Scalable patient-derived 3D colorectal cancer organoids in high throughput applications

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Introduction

Many oncology drugs fail at the later stages of the drug development pipeline and in clinical trials, despite promising data in vitro. Three-dimensional (3D) cell models, such as patient-derived organoids (PDOs), offer a promising solution to this problem. Cells grown in 3D can better mimic cell-cell interactions and the tissue microenvironment. Studies show that patients and their derived organoids respond similarly to drugs, suggesting the value of using PDOs to improve therapeutic outcomes. However, challenges such as assay reproducibility, scalability, and cost have limited the use of PDOs in mainstream drug discovery pipelines.

To address challenges associated with the use of PDOs in large-scale applications, we have

1. Developed a semi-automated process for the controlled production of PDOs. The bioreactor maintains an environment that ensures constant delivery of nutrients and growth factors to the culture while preventing the accumulation of toxins. This method results in the large-scale production of assay-ready organoids that are uniform in size and have high viability.
2. Developed automation methods to streamline organoid-based assays.
3. Developed an image-based deep-learning model for assay analysis.
4. Show the use of a high dimensionality approach for organoid profiling.

We find that most of the compounds showed cytostatic effects on the CRC organoids while only doxorubicin showed additional cytotoxic effects as shown by viability assay. Doxorubicin-treated organoids had the most significant reduction in size, with a greater number of dead cells compared to controls. Multi-parametric data mining of the dataset revealed additional hits that were not found in the viability assay, this suggests the advantages of leveraging high-content data extracted from image-based screens.

Overall, our results show the utility of PDOs in high-throughput drug discovery applications using automation with high-content imaging.

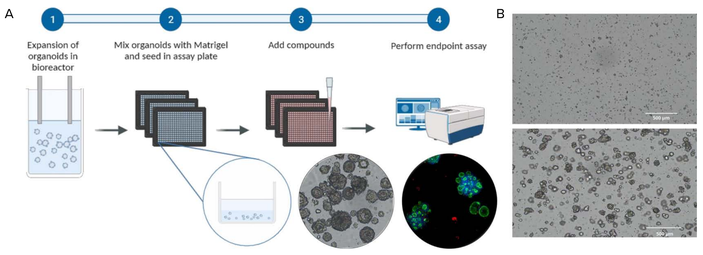


Figure 1. General workflow for organoid-based screens. A) 1. Organoids expanded in bioreactor are cryopreserved in vials until ready for use. 2. Organoids are thawed, mixed with Matrigel and seeded in a 384W black walled plate. 3. After 48 hours, compounds are added. 4. Organoids are imaged at regular intervals to capture the compound effects using brightfield imaging. For end-point readouts, assays for viability such as with Calcein AM dyes or ATP quantitation may be used. B) Representative images of organoids (bottom) expanded in the bioreactor. The top panel shows single cells that were seeded in the bioreactor.

Materials and methods

CRC organoid culture

Figure 1 shows the general workflow used here. Colorectal cancer organoids (Line ISO68) were handled according to manufacturer's instructions. Briefly, organoids were thawed quickly at 37°C, gently resuspended, and washed in media. Organoids were resuspended in Matrigel and then seeded in 384 well plate, at 200 organoids per well (10µl) with the Hamilton STAR liquid handler. Complete media supplemented with ROCK inhibitor was added to organoid culture for 48 hours to improve recovery.

Organoids were then treated with selected compounds for 5 days (6 point titrations with 4-fold dilutions) and in quadruplicates. Compound (highest concentration used): 5-Fluorouracil (5FU) (100µM), adavosertib (10µM), CA074 (100µM), cisplatin (20µM), doxorubicin (60µM), romidepsin (10µM), temsirolimus(10µM) and trametinib (20µM). Negative (DMSO) and positive (doxorubicin 100µM) controls were also included.

Automation setup for organoid culture

An automation workcell consisting of a liquid hander (LH), incubator, and high-content imager was used for organoid seeding and the monitoring of organoids in culture post-compound addition (Figure 2). For organoid seeding, CRC-Matrigel suspension was prepared on ice in a 96W compound plate. The plate was loaded on the LH deck in a pre-chilled position (IHECO CPAC). The 8-channel multiprobe head was used to dispense 10µl of suspension to a pre-chilled 384W plate. Plate with cells were then incubated at 37°C for 30min before the addition of media.

The Genera scheduling software (RETISOFT) is used to execute routine monitoring of the organoids in culture. The protocol involves the retrieval of the plate from the incubator, transport of the plate to the ImageXpress® Confocal HT.ai High-Content Imaging System (Molecular Devices) for imaging of organoids (z-stack acquisition, best-focused projection, 4X in bright field), and placement of plate back in the incubator using the PreciseFlex400 robotic arm (Brooks).

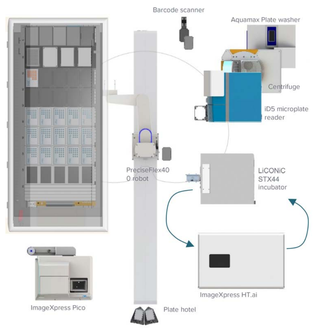
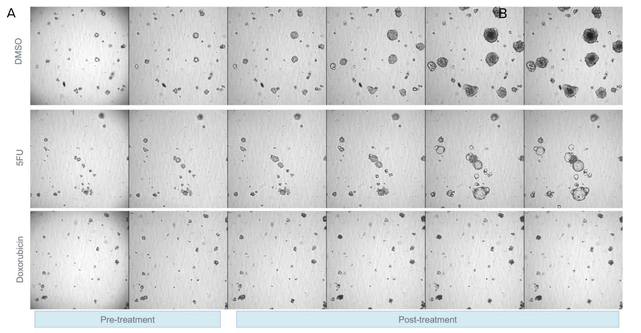


Figure 2. Layout of the automation workcell. The curve arrows show an example of the process to monitor cells in culture where plates are moved from the incubator to the ImageXpress Confocal HT.ai for imaging in brightfield and then back to the incubator.

Results

Effects of anti-cancer compounds on colorectal cancer organoid growth

Selected anti-cancer compounds added to the organoids 48hrs after seeding and their effects on CRC growth over the next 5 days were monitored (Figure 3A). To quantify the change in organoid sizes, a deep-learning based segmentation model was trained to identify all organoids throughout the experiment (Figure 3B, C). Untreated organoids increase in size over time in culture. In contrast, compounds that inhibited growth showed little change in size, suggesting cytostatic effects.



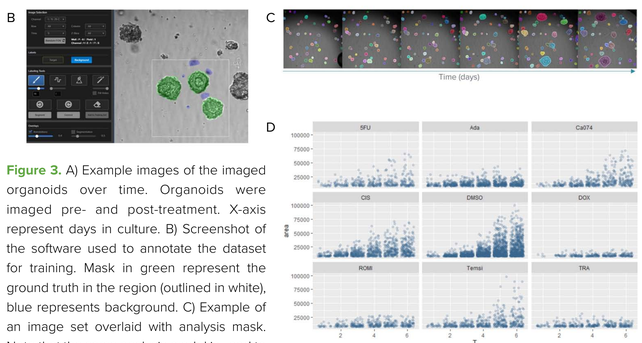


Figure 3. A) Example images of the imaged organoids over time. Organoids were imaged pre- and post-treatment. X-axis represent days in culture. B) Screenshot of the software used to annotate the dataset for training. Mask in green represent the ground truth in the region (outlined in white), blue represents background. C) Example of an image set overlaid with analysis mask. Note that the same analysis model is used to analyze the images from different timepoints. D) The 2D projected area of each organoid is shown as a scatter plot over time in culture. Compounds are added on day 2.

5-Fluorouracil (5FU, 100µM), adavosertib (Ada, 10µM), CA074 (100µM), cisplatin (CIS, 20µM), doxorubicin (DOX, 60µM), romidepsin (ROMI, 10µM), temsirolimus(Temsi, 10µM) and trametinib (TRA, 20µM)

Effects of anti-cancer compounds on colorectal cancer organoid viability

To determine if the compounds affected cell viability, organoids were stained with Calcein AM (live cell marker), Ethidium homodimer (dead cell marker) and Hoechst (nuclei dye). Organoids were imaged and analysis was carried out to quantify organoid viability (Figure 4).

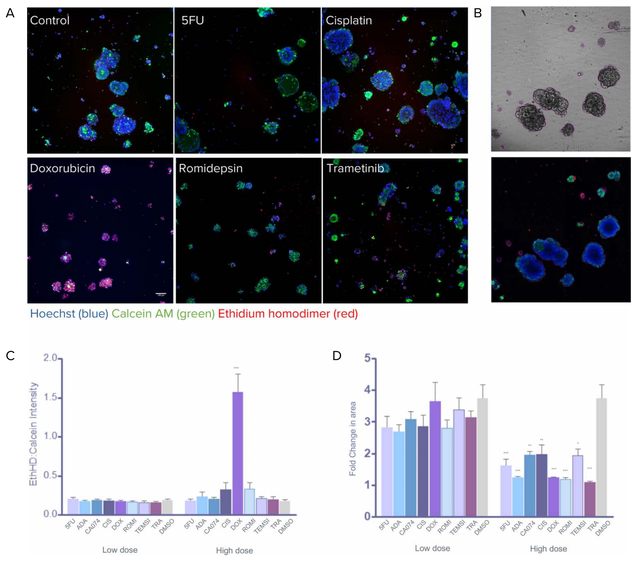


Figure 4. A) Viability assay was carried out on CRC organoids five days post-treatment. Shown here are example images of organoids from the various treatment groups (treated with the highest concentrations). B) An example image with overlay of the segmentation mask shown as outlines. Organoids were segmented in the brightfield channel (top), the fluorescent intensities of the other channels (bottom) were extracted from the mask. C) Viability was quantified by calculating the average ratio of Calcein AM to ethidium homodimer intensity per organoid. 2-way ANOVA was performed on the data, Bonferroni post-hoc test to compare treatments to DMSO. D) To quantify organoid growth over time, the total area of organoids on day 5 post-treatment was normalized to the total area of pre-treated organoids. Control organoids show an average of 3.5 fold growth. Growth inhibition was significant in treated organoids (highest concentration). (Based on 2-way ANOVA test)

Results

Image-based, morphological profiling of CRC organoids

Image-based profiling has been used to characterize cellular phenotypes in response to chemical or genetic perturbations. To test if the same approach can be applied to organoids, compound treated CRC organoids used in the viability assay were stained with mitotracker and fixed. Post fixation, organoids were additionally stained with phalloidin to visualize actin organization (Figure 5). Organoids were imaged and analyzed, and measurements from all channels were extracted for further analysis.

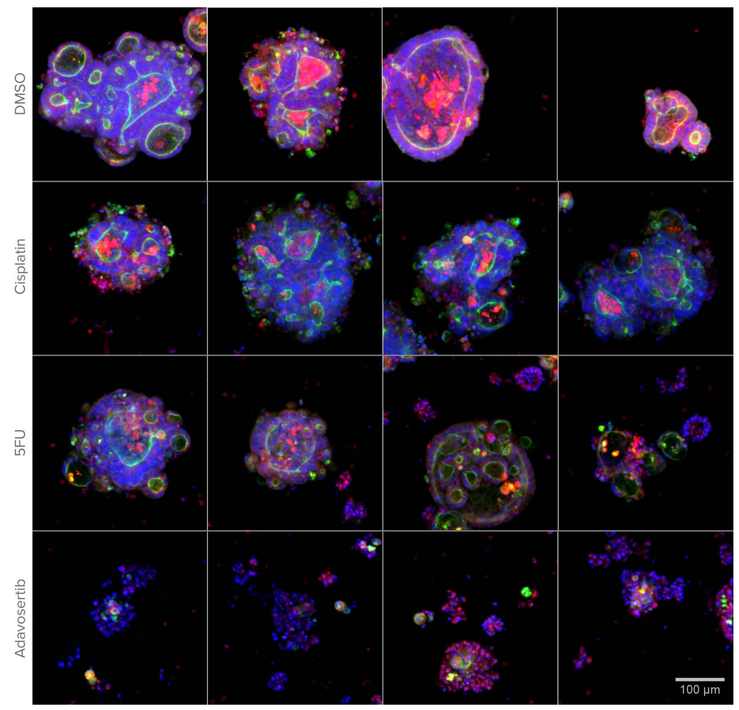


Figure 5. Examples of organoid images from control and treated groups. Note the morphological heterogeneity intrinsic in cancer organoids. Hoechst (blue), phalloidin (green), mitotracker (red)

Multiparametric analysis of CRC organoids

To glean additional insights into compound-induced phenotypes, we extracted all image-based measurements (including size, texture, and intensity) and uploaded them into StratoMineR, a cloud-based data analytics platform (Core Life Analytics) for further analysis (Figure 6).

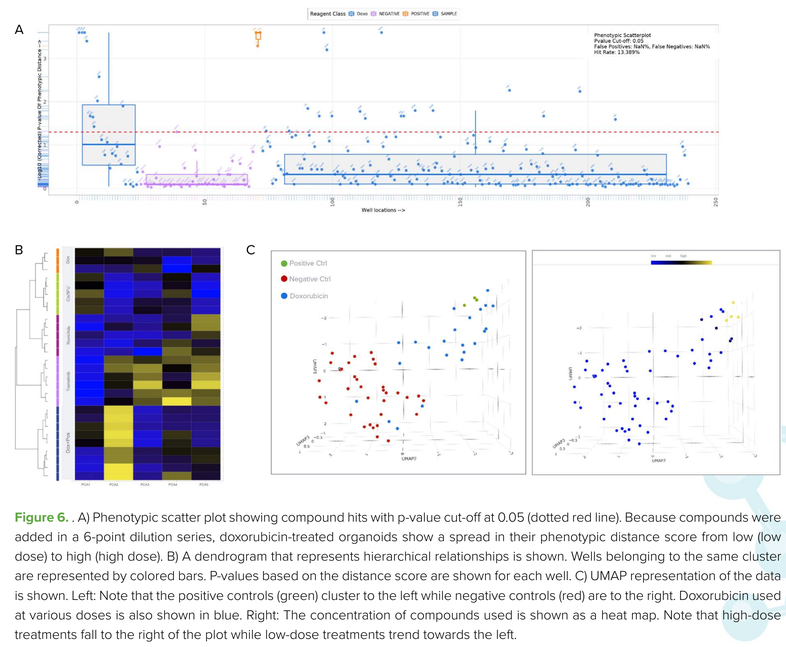


Figure 6. . A) Phenotypic scatter plot showing compound hits with p-value cut-off at 0.05 (dotted red line). Because compounds were added in a 6-point dilution series, doxorubicin-treated organoids show a spread in their phenotypic distance score from low (low dose) to high (high dose). B) A dendrogram that represents hierarchical relationships is shown. Wells belonging to the same cluster are represented by colored bars. P-values based on the distance score are shown for each well. C) UMAP representation of the data is shown. Left: Note that the positive controls (green) cluster to the left while negative controls (red) are to the right. Doxorubicin used at various doses is also shown in blue. Right: The concentration of compounds used is shown as a heat map. Note that high-dose treatments fall to the right of the plot while low-dose treatments trend towards the left.

Conclusions

- Organoid technology holds significant promise in transforming drug discovery. The combination of large-scale organoid production with automation-based screening workflows will help overcome some of the current barriers to organoid use.
- Image-based readouts along with morphological profiling can help with understanding complex phenotypes associated with PDOs.

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