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Single-Cell RNA Sequencing in Tumor-Infiltrating T Cells Research

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ABSTRACT

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Introduction

During the development of next-generation sequencing, RNA sequencing has become an indispensable deep-sequencing technology for measurement of levels of transcripts and isoforms [1]. But traditional RNA-seq from tissue and/or cells cannot easily resolve specific cell types. Now, exciting new applications are being explored, RNA-seq meet the greatly chance for its new technology that is single-cell RNA sequencing(scRNA-seq). Unlike traditional bulk RNA sequencing analyzing gene expression in bulk level, single-cell RNA sequencing can detect the global transcriptome of thousands of isolated cells on single cell level [2]. The applications of single-cell sequencing are widely, such as cancer research, developmental biology [3] and neurosciences [4]. In this review, we focus on the application of scRNA-seq on tumor-infiltrating T cells research. scRNA-seq can give us a map of tumor microenvironment by decomposition of complex tumor tissues into functionally distinct cell types and reveal cell types that are unknown in what were considered well-studied tumor diseases. scRNA-seq increase our understanding of the tumor-infiltrating T cells and potentially help us identify new immunotherapy targets.

The steps of scRNA-seq method can borrow from earlier bulk RNA-seq protocols. Single lymphocytes can isolate from peripheral blood, tumor, and adjacent normal tissues from patients. Most labs have access to flow-cytometry instrumentation and use microtiter plates containing lysis buffer [5]. For higher-throughput experiments can refer to droplet-microfluidic isolation, such as Drop-Seq [6] or InDrop [5]. Each single cell that tagged with Unique Molecular Identifiers (UMIs) is reverse transcribed in order to produce cDNA, and the cDNA is used as the input for RNA-seq library preparation. The transcriptional profiles of these individual cells, coupled with assembled T cell receptor (TCR), are sequenced [7]. Using unsupervised algorithms to cluster cell types and then assigned to cell types according to aggregated cluster-level expression profiles and delineate their developmental trajectory [8]. Most study may focus on analyze the cell-type composition and study dynamics of mixed cell population in various biological contexts. Tumor-infiltrating lymphocytes are highly heterogeneous, because of a variety of compositions of cell-type and gene expression profiles on tumor microenvironment. T cell patterns are distinct in both tumors and adjacent normal tissue. Yannick Simoni et al. reported that in tumor microenvironment CD8+ T cells are phenotypically heterogeneous within a tumor and across patients, and bystander CD8+ T cells are abundant and distinct in human tumor infiltrates [9].

The state of tumor-infiltrating T cells can be divided into cytotoxic, bystander cytotoxic, exhausted and dysfunctional state. The functional of different state T cells within tumors remain unknown. Analysis of paired single-cell RNA and T cell receptor sequencing data, Hanjie Li et al. reported a gradient of dysfunctional T cell are associated with tumor reactivity and are the major intratumoral proliferating immune cell compartment on melanoma [10]. Tirosh et al. revealed T cell exhaustion signature may connect to T cell activation and clonal expansion on melanoma turmors [11]. These findings provide evidence that dysfunctional T cells may be a driver of tumor reactive, equally to cytotoxic T cells. Tumor microenvironment have differential impact on T cell dysfunction across tumor

types. We need scRNA-seq to describe tumor infiltrates. The transcriptomes of T cell subset help to identify previously unknown marker for prognosis. For example, Xinyi Guo et al. reported a ratio of pre-exhausted to exhausted T cells are relative to better prognosis of lung adenocarcinoma [12]. Peter Savas et al. demonstrated that tumor-infiltrating lymphocytes in breast cancer contains several CD8+ T cells with features of tissue-resident memory expressing high levels of immune checkpoint molecules and effector proteins, which are associated with good prognosis in breast cancer [13]. Chuanhong zheng et al. reported primary CD8+ T cells over-expressing LAYN results in inhibition of interferon-gama production, which suggesting LAYN is linked to the suppressive function of tumor Treg and exhausted CD8 T cells [14]. Overall, these findings provided an exciting vision of how we use scRNA-seq to discover tumor immune markers and understand their roles in regulating immune response and tissue-specific functions.

The development and migration of T cells within tumors remain unknown. scRNA-seq has also been instrumental in resolving details of the trajectory and regulation of T cells. T Cell Receptor (TCR) clonotypes determine the developmental trajectories of T cells and reveal phenotypic diversity. Tumor antigen specific TCR clusters also are key components in anti-tumor immune response. scRNA-seq of TCR gene repertoires are useful for to reveal the intrinsic heterogeneity among antigen-specific T cells and their function in tumor response [7]. David Redmond et al. reported a method to identification and assembly of full-length rearranged V(D)J T cell receptor sequences from scRNA-seq data [15]. Combined with TCR analysis, EIham Azizi et al. yielded an immune map of breast cancer that points to continuous T cell activation and differentiation trajectories [16]. In short, identifying clonal TCRs at single-cell levels allows us to discover their developmental trajectory in various T cell clusters, and deduce their activation status in tumor microenvironment.

In conclusion, scRNA-seq measures the expression levels of genes in cells in a comprehensive, sensitive and accurate way. scRNA-seq is aiding in the discovery of the heterogeneity of tumor-infiltrating lymphocytes, unreported subpopulations and states, potential biomarkers, tumor antigen-specific TCR clusters and their relationship to physiology and disease.

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