Utilisation of a Novel 3D Culture Technology for the Assessment of Chemo-Resistance in Non-Small Cell Lung Cancer

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Introduction

The key mechanisms that underlie chemo-resistance in lung cancer have yet to be fully elucidated. A significant limiting factor in these studies is the seeming lack of biologically relevant cellular models available for basic laboratory research. To address these issues, many are now turning to 3D-based cellular assay systems that permit the formation of multicellular structures (MCS). Depending on their size, the internal microenvironment of these structures mimic more closely that of those *in vivo*. In the majority of cases, MCS with a diameter larger than 100 µM exhibit an asymmetry in cellular proliferation and viability, with proliferating tumour cells at the periphery; cell-cycle arrested cells at larger distances from the surface. Regions of necrosis associated with reduced oxygen tension and hypoxia have often been reported[1], as illustrated by Figure 1. This study compared models of non-small cell lung cancer (NSCLC) in 3D culture with those grown in two-dimensional (2D) culture.

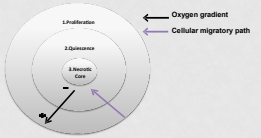

Figure 1: Cellular gradients observed in 3D MCS.

Methods

Happy Cell Advanced Suspension Medium™ (ASM) was chosen as a method to culture NSCLC MCS. This polymer-based formulation was selected for its ease of use, as well as its compliance with liquid handling, high content imaging and analysis (HCSA) and high throughput screening (HTS) systems. An isogenic NSCLC cell line model of cisplatin resistance (H460), were cultured in both 2D and 3D cell culture systems. Cisplatin, which induces apoptosis by targeting DNA, is a mainstay in lung cancer treatment. However, intrinsic and acquired cisplatin resistance is an increasing problem in lung cancer management. This model included both cisplatin sensitive (Parental - Pt) and cisplatin resistant (CisR) sub-types. Cell lines were cultured in two-dimensional (2D) monolayers and three-dimensional (3D) MCS, utilising Happy Cell ASM for the latter. The IC50 values had previously been elucidated for cisplatin and a positive control was selected. All cells were cultured in a range of cisplatin concentrations for 72 hours. Subsequently, viability assays (Cell Titre Glo) were conducted in order to compare the response of Pt and CisR cells to cisplatin in both 2D and 3D culture systems. Morphological analysis was performed via high content analysis (HCA) using the IN Cell 2000 (GE Healthcare).

Results

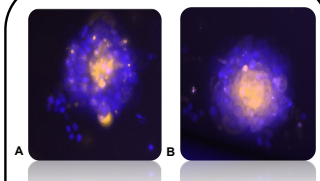

Figure 2: H460 PT (A) and CisR (B) 3D MCS necrotic core highlighted using Cell Viability Kit (GE Healthcare). Image taken using Cytell Imaging system (GE Healthcare) (10X magnification).

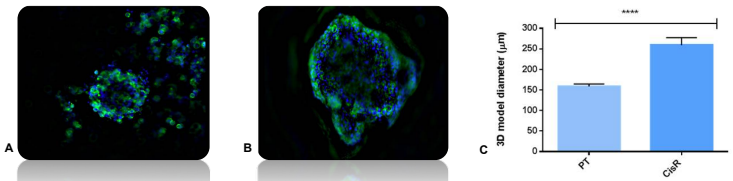

Figure 4: H460 PT (A) and CisR (B) 3D MCS stained with Hoechst nuclear stain (1:1000) (blue) and cytoskeleton stain phalloidin 488 (1:200) (green). Images taken with IN Cell Analyzer 2200 (GE Healthcare). PT MCS measured approx. 158.5 µm (range: 125-254) and CisR approx. 259.4 µm (range: 156-436) indicating a significant difference in diameter between H460 PT and CisR 3D MCS (C). *Mean ± SEM, n = 20.* ****p<0.0001, based on a paired student's *t* test.

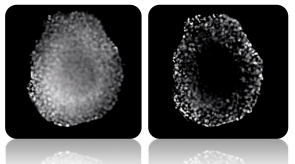

Figure 3: Z stack through a H460 3D MCS stained with Hoechst nuclear stain (1:1000), possibly identifying a hollowed necrotic core. Work is ongoing to further characterise the structure of the MCS. IN Cell Imaging System (GE Healthcare) (20x magnification).

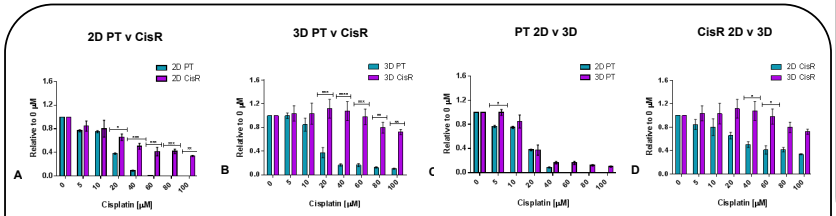

Figure 5: CellTiter-Glo viability data comparing cisplatin dose response curves of H460 PT and CisR cell lines cultured in both 2D and 3D. *Mean ± SEM, n = 3.* *p<0.05, **p< 0.01, ***p<0.001, ****p<0.0001, Cisplatin vs. UT (0µM) based on 2way ANOVA analysis. Experiments are ongoing.

Proteomic analysis – 3D v 2D (H460 PT)

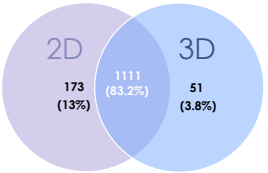

Figure 6: Number of proteins in common and unique to each sample.

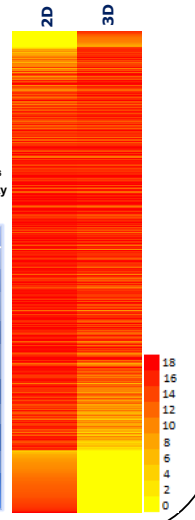

Figure 7: Heat map representation of protein expression in 2D and 3D samples. 1337 proteins represented. Scale:10^6 based on total Intensity Chi^2 analysis.

Table 1: 2D upregulated pathways
Translation
Cell cycle
RNA processing
Protein localisation
Protein transport
Proteolysis
Macromolecule catabolic process
Macromolecule complex assembly
Regulation of cellular protein metabolic process
mRNA processing
RNA splicing
Cellular response to stress
Cellular macromolecule localisation

Table 2: 3D upregulated pathways
Oxidation reduction
Apoptosis
Macromolecular complex assembly
Proteolysis
Generation of precursor metabolites and energy
Macromolecular catabolic process
Autophagy
Intracellular transport
Response to organic substance
Modification-dependant protein catabolic process

Discussion

Preliminary data suggests that at equivalent cisplatin concentrations the CisR cell line, in both 2D and 3D, conveys greater resistance to chemotherapy compared to the parental line. This is to be expected due to the intrinsic resistance inferred by the CisR cell line (Fig. 5A, 5B). However, when compared to monolayers the H460 3D MCS exhibit greater resistance in the Parental and CisR cell lines (Fig. 5C, 5D). We also observed that the CisR MCS appeared to be more tightly packed structurally than the PT MCS (Fig. 3). This could be a potential contributing factor to their chemo-resistant properties by inhibiting penetration of the drug into the MCS. Imaging experiments have also demonstrated that these 3D structures have a central necrotic core (Fig. 2). This is a feature of the asymmetric growth patterns associated with these 3D structures; that being a decrease in viable cells as you move inwards from the periphery of the MCS.

Conclusion

We have verified Happy Cell ASM as a novel system for generating 3D multicellular structures, and its potential for HTS and HCSA. When treated with cisplatin, H460 MCS exhibited more resilience to its cytotoxic effects compared with 2D cultures. As it has been argued that MCS and their microenvironment are more reflective of the *in vivo* situation, MCS may provide a more accurate *in vitro* model to elucidate mechanisms of drug resistance. Therefore, aiding in the identification of novel targets to re-sensitise patients to therapy and to identify mechanisms of chemo-resistance.

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References

1. Mueller-Klieser W. Tumor biology and experimental therapeutics. *Critical Reviews in Oncology/Hematology.* 2000;36:123-139.

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