HIGH CONTENT SCREENING AS A DRUG DISCOVERY PLATFORM

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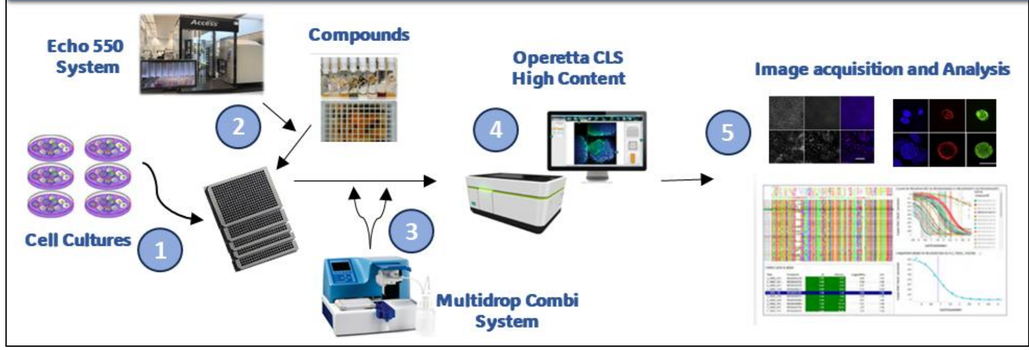
1. INTRODUCTION

High-Content Screening (HCS) technology has revolutionized the **early drug discovery** field in the last years. HCS 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 lead compounds across multiple drug classes through the automatized acquisition of confocal fluorescent images. HCS facilitates the characterization of cellular phenotypes altered by the test compounds, which allows a more profound understanding of drug effects and a better integration of disease-relevant screens.

MEDINA is a private non-profit Research Organization with a major focus on the discovery of novel molecules with relevant biochemical properties that can be applied to the development of new drugs. Although MEDINA´s high throughput screening platforms cover diverse approaches, including agriculture, cosmetics, or industrial exploitation. Here, we present a proof of concept for the use of the HCS technology in the **discovery of anti-tumoural** compounds with oxidant, apoptotic and cytotoxic effects. For that, we have developed relevant 2D and 3D cell culture models compatible with this technology.

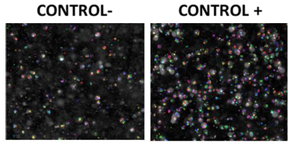
2. METHODOLOGY



3. CASPASE ACTIVITY

Caspase-3 (CASP3) is a major mediator of apoptosis activated during cellular exposure to cytotoxic drugs, radiotherapy, or immunotherapy. It is often used as a marker for efficacy of cancer therapy. Furthermore, drugs targeting caspase-3 may not only increase the sensitivity of cancer cell to chemotherapy and radiotherapy, but also inhibit cancer cell invasion and metastases.

Figure 1. Segmentation of caspase 3/7 active cells in control – (vehicle) and control + (Staurosporin 5 µM) in PC-3 cell line as example.



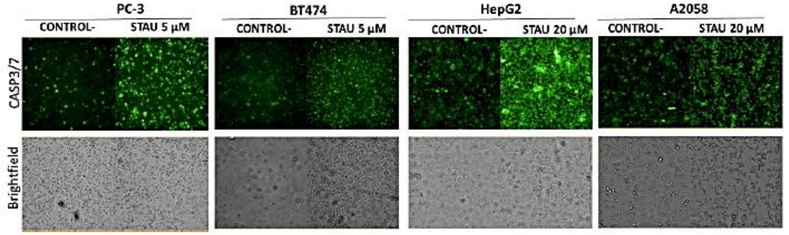


Figure 2. Images of control – (vehicle) and control + (Staurosporine) of PC-3, BT474, HepG2 and A2058 cell lines. To activate CASP3, Invitrogen™ CellEvent™ Caspase-3/7 Detection Reagent (ThermoFisher) was used. This reagent is made up of four amino acid peptide (DEVD) conjugated to a nucleic acid-binding dye that is non-fluorescent when not bound to DNA. Upon activation of caspase-3/7 in apoptotic cells, the DEVD peptide is cleaved and the free dye can bind DNA, generating a bright green fluorescence.

4. ROS PRODUCTION

ROS (reactive oxygen species) are intrinsic to cellular functioning and are present at low levels in normal cells. High levels of ROS cause damage to cell components, membranes and organelles, leading to cell death. Extensive studies have revealed that anticancer therapies that manipulate ROS levels show promising in vitro as well as in vivo results.

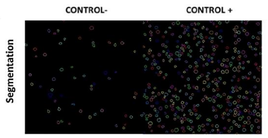


Figure 4. Segmentation of ROS positive cells in control – (vehicle) and control+ (menadione 50 µM) in PC-3 cell line as example.

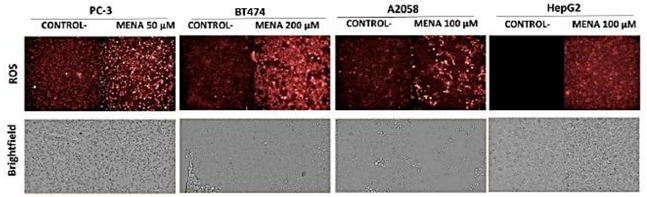
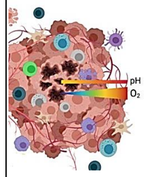


Figure 5. Images of control – (vehicle) and control + (menadione) of PC-3, BT474, A2058 and HepG2 cell lines. The CellROX™ Deep Red reagent (ThermoFisher) is a cell-permeant dye with absorption/emission maxima of ~644/665 nm. CellROX™ Deep Red reagent is non-fluorescent while in a reduced state and becomes fluorescent upon oxidation by reactive oxygen species with emission maxima ~665 nm that is measurable by fluorescent imaging. A positive control was created by treating the cells with menadione, which promotes ROS generation within the cells.

5. 3D CELL CULTURE (SPHEROIDS)



Tumor enviroment

Recent advances in 3D cell culture have attempted to bridge the gap between preclinical and clinical results. In particular, multicellular tumor spheroids (MCTS) have emerged as a promising in vitro model for cancer research because of their high throughput, low cost and increased physiological relevance compared to 2D culture. MCTS are defined as 3D spherical clusters of malignant cells and have been shown to mimic many features of solid tumors *in vivo*, such as cell–cell and cell–extracellular matrix (ECM) interactions, increased drug resistance, cell polarity and nutrient diffusion gradients. MCTS are introduced in the anti-tumour drug screening process at MEDINA as they mimic *in vivo* physiology incomparably better than the standard 2D culturing.

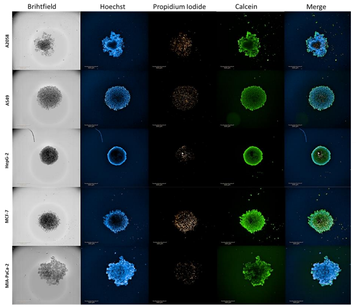


Figure 6. A2058, A549, HEPG-2, MCF-7 and MIA-PACA spheroids. The 2D cell lines were incubated with magnetic nanoparticles (Greiner Bio-one). After 24h, the cells were detached and seeded in a 384-well plate, then the spheroids were generated. After treatments, spheroids were stained with hoechst (blue-nuclei), calcein-AM (green-live cells) and propidium iodide (red-dead cells).

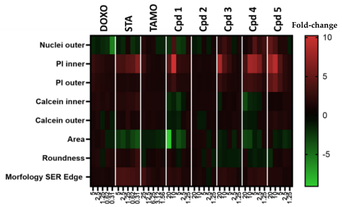


Figure 7. Heatmap of parameter comparison between known cytotoxic agents and compound tested MCF-7 spheroids. Fold-changes of compounds with regard to controls (DMSO 0.5%) for eight parameters (outer nuclei, inner PI, outer PI, inner calcein, outer calcein, area, roundness and morphology SER Edge) are shown. Parameter analysis performed by Harmony software (Perkin Elmer).

CONCLUSIONS

HCS is a potent phenotypic drug discovery strategy that characterizes small-molecule effects through the quantification of features that depict cellular changes among or within cell populations, thereby generating valuable data sets for subsequent data analysis which allows a more profound understanding of drug effects and a better integration of disease-relevant screens.

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