Rapid assessment of viability in patient-derived colorectal cancer organoids

Cathy Olsen, Prathyushakrishna Macha, Angeline Lim, Kirsty Greenow, Oksana Sirenko | Molecular Devices, LLC

Overview

Using patient-derived colorectal cancer organoids, we assessed the differential effects of known anti-cancer compounds on organoid size and morphology using both imaging and a microplate reader-based viability readout. Here we demonstrate the usefulness of label-free imaging and the ATP-based viability assay for rapidly gauging drug response. This streamlined readout may be used to guide identification of potential therapeutics early in the screening process, which could then be followed up with more intensive high content imaging or other analyses.

Introduction

Cancer cell lines grown in 2D have long served as experimental surrogates for cancers. In recent years, the 3D culture of cancer cells, often alongside other cell types in formats where they can form multi-layered structures, is enabling new models for cancer research that are considered more biologically relevant. Cancer organoids derived from patient tissue offer researchers a highly relevant disease model system, as these organoids and the patients from which they were derived have been shown to respond similarly to drugs. Recently, these models have become more widely utilized, thanks to standardization and scalability that have made available large numbers of uniformly sized and highly viable organoids.

The characterization of organoid responses to candidate drug treatment is a powerful research tool that provides a wealth of detailed information, but screening numerous compounds requires significant effort and hands-on time. We demonstrate here methods for analyzing organoid viability that allow rapid identification of effective drug candidates and can be combined with more complex downstream image analysis.

CRC organoid culture & analysis

Seeding and compound treatment

Colorectal cancer organoids (line ISO68) were thawed quickly, rinsed in media, and suspended in a solution of media + 50% Matrigel (growth factor-reduced). They were then seeded into 384-well white-walled, clear-bottom microplates at 250 organoids per well, in a volume of 10 µL per well. Organoids were incubated in media containing ROCK inhibitor for 48 hours for optimal recovery. Quadruplicate wells were then treated with selected compounds, each in a four-fold dilution series.

Assays for compound effects

Immediately prior to compound addition, and after five days of compound treatment, transmitted light images were acquired on an ImageXpress® Micro Confocal High-Content Imaging System and analyzed to obtain area covered by organoids using the Custom Module Editor in MetaXpress® High-Content Image Acquisition and Analysis Software. Fluorescent viability staining and imaging were performed on a subset of organoids.

5-day compound-treated organoids were assayed using the CellTiter-Glo® 3D Cell Viability Assay (Promega), with results detected using a SpectraMax® i3x Multi-Mode Microplate Reader.

Organoid culture & assay workflow

A combined approach to assessing drug effects

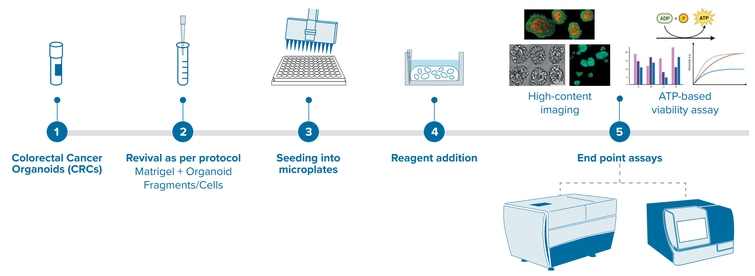


Figure 1. Overview of the experimental approach from organoid seeding (day 0) and compound addition (day 2), to analysis of results by imaging and luminescent microplate reader viability assay (day 7).

Label-free image analysis

Drug effects assessed by TL image analysis of area

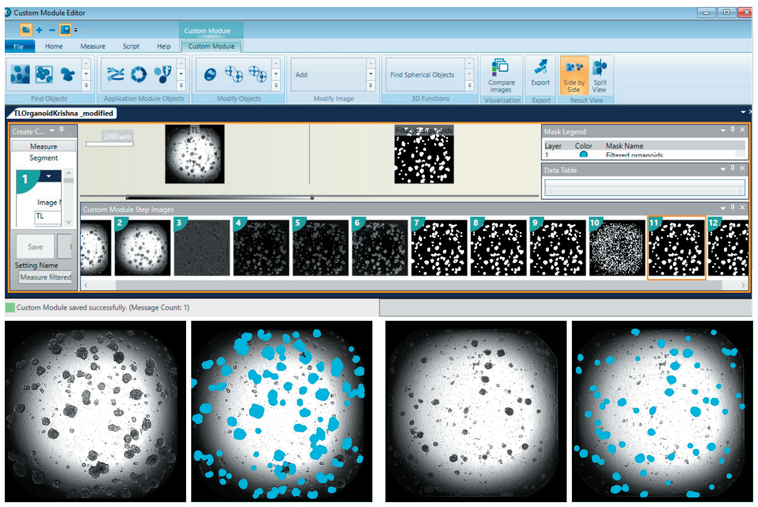


Figure 2. The Custom Module Editor was used to analyze TL images, identifying organoids through a multi-step process and applying masks to calculate the total area covered by organoids in each image. Left, control; right, treated.

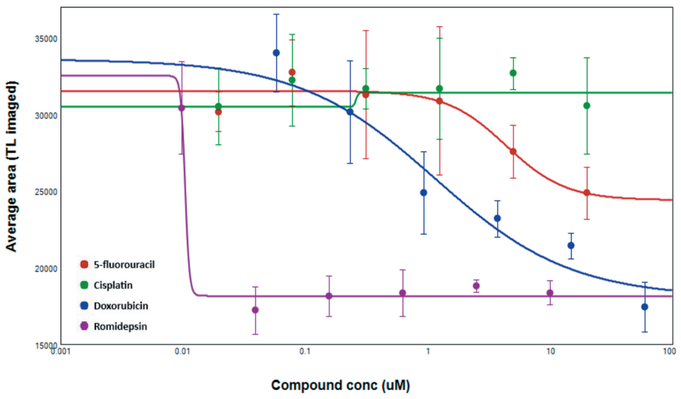


Figure 3. Concentration-response curves. Average area calculated from TL images was plotted against compound concentration using a 4-parameter curve fit.

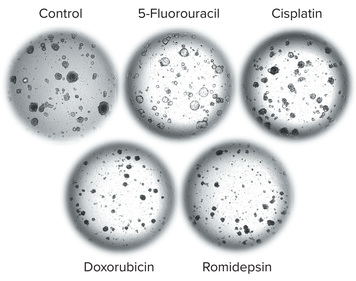


Figure 4. TL images of organoids treated with the highest concentrations of the indicated compounds (5-FU 20 µM, cisplatin 20 µM, doxorubicin 60 µM, romidepsin 10 µM).

Organoid viability assay

Luminescent ATP cell viability assay

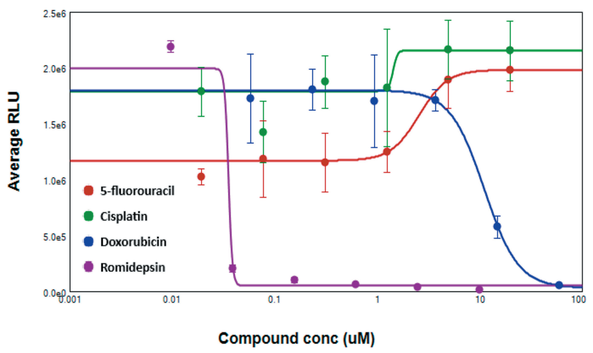
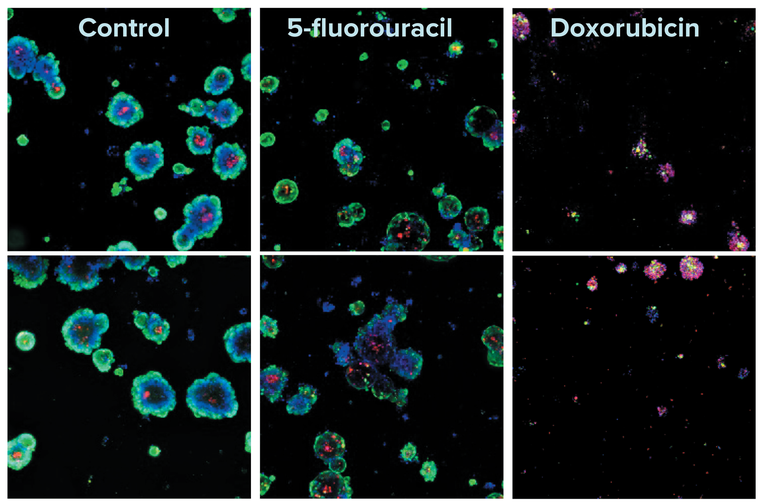


Figure 5. 5-day compound-treated organoids assessed for viability using the CellTiter-Glo 3D assay. A simple add-mix-read format produced results in about 30 minutes.

Fluorescence imaging of organoids

Area analysis of treated, labeled organoids



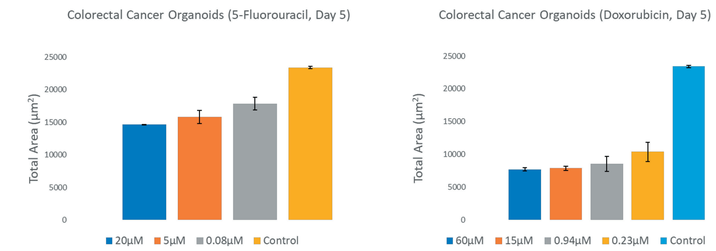


Figure 6. 5-day compound-treated organoids stained with DAPI (nuclei), calcein AM (viable cells), and MitoTracker Deep Red (mitochondria). Plots show total area for treated vs. control organoids.

Conclusion

Patient-derived organoids give researchers the opportunity to explore the possibilities of personalized treatment. This exploration often involves time-consuming imaging and analysis that provide a wealth of information but may be less practical for initial screening of large numbers of compounds. Using label-free imaging and plate reader-based viability analysis can serve as preliminary approaches to identifying potential drugs with measurable effects on treated organoids.

HUB Organoid Technology used herein was used under license from HUB Organoids. To use HUB Organoid Technology for commercial purposes, please contact bd@huborganoids.nl for a commercial use license.

MOLECULAR DEVICES

For Research Use Only. Not for use in diagnostic procedures.
©2023 Molecular Devices, LLC. All Rights Reserved. The trademarks mentioned herein are the property of Molecular Devices, LLC or their respective owners.
6/23 2573A

Poster 2573A_Rapid assessment of viability in patient-derived colorectal cancer organoids (39x39).indd 1 6/16/23 1:37 PM