A walkaway solution for assessing drug effects in patient-derived colorectal cancer organoids

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Overview

Cancer cell lines grown as monolayer cultures (2D) have long served as convenient 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. Standardization and scalability enabling the production of large numbers of uniformly sized and highly viable organoids, as well as automation of organoid culture and assays, have led to their increased use in drug screening.

Characterization of organoid response to candidate drug treatment is a powerful research tool that provides a wealth of detailed information, but screening many compounds requires significant effort and hands-on time. Streamlining the process is important for rapid identification of compounds that can be followed up with more time-consuming studies. The analysis of key parameters such as cell viability allows rapid identification of effective drug candidates and can be combined or followed up with more complex image analysis. Results from viability assays arrive faster via automation of reagent and plate handling, as well as data analysis.

Here we worked with colorectal cancer organoid lines derived from patient tissues. Organoids were placed in 384-well microplates, either manually or using an automated liquid handling system, and treated with selected anti-cancer compounds, including romidepsin, cisplatin, doxorubicin, and trametinib, for 3 or 5 days. Organoid number and overall morphology were assessed by label-free transmitted light imaging, then organoids were lysed and assayed for viability using a luminescent ATP assay, with automation of liquid handling and microplate transport to decrease hands-on time. The cell viability assay was used to quantitatively analyze drug responses, which were combined with automated image analyses for characterization of compound effects on organoid size and morphology. We demonstrated the usefulness of the automated microplate reader-based ATP assay for rapidly gauging drug response in patient-derived organoids.

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 uL 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.

Organoid culture & assay workflow

Automating the assessment of drug effects

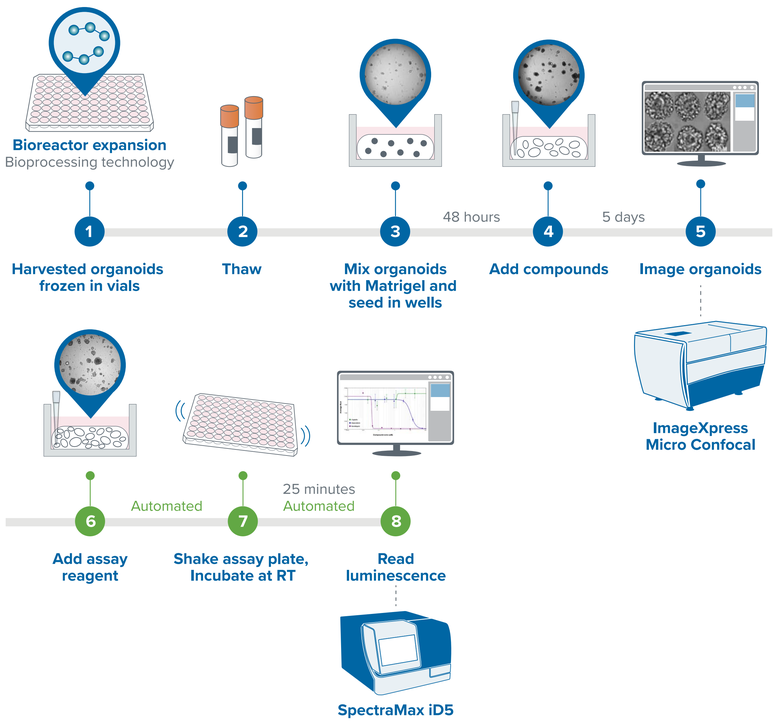

Figure 1. Overview of the experimental process from organoid seeding (day 0) and compound addition (day 2) to results by imaging and luminescent microplate reader viability assay (day 7).

Luminescent ATP cell viability assay

The response of organoids to compounds after five days of compound treatment was investigated using the CellTiter-Glo® 3D Cell Viability Assay (Promega), with results detected using a SpectraMax® iD5 Multi-Mode Microplate Reader. A Hamilton Microlab® STAR™ Liquid Handler was used to automate the addition of assay reagent to the wells and to shake the microplate to ensure organoid lysis. After a 25-minute incubation at room temperature, a PreciseFlex 400 sample handler (Precise Automation) and Genera scheduling software (Retisoft) were used to transfer the microplate from incubator to liquid handler, then to the SpectraMax iD5 reader. A preconfigured protocol in SoftMax® Software was used to generate data and analyze results automatically.

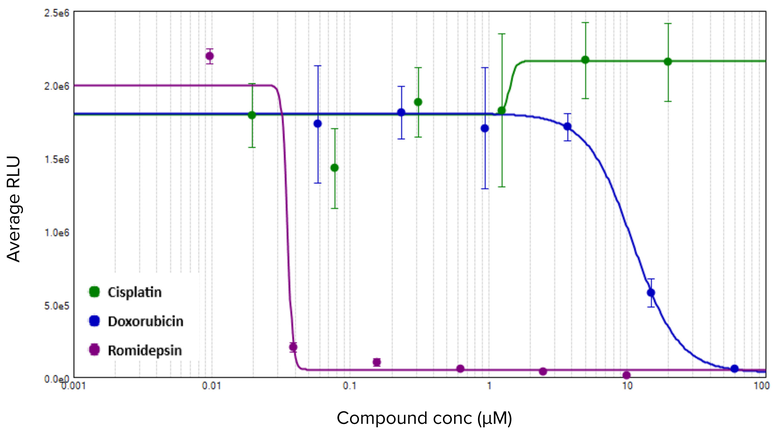

Figure 2. 5-day compound-treated organoids assessed for viability with the automated cell viability assay. An add-mix-read format, in combination with automation of reagent handling, assay detection, and analysis produced results in about 30 minutes.

Organoid viability assay

TL imaging readout for compound response

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. Results were plotted with a 4-parameter logistic using SoftMax Pro software.

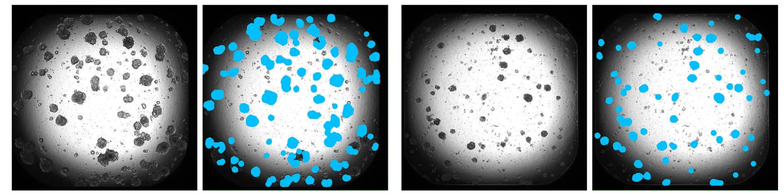

Figure 3. The Custom Module Editor was used to analyze TL images, identifying organoids through a multi-step process and applying masks to calculate the total area covered by organoids in each image. Left, control; right, treated.

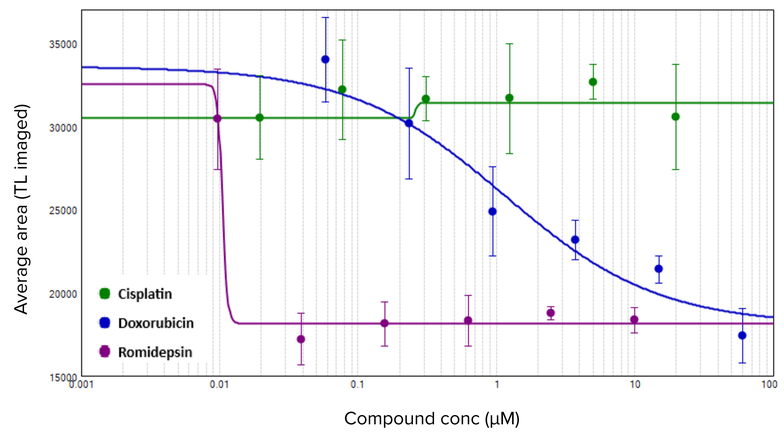

Figure 4. TL image analysis (above) plotted as average area calculated from TL images vs. compound concentration using a 4-parameter curve fit.

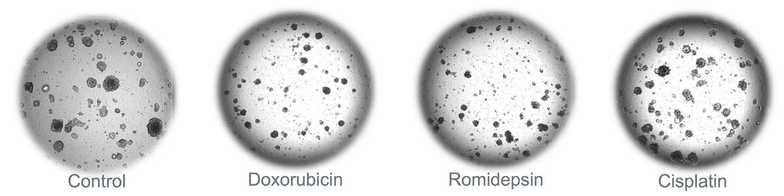

Figure 5. TL images of organoids treated with the highest concentrations of indicated compounds (cisplatin 20 µM, doxorubicin 60 µM, romidepsin 10 µM).

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 automating plate reader-based viability analysis can serve as preliminary approaches to speed the identification of potential drugs with measurable effects on treated organoids.

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