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Single-cell insights into transcriptomic diversity in immunity

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Abstract

Our body is composed of a diverse set of cells that together perform numerous functions in a highly coordinated manner, allowing a timely response to external cues and to inter-cellular signals. Emerging single-cell technologies enable investigation of these intricate processes at an unprecedented level.
Here, we discuss recent work on cellular differentiation and heterogeneity, and describe novel experimental and computational tools that enable this research. As an example of cellular differentiation, we focus on T cell development in response to diverse infections and in immune pathologies. We then describe how single-cell studies have contributed to our understanding of transcriptional variability in innate immune response, and how this variability might be important in achieving a balanced immune response.
Future single-cell studies will likely include spatial analyses and lineage tracing strategies, holding great potential to further our understanding of cell behaviour in steady-state and pathological conditions.

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Introduction

The human body contains between 3–4 × 10^{13} cells [1,2]. While all these cells share the same genetic information, they differ greatly in their function, physiology and morphology. The remarkable diversity of cells types is the product of development and differentiation of progenitor cells into numerous cell types, which carry out their specialized functions through expression of an array of cellular modules [3–6]. Understanding the regulatory processes that give rise to different cell types, are key questions in a variety of research fields – from embryonic development and immunity, to cancer evolution, and cellular ageing.

Recent advances in omics technologies, especially those that enable investigation at single-cell resolution, have given detailed insights into the genomic, epigenomic and transcriptomic nature of many important developmental processes. For example, a detailed spatial and temporal profiling of transcriptomes of nematode embryos has elucidated the evolutionary history of the three germ layers [7].

Other single-cell studies have given important insights into cellular heterogeneity – subtle differences between cells that cannot be detected using techniques that probe cell populations as a whole, such as bulk RNA sequencing. For example, recent analysis of individual cells from glioblastoma tumours, has revealed a previously unappreciated diversity of transcriptional programmes in brain tumours that is thought to be important for tumour development and treatment [8].

The present review aims at covering some of the latest and most significant advances of single-cell omics and how they have contributed to our understanding of cell differentiation and heterogeneity. We will focus on transcriptomics (single-cell RNA sequencing, or scRNA-seq), arguably the most mature and widely used approach in cell differentiation studies. Single-cell genomic and epigenomic approaches have been thoroughly reviewed elsewhere [9,10]. We will start with a survey of the current state of the art techniques and how they can be used to study cellular differentiation. We will then focus on the immune system and demonstrate how single-cell technology has been utilised to explore important aspects of innate and adaptive immunity.

An overview of scRNA-seq methods to investigate cell differentiation

Since the first publication on scRNA-seq [11], single-cell transcriptomic approaches have been undergoing

an exponential maturation period, both on the experimental and analytical side.

The throughput of scRNA-seq methods has dramatically increased from tens of cells up to tens of thousands, as capture of single-cells shifted from manually picking cells to automated microfluidics systems, single-cell sorting into 96 and 384-well plates, and more recently, to droplet-based microfluidics and micro-well methods [12].

In parallel, different methods of reverse transcription and amplification were also introduced, granting access to either full-length transcripts, 5′ or 3′ fragments [13]. In addition, an increasing number of protocols allow the integration of scRNA-seq with single-cell genomics [14,15], epigenomics [16] and CRISPR screenings [17–19]. Systematic comparisons of scRNA-seq protocols have now been published [20,21], suggesting that single-cell mRNA protocols are able to correctly assess the relative abundance of mRNA molecules present across cells. However, notable differences in lower detection limits were observed between protocols.

Computationally, scRNA-seq data has presented new challenges to the genomics community [22,23]. These challenges include, among others: (1) Technical differences between cells stemming from library quality and batch effects: low quality of libraries can be detected by a preliminary quality-control stage where quality of each library is assessed based on various criteria (such as total number of mapped reads or number of detected genes) [24]. Batch effects can be addressed by batch correction methods (these include traditional and newly developed methods to remove unwanted factors [25]). (2) Variable or inefficient capture of mRNA (or "dropouts") due to low amount of starting material, which can affect the ability to detect lowly-expressed genes. This can be addressed by methods that impute the data and restore its likely structure [26]. (3) Difficulties in discerning between technical noise and genuine biological heterogeneity, which will be discussed in detail in the last section of this review. In Table 1 we summarize several packages and their applications, which can help users analyse their data in a simple and efficient manner.

In general terms, gene expression data of a differentiation process with single-cell resolution has the potential to provide answers to five main objectives [12]: (1) Are there distinct clusters of cells within the general cell population (corresponding to either a variety of differentiation stages or to various, fully-differentiated, cell-types)? (2) Which genes characterize each cell population? (3) Which gene modules regulate differentiation? (4) How do cells progress through the differentiation process? (5) How is the differentiation process spatially reflected in the relevant tissue?

These questions can be addressed by utilising different techniques (see also Figure 1):

(1) Finding cell clusters can be achieved by applying dimensionality reduction techniques or clustering algorithms (reviewed in Refs. [25,27]).
(2) The biological roles of the identified clusters can then be evaluated based on genes that differentially expressed (DE) between clusters, using available methods to infer differential expression (reviewed in Refs. [25,27]).
(3) Measuring gene co-occurrence or gene–gene correlations across single-cells can be used to build co-expression networks that can yield insights into important gene modules and gene regulation involved in differentiation. With the recent integration of CRISPR screenings with scRNA-seq [17–19], there is now the potential to systematically perturb these networks and assess true regulatory relationships between their elements.
(4) Although single-cell data of a differentiation path is a static snapshot, differentiation is a dynamic and continuous process, encompassing a continuous range of cell-states along this process. Multiple algorithms have been recently developed to interpret such cell-to-cell differences and reconstruct trajectories of cell differentiation. Ordering cells along

Table 1

Representative examples of integrated pipelines to analyse scRNA-seq.

Name	Type	Applications	Ref. Numbers
SCDE	package	Differential expression; overexpression analysis	[78]
scater	package	Mapping; quality control; easy integration with popular tools	[92]
Seurat	package	Quality control; clustering; determination of highly variable genes; differential expression; spatial reconstruction	[34]
SINCERA	package	Clustering; differential expression; inference of regulatory networks	[93]
SimpleSingleCell	workflow	Mapping; quality control; clustering; determination of highly variable genes; differential expression	[94]

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