Applying machine vision to empower preclinical development of cell engager and adoptive cell therapeutics in patient-derived organoid models of solid tumors.

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INTRODUCTION

Cell engager and adoptive cell therapeutics have emerged as efficacious and durable treatments in patients with B-cell malignancies. Though many analogous strategies are under development in solid tumors, none have received approval. Preclinical development of these therapies requires cell labeling of immortalized cell lines and/or primary expanded T cells to distinguish target and effector cells. However, cell engager and adoptive cell therapies have limited evidence of reproducibility in primary patient-derived models such as tumor organoid (TO) cultures thus far. Here, we build upon our TO platform (Larsen et al., 2021, *Cell Reports*) to measure organoid specific responses to these therapies. Utilizing machine vision coupled with time-lapse-microscopy, we obtain multiparameter kinetic readouts of patient-derived TO cell killing and allogeneic MHC-matched primary peripheral blood mononuclear cells (PBMCs).

METHODS

The patient-derived TOs were co-cultured with PBMCs in the presence of engagers/activators and vital dyes and incubated in time course studies. Caspase dye was measured using fluorescent pixel intensities at different time points using high throughput imaging. A fully convolutional neural network was trained on 374 examples to segment out organoids from brightfield images comprised of organoids, immune cells and potential background artifacts. This segmentation mask was then transferred to registered caspase images to quantify tumor cell specific phenotypes in an automated manner.

TOs and Immune Cell Co-culture

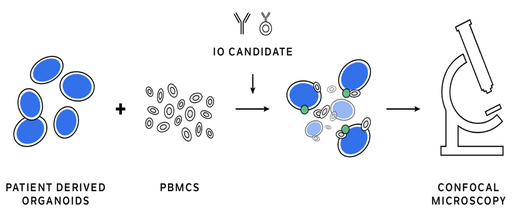

Figure 1. Patient derived TOs were identified based on the curated molecular profiles and co-cultured with HLA compatible PBMCs in the presence of commercially available EPCAM-CD3 bispecific antibody and Caspase-3/-7 Green Apoptosis assay reagent. The cells were imaged in brightfield and with caspase dye to monitor T cell activation and organoid killing using high content imaging over 24, 48, 74, and 96 hours.

RESULTS

Segmentation model architecture

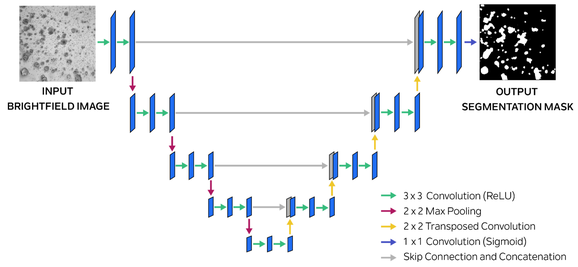

Figure 2. The model is based on the UNET architecture and generates a binary mask of organoids using an input brightfield image. The neural network isolates organoids with high accuracy (Dice score of 0.94±0.14 on 108 validation images) using morphology, size, shape, texture and other phenotypes.

Average Caspase Intensity Over Time

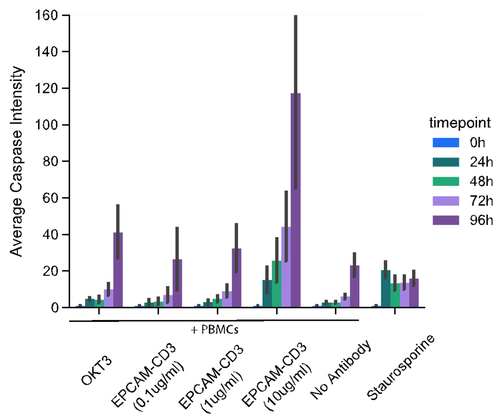

Figure 5. Caspase channel intensities are quantified for each of the organoids and aggregated across time and various treatments. The time-lapse imaging assay allows for quantification of the kinetics of engagers/activators in comparison to controls, resulting in accurate and precise technical reproducibility.

Neural Network Distinguishes TO Apoptosis

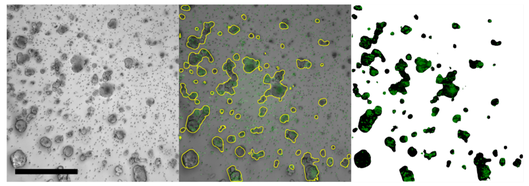

Figure 3. Brightfield (left), overlaid segmentation contours and caspase fluorescence (middle), and organoid-specific caspase (right). The predicted organoid mask is transferred over the caspase fluorescence image, masking out signal from background and PBMCs allowing for standardized automated detection of organoid-specific caspase signal. Scale bars are 0.5 mm.

Secreted Cytokines Profiling

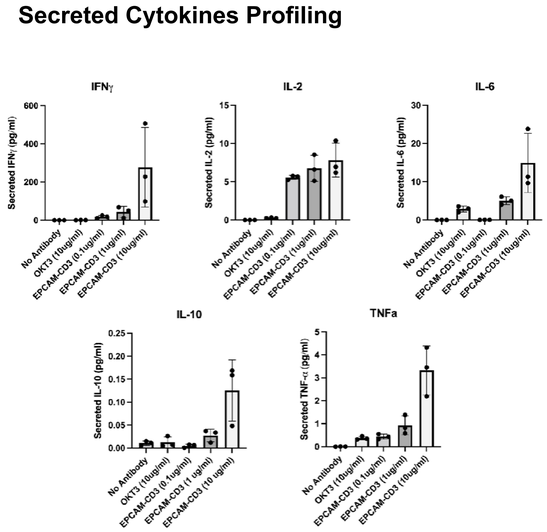

Figure 6. Multiplex profiling of secreted cytokines. The cell culture supernatants were harvested at different intervals of time and were analyzed for cytokines release using a custom V-PLEX biomarker panel from Meso Scale Discovery.

TO Apoptosis Over Time

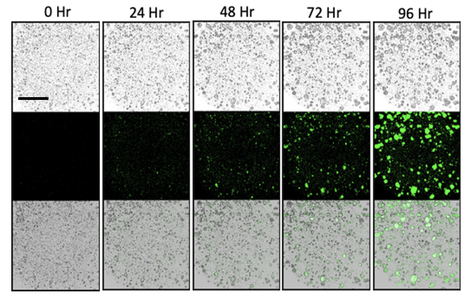

Figure 4. Whole well time lapse images consisting of brightfield (top), caspase fluorescence (middle), and overlaid (bottom) images. Scale bars are 0.5 mm.

PBMC Detection for Colocalization

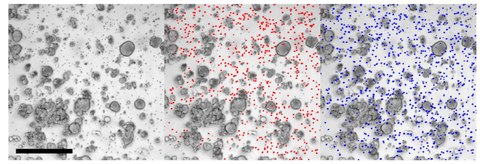

Figure 7. A separately trained UNET segments PBMCs from organoids and background, enabling colocalization and organoid-cell interaction studies. Prediction on right, ground truth in middle. Scale bars are 0.5 mm.

TO CAR-T Cells Co-culture

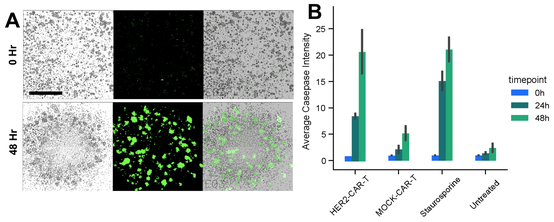

Figure 8. HER2+ TOs were co-cultured with HER2-CAR-T cells in the presence of Caspase-3/-7 Green Apoptosis Assay reagent and imaged for brightfield and Caspase dye to monitor organoid killing (A). Caspase channel intensities were quantified for each of the organoids and aggregated across time and various treatments (B). Scale bars are 0.5 mm.

CONCLUSIONS

We demonstrate the scalability and throughput of a machine vision TO immune co-culture platform across multiple unique patient-derived TO lines bearing a target of interest, enabling future discovery of biomarkers of therapeutic response and resistance.

Crucially, effector and target cells are accurately distinguished without the need for cell labeling with chemical or transgenic markers.

This assay forms a platform for co-localization of the organoids and immune cells over time, thus, enabling a spatiotemporal summary of dose-dependent efficacy of candidate therapeutics.

ACKNOWLEDGEMENTS

We thank Adam Hockenberry, Ph.D., Vanessa Nepomuceno, Ph.D. as well as the Tempus Scientific Communications and Design teams for data visualization guidelines and poster review.

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