Dissociating Tumor Tissue into Cells with S2 Genomics' Pan Tumor Reagent and the Singulator Platform

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Introduction /

Cancer is a complex disease that arises from alterations in the genetic material of cells, leading to uncontrolled growth and proliferation. Tumor sequencing has emerged as a powerful tool for understanding the genetic changes that occur in cancer cells and identifying potential therapeutic targets. However, tumors are heterogeneous, consisting of different types of cells with varying genetic profiles. Therefore, it is essential to dissociate tumors into the constituent cells and perform sequencing at the single cell level to obtain accurate information about the genetic changes driving the cancer. The conventional techniques for dissociating solid tumors are often intricate, require considerable time and expertise, and yield variable results. In this study, we showcase the efficacy of the Singulator™ platform as a means of isolating viable lung tumor cells in high yield and viability from patients diagnosed with adenocarcinoma and squamous cell carcinoma. Our approach incorporates the utilization of S2 Genomics' Pan Tumor Reagent, which enables robust and reliable tumor cell isolation.

Experimental Design /

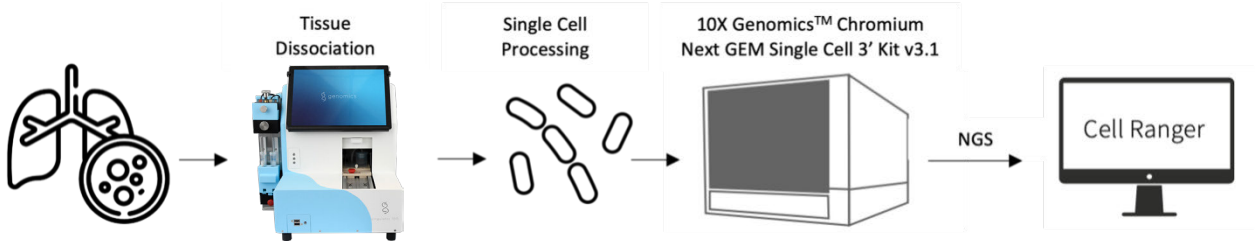


Figure 1. Experimental Design. Tumor tissues were dissociated into a single cell suspensions with a Singulator™ 100. The yield and viability of the cells were measured. A target number of 10,000 cells were prepared with the 10X Genomics™ Next GEM Single Cell 3' kit v3.1. Sequencing data were analyzed with 10X Genomics' analysis pipeline and Seurat.

Methods /

Sample Processing

Fresh human lung tumor tissues from 3 patients were procured from the Cooperative Human Tissue Network (CHTN) under an approved IRB protocol and received cold in RPMI buffer through overnight shipment. For patient pathology, refer to Table 1. The tumor samples were finely minced and processed in the Singulator 100 using the Mouse Lung Cell Isolation protocol, Tumor Isolation Reagent (100-247-099), and Cell Isolation Cartridges (100-063-178). The resulting cell suspensions were removed from the cartridge and centrifuged. Red blood cells (RBCs) were lysed with RBC Lysis Buffer, the suspension centrifuged, and supernatant removed. The tumor cells were further purified with a Percoll density gradient centrifugation and sequential filtration through cell strainers. Cell counts and viability were determined (AOPI staining, Nexcelom K2) to obtain the appropriate dilution for loading the samples into the 10X Genomics Chromium Controller (Table 2). The samples were processed using the 10X Genomics™ Next GEM Single Cell 3' kit v3.1. Images of the cells at 10x magnification are shown in Figure 2.

PATIENT	KIND OF TUMOR	AGE	RACE
1	Adenocarcinoma	69	White
2	Squamous Cell Carcinoma	62	White
3	Invasive Squamous Cell Carcinoma	76	White

Table 1. Patient Pathology.

SAMPLE	AVERAGE TITER	VIABILITY
Tumor Cells 1	5,880,000	97%
Tumor Cells 2	6,732,000	94%
Tumor Cells 3	5,065,000	76%

Table 2. Average Titer and Viability of Tumor Samples.

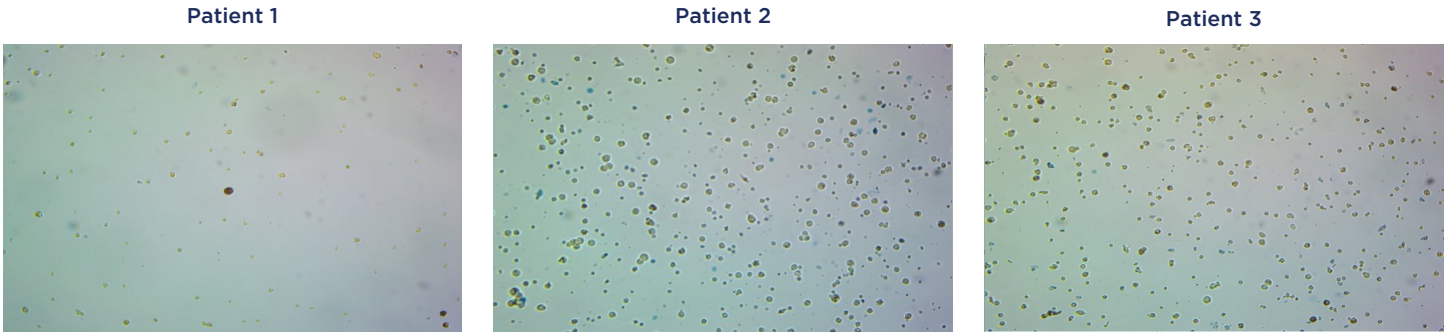


Figure 2. Human Lung Tumor Cells. Images of the cells at 10x magnification stained with Trypan Blue for visual assessment of sample quality prior to sequencing library preparation.

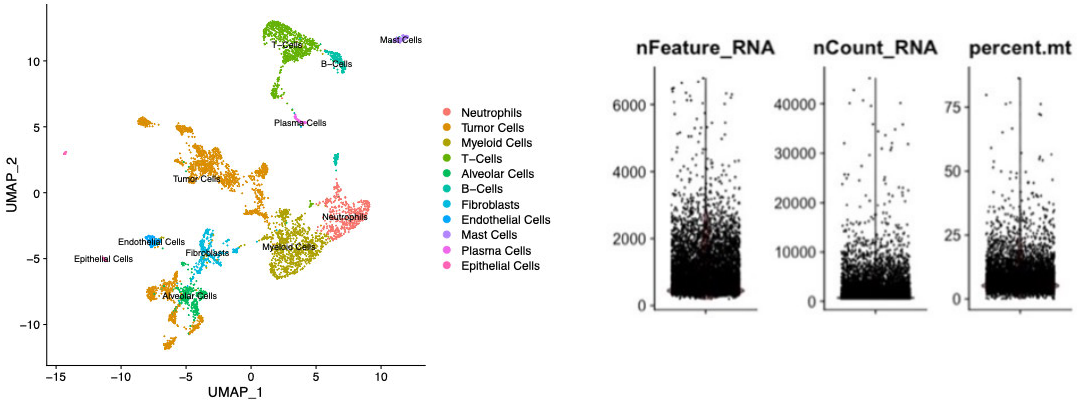
Sequencing and Data Analysis

The tumor samples' libraries were sequenced on an Illumina Novaseq™ 6000 by a third-party service provider. The data were analyzed with the 10X Genomics' Cell Ranger analysis pipeline and quality control filtering metrics were set to the manufacturer's default values. The data were independently clustered with Seurat v4.0 and the clusters were manually annotated using marker genes described in the literature[1,2,3].

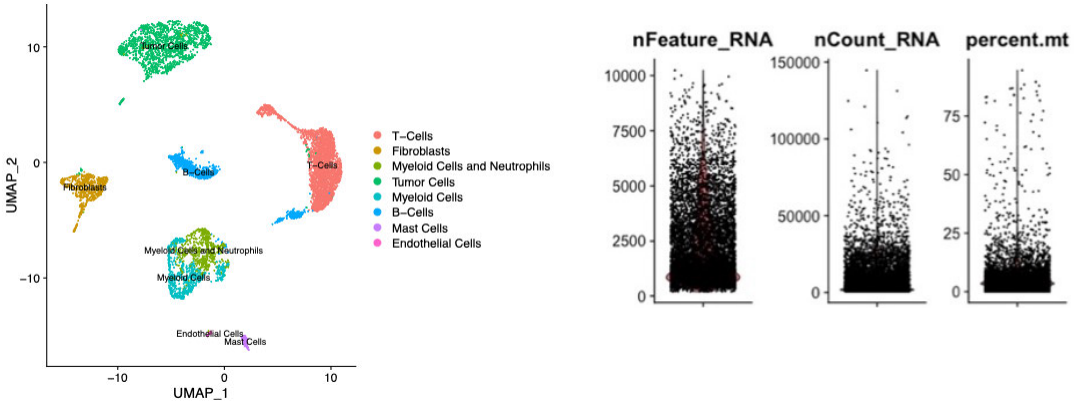
Results & Discussion /

8,085 tumor cells from Patient 1, 8,500 cells from Patient 2, and 6,458 cells from Patient 3 were analyzed and independently clustered with Seurat, annotated, and visualized as UMAPs. High quality isolations of the samples were confirmed with acceptable mitochondrial contamination, showing comparable results between tumor types (Figure 3). Broad clustering of cell types of each tumor type is shown in Figure 3. The clustering of Patient 1 with adenocarcinoma showed different cell types than Patients 2 and 3 with squamous cell carcinoma. Figure 4 shows the heat map of 17 biomarker genes used to distinguish adenocarcinoma and squamous cell carcinoma in lung cancers[4]. The expression of these 17 biomarkers is consistent with the results of pathology confirming the type of cancer tumor.

Patient 1: Cell Clustering and QC



Patient 2: Cell Clustering and QC



Patient 3: Cell Clustering and QC

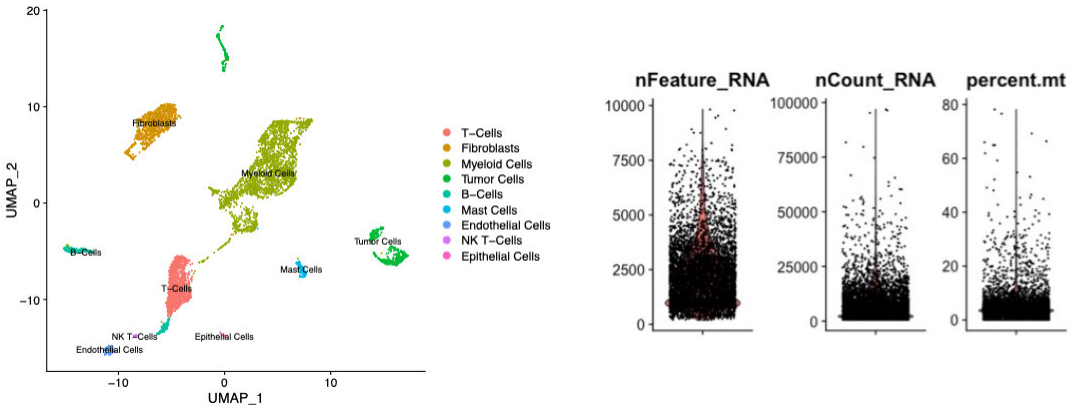


Figure 3. UMAPs and Violin Plots. UMAPs and Violin plots from scRNA-Seq data of tumor cells from isolated with the Singulator 100 showing nFeature RNA, nCount RNA, and percent mitochondrial contamination per cell across the individual patient samples and broad cell clustering.

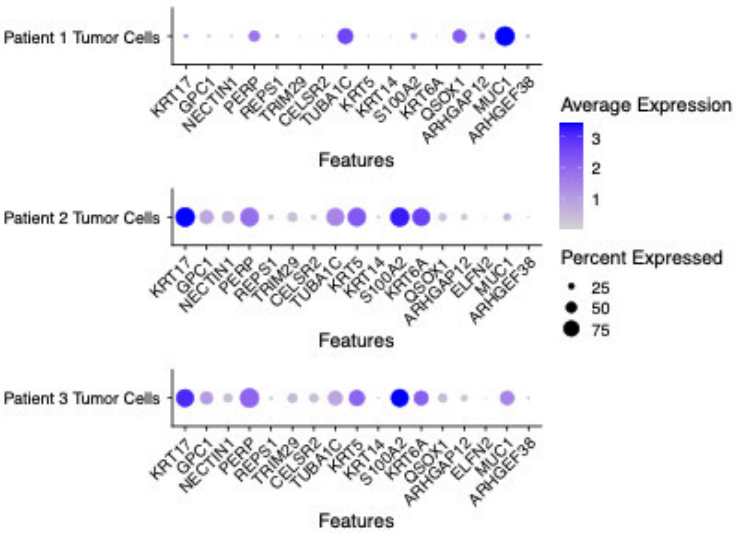


Figure 4. Squamous Cell Carcinoma vs. Adenocarcinoma Biomarkers. Heatmap of 17 biomarker genes for each patients' tumor cell clusters found to be upregulated in lung squamous cell carcinoma and adenocarcinoma. QSOX1, ARHGAP12, ELFN2, MUC1, AND ARHGEF38 corresponding to adenocarcinoma and other genes to squamous cells.

Conclusion /

Using the Singulator™ 100 system and S2 Genomics' Pan Tumor Reagent, we were able to successfully isolate tumor cells from patients with adenocarcinoma and squamous cell carcinoma and obtain single-cell sequencing data with broad cell-type representation. The Singulator 100 eliminates the technical biases, time-consuming protocol development, and technical challenges associated with traditional cell isolation methods, allowing for improved cluster resolution and cell-type representation, including large and fragile cell types.

References:

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