Characterization of patient-derived organoids cultured on a gas-rich, liquid-liquid interface

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INTRODUCTION

System Modeling tissues in vitro has long consisted of two-dimensional (2D) monolayer culture in flat, no- or well-plates. While these methods remain adequate for some applications, the advent of personalized cancer therapy has increased the need for culture methods that better imitate in vivo conditions—such as culture on a rigid substrate. Three-dimensional (3D) cell culture methods have been shown to provide a more physiologically relevant environment for cell growth and function. As a result, 3D culture models are one attractive solution for creating predictive, patient-specific tumor models in vitro. Typically grown as spheroids, these models still present limitations in terms of medium volume exchange per culture and cell survival and mobilization of tumor development.

Lena Biosciences has previously developed complex vascularized tissue models using patented 3D scaffolds and a perfusion system. Hepatocytes grown in this system demonstrated increased cell viability and recovery of cytochrome P450 enzyme activity compared to 2D controls. Integral to the perfusion system is a gas-rich, liquid-liquid interface between the cell culture medium and this synthetic liquid. H1299 NSCLC cells (left) were grown in a multi-well plate or atop the Blood Substitute in 6-well inserts. Photos were acquired at the time points indicated. On days 2 and 5, cell morphology and distribution were comparable. At day 8, the cells in the multi-well plate had become overgrown while the cells on the Blood Substitute maintained a more consistent spheroid shape. These results demonstrate the ability of the Blood Substitute to maintain cell viability and promote cell attachment in a 3D model.

METHODS

Cell Culture

Patent-derived NSCLC cells were plated at 10,000 cells/cm² in a humidified incubator at 37 °C for 24 hours in the manufacturer’s recommended medium. Cells were maintained in EMEM supplemented with 10% FBS. Patient-derived NSCLC cells were maintained in modified M87 medium1. Modified H1299 cells were a gift from Adam Arnst2. Cells were grown in a humidified incubator at 37 °C for 24 hours in the manufacturer’s recommended medium. Cells were maintained in EMEM supplemented with 10% FBS. Patient-derived NSCLC cells were maintained in a humidified incubator at 37 °C for 24 hours in the manufacturer’s recommended medium. Cells were maintained in EMEM supplemented with 10% FBS. Patient-derived NSCLC cells were maintained in a humidified incubator at 37 °C for 24 hours in the manufacturer’s recommended medium. Cells were maintained in EMEM supplemented with 10% FBS.

Culturing in PerfusionPal potentiates hepatic CYP activity for drug toxicity testing

Culturing in PerfusionPal potentiates hepatic CYP activity for drug toxicity testing. Primary human hepatocytes (BiOfy) were maintained in culture over a 7-day period (n=3 for all conditions) in either a multi-well plate (2D Control) or in Lena Biosciences’ PerfusionPal system (PerfusionPal) with a perfusion rate corresponding to 16 culture medium volume exchanges per 24-hour period. Baseline CYP activity (left) was significantly increased for all samples tested. Induced CYP activity (right) was similarly enhanced by the ntd-liquid interface. This enhanced CYP activity was a number of CYP enzymes in PerfusionPal were higher than the induced activity in 2D. Note the log scale for the left graphs. All data are normalized to their respective uninduced 2D controls. (*p<0.05, **p<0.01, ***p<0.001).

Growth of Patient-derived NSCLC cells on Blood Substitute induces spheroid formation

Growth of Patient-derived NSCLC cells on Blood Substitute induces spheroid formation. Patient-derived NSCLC cells demonstrate compromised growth in the absence of the Blood Substitute, an effect possibly enhanced by the medium additive. Patient-derived cells were plated in a multi-well plate or atop the Blood Substitute in the presence or absence of the medium additive. On day 1, the cells on the Blood Substitute appeared rounder and denser than the cells on the multi-well plates. Without the additive, the cells grew in the multi-well plate and began to form spheroids. On day 5, spheroid growth could be observed in all conditions. On day 7, the spheroids on the Blood Substitute were more consistent in size and shape, compared to the multi-well plate condition. The Blood Substitute allowed improved spheroid formation, although the presence of the additive seems to result in smaller, more consistent spheroids.

Cell response to the liquid-liquid interface is cell-type dependent

Cell response to the liquid-liquid interface is cell-type dependent. Lena Biosciences’ PerfusionPal system provides a simple method for culturing cells at a liquid-liquid interface between medium and Blood Substitute. PerfusionPal multi-well inserts (2x2 well or 4x4 well inserts, of which 4, 8, 16, 32, and 64 inserts (pore size) can be used with standard multi-well plates with Blood Substitute in the wells. Cells are seeded in medium on medium in a humidified incubator at 37 °C to promote cell attachment in the Blood Substitute surface.

CONCLUSIONS/FUTURE DIRECTIONS

• We have developed a modified application of our PerfusionPal/Blood Substitute and multi-well inserts to generate 3D cultures without the need for scaffolds.

• The superior gas exchange provided by the Blood Substitute results in a more physiologically relevant environment that facilitates in vivo-like tissue and spheroid growth.

• Future studies will incorporate freshly acquired tissue samples to demonstrate maintenance of in vivo cell characteristics to validate this system as a model for the study of cancer metastasis and pharmacological testing.

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