EXPLORING DISSOCIATED HUMAN TISSUES AS AN ALTERNATIVE TO FRESH TISSUE FOR MULTIPLE DOWNSTREAM APPLICATIONS

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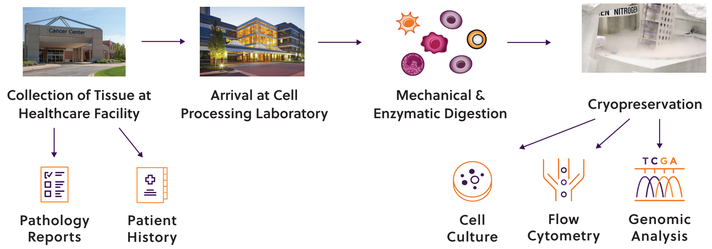
INTRODUCTION

The acquisition of fresh tissue is often an impediment to significant research advances. To provide an alternative to this issue, we have established cryopreserved dissociated human tissues as a viable specimen source with many applications. These single cell suspensions remain viable during the cryopreservation process and can be advantageous for multiple downstream applications, including immunophenotyping, cell culture, and sequencing, among others. Specifically, we have performed multiparametric flow cytometry on over 500 dissociated human tissues (tumor and normal) across 10 different oncology indications. As expected, we observed significant heterogeneity among the cellular composition for each individual tumor type. Across indications, however, trends were observed with regard to percentages of tumor and immune cell populations. Our data, case studies, and observed trends will be discussed.

GENERATION OF DISSOCIATED TISSUE



Tissue is collected following surgical resection and shipped to the central Cell Processing Lab. The tissue undergoes mechanical and enzymatic dissociation to a single cell suspension and is cryopreserved for long term storage in liquid nitrogen. Dissociated tissue cells are annotated with detailed pathology reports and patient data provided by the surgical site and are ready for downstream assays such as flow cytometry, cell culture, and genomic analysis.

DOWNSTREAM APPLICATIONS

In Vitro Cell Culture

FIGURE 1.

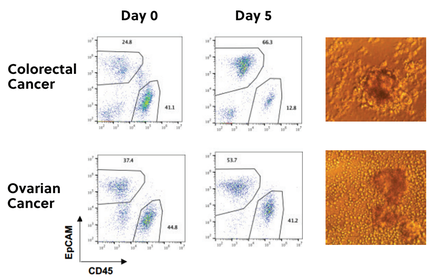


FIGURE 1. In vitro Cell Culture of Dissociated Tumor Cells.

Ovarian and colorectal dissociated tumor cells were analyzed for tumor (EpCAM+) and immune (CD45+) cells by flow cytometry. Single cell suspensions were then cultured in DTC Growth Media. Following 5 days in culture, cultures were examined for the presence of tumor and immune cells by flow cytometry. Cultures were also analyzed for the formation of tumor spheres by light microscopy.

Immunophenotyping via Flow Cytometry

FIGURE 2A. Representative Flow Cytometry Data from Dissociated Tumor

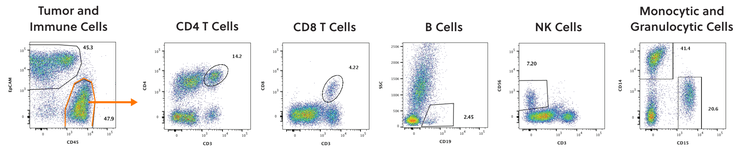


FIGURE 2B. Flow Cytometric Profiling of 500+ Unique Tumor Samples

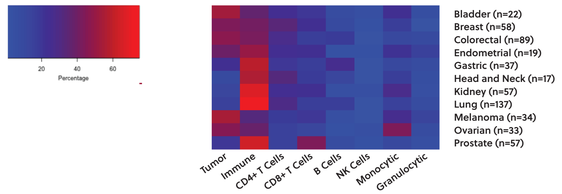


FIGURE 2C. Integration of Pathology Reports and Patient History: Lung Cancer

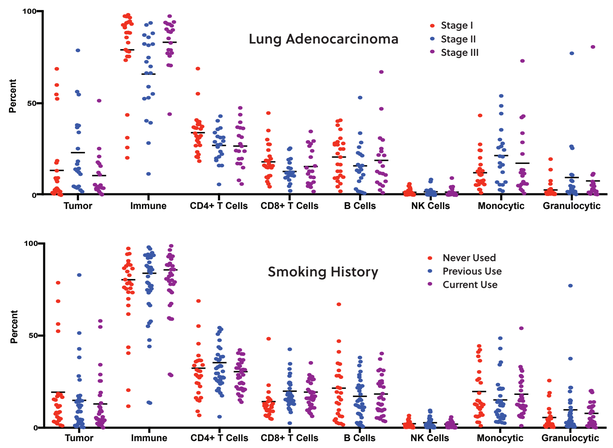


FIGURE 2. Immunophenotyping of Dissociated Tumor Cells by Flow Cytometry.

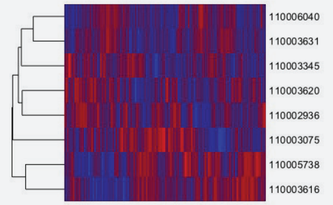
(A) Single cell suspensions of dissociated tumors were analyzed for tumor (EpCAM+) and immune (CD45+) cells by flow cytometry. CD45+ immune cells were further analyzed for CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+), B cells (CD19+), NK cells (CD3- CD56+), monocytic cells (CD14+) and granulocytic cells (CD15+). Data are representative of 560 unique samples across 11 different indications. (B) The average percentage of each cell population described in Figure 2A across 11 indications (left panel). Tumor and immune cells were calculated from total viable cells, while each immune cell subset was calculated as a percentage of CD45+ immune cells. (C) Lung adenocarcinoma dissociated tumor cells were subdivided by stage based on the pathology reports (top panel) or patient smoking history (bottom panel) and correlated with the flow cytometry data described in Figure 2A.

Genomic Analysis

FIGURE 3A. Whole Exome Sequencing Analysis

Donor	Indication	Total	Biallelic	Multiallelic	SNPs	INDELs
110003345	Lung	306101	302560	3541	257557	48544
110003620	Lung	278780	275696	3084	234989	43791
110003616	Melanoma	278227	275009	3218	235337	42890
110005738	Melanoma	264421	261360	3061	222973	41448
110003075	Ovarian	306066	302239	3827	256017	50049
110002936	Ovarian	340699	337235	3464	288397	52302
110006040	Bladder	307065	304425	2640	262345	44720
110003631	Bladder	317221	313953	3268	268901	48320

FIGURE 3B. Whole Transcriptome Analysis



Donor 110003345			
ID	Reference	Alternate	Gene
rs1558544	A	T	EGFR
rs1140475	T	C	EGFR
rs1050171	G	A	EGFR
rs2692456	G	A	EGFR
rs4947986	G	A	EGFR
rs759162	C	T	EGFR
rs6970262	A	G	EGFR
rs34723095	C	CAG	EGFR
rs10241326	T	C	EGFR
rs2293348	C	T	EGFR
rs4947987	G	C	EGFR
rs712831	T	C	EGFR
rs3752651	T	C	EGFR
rs13222549	G	C	EGFR
rs6964705	C	A	EGFR
rs148883365	A	AAC	EGFR
rs55678943	G	A	EGFR
rs2472520	G	C	EGFR
rs371572654	A	G	EGFR
rs141162488	TACAC	T	EGFR
n/a	C	A	EGFR
rs1076452	G	A	EGFR
rs55920948	G	GACAC	EGFR

FIGURE 3. Genomic Analysis of Dissociated Tumor Cells.

DNA and RNA were isolated from eight unique dissociated tumor samples across four different indications and analyzed by whole exome sequencing and RNASeq, respectively. (A) The total genomic alternations were compiled across the eight unique patient samples. The sample from lung cancer patient 110003345 was examined for genetic alterations across the EGFR locus. (B) Heat map of log2 FPKM values and clustering analysis from the genes expressed across all eight patient samples.

SUMMARY

- **Cryopreserved Dissociated Tissue Cells Represent an Alternative to Fresh Tissue**
  - High viability immediately after dissociation and following cryopreservation
- **Flow Cytometric Analysis Revealed Significant Tumor Heterogeneity**
  - All major cellular components are present
  - Indication-specific trends observed in immune cell composition
- **Cells Remain Viable in Short-Term Cultures**
  - Tumor cells aggregate into spheroid clusters
- **High Quality DNA and RNA Isolation**
  - Suitable for whole exome and whole transcriptome analysis
- **Tumor and Immune Cells can be Further Purified via Magnetic Separation**
  - High purity and viability of isolated cells
- **Integration of Large Datasets Enables In-depth Characterization**

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