and passage 2 for B028 and grown in T-25 cell culture flasks. Prior to their use, the cell culture flasks were coated with collagen solution and incubated at 37 degrees Celsius for 30 minutes, after which the collagen was aspirated, and the flasks were washed with media and aspirated. B011 and B028 cell cultures were grown in Gibco Dulbecco's Modified Eagle

Medium (DMEM) with addition of supplemental nonessential amino acids, sodium pyruvate, fetal bovine serum, and penicillin and incubated at 37 °C. Cultures were observed under a microscope and determined ready for experimental use once reaching 60-70% confluence. To expand the cell culture, media was aspirated and the cells were washed with phosphate buffered saline which was then aspirated. 1 mL of trypsin was added, and the flasks were incubated for 8 minutes. Mobility of cells were observed under a microscope and 4 mL of DMEM was then added to the flask. The concentrations of cells were determined using a cell counter, and 90 µL of volume containing 5000 cells were added to 96-well flat bottom plates for 2D cultures or 96-well U-bottom plates with 1.5% Matrigel for 3D organoid cultures. 2D cultures were grown for 24 hours prior to drug treatment and organoid cultures were grown for 72 hours prior to drug treatment.

Preparation of super-enhancer inhibitor agents

The powder form of three super-enhancer inhibitors (triptolide, THZ1, and THZ2) were obtained from MedChemExpress and stock solutions of the drug aliquots were prepared in DMSO and stored in - 80 °C. Serial dilutions were made in 96-well plates to achieve 10x the final concentration and 10 µL of drug were transferred to the culture plates to achieve a final volume of 100 µL. Triplicates were created for each condition. Following treatment, cells were incubated at 37 °C for 72 hours.

Cell viability assay

Following 72 hours of drug treatment, the CellTiter-Glo® Luminescent Cell Viability Assay was performed to determine the relative number of viable cells. The standard CellTiter-Glo® substrate was used for 2D cultures and the 3D CellTiter-Glo® substrate was used for the organoid cultures. Addition of the substrate results in cell lysis and generates a luminescent signal proportional to the amount of ATP present, which is directly proportional to the number of viable cells at the time of lysis. Following the mixture of 100 µL CellTiter-Glo® substrate per the protocol, the luminescent signal was recorded, and the data was analyzed to determine the half maximal inhibitory concentration (IC_{50}).

Data analysis and statistical methods

The statistical analysis for the cell viability assay was carried out using GraphPad Prism Software to plot the dose-response curve for each drug and to calculate the 95% confidence interval of the IC_{50}.

Results

The 2D B011 cultures demonstrated a lower IC_{50} compared to their 3D counterparts for all 3 super-enhancer inhibitors; triptolide, THZ1, and THZ2. The 2D B011 that were treated with THZ1 demonstrated an IC_{50} of 13.34 nm while the 3D B011 cells demonstrated an IC_{50} of 57.45 nm (figure 1). Furthermore, the 2D B011 that were treated with THZ2 demonstrated an IC_{50} of 61.18 nm while the 3D B011 cells demonstrated an IC_{50} of 182 nm (figure 2). The 2D B011 cultures treated with triptolide also exhibited less resistance to drugs as their 3D counterparts. The 2D B011 cultures treated with triptolide has an IC_{50} of 11.85 nm while the 3D B011 cultures demonstrated an IC_{50} of 26.29 nm (figure 3). The B028 cell line did not grow as well as the B011 cell line and the luminescence results demonstrated unviable cells. However, THZ1 was successfully tested in the B028 cell line and 2D B028 cultures demonstrated an IC_{50} of 9.46 nm while the 3D B028 cultures demonstrated an IC_{50} of 71.88 nm (figure 4). Overall, the 2D cultures of the B011 cell line demonstrated greater sensitivity to all 3 tested drugs when compared to their 3D culture counterparts. The B028 cell line also demonstrated this pattern with THZ1, but the assays for the other drugs were inconclusive.

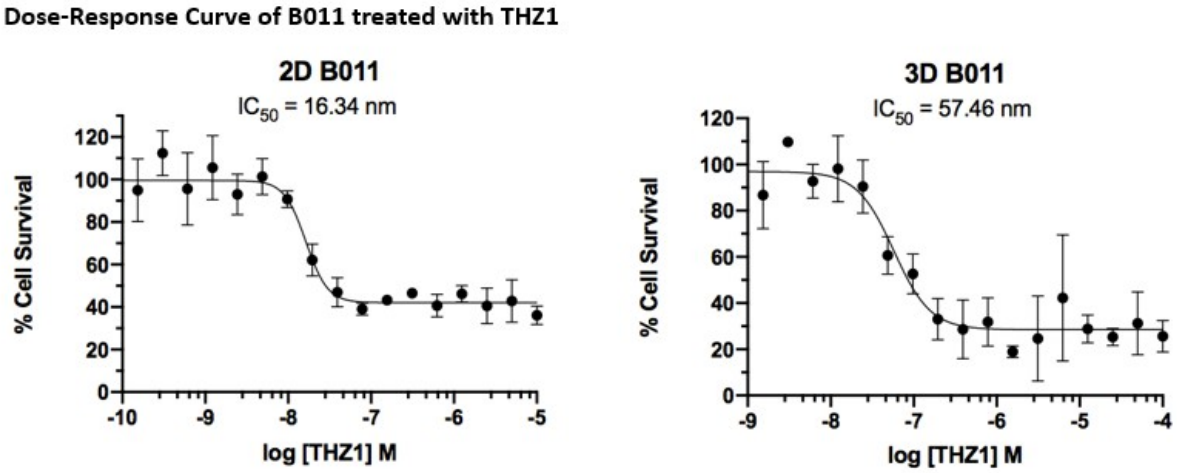

Figure 1: B011 cells grown in a 2D monolayer demonstrated an IC_{50} of 16.34 nm while the 3D cultures demonstrated an IC_{50} of 57.46 nm when drugged with THZ1 for 72 hours. A total of 5000 cells were seeded for each condition. The x-axis represents the logarithmic transformation of THZ1 concentration and the Y-axis represents percent cell survivability. Error bars are standard error of the mean (SEM) of the triplicate drug treatments.

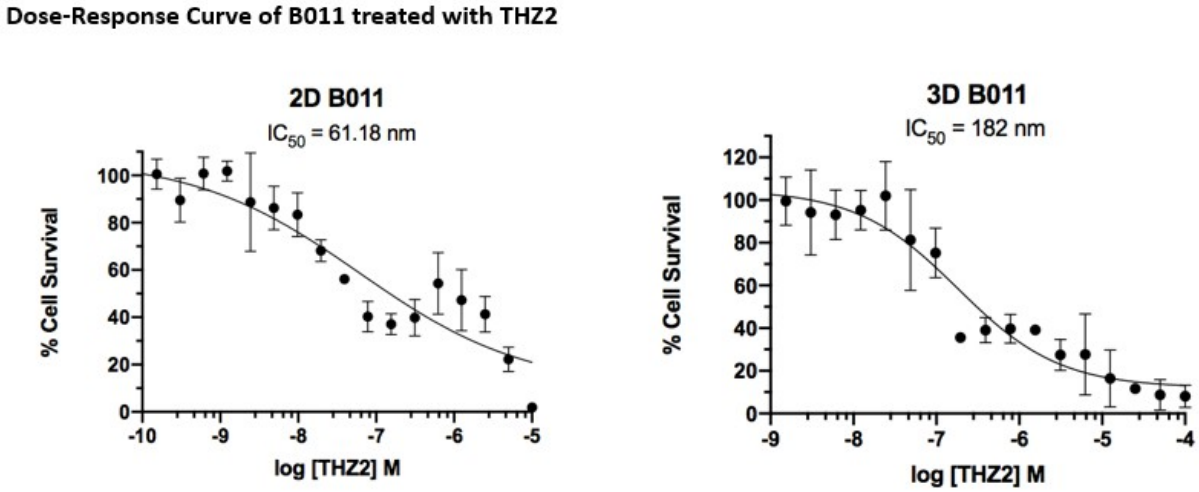

Figure 2: B011 cells grown in a 2D monolayer demonstrated an IC_{50} of 61.18 nm while the 3D cultures demonstrated an IC_{50} of 182.00 nm when drugged with THZ2 for 72 hours. A total of 5000 cells were seeded for each condition. The x-axis represents the log of THZ2 concentration and the Y-axis represents percent of cell survivability. Error bars are standard error of the mean (SEM) of the triplicate drug treatments.

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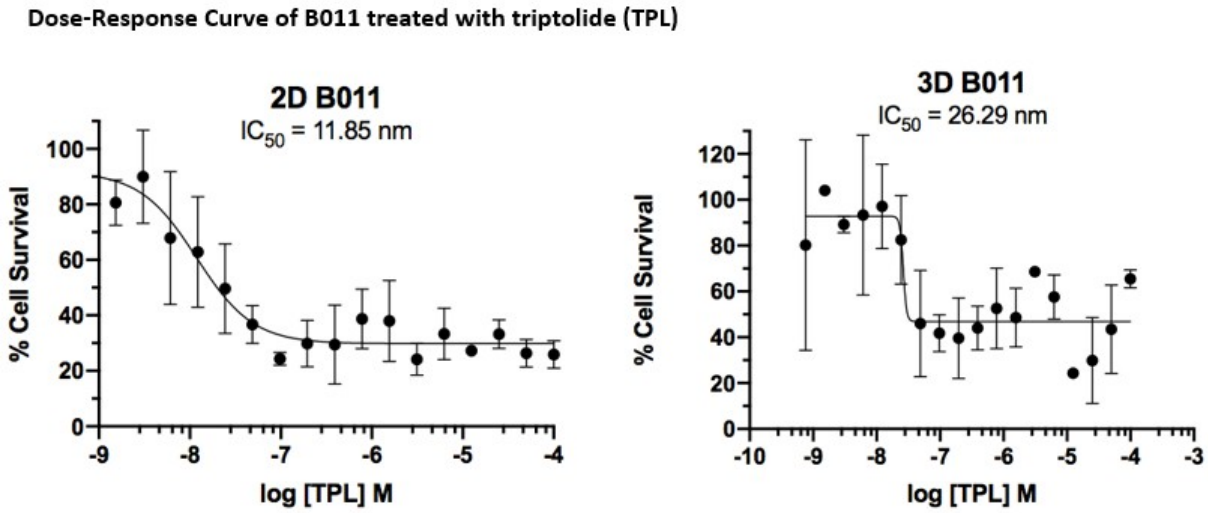

Figure 3: B011 cells grown in a 2D monolayer demonstrated an IC_{50} of 11.85 nm while the 3D cultures demonstrated an IC_{50} of 26.29 nm when drugged with triptolide for 72 hours. A total of 5000 cells were seeded for each condition. The x-axis represents the log of triptolide concentration, and the Y-axis represents percent of cell survivability. Error bars are standard error of the mean (SEM) of the triplicate drug treatments.

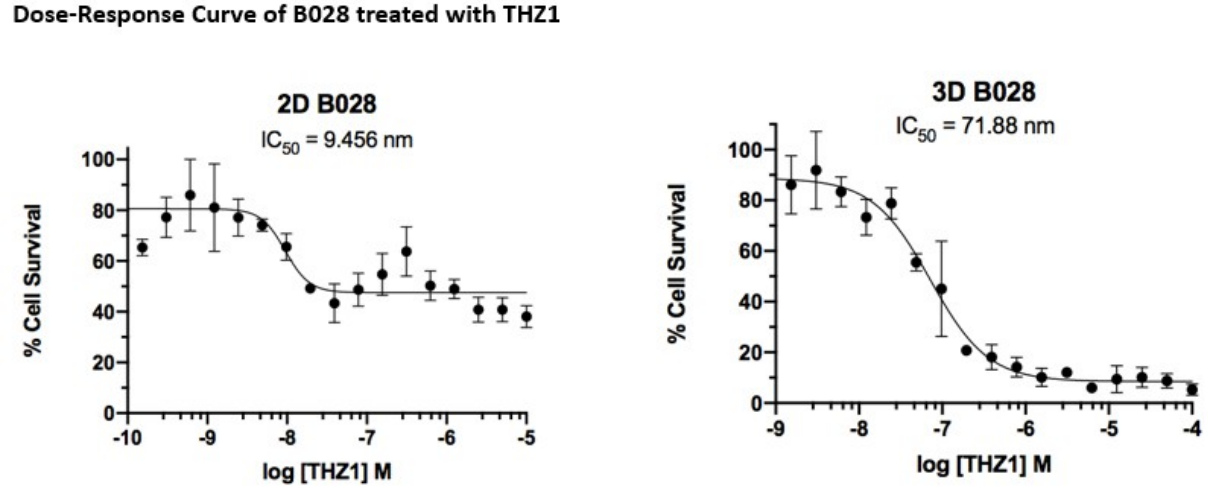

Figure 4: B028 cells grown in a 2D monolayer demonstrated an IC_{50} of 9.46 nm while the 3D cultures demonstrated an IC_{50} of 71.88 nm when drugged with THZ1 for 72 hours. A total of 5000 cells were seeded for each condition. The x-axis represents the log of THZ1 concentration, and the Y-axis represents percent of cell survivability. Error bars are standard error of the mean (SEM) of the triplicate drug treatments.

Discussion

The 3D B011 cell cultures demonstrated larger IC_{50}, indicating a greater resistance to treatment with all three super-enhancer inhibitors when compared to their 2D counterparts for each experiment. The 3D

B028 cell cultures demonstrated greater resistance to treatment with THZ1 but did not produce viable results when treated with THZ2 and triptolide. These results support the finding that super-enhancer inhibitors demonstrate activity against PDAC cell cultures (4), and show that 3D PDAC cell culture models are more resistant to treatment than their 2D counterparts.

There are a few notable limitations for this study. Although both 2D and 3D culture methods were initiated with 5000 cells per well, the 2D culture was grown for 24 hours prior to drug introduction while the 3D culture was grown for 72 hours prior to drug introduction. The longer growth time for 3D cultures were necessary to form viable spherical structures, but may have allowed for the opportunity for the difference in cell numbers to be significant between the two cultures, which may influence the luminescence results. However, both culture methods were initiated during the log growth phase of the cell cycle, which was demonstrated by a 60-70% confluence in their stock culture flasks. To address this potential pitfall, future experiments should be designed to test the IC_{50} in cultures with multiple different starting cell counts to see if the IC_{50} of 3D cultures remains higher than the IC_{50} of 2D cultures regardless of the starting cell count. Another limitation of this study was that viable results were not obtained for the B028 cell line with triptolide or THZ2. This was likely due to the use of different assay plates, which required additional pipetting steps and transfer of cells from their growth plates to the assay plates.

The results for this study potentially have clinical significance as they demonstrate that the same PDAC cell line, grown under different conditions, exhibit different sensitivity to therapeutic drugs. Given the more realistic physical and biochemical profile of 3D cultures, these findings suggest that 3D culture methods for PDAC may be superior to their 2D culture when testing the inhibitory effects of novel therapeutic agents for pancreatic cancer.

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