Miniatrised 3D Pancreatic Cancer Assay for Drug Discovery

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Summary: The semi-automated workflow presented here utilises automated liquid handling robotics, plate readers and high content screening platforms to reduce well variability and increase assay robustness, while also reducing reagent costs. The physiologically relevant 3D cell model is used to examine drug interactions and provide insights into drug penetration and resistance effects of the tumour microenvironment. Chemotherapeutic drug sensitivity, assay reproducibility and suitability for HTS in these miniaturised formats was assessed across multiple pancreatic cancer cell lines.

Introduction: Pancreatic cancer continues to have one of the poorest prognoses amongst all cancers, with a 95% mortality rate. Standard of care chemotherapy has failed to provide significant clinical benefits, which has led to the development of targeted agents against validated signalling pathways. However, so far the approach of targeted agents alone, or in combination with traditional chemotherapeutics, has failed to improve the prognosis for pancreatic cancer patients. Models that improve the predictive potential of drug discovery programs and gain greater insights into the complexity of tumour biology are urgently needed. Current in vitro models involving cell monolayer cultures are unable to recapitulate the biological complexities of the in vivo tumour microenvironment and may be poor predictors of drug efficacy.

Methods: Ice cold Growth Factor Reduced Matrigel (GFR Matrigel) was diluted in RPMI and automatically dispensed (1.5µl per well for 1536 and 15µl for 384) onto the bottom of the well of PerkinElmer Cell Carrier microplates using an Agilent Bravo. The pancreatic cancer cell lines AsPC-1, BxPC-3 and PANC-1 were examined. Optimised cell seeding densities were calculated for each cell line and cells were dispensed onto the bed of Matrigel and allowed to form 3D structures over 72 hours. Automatic media renewal and drug dispensing was performed on the Agilent Bravo at 48h interval periods. Either resazurin or Calcein AM was dispensed and incubated until optical signal was reached in each detection method. Metabolic activity was calculated from fluorescent values detected on an Envision plate reader and cell viability was determined by image analysis protocols from an PerkinElmer Opera or Operetta high content imaging platforms.

Results: 3D cell cultures were assessed in both 384 well and 1536 well format with assay performance and sensitivity of reference chemotherapy agents calculated. Figure 2 highlights an image montage of the Calcein AM cell viability assay, in which a dose response of the standard of care chemotherapy agent gemcitabine effects 3D (micro-tumour like) structure morphology and cell viability.

Conclusions: The miniaturised 3D cell culture based assays examined here demonstrate it is possible to generate reproducible, cost effective 3D culture systems for use in future high throughput and high content analysis applications, using standard lab consumables and automated imaging equipment. Insights into drug resistance mechanisms can be assessed in a more physiological relevant model that may better reflect the in vivo tumour microenvironment. These culture system may ultimately provide better predictive data on novel compounds and insights into pancreatic cancer tumour penetration of current chemotherapy drugs.