Exploring tumor heterogeneity of chronic lymphocytic leukemia using single cell multiomics

BD FACSMelody™ Cell Sorter and BD Rhapsody™ Single-Cell Analysis System enable targeted mRNA and protein expression profiling in single cells

Features

- Simultaneous analysis of single cell gene expression and surface protein on human samples
- Integrated workflow featuring BD® Human Single-Cell Multiplexing Kit to reduce reagent cost and improve data quality
- Sorting human samples using the BD FACSMelody™ cell sorter prior to single cell workflow, allowing enrichment of cell types of interest

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults characterized by proliferation and accumulation of neoplastic B cells in the peripheral blood, bone marrow and secondary lymphoid organs. Numerous studies have revealed the heterogeneity and complexity underlying pathogenesis of CLL. While bulk RNA-seq studies provide frameworks to understand CLL pathogenesis, transcriptome data from a complex mixture of cells derived from bulk tumor samples fails to accurately elucidate the intratumoral heterogeneity of CLL. The emergence of single cell RNA sequencing technology provides an unprecedented opportunity to discover disease-specific signatures and reveal the heterogeneity of CLL.

BD

Figure 1

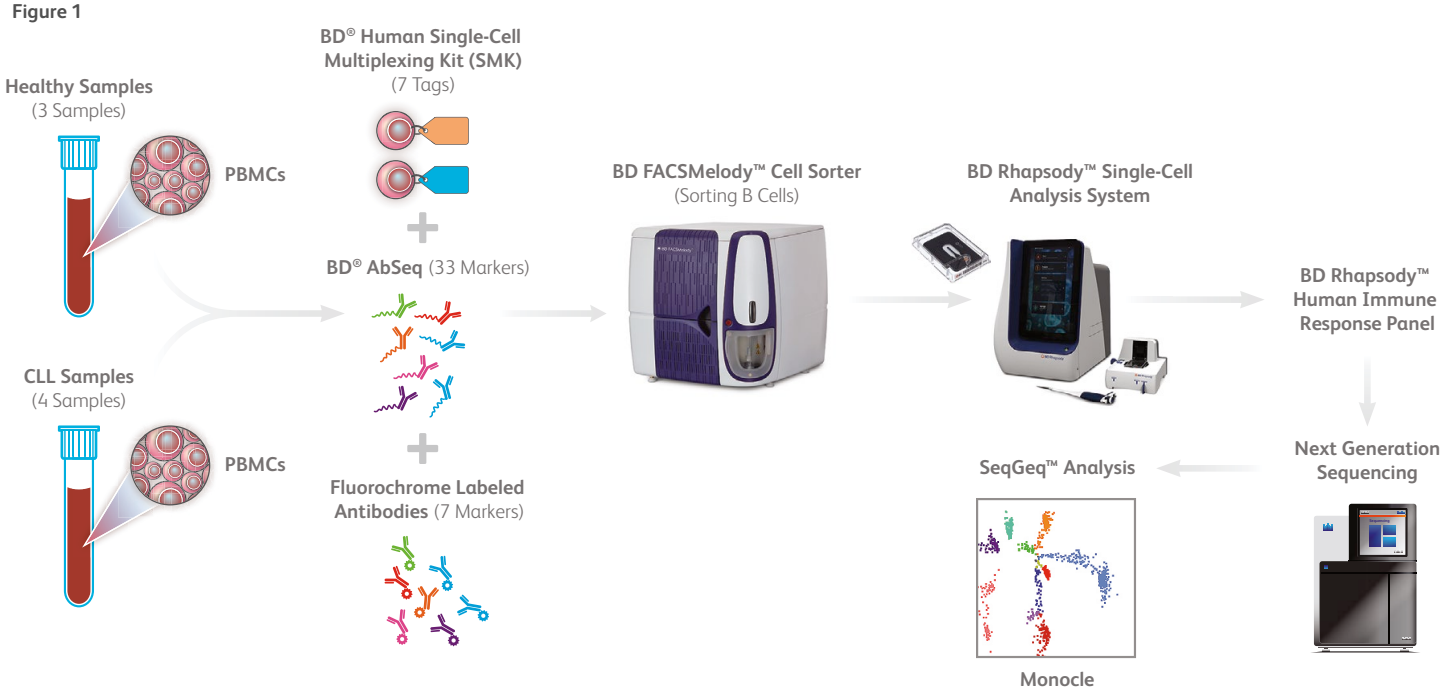

Figure 1. Workflow of single cell multiomics on healthy and chronic lymphocytic leukemia samples using BD Rhapsody system

Peripheral blood mononuclear cells (PBMCs) from a total of three healthy donors and four CLL samples were simultaneously stained with BD SMK, fluorochrome-labeled antibodies and 33 BD AbSeq markers. CD19$^+$ cells from different samples were then sorted using the BD FACSMelody cell sorter and pooled together prior to being loaded on the BD Rhapsody system. cDNA was synthesized and processed for library preparation using targeted BD Rhapsody Immune Response Panel before sequencing. Data was analyzed using BD Rhapsody™ Analysis Pipeline and SeqGeq™.

This data sheet provides an example of simultaneous measurement of protein and mRNA expression at single cell resolution using BD Rhapsody™ Single Cell Analysis System on healthy and clinical research CLL samples. As shown in Figure 1, samples were labeled simultaneously with a panel of 33 BD AbSeq antibody-oligonucleotide conjugates (Table 1, right panel), fluorochrome-conjugated CD19 and negative cell lineage antibodies for sorting (Table 1, left panel) and BD® Human Single-Cell Multiplexing Kit for sample multiplexing. CD19$^+$ cells from different samples were then sorted by BD FACSMelody™ cell sorter and pooled together prior to being loaded to BD Rhapsody™ system. Subsequently, the captured cells were profiled using BD Rhapsody™ Human Immune Response Panel (399 genes).

Table 1. List of fluorochrome-conjugated and AbSeq antibodies used

BD Fluorochrome-labeled Antibodies Used for Sorting

Marker	Fluorochrome
CD3	APC
CD14	APC
CD16	APC
CD19	PE
CD41a	APC
CD56	APC
CD235a	APC

BD AbSeq Antibodies Used for Single Cell Multiomics

AbSeq Marker			
CD4	CD24	CD80	CD184
CD5	CD27	CD81	CD275
CD7	CD33	CD86	CD279
CD8	CD34	CD90	CD294
CD9	CD38	CD103	IgD
CD10	CD40	CD117	IgG
CD11c	CD45	CD123	
CD19	CD45RA	CD127	
CD20	CD69	CD133	

t-SNE analysis was performed on four CLL samples and three healthy donor samples using mRNA expression, surface protein expression or a combination of both. t-SNE plot generated based on AbSeq data showed higher level of separation among different samples compared to using RNA-seq data alone. A combination of both mRNA and AbSeq data greatly improved the resolution of different samples (Figure 2A). Four CLL samples formed distinct clusters individually whereas the healthy samples overlapped to a greater extent in the t-SNE plot (Figure 2A, right), suggesting the clinical course of CLL patients can be heterogeneous. CLL is characterized by clonal expansion and accumulation of CD5$^+$ B lymphocytes. Additionally, malignant B cells in CLL patients can also express CD11c at higher levels. BD AbSeq reagents revealed higher expression of CD11c and CD5 in CLL samples compared to healthy donors, correlating to pathogenesis of CLL (Figure 2B). On the other hand, low CD10 and CD20 expression were observed in CLL samples compared to healthy donors, which is consistent with published literature (Figure 2B).

Figure 2A

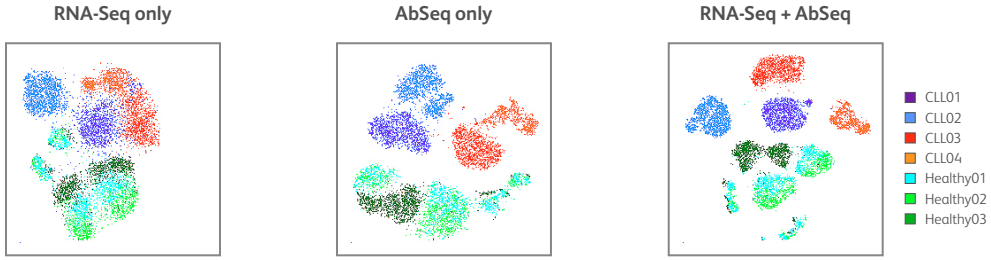

Figure 2B

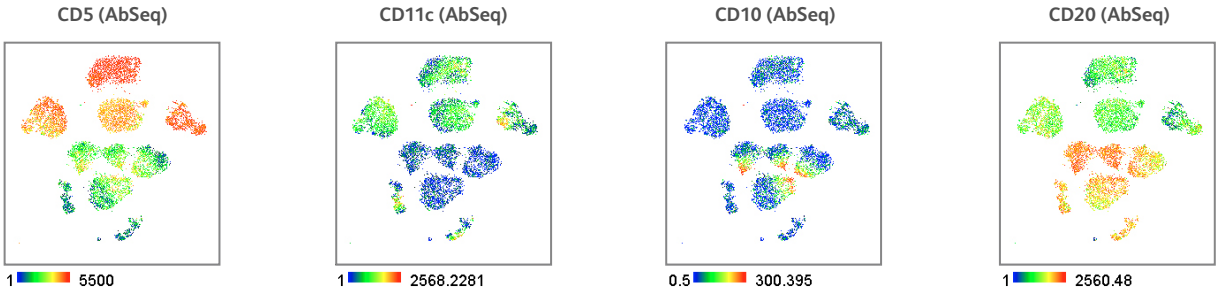

Figure 2. Multiomic analysis of CLL samples and healthy donors

A. t-SNE clustering of different samples using mRNA or surface protein or a combination of both. **B.** t-SNE coordinates of key markers associated with CLL disease progression based on BD AbSeq.

To understand the gene signatures underlying the distinct clustering between healthy and CLL samples, we performed a differential expression analysis using single cell mRNA and AbSeq data. We identified markers that were decreased in CLL samples compared to healthy samples, Figure 3, Group 1. Genes or proteins that were differentially increased in all CLL samples are listed in Group 2 (all CLL), while Group 3 lists those which are up in some CLL samples. CD5, the marker aberrantly expressed in CLL patients, was higher in CLL03 and CLL04 compared to other samples. We also discovered a subset of markers that were specifically associated with CLL04 (Group 4), such as CD11c. Interestingly, not all cells produce CD11c at the same level, suggesting intratumoral heterogeneity of CLL. It is reported that CD11c expression can be associated with harmful clinical complications and secondary tumors in CLL patients. However, average expression of CD11c might be low when bulk RNA-seq is utilized. Single cell multiomics has the potential to overcome this caveat and provide useful information on disease progression.

Figure 3

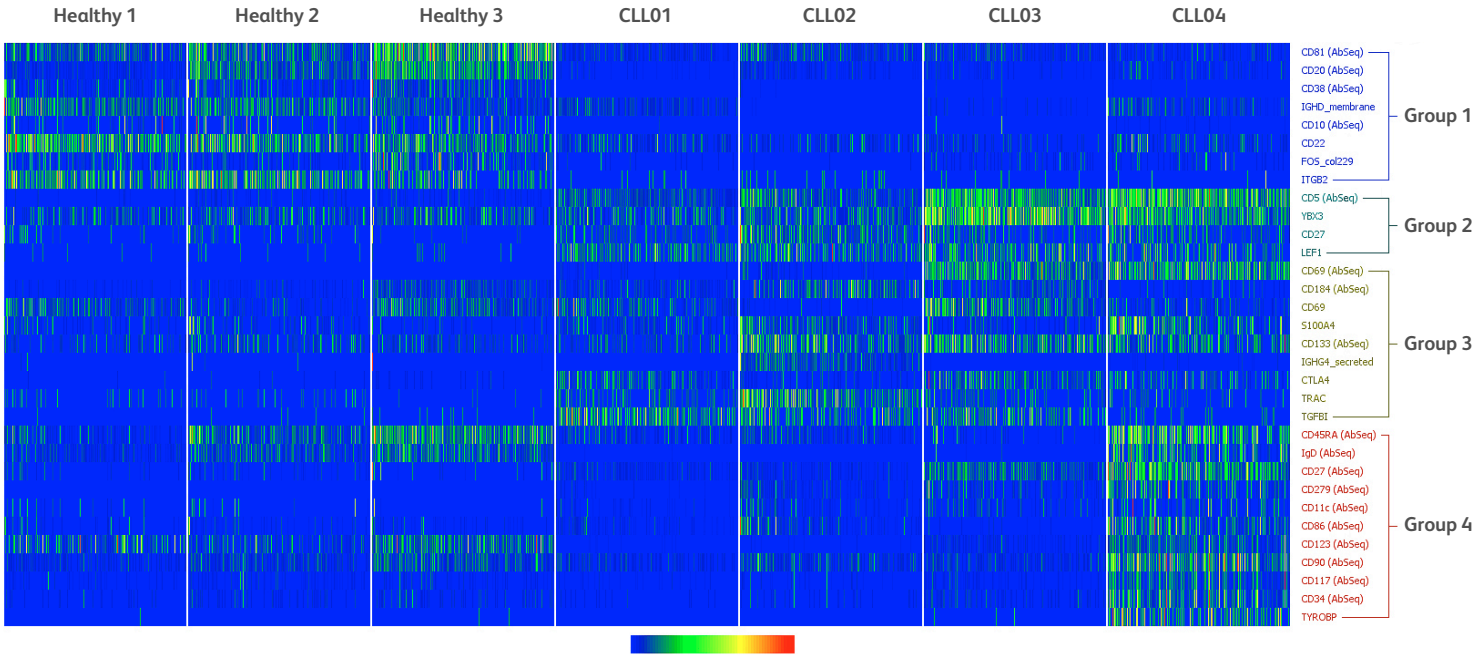

Figure 3. Heatmap of representative differentially expressed genes or surface proteins in CLL and healthy samples at single cell level

Expression of genes or proteins (indicated as AbSeq) was lower (Group 1) or higher (Group 2 and 3) in CLL samples compared to healthy samples. A subset of genes or proteins whose expression was specifically associated with CLL04 is also listed (Group 4).

Ordering information

Systems and software
Description
BD Rhapsody™ Single-Cell Analysis System
BD FACSMelody™ Cell Sorter
SeqGeq™ Software

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

23-21772-00

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