High Content Analysis of 2D and 3D cellular models for target and phenotypic drug discovery

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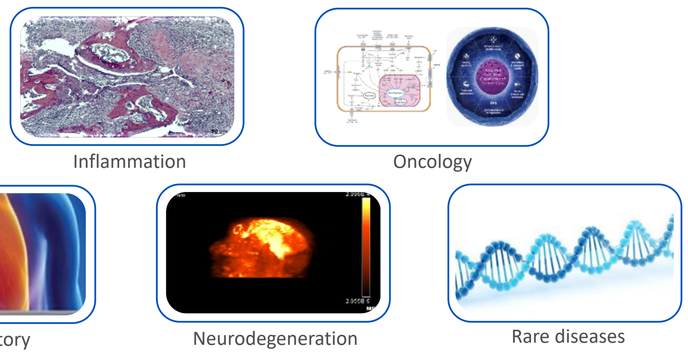
1 Introduction

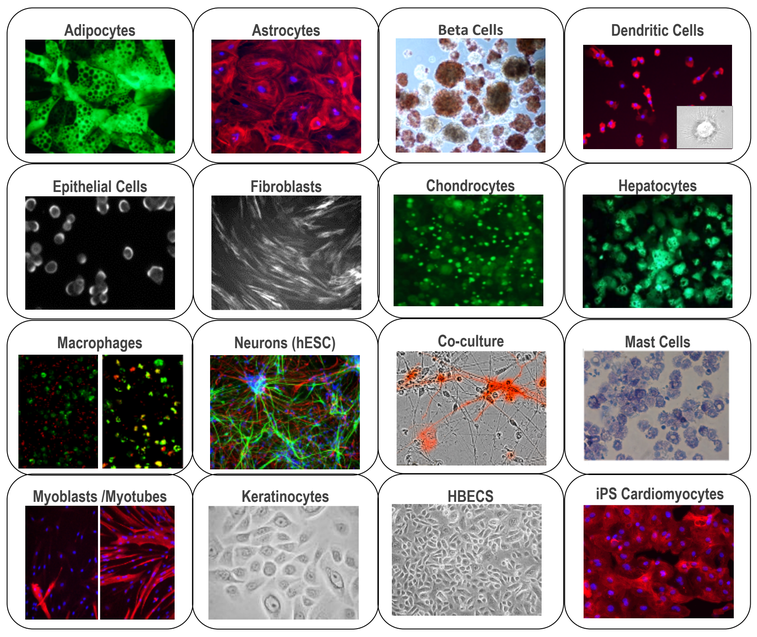
Charles River Laboratories (CRL) has developed many disease-relevant cellular models to screen small molecules and genetic modifiers for target and phenotypic based drug discovery. Our expertise and portfolio in bespoke cellular model development covers several therapeutic areas (e.g. inflammation, oncology, neurodegeneration and rare diseases) and a wide variety of target classes.

Complex cellular models derived from primary cells, patient derived embryonic stem cells and induced pluripotent stem cells have been adapted for phenotypic screening. Models have been further developed to produce physiologically relevant multicellular structures, such as 3D liver spheroids and neuronal co-cultures.

Such validated High Content (HC) assays can be applied to both target and phenotypic based discovery platforms to support preclinical drug discovery and improve translation of targets and compounds to the clinic.



2 Large panel of primary cell based assays



3 Algorithms developed for numerous therapeutic areas

Formats	Assays developed	Therapeutic area(s)
Anchorage independence and colony forming assays (in 3D)	Multiple cell lines (3D), Clonogenic assays (2D)	Oncology
Apoptosis, cell death, DNA damage response, cellular stress and degeneration	Early and late stage apoptosis markers, mitochondrial function, nuclear condensation, DNA damage assay and downstream signaling assays, foci, stress granules, protein inclusions	Neurodegeneration Fibrosis, Oncology
Cell cycle	Cell cycle progression and S phase	Oncology, Obesity
Autophagy and protein aggregation	Inclusion readout, aggregates	Neurodegeneration
Cell motility and migration	Scratch wound assays	Oncology, Fibrosis
Cytoskeletal rearrangements	Changes in cell morphology (multiple markers/cell lines) hypertrophy, hyperplasia, F-actin alterations	Fibrosis, Respiratory
Post translational modification	Multiple assays for signalling events (phosphorylation and acetylation)	Neurodegenerative, Oncology, metabolism
Real time imaging	Calcium flux in neurons and cardiomyocytes, neurite outgrowth and retraction, phagocytosis	Safety toxicity Neurodegenerative disease
Marker expression	Multiple assays to assess transduction efficiency, epigenetic target and biomarker	Virus production, Neurodegeneration, Oncology
Differentiation	FISH (RNA), TG-ase Calcium deposition, myotube formation, neurite outgrowth	Fibrosis, Psoriasis Osteogenesis, Muscular dystrophy, Neurodegeneration
Neurite outgrowth	Multiple endpoint and real time formats	Neurodegeneration
Receptor internalization and degradation	Nuclear receptor degradation, receptor internalisation, ligand binding	Oncology, Neurodegeneration
Sub-cellular localization	Protein trafficking, Inclusions in cytoplasm/nucleus	Neurodegenerative disease
Translocation of transcription factors	Multiple cytosolic/nuclear translocation assays	Oncology, inflammation

3 Examples of High content based assays

PRIMARY RAT NEURON CORTICO-STRIATAL CO-CULTURES

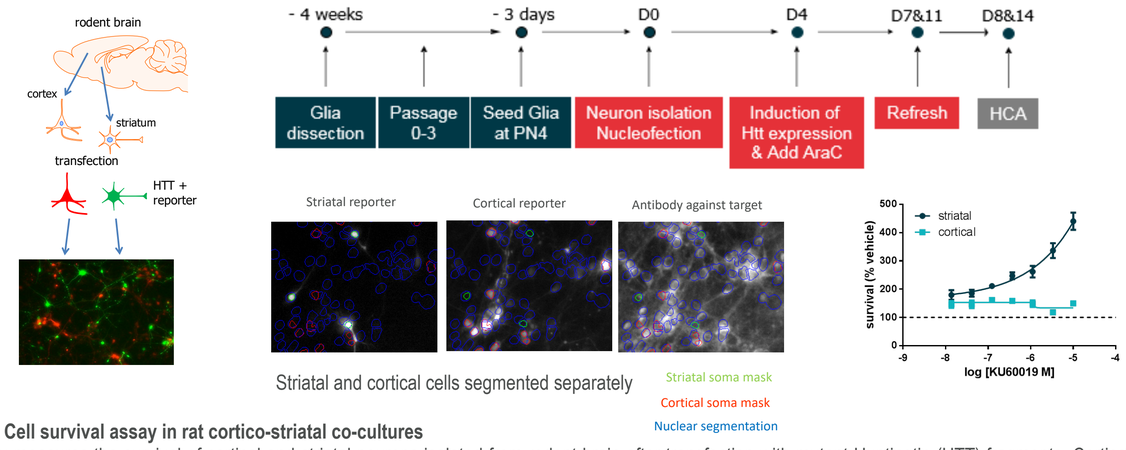


Figure 1. Cell survival assay in rat cortico-striatal co-cultures
The assay measures the survival of cortical and striatal neurons isolated from rodent brain after transfection with mutant Huntingtin (HTT) fragments. Cortical and striatal neurons are transfected separately with different fluorescent reporter alongside with a mutant HTT fragment expressing plasmid. Striatal and cortical cell survival is measured by high content analysis of the number of fluorescent cells remaining after a certain time window, and compared to cells transfected with an empty vector plasmid (instead of the mutant HTT vector). Compounds demonstrating a neuroprotective effect in this assay will result in an increase in the number of fluorescent striatal and/or cortical cells (ex: KU60019).

FIBROBLASTS-TO-MYOFIBROBLASTS TRANSITION (FMT) ASSAY

Figure 2. FMT assay. (A) Lung-derived primary human bronchial fibroblasts were seeded and refreshed before addition of small molecule compounds to screen and the TGF-β1 trigger. **(B)** Imaging results: after 3 days of incubation, cells are stained with DAPI-labeled αSMA and imaged via high-content analysis. **(C)** Imaging analysis: quantification of αSMA expressing myofibroblasts resulted in concentration response data shown for trigger and reference control. SB525334 inhibits the formation of myofibroblasts promoted by the TGF-β1 trigger.

REPROGRAMMING OF IPSCS AND FIBROBLASTS TO MYOTUBES

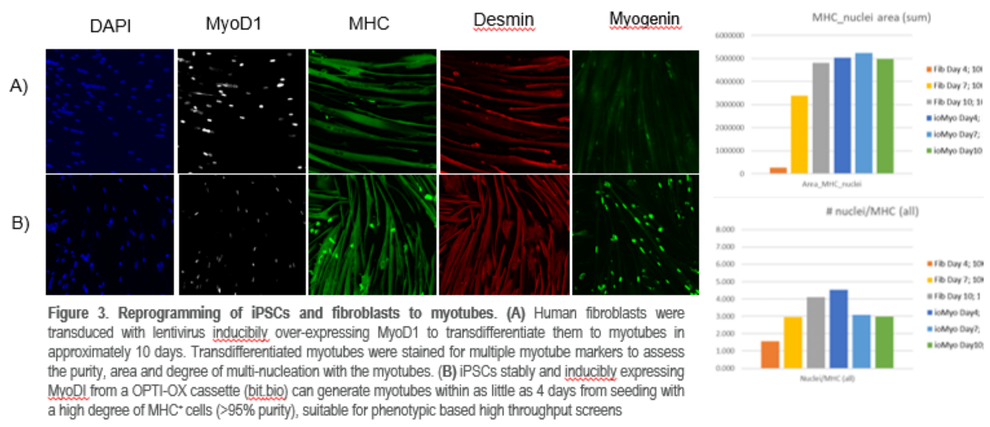


Figure 3. Reprogramming of iPSCs and fibroblasts to myotubes. (A) Human fibroblasts were transduced with lentivirus inducibily over-expressing MyoD1 to transdifferentiate them to myotubes in approximately 10 days. Transdifferentiated myotubes were stained for multiple myotube markers to assess the purity, area and degree of multi-nucleation with the myotubes. **(B)** iPSCs stably and inducibly expressing MyoDI from a OPTI-OX cassette (bit.bio) can generate myotubes within as little as 4 days from seeding with a high degree of MHC+ cells (>95% purity), suitable for phenotypic based high throughput screens

REPROGRAMMING of iPSC HUMAN GLUTAMATERGIC NEURONS

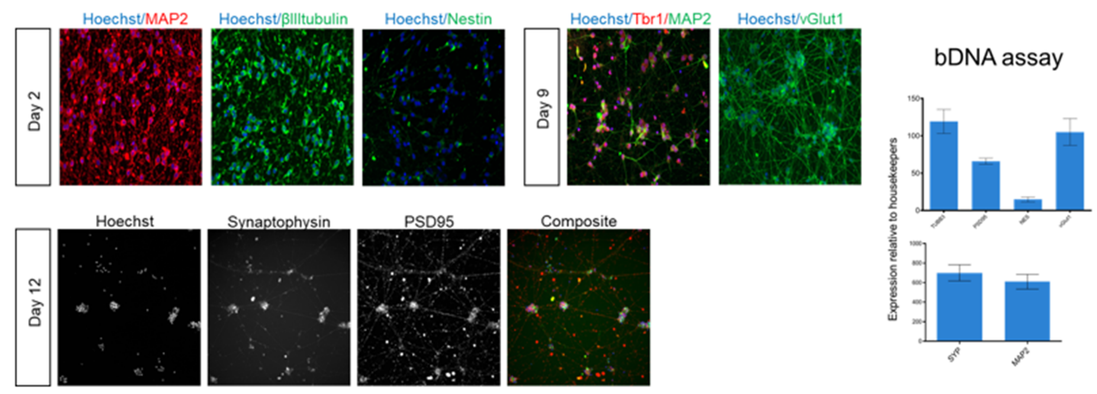


Figure 4. Representative images of eNeuron/glut neurons at day 2, 9 and 12 of differentiation. Cells show presence of pan-neuronal markers already after 2 days of differentiation and Tbr1 and vGlut1 at day 9. Synaptic markers PSD95 and Synaptophysin were detected at day 12 of differentiation. Branched DNA assay confirmed gene expression profile of key neuronal and synaptic markers.

IMMUNO-ONCOLOGY 3D SPHEROID T CELL CYTOTOXIC ASSAY

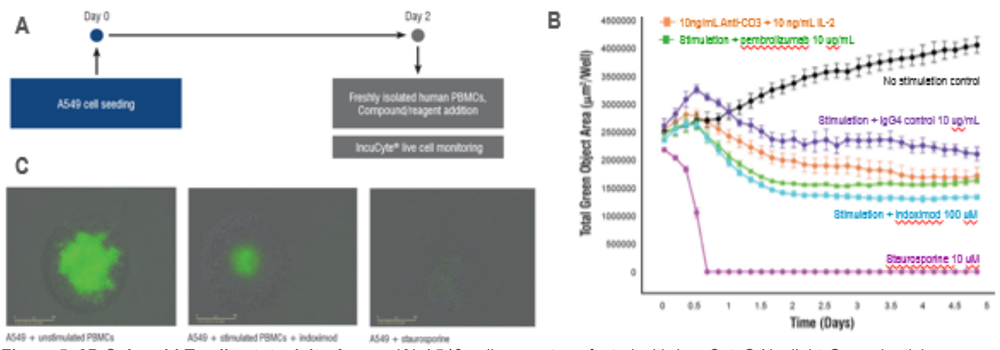


Figure 5. 3D Spheroid T cell cytotoxicity Assay. (A) A549 cells were transfected with IncuCyte® Nuclight Green lentivirus reagent to generate a stable cell line. Cells were seeded in ultra-low attachment plates to enable spheroid formation, and treated after 2 days of culture with activated T cells in the absence and presence of test compounds or therapeutic antibodies. This method uses the IncuCyte® live-cell analysis system to generate data in the form of presentation-ready and time-lapse graphs and movies. **(B)** Example of data generated with IncuCyte® over a period of 5 days after treatment. **(C)** Examples of live cell imaging acquired with IncuCyte®

4 Conclusion

Our extensive experience in the development of complex cellular assays involving co-culture, 3D and organoid systems, execution of high-throughput phenotypic screenings involving multi-parametric readouts, design of quantitative image segmentation algorithms, and experience in a wide variety of disease models allows us to approach with confidence any preclinical drug discovery program.