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 150 µm exhibit an asymmetry in cellular proliferation and viability, with proliferating cells at the periphery of the MCS. In the majority of cases, MCS with a diameter larger than 150 µm exhibit an asymmetry in cellular proliferation and viability, with proliferating cells at the periphery of the 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 resistance to cisplatin 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, utilizing 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 Titer 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 stained with Hoechst nuclear stain (1:1000) (blue) and cytoskeleton stain phalloidin 488 (1:200) (green). Images taken with IN Cell Analyzer 2300 (GE Healthcare). PT MCS measured approx. 158 ± µm (range 125-254) and CisR approx. 258 ± µm (range 138-435) indicating a significant difference in diameter between H460 PT and CisR 3D MCS (C). Mean ± SEM, n = 3. **p<0.01, ***p<0.001, ****p<0.0001, Cisplatin vs. UT (0 µM) based on 2way ANOVA analysis. Experiments are ongoing to further characterise the structure of the MCS.

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, SB). 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 chemoresistant 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 resistance 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


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