Establishment of a TME-Resembling In Vitro Assay System Using Freshly Isolated Human Tumoroids

Ryotaro Tabata, Satoko Unno, Masahiro Ogawa, Masashi Yamasaki, Yasushi Fujitani, Masayuki Goto
Axcelead Drug Discovery Partners, Inc., Kanagawa, Japan

Introduction

Conventional single-cell and 2D multicellular culture systems have been widely used in anticancer drug development, but they fail to adequately recapitulate the complex and heterogeneous tumor microenvironment (TME), limiting their ability to predict clinical efficacy.
In this study, we established a patient-derived tumoroid assay system from fresh surgical tumor tissues within 24 hours, verified the retention of tumor-associated cell types such as CAFs and TILs, and conducted pharmacological evaluations of existing drugs.

Highlight of this study

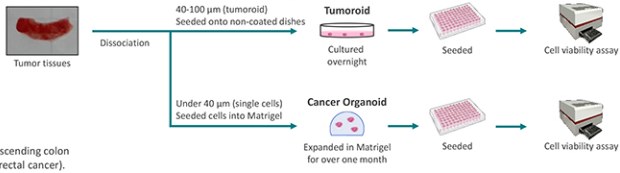
Patient-derived tumoroid and cancer organoid assay systems were successfully established.
Tumoroids retained diverse cell populations, including immune cells, CAFs, and endothelial cells, more faithfully reflecting the tumor microenvironment (TME) than cancer organoids. However, they showed little proliferation and were used without passaging.
Cancer organoids could be expanded for over one month through serial passaging, but they consisted predominantly of cancer cells with reduced stromal and immune components.
Cancer cells in both models showed proliferative activity (Ki67 staining).
In both models, treatment with 5-FU resulted in a dose-dependent reduction of cell viability.

Material & Methods

Fresh human tissue acquisition workflow

Fresh tumor tissues were obtained from four treatment-naïve colorectal cancer patients: Donor #13 (female, 77 years, ascending colon cancer), Donor #21 (colon cancer), Donor #25 (male, 66 years, ascending colon cancer), and Donor #32 (male, 58 years, rectal cancer).
All procedures were approved by the relevant ethics committees, and written informed consent was obtained from all patients.

Dissociation & Cell viability assay workflow



Results

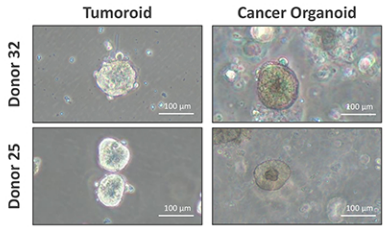


Figure 1 Tumoroids and cancer organoids reproducibly established from independent donors.
Tumor tissues (2 donors) were processed into tumoroids within 24 h of surgical resection or cultured in Matrigel to generate cancer organoids. Representative phase-contrast images show that both tumoroids and organoids could be consistently established from different donors.

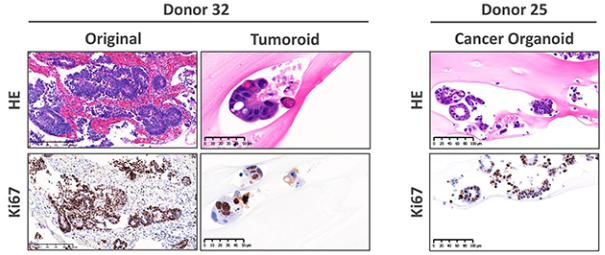


Figure 2 Cancer cells in tumoroid and cancer organoid showed proliferative activity.
Original tumor tissue, tumoroid (same donor), and cancer organoid (different donor) were analyzed by hematoxylin–eosin (HE) staining and Ki67 immunohistochemistry. Histopathology showed organized epithelial structures in cancer organoid, whereas tumoroid appeared as compact aggregates. Ki67 staining suggested that cancer cells in both models have proliferative activity.

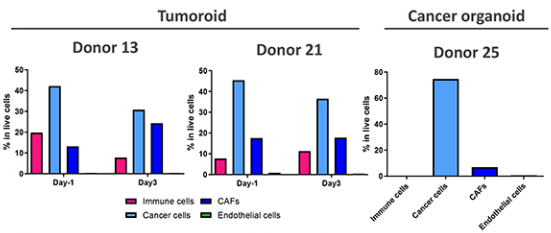


Figure 3 Coexistence of diverse cell populations in tumoroids.
Cell populations were quantified by flow cytometry after gating on live single cells. Immune cells were defined as CD45⁺, cancer cells as EpCAM⁺CD90⁺ or EpCAM⁺CD90⁻, CAFs as EpCAM⁻CD90⁺, and endothelial cells as EpCAM⁻CD90⁻CD31⁺. Tumoroids (2 donors) were analyzed at multiple time points (Day -1 and Day 3), whereas cancer organoid was evaluated after more than one month of serial passaging.

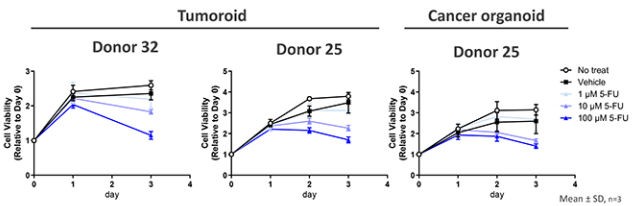


Figure 4 5-FU reduced cell viability in the tumoroid and cancer organoid.
Cell viability was assessed using the RealTime-Glo™ MT Cell Viability Assay following treatment with 5-FU. Tumoroids and cancer organoids were seeded on Day –1. RealTime-Glo reagent was added on Day 0, and luminescence measured 1 h later was used as the Day 0 baseline. 5-FU was then added, and viability was subsequently measured on Days 1, 2, and 3. Viability values are shown relative to the Day 0 baseline.

Conclusion

Cancer cells proliferated, while non-cancer cell populations, including immune cells, progressively decreased in cancer organoid. In contrast, tumoroid preserved CAFs, endothelial cells, and immune cells, enabling drug efficacy assessment in a physiologically relevant tumor microenvironment. Therefore, compared with cancer organoid, the tumoroid assay system represents a more valuable platform for predicting the clinical efficacy of novel anticancer drugs in humans.

Acknolegement:
We sincerely thank Drs. Masatoshi Yamada, Taku Miyamae, and Hirohito Kobayashi at Ebina General Hospital; Drs. Keiichi Yoshida, Jiro Okami, and Masayoshi Yasui at Osaka International Cancer Institute; and Satoshi Suzuki at HAB Research Organization for kindly providing human tissue samples.

COI disclosure information: We have no financial relationship to disclose for our presentation contents.

ryotaro.tabata@axcelead.com
masashi.yamasaki@axcelead.com
https://axcelead-us.com/