Deep characterization of tumor infiltrating leukocytes using a combination of flow cytometry and single-cell multiomics

Abstract #2788

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Summary

Cancer immunotherapies have the potential to induce durable anti-tumor immune responses; however the efficacy of these treatments varies among patients and partially depends on the complex interplay between malignant cells and non-malignant cells within the tumor microenvironment. In the tumor microenvironment, T cells play a crucial role in eliminating tumor cells and orchestrating anti-tumor immune responses; in contrast immunosuppressive cell types such as myeloid-derived suppressor cells can support tumor progression. Here we harnessed recent advances in single-cell multiomics that allow high-resolution functional characterization of intratumoral immune cells to improve our understanding of their dynamic relationships. Specifically, we established a workflow to dissociate a solid tumor followed by in-depth dissection of the immune composition of the tumor microenvironment using both flow cytometry and the BD Rhapsody™ Single-Cell Analysis System. We characterized the cellular composition of the tumor microenvironment using flow cytometry analysis. Importantly, by examining the expression of mRNA and surface protein markers at single-cell resolution, we unraveled the complexities of the tumor microenvironment, elucidated cellular heterogeneity and identified cell-type specific gene signatures. We propose this workflow can be adopted to understand the transcriptional and proteomic phenotypes of tumor infiltrating leukocytes for development of novel immune therapeutic strategies in cancer research.

Methods

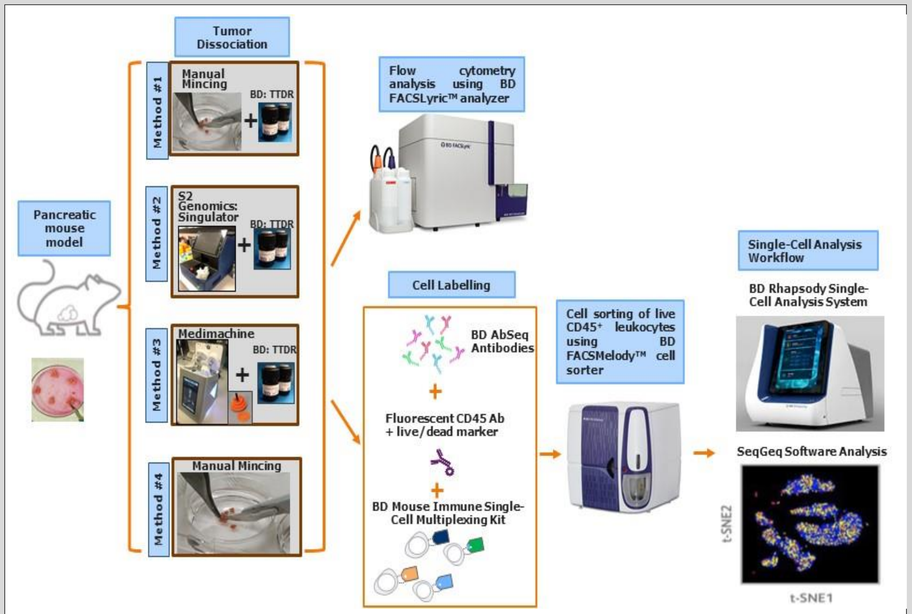

Table 1. 29 plex BD® AbSeq Panel

CD4	Ly-6A_Ly6E	CD197	TIGIT
CD11c	CD103	CD326	CD11a
CD25	CD49b	CD54	CD137
SiglecF	CD335	CD14	CD366
Ly6G/C	CD274	CD278	CXCR2
CD28	CD184	CD273	VISTA
CD117	CD223	CD357	CD279
CD83			

Table 2. Flow cytometry panel

Fluorochrome	Marker
BV421	MHC II
BV510	Live Dead
BV605	CD4
	CD49d
BV711	CD8
	F4/80
BV786	CD19
FITC	CD11b
PerCP-Cy5.5	CD45
PE	CD3
PE-Cy7	Ly6C
APC	CD326 (EpCAM)
Alexa Fluor™ 700	Ly6G
APC-Cy7	CD11c

Figure 1. Experimental overview of single-cell multiomic analysis on sorted leukocytes from dissociated mouse tumor. A mouse pancreatic tumor was divided into four pieces (comparable in weight) and dissociated by four different methods including manual mincing, manual mincing with BD Horizon™ Dri Tumor & Tissue Dissociation Reagent (TTDR), Singulator™ 100 from S2 Genomics with TTDR and BD® Medimachine System with TTDR. A small portion of all samples were taken for flow cytometry analysis. A majority of dissociated cells were co-stained with BD® AbSeq Antibody-Oligo Conjugate Panel, BD® Mouse Immune Single-Cell Multiplexing Kit, live/dead marker and fluorescent CD45. Live CD45+ cells were sorted from each sample using BD FACSMelody™ Cell Sorter and pooled together for single-cell capture using BD Rhapsody™ Single-Cell Analysis System.

Results

Flow cytometry analysis of the tumor infiltrating leukocytes

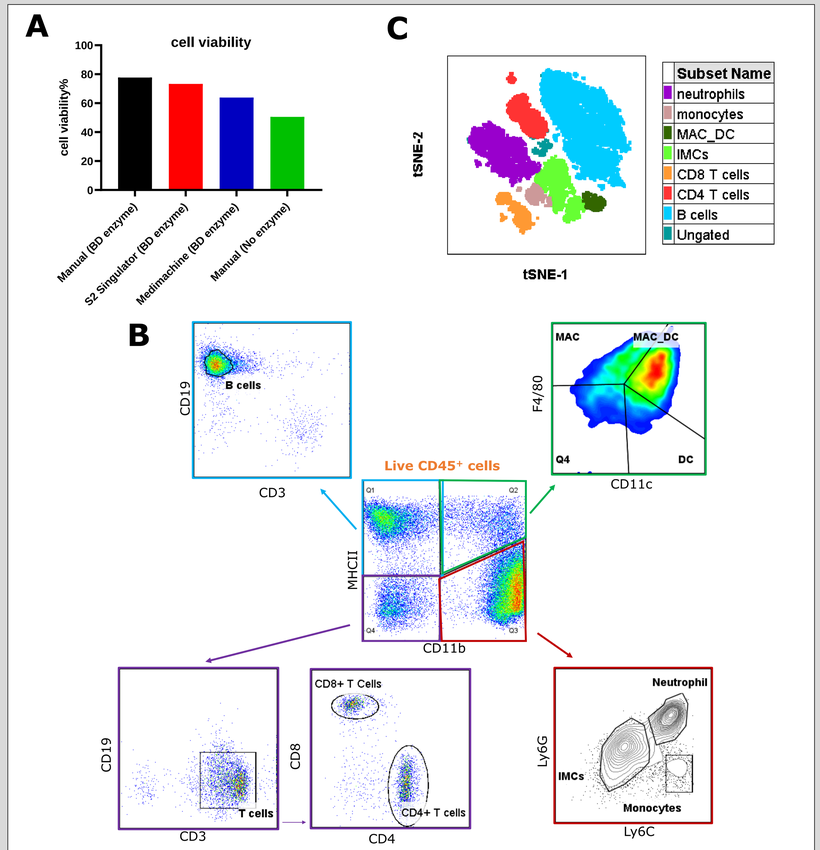

Figure 3. Flow cytometry analysis of tumor infiltrating leukocytes. A) Cell viability from different dissociation methods. **B)** Representative gating strategy to identify different immune cell types using the flow cytometry panel from Table 2. **C)** tSNE analysis to identify different immune cell populations using total cells from four different dissociation methods. MAC, macrophages; DC, dendritic cells; IMCs, immature myeloid cells.

Similar molecules/cell detected from different dissociation methods

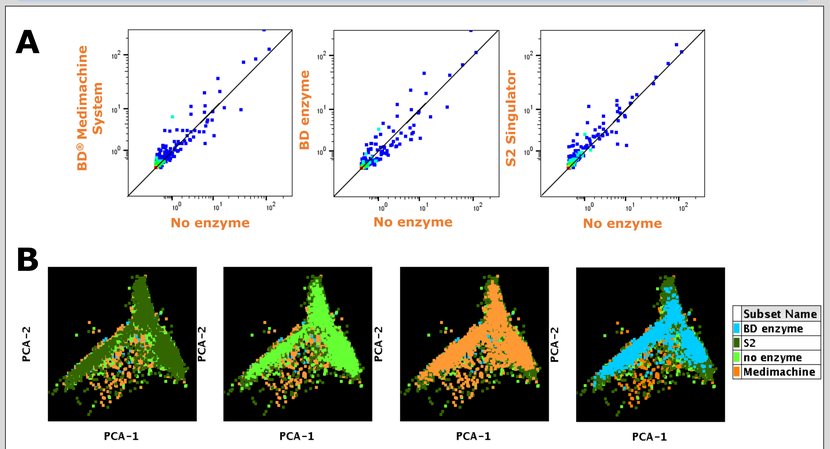

Figure 4. Marker detection using BD Rhapsody™ Single-Cell Analysis System. A) Correlation plots indicate that similar molecules/cell per gene or AbSeq marker were detected in tumor infiltrating leukocytes from different dissociation methods. **B)** Principal component analysis (PCA) of all leukocytes suggests that there is no batch effect in single cell marker expression from different dissociation methods.

Single-cell multiomic analysis of the tumor infiltrating leukocytes

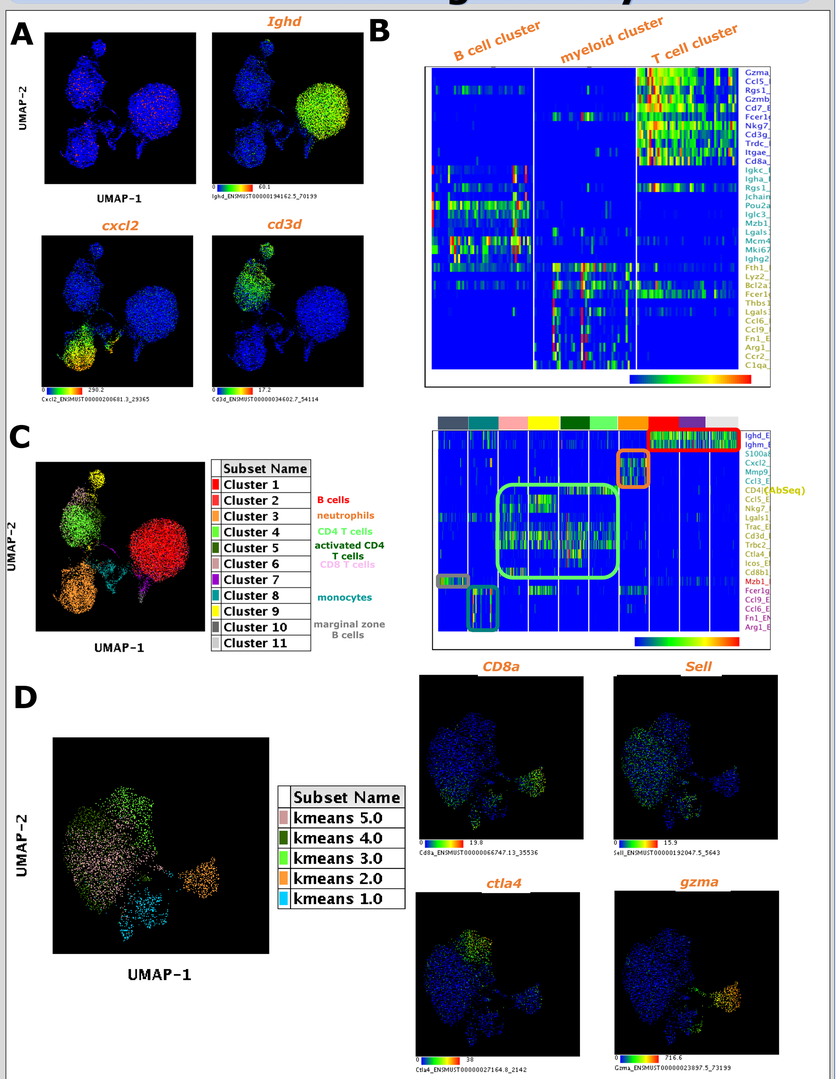

Figure 5. In-depth characterization of tumor infiltrating leukocytes using single-cell multiomic analysis. A) Dimensional reduction by UMAP and expression of key cell type markers. UMAP was performed using a targeted gene panel including around 400 genes and all AbSeq markers. **B)** Identification of marker signature of B cells, T cells and myeloid cells. **C)** Seurat analysis reveals 11 different clusters and their associated marker signature. **D)** Kmeans analysis on total T cells uncovers *ctla4* high cluster and *gzma* expressing CD8 T cell cluster.

Conclusions

- A comprehensive workflow was presented in this study to showcase solid tumor dissociation using TTDR reagent and other instruments followed by single-cell multiomic analysis on sorted tumor infiltrating leukocytes using BD Rhapsody™ Single-Cell Analysis System.
- Different immune populations in the tumor microenvironment can be identified using flow cytometry analysis on the BD FACSLyric™ analyzer.
- The molecules/cell detected for genes and protein markers at the single-cell level are comparable across different tumor dissociation methods.
- The tumor immune composition was characterized and the marker signatures associated with different immune cell subclusters were revealed in details using single-cell multiomic analysis.