Applications of Cryopreserved Dissociated Human Tumor Cells

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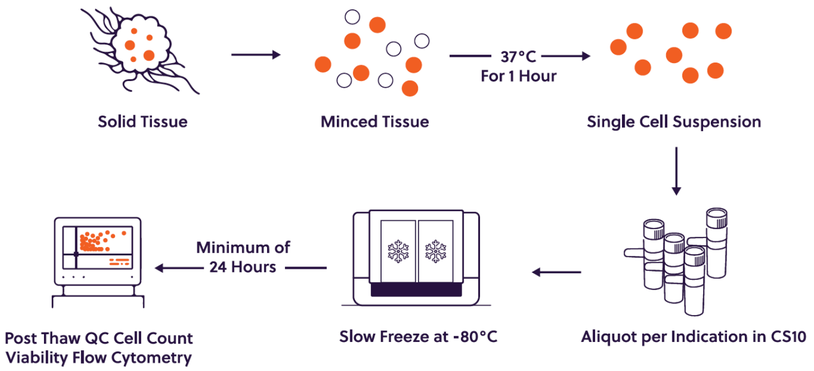
INTRODUCTION

The acquisition of fresh human tissue is often an impediment to significant research advances. As an alternative, Discovery Life Sciences offers cryopreserved dissociated human tissues as a viable specimen source for many downstream applications, including immunophenotyping, bulk and single cell sequencing, and single cell functionality. Extensive cellular profiling of these samples by flow cytometry revealed indication-specific trends in the composition of the tumor microenvironment. Additionally, these specimens provide the opportunity to screen for novel biomarker expressions including the expression patterns of PD1/PDL1. These specimens are amendable to the 10X Genomics single cell pipelines, and workflows have been established to optimize sample preparation into these assays. Finally, as viable cell suspensions, dissociated human tissues can be utilized in both short term and long-term cultures, and the functionality of tumor-infiltrating T cells can be evaluated using IsoPlexis Single Cell Proteomics pipelines. Collectively, cryopreserved dissociated human tissue allows for multifaceted analysis of the next generation of cancer therapies.

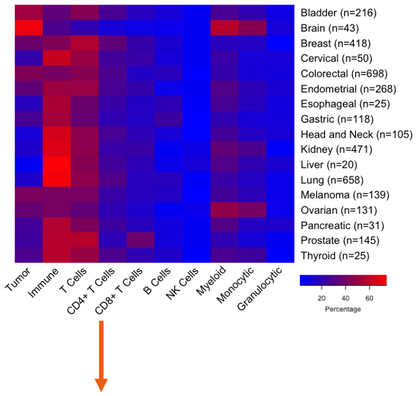
METHODS & MATERIALS



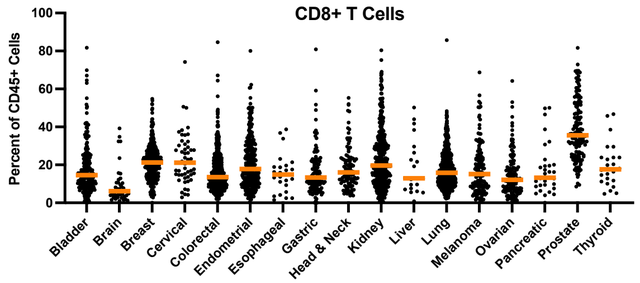
Fresh surgical resection tissue is received at central laboratory facilities within 72 hours of resection. Tissue is minced and then enzymatic and mechanically dissociated to the single cell level. Single cell suspensions are cryopreserved in CryoStor® CS10 and stored long-term in liquid nitrogen. One vial from each lot is thawed to determine cellular viability and composition.

RESULTS

Evaluation of Cellular Composition of Solid Tumors

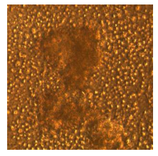


Flow cytometric analysis was performed on over 4500 unique solid tumors. The heat map displays the average percentage of each cell population in each indication. Tumor and immune cells are gated from total live cells, while all immune cell subsets are displayed as a percentage of immune cells. While these averages demonstrate some indication-specific trends, each patient sample is unique with a variability of percentages observed.

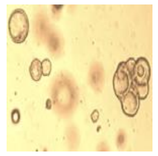


In Vitro Culture and Single Cell Functionality

Spheroid

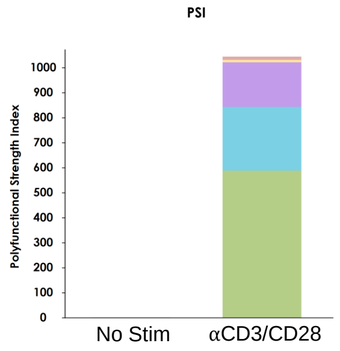


Organoid



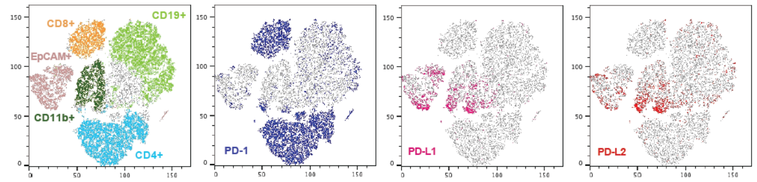
Dissociated tumor cells were cultured in ultra-low attachment conditions to initiate spheroid development, which were viable for 3-5 days in culture. Colorectal DTCs were cultured in an ECM-based hydrogel (Matrigel ®) for long term organoid cultures. Organoid cultures could be propagated long term through multiple passages (minimum 9) and undergo cryopreservation. CD3+ TILs were isolated from DTCs and stimulated with or without anti-CD3/CD28. Single cell functionality was evaluated is the IsoPlexis Single Cell Human Adaptive Immune Secretome panel. Stimulation resulted in robust induction of cytokine secretion.

CD3+ TILs



Target and Biomarker Identification

Flow Cytometry



Single Cell RNA Sequencing

PD-1, PD-L1, and PD-L2 protein and mRNA expression was analyzed by flow cytometry and 10X Genomics single cell gene expression, respectively, in non-small cell lung cancer dissociated tumor cells. PD-1 expression was largely restricted to T cells, while PD-L1 and PD-L2 expression was observed in tumor and myeloid cells.

CONCLUSIONS

- Cryopreserved dissociated tumor cells alleviate the logistic demands of acquiring fresh tissue
- The complex tumor heterogeneity is maintained following dissociation and cryopreservation.
- Indication-specific trends in cellular composition of the tumor microenvironment are observed.
- Drug targets and biomarkers can be evaluated at the single cell level to determine cell-specific expression patterns.
- Cytokine secretion of tumor infiltrating T cells can be evaluated at the single cell to understand immune functionality.
- *In vitro* tumor models, including short-term spheroid and long-term organoids, can be initiated to evaluate new therapies.