m3DinAI-HCS DrugQuest: Unveiling Tumor Therapies in 3D cultures through High Content Screening and AI Innovation.

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High-Content Screening (HCS) technology combines cell biology and molecular tools with automated high resolution microscopy and robotic handling. This new type of cellular phenotypic screening enables the identification of hit compounds across multiple drug classes through the automatized acquisition of confocal brightfield or fluorescent images.

With a major focus on cancer research, in this study, we have utilized the HCS technology to develop a drug discovery pipeline that allows to rapidly determine the effectiveness of first-line chemotherapeutic drugs on 3D cancer cell spheroids using brightfield images. Specifically, we have focused on triple negative breast cancer (TNBC), which is the most aggressive type of breast cancer and lacks targeted therapy. Although cell monolayers have been used historically to model cancer diseases, 3D cell culture models are preferred for cancer drug discovery, since they mimic the complex architecture of tumors and represent better their physiological conditions, such as complex cell-cell interactions, gradients of oxygen, nutrients, and metabolites. However, reproducibility and scalability of 3D cultures has been a challenge using standard techniques. Here, we have optimized a 3D bioprinting platform in 384-well format using the *384-well bioprinting kit NanoShuttle*™-PL (Greiner Bio-one), which combined with our proprietary algorithm “m3DinAI-HCS DrugQuest” based on artificial intelligence, allows the quick identification of hit compounds with cytotoxic effects on cancer spheroids. The TNBC cell lines HCC1806 and BT-549 were cultured in 2D or used to bioprint spheroids in 384-wells using the NanoShuttle technology as indicated by the manufacturer with optimizations. Monolayers and spheroids were treated with different doses of tamoxifen, doxorubicin, vincristine, docetaxel, staurosporine, 5-fluorouracil, paclitaxel, and cisplatin for 24 h, 48 h, and 72 h. Cytotoxicity in 2D cultures was measured via MTT assays and analyzed with the Genedata Screener software. For 3D cultures, brightfield images were acquired with the Operetta CLS High Content Analysis System (Revvity) and analyzed with the Harmony 5.2 software (Revvity). The Harmony data was further integrated by our m3DinAI-HCS DrugQuest algorithm into a KNIME workflow that uses selected image features to train a decision tree. Drug cytotoxicity scores were determined according to the 24 features that yielded most information in the decision-making process, including spheroid size, density, compaction, texture, cell roundness, number of particles, etc. Considering 100 the maximum toxicity score, we have concluded that score values below 15 indicate no cytotoxic effect and intermediate values (16-99) represent different levels of drug effectivity in TNBC spheroids.

The results of this study reveal differences in cytotoxicity in 2D vs. 3D cell cultures and demonstrate the soundness of the m3DinAI-HCS algorithm to triage hundreds of chemotherapeutic drugs simultaneously within days in TNBC spheroids.