Exploring morphology and drug targets in pancreatic cancer with 3D cell culture models

TB Shelper and VM Avery
Discovery Biology, Eskitis Insititute for Cell and Molecular Therapies
Griffith University, Nathan, Qld , 4111

Introduction

Pancreatic cancer continues to have one of the poorest prognoses amongst all cancers, with a 5 year survival rate of less than 5%[1]. Drug discovery has traditionally relied on 2D cell culture based systems. Utilising 3D cell culture based systems in drug discovery programs has the potential to improve success rates and lower costs of bringing new therapeutics to market.

AIMS: To determine whether *in vitro* 3D cell culture models can be used to more accurately study cellular and drug interactions in pancreatic cancer.

The current challenge is to produce a 3D cell culture model that accurately reflects the biological complexity of human tumour growth. There exists no 'one type fits all' 3D cell culture system, with a range of models being developed over many broad research fields. There has been increased focus on 3D tumour models over the last 10 years with the ultimate goal being the development of a 3D *in vitro* cancer model that better mimics *in vivo* tumour biology[2].

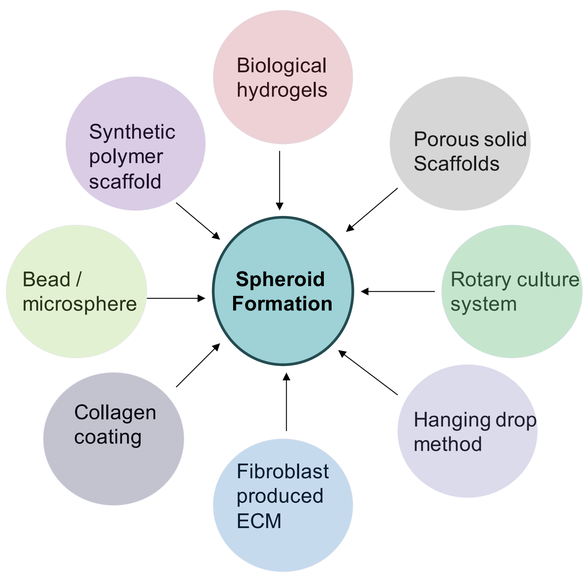

Figure 1. 3D cell culture technolgies suitable for cell based screening.

The data presented here evaluates morphology and biomarker expression of a selection of pancreatic cancer cell lines on commercially available ECM's. Biological hydrogels were chosen as they are one of the most widely used and documented artificial ECM's with the benefit of being biologically relevant, providing a rich environment for cell attachment and suitability for automated cell based assays. However they are expensive, involve labour intensive cell culture and have variable and undefined components across batches.

Comparisons of commercial biological based hydrogels have been investigated for spheroid production and biological relevance. Morphology and expression of a variety of biomarkers is compared between 2D and 3D cultures. Cell surface receptors involved in intercellular interactions and cellular-extracellular interactions such as cadherins, integrins, selectins, and adhesion molecules can be used to evaluate the 3D growth of the cultures. Data presented here will examine E-cadherin, the polarity marker GM130 and IGF-1 receptor.

E-cadherin is a transmembrane protein involved in cell adhesion and plays an important role in cell-cell interactions, its expression levels have been linked to invasiveness and tumour progression in human cancer[3]. The IGF-1R pathway has been implicated specifically in pancreatic cancer and recent novel treatments have targeted this receptor[4].

For a 3D cell culture system to be incorporated into a drug discovery program the cultures must produce consistent growth rates and reproducible spheroid formation for automatic data analysis programs to be utilised during drug screening.

Materials & Methods

Cell Culture

To create the *in vitro* 3D cell cultures the following gels were examined: Matrigel™, Matrigel™ Growth Factor Reduced (GFR), Cultrex™ and Geltrex™ (GFR). A layer of appropriate basement membrane was coated onto the bottom of 384 well microplates before a dilution of cells was plated out.

A panel of pancreatic cell lines with different growth characteristics and metastatic potential were selected (Table 1.)

Cell lines	Metastasis potential	Differentiation in 2D culture	3D culture structure	Tumour origin
BxPC-3	High	moderate - poor	Round	Primary - late stage
MIA PaCa-2	-	moderate - poor	Mass	Primary - late stage
PANC-1	low	poor	Mass	Metastasis lymph
Su.86.86	High	moderate - poor	Round - Mass	Metastasis liver
AsPC-1	High	poor	Mass	Metastasis Ascites
Capan-1	-	well - polarised, duct like structures	Large Aggregate Mass	Metastasis liver

Table 1. Pancreatic cell lines.

All cell culture work was performed in 384 micro plates to maintain future drug screening assays parameters. 2D cell cultures were run in parallel to compare morphology with cells grown in matrices. Media changes were performed every 3 days for live cell imaging plates using RPMI supplemented with 10% FBS.

Liquid handler automation

The Bravo™ 384 well liquid handler from Agilent was examined for suitability for 3D cell culture maintenance and immunofluorescence staining protocols. The automated pipetting functions reduced loss of cells for media change and wash steps in the 3D cultures.

Microscopy

DIC images of 3D cultures were taken over a period of several days, with the average spheroid area and diameter taken at regular intervals. All images were taken on an Olympus IX81 microscope linked to CellR imaging software. High detail Confocol IF images were taken on an Olympus FV1000 following staining with the nuclear hoesht stain, actin stain phalloiden and appropriate antibody. The High throughput and content analysis microscopes PE Opera™ and GE Incell™ were used for automated plate reads and evaluated for HTS suitability.

Image analysis

The large amount of data produced with 3D images requires a robust database and analysis system in place. For image analysis several commercial programs were examined for suitability including PE Acapella, GE Incell investigator, PE Velocity, Bitplanes Imaris as well as the public domain software ImageJ. DIC images were analysed using ImageJ scripting to determine size and consistency of plating protocols.

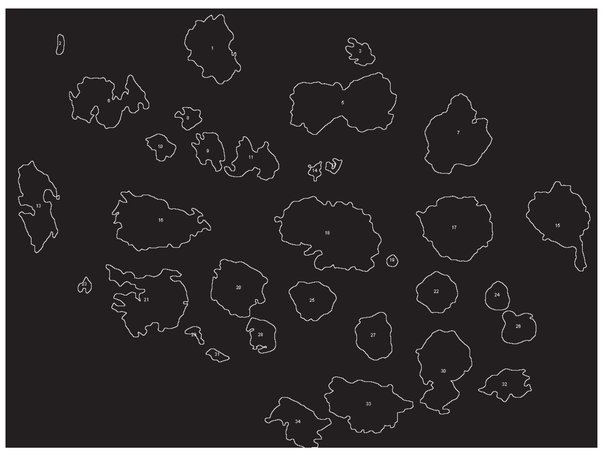

Figure 2. Example of Image 'analyse particle' scripting in ImageJ.

Results

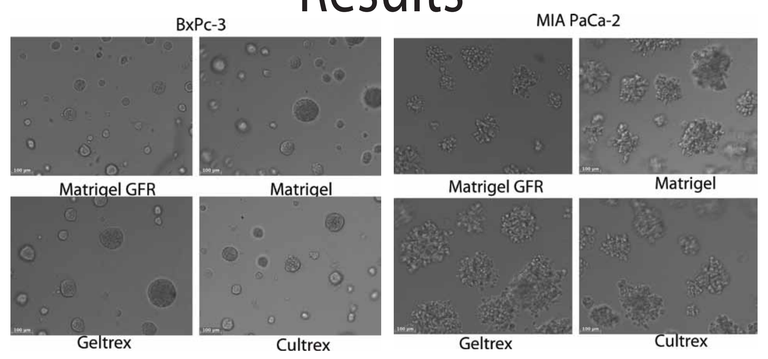

Figure 3. BxPc-3 and MIA PaCa-2 cell lines grown in different matrices at Day 4. There was no morphological difference detected between the different gels. All matricies had reproducible spheroid formation with the Matrigel growth factor reduced ECM having slightly reduced growth rate.

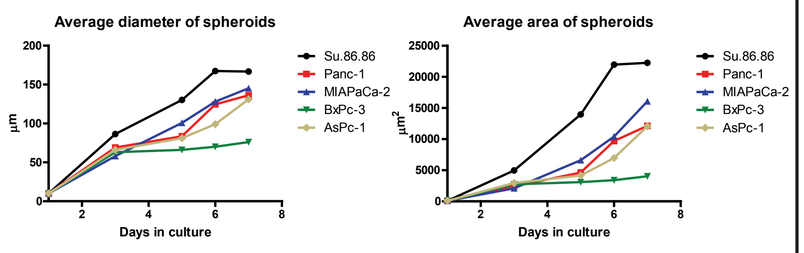

Figure 4. The suitability of Matrigel (GFR) for a use in HTS programs was evaluated using average spheroid size and diameter as indicators of reproducibility. Spheroid production was reproducible until day 7 after which some spheroids begin to aggregate together making automatic data analysis inaccurate.

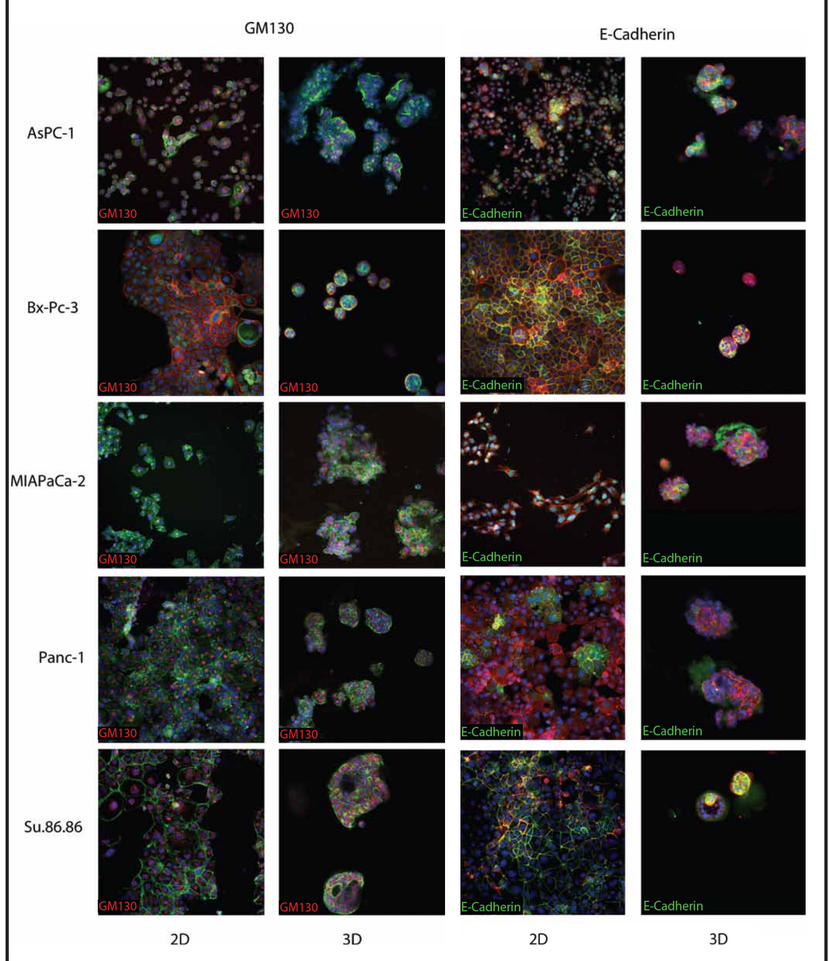

Figure 5. Representative immunostaining of 2D monolayer and 3D spheroid structures at day 4 and Day 8 respectively. Alexa488 and 594 labelled phallioden was used to detect F-actin (green channel for GM130 slides and Red channel for E-cadherin slides) and Hoechst stain to label nuclei (blue channel).

Immunoflourescent staining with GM130 showed specific orientation of cells in 3D spheroids while 2D monolayers had no distinct orientation. E-cadherin and IGF-1R staining revealed reduced intensity in 3D, with detection in some spheroids only present on the exterior surface. Further investigation is needed to determine if these markers are downregulated or antibody penetration is an issue.

Conclusions

Matrigel provided a suitable platform for development of a 3D cell culture system for drug discovery. Optimal conditions for reproducible growth were established in 384 well format, with automation and data analysis on going. Preliminary results indicate observable differences in expression patterns between 2D and 3D cultures. Further clarification of observations presented here, in addition to cytotoxicity, metastasis and drug penetration assays will be undertaken.

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References

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3. Wijnhoven, B.P., Dinjens, W.N., and Pignatelli, M. (2000). E-cadherin-catenin cell-cell adhesion complex and human cancer. Br J Surg 87, 992-1005
4. Chames, P., Kerfelec, B., and Baty, D. (2010). Therapeutic antibodies for the treatment of pancreatic cancer. ScientificWorldJournal 10, 1107-1120.