Establishment of a Patient-Derived Organoid-Immune Cell Co-Culture Platform for Evaluating Immuno-Oncology Therapies

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Introduction

Tumor immunotherapy, known for its high specificity and favorable safety profile, inhibits tumor progression and prolongs patient survival by activating the immune system. However, the full potential of many immuno-oncology agents and the underlying mechanisms of therapeutic resistance remain incompletely understood.

The emergence of organoid technology has not only improved the accuracy of tumor modeling but also enabled co-culture with immune cells, thereby advancing the study of tumor-immune interactions and immunotherapy evaluation.

Here, we established a human tumor organoid-immune co-culture platform for assessing responses to multiple immunotherapies.

Methods

To measure antibody-dependent cellular cytotoxicity (ADCC) and T-cell-dependent cellular cytotoxicity (TDCC), we established a co-culture system with patient-derived xenograft organoids (PDXO) and allogenic PBMCs.

To model patient responses to immune checkpoint inhibitors and T-cell engagers, a co-culture model with patient-derived organoids (PDO) and tumor-infiltrating lymphocytes (TIL) was developed. To minimize graft-versus-host disease, the tumor and immune cells were derived from the same patients.

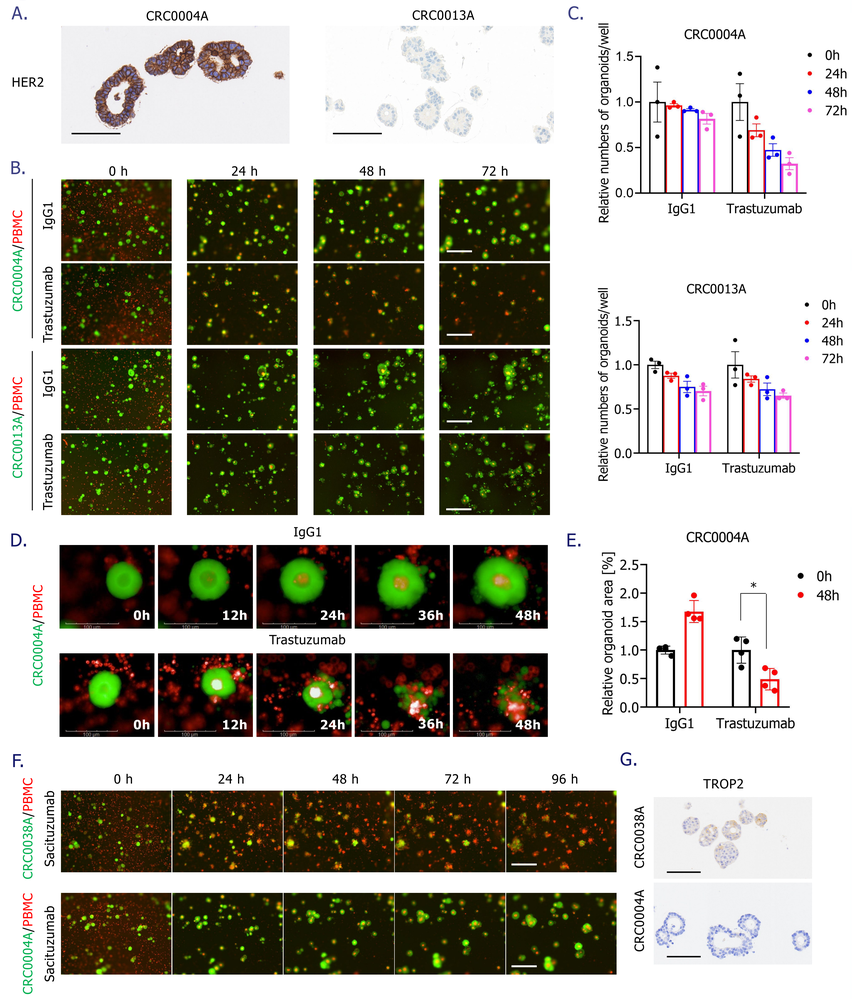
Immune cell recruitment, infiltration, and cytotoxicity were dynamically monitored using:

- GFP fluorescent tag transfection
- Fluorescent tracer dye for staining living and apoptotic cells
- Real-time 3D live-cell imaging
- 3D laser confocal microscopy imaging

Conclusion

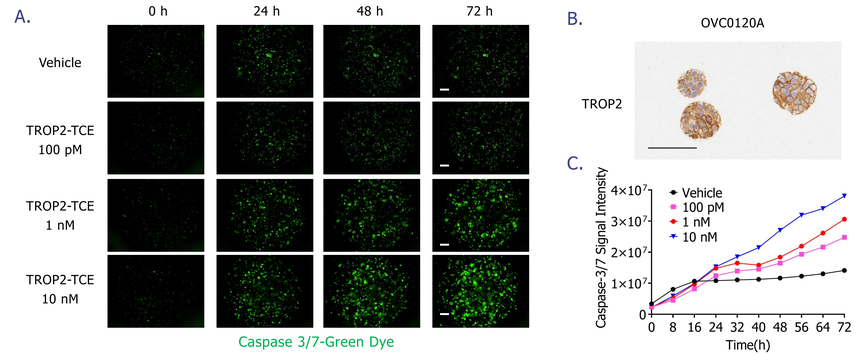
- We established a versatile tumor organoid-immune cell co-culture platform, consisting of a PDXO-allogeneic immune cell co-culture model and a PDO-autologous TILs co-culture model.
- This platform enables efficient evaluation and validation of diverse immunotherapies, including monoclonal antibody-mediated ADCC, TCE-mediated TDCC, and the therapeutic efficacy of immune checkpoint inhibitors.
- As a biomimetic *in vitro* model, it serves as a powerful tool for immuno-oncology drug screening, accelerates translational drug development, and provides a human-relevant alternative that circumvents the species-specific limitations and animal welfare concerns associated with conventional preclinical animal models.
- In addition, we are continuously working to construct more comprehensive tumor organoid models with enriched tumor microenvironment components, such as CAFs, vascular endothelial cells, and various immune cell populations, to support more efficient drug discovery for immunotherapies.

1 ADCC-eliciting antibodies significantly potentiate PBMC-mediated killing of target PDXOs



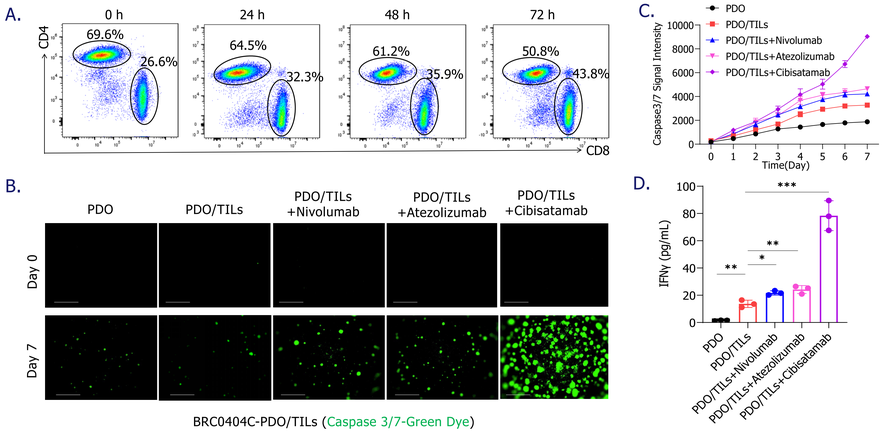
Assessment of ADCC in the PDXO-immune cell co-culture system. Immunohistochemical staining for HER2 **(A)** and TROP2 **(G)** in the indicated PDXO models. **(B)** 3D real-time live-cell imaging and **(C)** quantification of relative organoid numbers in CRC0004A-GFP or CRC0013A-GFP co-cultured with PBMCs and treated with 30 nM IgG1 or anti-HER2-mAb Trastuzumab. **D,** To better characterize the spatiotemporal dynamics of ADCC effect, confocal microscopy demonstrated that Trastuzumab elicited rapid PBMC recruitment toward HER2-positive PDXOs, along with extensive immune infiltration and robust cytotoxicity. **E,** Quantification data for Fig.D was based on PDXO size. Data are mean ± SD (n=4), p<0.05 (*). **F,** 3D real-time live-cell images of CRC0038A-GFP (TROP2+) or CRC0004A-GFP (TROP2-) co-cultured with PBMCs at 0, 24, 48, 72 and 96 h after treated with 30 nM anti-TROP2-mAb Sacituzumab. The E:T ratio was 10:1. PDXOs labeled by GFP tag, PBMCs labeled by CellTracker Red dye. D,G, Scale bars=100 μm. B,F, Scale bars=400 μm.

2 TROP2-targeted T-cell engager induces specific T-cell killing of PDXOs



Assessment of TDCC effect in the PDXO-immune cell co-culture system. **A**, Representative images of OVC0120A (TROP2$^+$) co-cultured with PBMCs (not labeled) and treated with TROP2/CD3-targeted T-cell engager (TCE) (Scale bar=400 μm). Caspase-3/7 Green Dye for apoptosis enabled real-time labeling of apoptotic PDXOs. **B**, Immunohistochemical staining of TROP2 in OVC0120A (Scale bar=100 μm). **C**, Quantification of apoptosis in OVC0120A.

3 Immunotherapies enhance autologous TIL-mediated anti-tumor responses



Efficacy of immunotherapies with PDO-TIL co-cultures. **A**, Flow cytometric analysis of CD4$^+$/CD8$^+$ subset dynamics over 3 days post-priming. **B**, PDOs from breast cancer were co-cultured with autologous TILs and treated with Nivolumab (anti-PD-1 mAb), Atezolizumab (anti-PD-L1 mAb), or Cibisatamab (CEA/CD3-targeted TCE), respectively. Meanwhile, Caspase-3/7 Apoptosis Dye was used to indicate the population of apoptotic PDOs. On day 7, the two immune checkpoint inhibitors significantly enhanced TIL-mediated killing of PDOs, despite the low expression levels of PD-1 and PD-L1 (data not shown). Cibisatamab elicited robust TIL-mediated antitumor cytotoxicity. Scale bar=100 μm. **C,** Quantification of apoptosis. **D,** ELISA measurements confirmed that IFN-γ secretion was positively associated with immune-mediated killing efficiency. Data are mean ± SD (n=3). p < 0.01 (**), p < 0.001 (***).

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