The Use of High Content Imaging and Viability Readouts for Screening with 3D Spheroids

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Introduction

Cell based assays have widely been used in drug discovery, however, it is becoming increasingly clear that the traditional 2D model of cell culture is not truly representative of how cells grow and respond *in vivo*. To address this disparity, 3D cell culture technology has made vast improvements in recent years and is fast emerging as a new approach to drug discovery allowing for a greater understanding of drug efficacy and toxicity before drugs move into the clinical trial phase.

Aurelia has implemented 3D cell culture techniques which can easily be adapted into an HTS format. Ultra-low attachment (ULA) plates allow spheroids to form naturally. This technology relies upon a cells innate ability to grow and interact in a 3D format to form a spheroid. This provides a better representation of *in vivo* cell physiology which is lost in the tradition monolayer format. Using this technology we were able to monitor drug potency and efficacy in either an imaging-based assay (on the CellInsight™ CX5 High Content Screening (HCS) Platform) or by using commercially available assays such as the CellTiter Glo® 3D viability assay (Promega).

Here we test two spheroid cultures derived from either a HCT116 (human colorectal carcinoma) or U87 (human glioblastoma) cell line. Each cell line produces a compact spheroid. We have used a number of readouts to generate a pharmacological profile of each spheroid type.

Methodology

HCT116 and U87 cells were cultured in DMEM media supplemented with 10% FBS in TC-treated flasks at 37°C, 5% CO_2. For spheroid formation, each cell line was seeded at 400 cells/well (40µl/well) in cell culture medium in 384-well ultra low attachment (ULA) microplates (Corning). The plates were incubated at 37°C, 5% CO_2 for a period of 4 days to allow the cells to cluster and form a spheroid. Compounds were added on day 4 at a 1:2 dilution (40µl/well) and incubated with cells for a further 4 days before measurements were taken.

The effect of each compound was determined in three different assays. Viability was measured using the CellTiter Glo® 3D Viability Assay (Promega). An equal volume of reagent was added to the culture medium. Plates were mixed for 5 minutes on an orbital shaker to ensure good cell lysis. The plates were allowed to equilibrate at room temperature for 20-25 minutes after which time luminescence was determined using the Enspire® Plate Reader (PerkinElmer). The luminescent signal is directly proportional to the level of ATP within the cells and hence gives a measurement of metabolic activity and cell viability.

Over time, the size of the spheroid was monitored using brightfield microscopy on the CellInsight™ CX5 High Content Screening (HCS) Platform (Thermo Scientific). An algorithm was used to define the area of the spheroid using a 10x objective.

In addition to monitoring spheroid size, we also stained the spheroids with DRAQ7™, a far-red DNA stain that will only stain the nuclei of dead or permeabilised cells. Spheroids were stained with DRAQ7™ at a 1:2 dilution to give a final concentration of 1.5µM.

Summary and Conclusion

- All compounds tested affected cell viability in a dose dependent manner (CellTiter Glo® 3D Viability Assay). While there was some variation in potency, not all compounds were able to restore viability to untreated levels within the concentration range tested.
- Further investigation with a DRAQ7™ dead cell stain revealed that those compounds which showed reduced viability also appeared to have a necrotic core. This effect was also dose dependent.
- Growth inhibition was determined by calculating spheroid area. We were able to monitor growth inhibition in a dose- and time- dependent manner in both spheroid types tested. Only nocodazole and paclitaxel increased spheroid size. They appeared to cause shedding of peripheral cells and a loss of the tight spheroid morphology.
- Taken together, we were able to build a pharmacological profile for each compound tested.

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CellTiter Glo® 3D Viability

Spheroids were allowed to form in ULA plates for 4-days, this was an optimum time for cell compaction prior to drug treatment. To assess cellular viability, spheroids were cultured and then treated with an increasing concentration of compound. Each cell line showed a dose-dependent decrease in viability with an increasing concentration of compound.

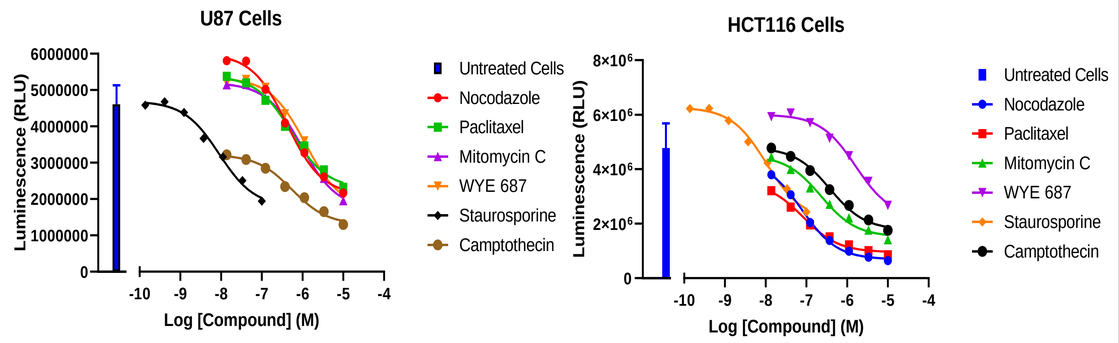

Figure 1: Cell viability as determined by the presence of ATP in U87 or HCT116 spheroids. Data is expressed as a percentage of the untreated control.

	Nocodazole	Paclitaxel	Mitomycin C	WYE 687	Staurosporine	Camptothecin
U87	416nM	535nM	935nM	1165nM	9nM	553nM
HCT116	78nM	84nM	229nM	1622nM	10nM	376nM

Table 1: Estimated IC_{50} values determined in the CellTiter Glo® 3D Viability assay in spheroids derived from U87 or HCT116 cells.

Growth Inhibition

Spheroid size was quantified on the CellInsight™ CX5 High Content Screening (HCS) Platform using an overlay on the brightfield imaging channel.

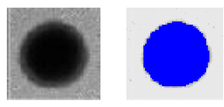

Figure 2: HCT116 derived spheroid imaged in brightfield. The blue mask which overlays the image details the size quantification.

Prior to treatment, spheroid sizes showed an initial decrease due to cell compaction. After this time, in the absence of compound, spheroids grew in a linear manner up to day 8 of the experiment. Compounds induced a dose-dependent inhibition of growth as characterized by a reduction in the spheroid size. This was consistently seen in the U87 cell line. In comparison, compounds paclitaxel and nocodazole induced a looser cytoskeletal organization in HCT116 spheroids at higher concentrations.

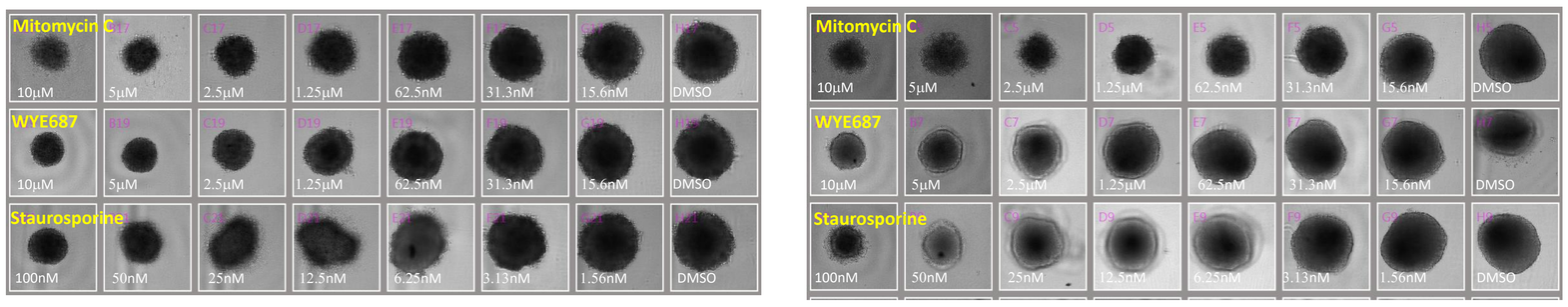

Figure 3: Brightfield images of spheroids consisting on U87 cells (left panel) or HCT116 cells (right panel).

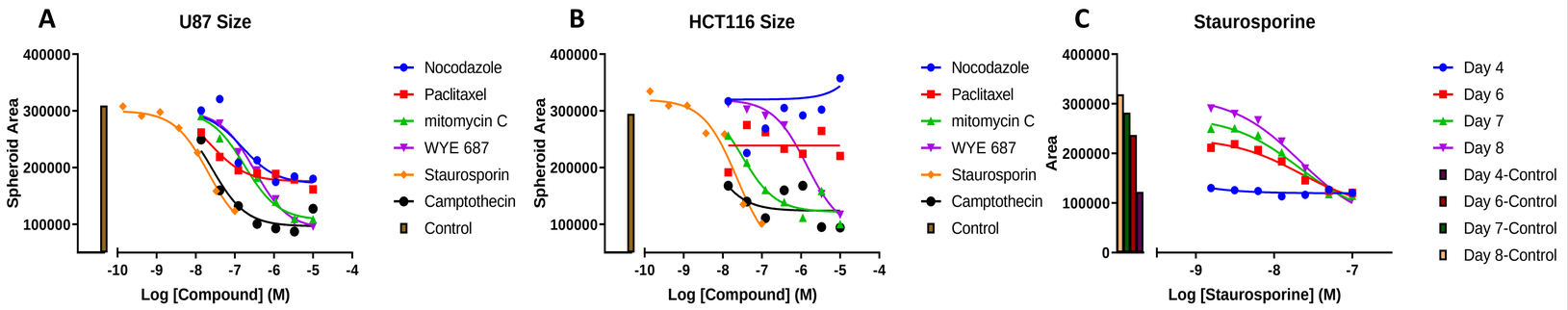

Figure 4: Spheroid area (in pixels) plotted against compound concentration in the presence of U87 (A) or HCT116 (B) cells, data is taken from an 8-day time point. The cytotoxic effects of staurosporine can be seen over time (C).

	Nocodazole	Paclitaxel	Mitomycin C	WYE 687	Staurosporine	Camptothecin
U87	154nM	26nM	177nM	315nM	21nM	26nM
HCT116	-	-	31nM	1407nM	23nM	4nM

Table 2: Estimated IC_{50} values derived from the spheroid size at day 8 derived from spheroids comprising of U87 or HCT116 cells.

Spheroid Viability

Spheroid structure is characterized by a mixed population of cells whereby a hypoxic core is surrounded by a viable and replicating collection of cells on the periphery. In the absence of compound, DRAQ7™ staining increased in untreated spheroids over time.

Figure 5: HCT116 derived spheroid. The hypoxic core is stained with DRAQ7™ (red), a far-red dye that will only stain dead cells. Viable cells are stained with Calcein AM (green).

Spheroids were treated with compounds early in their growth phase prior to the formation of distinct necrotic core. Compounds induced a gradual increase in DRAQ7™ staining over time in a dose-dependent manner. Treatment with nocodazole or paclitaxel significantly increased DRAQ7™ staining, this may be due to the loose nature of the spheroids in the presence of compound.

Figure 5: DRAQ7™ staining of U87 spheroids (left panel) or HCT116 spheroids (right panel) at day-8 in the presence of increasing concentrations of compound.

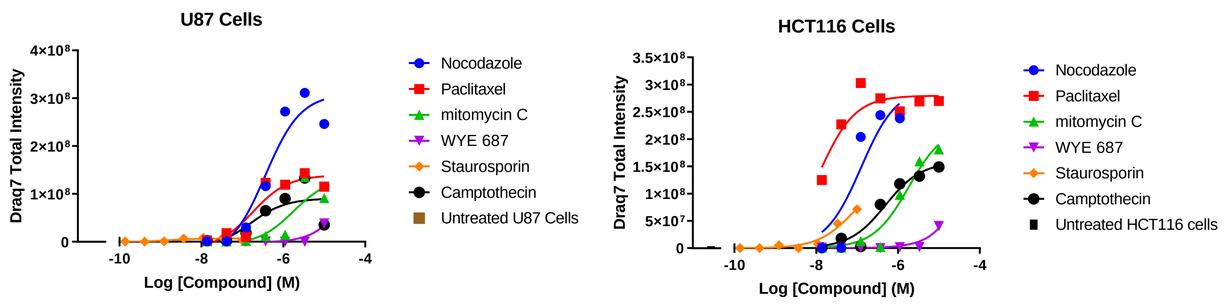

Figure 4: DRAQ7™ intensity plotted against compound concentration using either U87 or HCT116 cells.

	Nocodazole	Paclitaxel	Mitomycin C	WYE 687	Staurosporine	Camptothecin
U87	476nM	233nM	2107nM	-	-	219nM
HCT116	54nM	125nM	1857nM	-	-	495nM

Table 3: Estimated IC_{50} values derived from DRAQ7™ intensity in spheroids - comprising of U87 or HCT116 cells.