T cell-induced morphological change analysis of colorectal cancer organoids using AI

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Introduction

Immunotherapy is increasingly popular as a type of cancer treatment. Chimeric antigen receptor (CAR) T-cell therapy is an approach to arm the immune system to treat cancer by genetically altering T-cells so they can target and destroy cancer cells. Although much success has been achieved with immunotherapy for treatment of blood cancers, the application in solid tumors remains challenging. One of the barriers is intrinsic to the solid tumor microenvironment (TME) where suppressive cytokines limit the tumor killing ability of T-cells. Thus, TME plays a critical role in the response to cancer treatment and understanding the role the TME plays is essential to enhance CAR T-cell efficacy.

The benefit of using 3-dimensional (3D) patient-derived organoids (PDO) is that they model more of the physical and chemical cues present within the TME that are lacking in traditional 2D monolayer cultures. Studies show that PDOs show similar responses to drugs as original tumors, suggesting the value of using PDOs to improve therapeutic outcomes. Thus, PDOs represent a superior preclinical model system compared to 2D models through their inherent heterogeneity, long-term stability, applicability for high-throughput screens, and enhanced capacity to capture tumor characteristics.

Despite the benefits associated with the use of PDOs, there are significant barriers to their widespread adoption in drug discovery due to costly and highly labor-intensive growth and maintenance. To address the challenges associated with the use of PDOs in large-scale applications, a semi-automated bioreactor was developed for the large-scale expansion of assay-ready organoids. Here, we developed a workflow to quantify the efficacy of T-cells in solid tumors using PDOs. Using bioreactor-expanded patient-derived colorectal cancer organoids (CRCs), activated human peripheral blood mononuclear cells (PBMCs) were added to CRCs in a 384-well microtiter plate and monitored every 4 hours for 3 days using high-content imaging. To quantify the morphological changes induced by T-cells, we first segmented each organoid with the transmitted light (TL) channel using a deep-learning U-net model and extracted features from each organoid. The resulting features were then classified using a trained Random Forest model that differentiates the morphologically modified organoids from the intact organoids. Using this approach, we found that, compared to control wells, the percentage of modified organoids in stimulated PBMC-treated wells increased rapidly—within around 2 days—and then reaches a fluctuated plateau, suggesting the potential of a live T-cell efficacy assessment approach with or without labeling. The results demonstrate the utility of the bioreactor-expanded organoids with the analysis approach in largescale T cell-based screens.

Methods

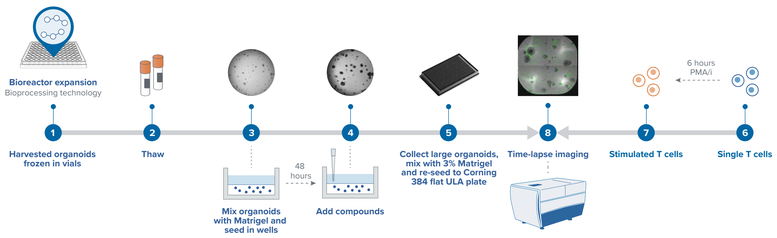

Figure 1. T-cell and CRC PDO interaction workflow.

- Bioreactor-expanded patient-derived CRCs were first mixed with 80% Matrigel and grown for 48 hours before collection. The collected large organoids were mixed with 3% Matrigel before seeding into a 384-well flat-bottom plate with ultra-low attachment (Corning). The thawed PBMC/T-cells were stimulated in PMA/i for 6 hours before adding to the CRC organoids for co-culture.
- We used the ImageXpress® Confocal HT.ai High-Content Imaging System (Molecular Devices) equipped with spinning disk confocal and sCMOS camera to perform timelapse live cell imaging every 4 hours.
- Timelapse images were exported into a format compatible with IN Carta® Image Analysis Software (v. 2.4). The embedded modules SINAP and Phenoglyphs were used to segment and classify the organoids into two classes, i.e., morphologically intact or modified organoids.

Methods

Automation setup

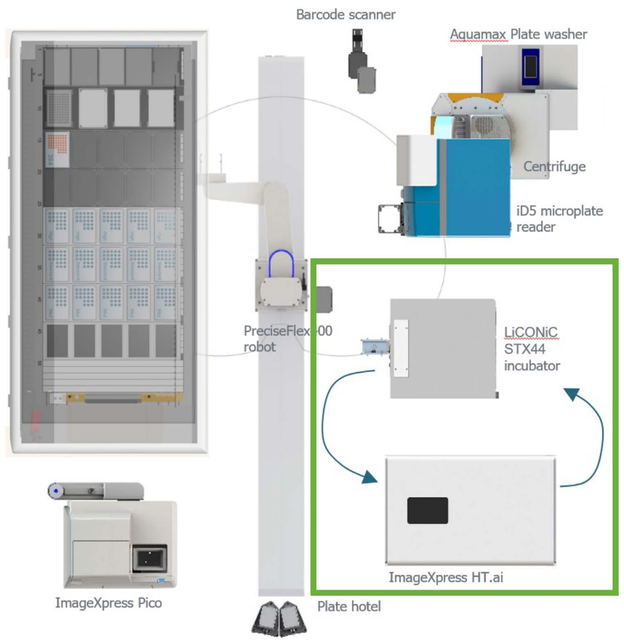

Figure 2. Layout of the automated workcell consisting of an incubator and a high-content imager (Figure 2, green box) was used for monitoring the co-culture of organoids and T-cells. The Genera scheduling software (RETISOFT) was used to execute routine monitoring of the organoids in culture. The protocol involved the retrieval of the plate from the incubator, transport of the plate to the ImageXpress Confocal HT.ai to image the organoids every 4 hours (z-stack acquisition with step size 10µm, 10X), and placement of the plate back in the incubator using the PreciseFlex400 robotic arm (Brooks).

Results

Deep learning-based segmentation (SINAP)

SINAP is established on the convolutional neural network (CNN) model and orchestrated in a way that even a data analytics beginner can easily grasp to do deep learning-based segmentation. We divided the morphological types of organoids into two categories: I. intact organoids with bubbly structure which included those at the early timepoints treated with stimulated or non-stimulated T-cells with light appearance in TL channel (Figure 3A–3B) and those at the late timepoints treated with non-stimulated T-cells but with dark appearance in TL channel (Figure 3C–3D), II. modified organoids that were losing their bubbly structure at the late timepoints treated with stimulated T-cells with dark appearance in TL channel (Figure 3E–3F). This implies that stimulated T-cells impact the overall structure of the CRC organoids, while non-stimulated T-cells have little effect. Thus, we were able to quantify the percentage of organoids of stimulated T cell-induced morphological change as a measure of T-cell efficacy.

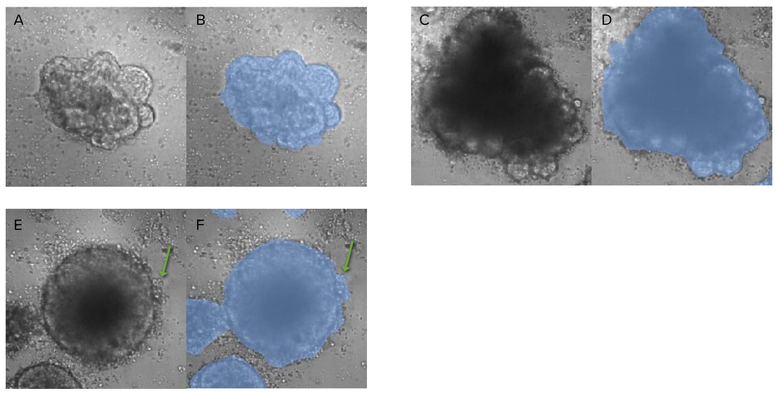

Figure 3. A. Representative TL image and B. the corresponding segmentation of intact organoids at the first timepoint. C. Representative TL image and D. the corresponding segmentation of intact organoids at the last timepoint treated with non-stimulated T-cells. E. Representative TL image and F. the corresponding segmentation of modified organoids at the last timepoint treated with stimulated T-cells.

Results

Machine learning-based classification (Phenoglyphs)

To classify thousands of organoids in TL channels from 22 timepoints into two classes of morphologically modified organoids and intact organoids, we loaded object-level features into the Phenoglyphs module. The Phenoglyphs classifier uses Random Forest as its underlying algorithm and enables image-based labeling to build the training set. We then graphed the percentage of modified organoids throughout the time. Figure 4F shows that for stimulated T cell-treated wells, the percentage of morphologically modified organoids increases rapidly at an average growth rate of 1.38%/hour within 52 hours after treatment and then begins to saturate at an average growth rate of 0.09%/hour (Figure 4F, purple dotted line). This implies that stimulated T-cells have more effect on organoids' morphology within 52 hours.

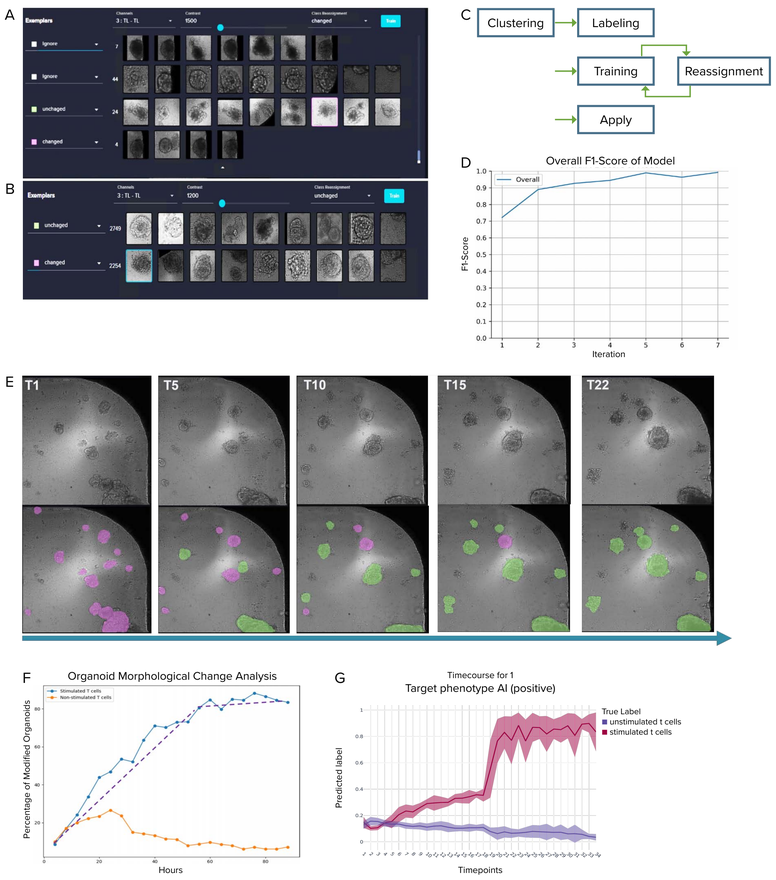

Figure 4. A. We generated 50 clusters with the built-in k-means++ algorithm, where clusters of interest were labeled as Changed and Unchanged classes with minor reassignment of images. B. The model was first trained with the labeled classes (ground truth) and the resulting classification can be improved with the reassignment of the organoids of classification error. C. The iterations of the cycle of Class Reassignment to correct the classification error and Training using the now expanded ground truth was then repeated . D. We stopped the loop when the model quality metric F1 score reaches 1 and the exemplar images are satisfactorily classified in both classes. Total 125 objects were labeled as ground truth when we stopped the training loop. E. The representative images of classified organoids showing the increased number of modified organoids throughout the time (Pink: intact organoids, Green: modified organoids). F. Percentage of morphologically modified organoids summarizing all corresponding wells that are either treated with stimulated T-cells (10 wells) or non-stimulated T-cells (5 wells). G. Similar trend observed when we treated spheroids with stimulated T cells.

Conclusion

- We successfully implemented a novel T-cell workflow to automate time-lapse high-content imaging.
- CRC organoid segmentation that included various morphologies was successfully achieved using a customized segmentation model.
- CRC organoids were successfully classified according to the morphological characteristics using Phenoglyphs.
- The whole system—along with the percentage analysis of morphologically modified organoids across time—can be used as a successful live T-cell screening approach.

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