Flexomics

White Paper

Identification of Neoantigen-Specific TCRs using Flexomics' Multi-Modal Platform

In this study, we demonstrate the collection and integrated analysis of multiple data types from single cells to directly identify T-cell receptor (TCR) sequences that recognize specific tumor-associated antigens (TAA). Here, we used the Flexomics 8-site chip to co-culture antigen presenting cells (APCs) with T-cells, identify activated T-cells and cytokine secretion events with fluorescent markers, extract TCR sequences using long-read sequencing, and classify the transcriptomic response using targeted short-read sequencing. Finally, all data types are traced to the individual cell of origin using Flexomics' proprietary barcode mapping system. Analysis of integrated data identifies TCRs that elicit the most favorable response.

Introduction

Neoantigens — novel proteins arising from cancer-specific genetic mutations — represent a promising frontier for targeted cancer therapies. Unlike normal proteins, these tumor-specific antigens are recognized by the immune system as foreign, triggering a targeted immune response against cancerous cells. Understanding the interactions between neoantigens and the T cells that mediate an immune response is critical for advancing both personalized immunotherapies and next-generation cancer treatments. In particular, identifying the specific TCR clonotypes expressed by antigen-activated T cells is essential for leveraging cell interaction data in therapeutic development.

A major challenge to identification of TAA-specific TCRs is obtaining the required data types, combined, *at single-cell resolution*. Current approaches are able to detect the TCR repertoire expressed by a population of activated T-cells, but connection of critical TCR sequences to specific functional and transcriptomic readouts are often made using inferrence rather than direct association in individual cells.

In this study, we demonstrate the utility of Flexomics' FLEX chip to collect and integrate imaging and sequencing data at single cell resolution to identify and classify the TCR-mediated response to neoantigen presentation in each individual cell.

Methods

T2 antigen presenting cells were loaded with MART-1 TAA peptides and co-cultured on a Flexomics picowell chip with CD8+ T-cells derived from CMV+ or CMV- donors. After overnight co-culture, cells were assayed for IL2 and IFNγ secretion directly on the chip using a modified version of Miltenyi's cytokine secretion catch assay. Finally, cells were stained for T-cell activation markers (CD69 and CD137), and the chip was imaged to collect functional and phenotyping data.

For NGS workflows, imaged cells were lysed directly on the chip and RNA was captured on well-specific barcoded beads. Barcoded cDNA was synthesized from the recovered RNA, and amplified cDNA was used as template in two separate downstream RNA-seq workflows. To identify expressed isoforms of TCR alpha/beta genes, multiplex PCR was performed on full-length cDNA and used as template for long-read sequencing. To further characterize cellular responses to TAA recognition, a panel of 17 immune response genes was analyzed in a targeted RNA-seq assay.

For analysis, imaging and sequencing data for each cell were combined based on their assigned well-of-origin. For imaging data, cells were mapped to picowells using Flexomics' fiducial reference markers and fluorescence-based object coordinates. For sequencing data, the spatial map of barcode beads was used to link each captured RNA transcript to its well-of-origin.

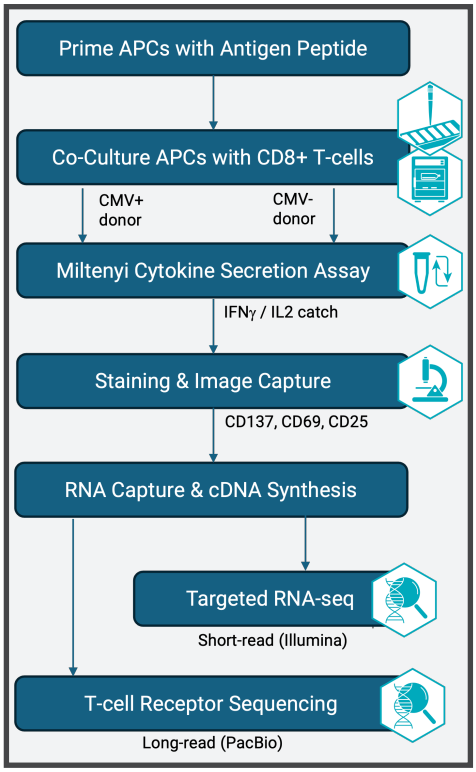

Figure 1. TAA-responsive TCR identification workflow using the Flexomics 8-site FLEX chip.

Results

The FLEX chip allows direct linkage of cell phenotyping fluorescence data (e.g. cell surface marker or secreted protein intensity) to transcriptome data at individual cell level. In this study, we applied this capability to identify TAA-activated T-cells and determine the TCR clonotypes responsible for the response. To further characterize the T-cell response to TAA, we measured expression of 17 genes associated with T-cell activation.

T-cells were co-cultured with antigen presenting cells directly on the FLEX chip to allow observation and tracking of individual cell-cell interactions. Activated T cells were identified based on immunofluorescent signals from CD69 and CD137 (markers of early and late activation, respecively) and IL2 and IFNG (proteins secreted in response to activation). Since only select TCR clonotypes are able to recognize and respond to each antigen, most T-cells are negative for activation markers and secreted proteins.

Generally, IL2 secretion more often corresponded with CD69 expression, and IFNG secretion with CD137, consistent with early and later stages of activation, respectively (Fig. 2).

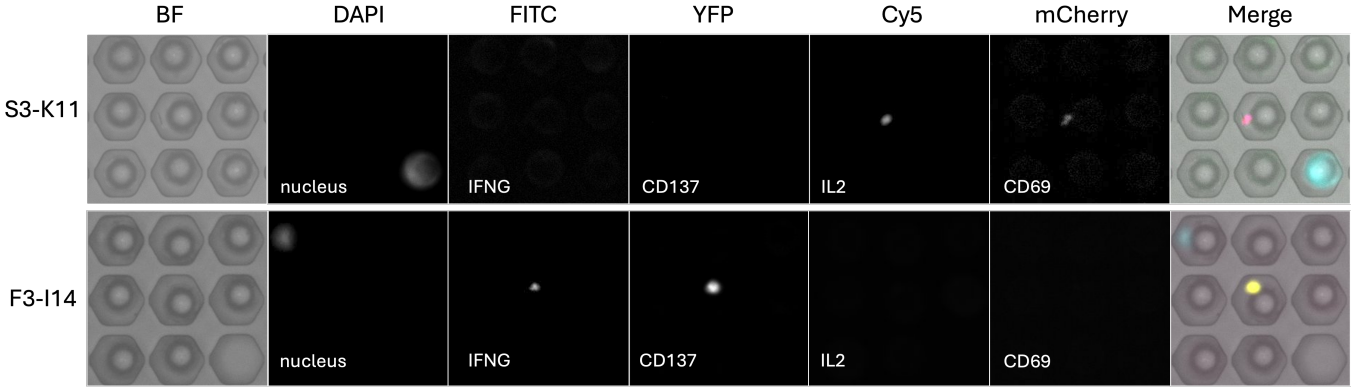

Figure 2. Example wells prioritized for downstream TCR-seq and gene expression anallysis. Wells were selected based on fluorescence signal from activation markers CD69 or CD137 along with secretion of cytokines IL2 or IFNG.

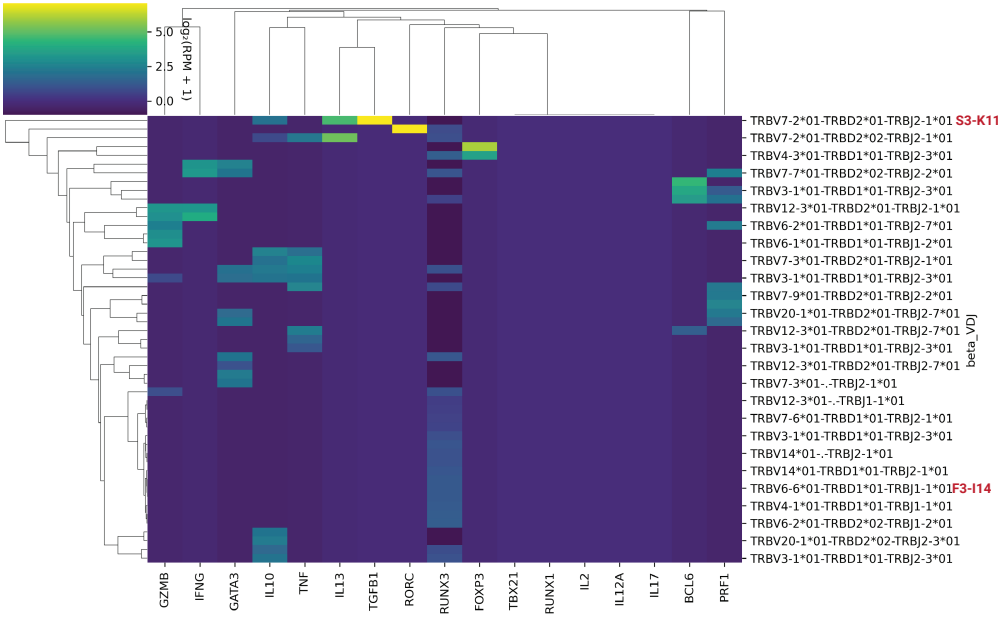

Figure 3. Hierarchical clustering of TCR clonotypes based on transcription signatures.

TRA and TRB VDJ regions were identified from single cell TCR-seq and linked to the cell's transcriptomic response signature using well barcode information. TCR clonotype, gene expression, and functional phenotypic data were linked together by mapping the molecular barcode to the well-of-origin.

Example linkage data is shown for wells S3-K11 and F3-I14 in Figs. 2 and 3.

Cells showing strong activation signatures in the imaging data were cross-referenced with their NGS profiles to retrieve paired TRA and TRB clonotypes. Each cell's clonotype was then mapped to its transcriptomic signature, allowing direct linkage between TCR identity, gene expression, and functional phenotype (Fig. 3). This integrated view revealed clonotype-specific activation patterns and transcriptional programs associated with antigen recognition.

To further characterize the immune response to neoantigen presentation, we compared the transcriptomic signatures of T-cells from two independent donors after exposure to MART-1 or NY-ESO-1 tumor antigens. Several key immune response genes, including IFNG (Fig. 4), showed distinct, donor-specific upregulation in a subpopulation of T-cells in response to MART-1, but not NY-ESO-1, stimulation. These findings highlight the utility of the platform as a critical tool for high-value biological applicaitons such as neoantigen-reactive TCR discovery.

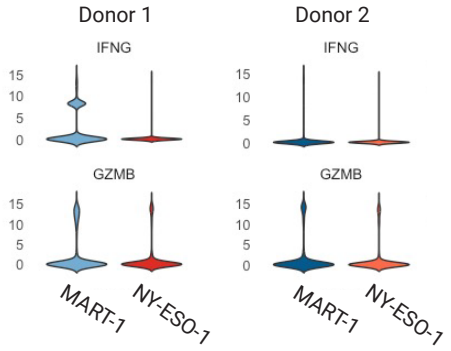

Figure 4. Distinct transcriptional activation patterns observed in T-cells across different donors. Transcripts from 17 genes were selectively sequenced and quantified at single-cell resolution. Expression levels were compared across donors to identify populations of TAA-responsive T-cells in each donor.

Discussion

Together, these results demonstrate how the Flexomics platform enables a comprehensive view of T-cell response to tumor-associated antigens. Unlike traditional technologies that capture only one aspect of the immune response (e.g. TCR sequencing or cytokine profiling) the FLEX chip enables researchers to combine fluorescent phenotyping, functional analysis, gene expression profiling, and TCR clonotype determination within a single workflow.

By linking these data types at single-cell resolution, Flexomics provides researchers with the power to pinpoint which TCR clonotypes drive specific functional responses. Because the multi-modal data types are gathered from the same cell of origin, the linked data go beyond inferrence to capture true biological relationships and mechanisms. This integrated approach not only accelerates TCR discovery and validation but also delivers deeper, more accurate insights into immune activation dynamics.

These capabilities demonstrate the FLEX chip's potential as a next-generation platform driving innovation in immunotherapy discovery and development.