Introduction

Increasingly, the cancer research field is recognizing the need to transition to more physiologically relevant 3D screening systems that better replicate the cell-cell interactions, microenvironment and mass transfer properties of in vivo tumors. 3D systems have shown in several studies to be more predictive of drug efficacy than traditional culture methods. However, with improved physiological relevance comes increased heterogeneity that can complicate cell culture practices and confound standard assay readouts. Furthermore, many 3D culture systems are laborious, time-consuming and low throughput. AggreWell™ plates are microwell-based 3D cell culture devices that produce mass-size-controlled cancer spheroids per well from a single pipetting step (Figure 1). Within each imaged well, every spheroid can be individually identified, indexed, and analyzed to provide multiple repeated measures per treatment condition to yield more representative and predictive data.

Methods

Herein, we document the use of AggreWell™ to perform a 3D drug screening study using cancer spheroids of the MCF7 breast cancer cell line. Briefly, 500 cell cancer spheroids were made by seeding 6000 cells in AggreWell™ 24-well plates (Cat #3460) in serum-free MammoDiff™ (Cat #05620) media. 24 hours after seeding, spheroids were exposed to drug via 50% media exchange. Drug compounds were selected to target the luminal, basal, and milk epithelial cell lineages present in breast tissue (Figure 2A) to assess the sensitivity of MCF7 cells to these treatments. EC50 ranges for each drug were determined for concentration ranges up to 100 µM and 20 µM for Tamoxifen (TMX) and Lapatinib (LTB) respectively. DAPT up to concentrations of 200 µM had no effect beyond that of vehicle. For multi drug treatments, concentrations of 50 µM and 10 µM were used for TMX, LTB, and DAPT respectively. Treated spheroids were imaged, dissociated and quantified for cell viability by ADAPTI staining and analysis in a NIC-250 NucleoCounter (Chemomet). Image analysis was performed to measure morphometric parameters for each of 16 spheroids per well. The resulting data set was interrogated using Principal Component Analysis (PCA) to identify parameters predictive of drug-treatment effects and grouped to identify combinations that permit treatment classification.

Results

Figure 4: Synergistic Effects of Multi Drug Treatments in MCF7 Spheroids. LTB and DAPT show little effect alone, but demonstrate synergistic reductions of cell viability when treated in combination with TMX. 3-drug treatment displays further viability reductions. As DAPT has no effect in single drug treatment, this suggests that the combination of TMX and LTB is targeting a separate portion of the population to DAPT. Data presented as (A) percent viability and (B) total viable and dead cells.

Summary

AggreWell™ is a microwell based cell culture system that:

- Is an efficient tool for generating 3D spheroids
- Provides a standardized method for performing 3D drug screens
- Provides multiple individually identifiable and measurable spheroids per treatment condition
- Allows determination of EC50 or IC50 curves via cell-based assays including viability

References

Gangadhara et al. BMC Cancer, 16(1):345, 2016. (24.2% viable) cultures. Representative images show clear resulting in increased measure area.


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