Full-length TCR and BCR repertoire analysis, integrated with mRNA and surface proteins characterization, in a tumor mouse model

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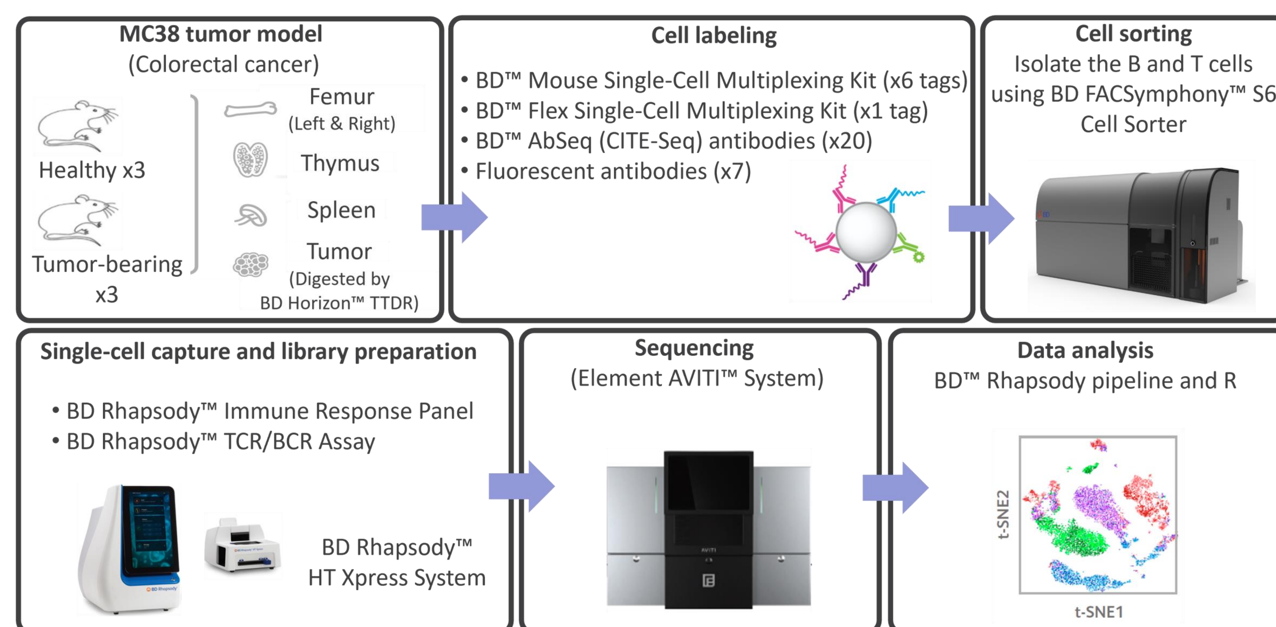
Abstract

Full-length TCR and BCR analysis with single-cell multiomic assays provides an understanding of immune responses, offering insights into the complete receptor structure and functionality that is crucial for accurately assessing the diversity and specificity of immune repertoires. Studying mouse immune cell heterogeneity can be achieved by simultaneous mRNA and surface protein profiling. However, when coupled with full-length TCR and BCR analysis, this integrated approach provides a more comprehensive understanding of immune responses and cellular function in healthy and diseased conditions.

In this study, we used the MC38 colon carcinoma model to study T and B cell heterogeneity and clonotype diversity. We stained single-cell suspensions prepared from bone marrow, thymus, spleen, and tumor tissues with 20 BD™ AbSeq (CITE-Seq) Antibody Oligos and sample-multiplexing tags. We then loaded 50,000 isolated T and B cells into two lanes of a BD Rhapsody™ HT Xpress Cartridge for single-cell capture by the BD Rhapsody™ Single-Cell Analysis System. To aid in the simultaneous analysis of clonality and gene expression, we carried out library preparation using the BD Rhapsody™ TCR/BCR Multiomic Assay and BD Rhapsody™ Mouse Immune Response Panel. The combination of mRNA and protein expression confirmed the elevated expression of exhaustion and activation markers in tumor-infiltrating lymphocytes (TILs). We identified high-frequency clonotypes within TILs and tracked their frequency across other tissues. Tumor-burdened mice splenic T cells showed shared TCR combinations with TILs, which were absent in healthy tissue. The results show the ability to successfully profile T and B cell receptors using the full-length BD Rhapsody™ TCR/BCR Multiomic Assay in a mouse tumor model and the use of transcriptome, protein and TCR/BCR clonal information to investigate immune cell characteristics across multiple samples.

Methods

Figure 1 Workflow of cell staining and sorting followed by single-cell analysis using the BD Rhapsody™ system

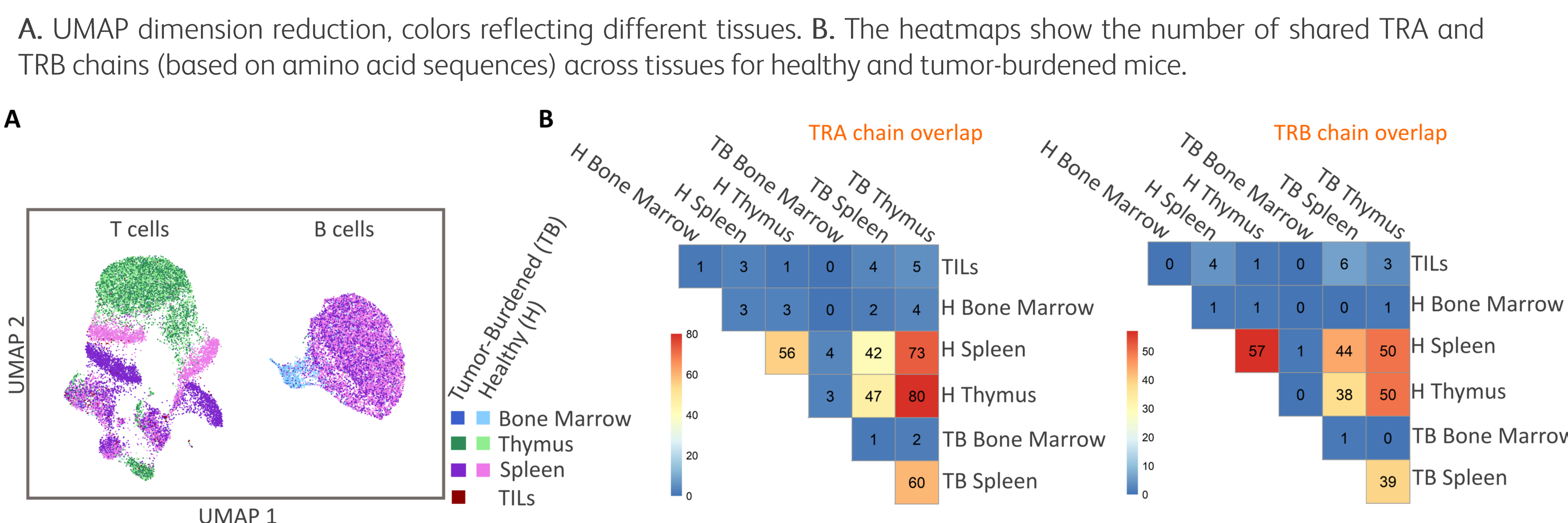


Eight-week-old female C57BL/6 mice were injected with MC38 tumor cells (tumor-burdened, n=3) or used as the control group (healthy mice, n=3). Four weeks after the implantation, the mice were euthanized and the left and right femur, spleen, thymus, and tumor tissues were collected. Single-cell suspensions were prepared from the healthy tissues while BD Horizon™ Dri Tumor & Tissue Dissociation Reagent (TTDR) was used to dissociate single cells from tumor tissues. RBCs were lysed with BD Pharm Lyse™ Lysing Buffer. All samples were simultaneously stained with fluorescent antibodies, BD™ AbSeq panel (CITE-Seq), and sample-multiplexing tags. 6 samples (3 tissues from tumor-burdened mice and 3 tissues from control mice) were stained using 6 BD™ Mouse Single-Cell Sample Multiplexing Kit (1 sample tag per tissue type) and the tumor tissue was stained using the BD™ Flex sample-multiplexing kit. Then all 7 samples were pooled in a tube, and T and B cells were sorted using a BD FACSymphony™ S6 cell sorter. Sorted T and B cells were then pooled and loaded on two lanes of a BD Rhapsody™ 8-Lane Cartridge for single-cell capture using the BD Rhapsody™ HT Xpress System. BD™ AbSeq (CITE-Seq), Sample Tag (Cell Multiplexing), targeted mRNA (BD Rhapsody™ Mouse Immune Response Panel), and TCR/BCR libraries were sequenced on the Element AVITI™ system. The sequencing data was processed using the BD Rhapsody™ Sequence Analysis Pipeline and further analyzed in R v4.2.2 by package Seurat v4.3.0.

Results (1)

Figure 2. Unsupervised cell clustering and repertoire overlap analysis across all tissues

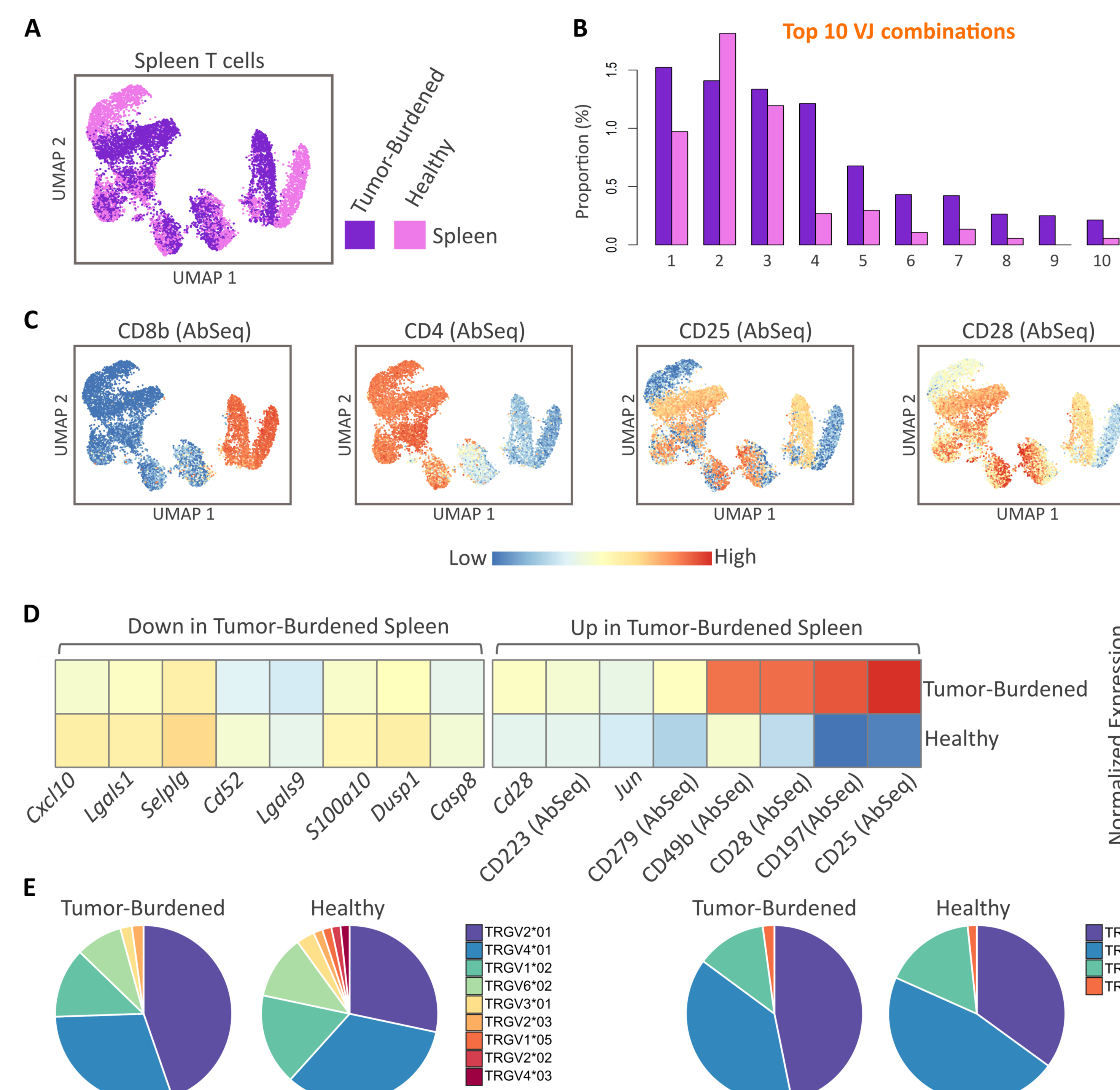
A. UMAP dimension reduction, colors reflecting different tissues. B. The heatmaps show the number of shared TRA and TRB chains (based on amino acid sequences) across tissues for healthy and tumor-burdened mice.



UMAP analysis was performed using protein and mRNA expression on approximately 27,000 high-quality cells (Figure 2A). CD3 and CD19 protein expression confirmed the successful sorting of B and T cells. Additionally, TCR repertoire overlap analysis (alpha and beta chains) was performed across all tissues (Figure 2B).

Figure 3. Multiomic analysis of healthy and tumor-burdened mice spleen T cells

A. Unsupervised clustering of T cells in spleens from both tumor-burdened and healthy mice. B. The frequency of the top 10 VJ combinations in tumor-burdened mice spleen T cells compared to healthy mice spleen T cells. C. Relative expression of CD4, CD8, CD28, and CD25 surface proteins assessed using BD™ AbSeq antibodies D. Differentially expressed mRNA and proteins between tumor-burdened and healthy mice spleen T cells. E. Distribution of V and J gene usage of γδ T cell receptors in tumor-burdened and healthy mice spleen T cells.

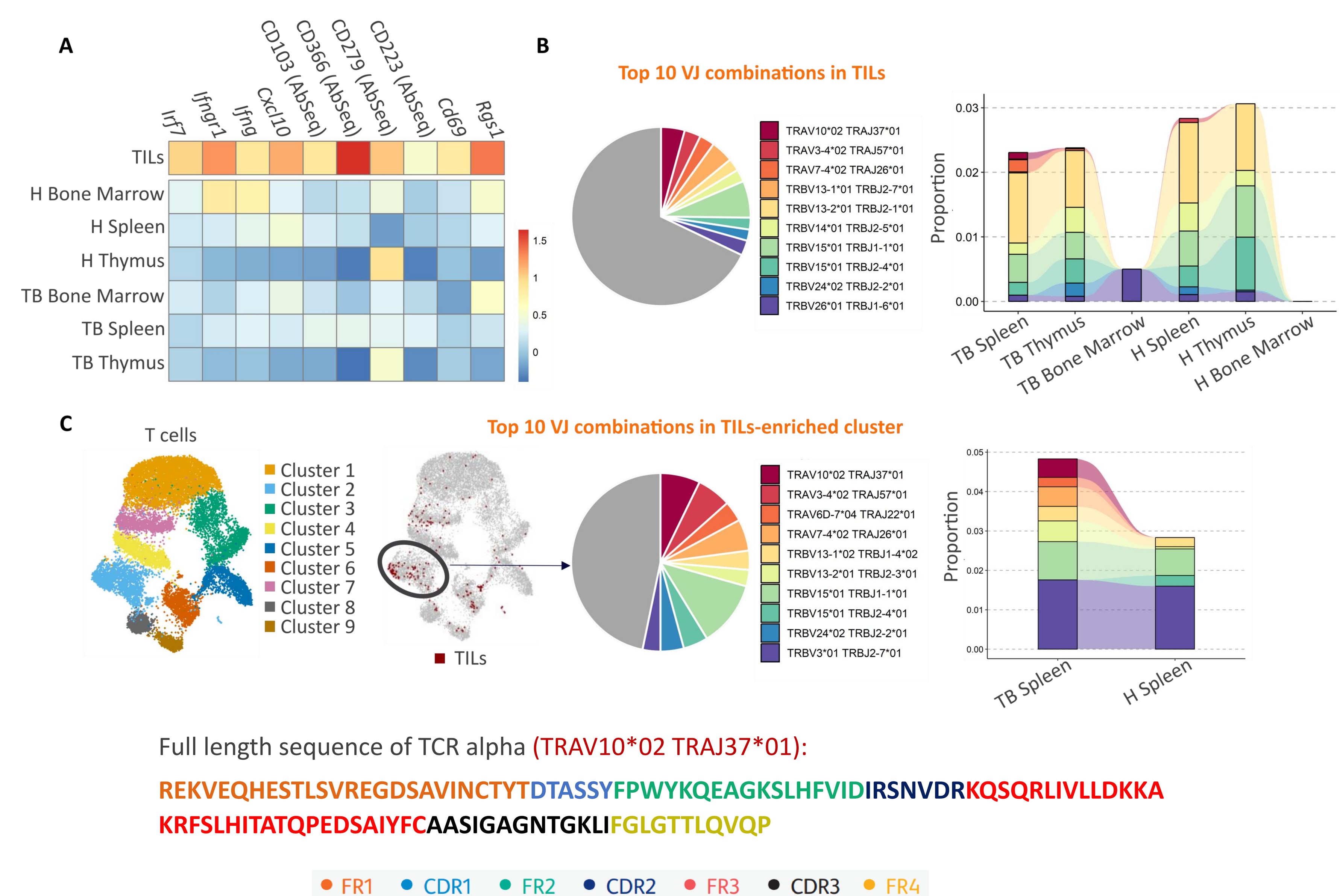


Based on mRNA and protein expression, unsupervised clustering was performed for approximately 8,000 T cells from healthy and tumor-burdened mice spleen (Figure 3A). CD4⁺ and CD8⁺ T cells from the tumor-burdened mice showed upregulation expression of genes and surface proteins associated with T cell activation (CD25 and CD28) and exhaustion (CD279; PD-1 and CD223; LAG-3) compared to healthy mice spleen (Figure 3C and D). The combination of gene expression and cell surface protein using BD™ AbSeq (CITE-Seq) antibodies yielded an enhanced resolution of T cell activation and exhaustion state. Also, the frequency of the top 10 TCR VJ combinations (alpha and beta chains) in T cells were derived from tumor-burdened mice spleen and then compared to that of healthy mice spleen (Figure 3B). Additionally, the analysis of V and J gene usage ratios in γδ T cell receptors showed differences between the two groups (Figure 3E).

Results (2)

Figure 4. Gene expression, surface protein, and single-cell TCR analysis in TILs

A. Heatmap of top differentially expressed mRNAs and proteins in TILs, compared to the T cells from other tissues. B. Pie chart shows the distribution of top 10 V and J gene usage in TCR repertoire of TILs, and the bar plot illustrates the frequency of these combinations across other tissues. C. Pie chart shows the distribution of the top 10 VJ gene combinations in TCR repertoire of TILs in cluster 2, which has a high frequency of TILs. The bar plot illustrates the frequency of these combinations across tumor-burdened and healthy mice spleen T cells. VJ tracking plots were generated by Immunarch v1.0.0.



To investigate the characteristics of the TILs, differential mRNA and protein expression analysis was performed across different tissues (Figure 4A), which revealed upregulation of T cell activation marker (*Cd69*), tissue-residency (*Rgs1*) and cytokine and chemokine mRNA (*Ifng* and *Cxcl10*) in TILs. TILs also showed elevated protein expression of T cell exhaustion markers such as CD223 (LAG-3) and CD279 (PD-1). The frequency of the top 10 VJ combinations in TILs were tracked across other tissues (Figure 4B). Zooming in on the TILs cluster (Figure 4C, left), the frequency of the top 10 VJ combinations from this cluster were tracked across the spleen T cells in this cluster. Tumor-burdened mice spleen T cells showed shared TCR combinations with TILs, which were absent in healthy spleen T cells (Figure 4C,right).

Conclusions

- In summary, full-length T and B cell receptors were successfully profiled using the full-length BD Rhapsody™ TCR/BCR Multiomic Assay in a mouse tumor model. The comprehensive analysis of the transcriptome, surface protein, and TCR repertoire at the single-cell level using the BD Rhapsody™ Analysis System enables the investigation of immune cell characteristics and clonotype diversity across multiple samples.
- The combination of mRNA and protein expression validated the elevated expression of exhaustion and activation markers in tumor-infiltrating lymphocytes (TILs).
- By detecting the most frequent clonotypes in TILs and monitoring their frequency across other tissues, we revealed shared TCR combinations between TILs and splenic T cells from tumor-burdened mice, absent in healthy tissue.

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