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Review

Exploring viral infection using single-cell sequencing

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ABSTRACT

Single-cell sequencing (SCS) has emerged as a valuable tool to study cellular heterogeneity in diverse fields, including virology. By studying the viral and cellular genome and/or transcriptome, the dynamics of viral infection can be investigated at single cell level. Most studies have explored the impact of cell-to-cell variation on the viral life cycle from the point of view of the virus, by analyzing viral sequences, and from the point of view of the cell, mainly by analyzing the cellular host transcriptome. In this review, we will focus on recent studies that use single-cell sequencing to explore viral diversity and cell variability in response to viral replication.

Contents

1. Introduction

Cells are the basic biological units of living organisms. They are structurally grouped by function into tissues and organs, and they form the most basic component of these structures (at the transcriptome and proteome levels) and thus their identity. Despite sharing the same DNA content and being exposed to the apparent same conditions, cells display some level of functional hetero-

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geneity. This heterogeneity can be explained by extrinsic features, such as cell identity (cell type/subpopulation/lineage) and cell state/process (cell cycle, circadian rhythm), or by the intrinsic stochastic nature of gene expression (Battich et al., 2015; Satija and Shalek, 2014; Stoeger et al., 2016). These cell-to-cell variations can impact cell function, cell communication and proliferation or cell fate, and behavior of the cell population at large (Altschuler and Wu, 2010; Huang, 2009; Satija and Shalek, 2014). Moreover, rare cells or small fractions of cells can play an important biological role in specific disorders and environments (*e.g.* cancer cells), but their contribution can be masked by the larger cell population.

Single-cell technology evolved quickly in the last years, from a simple FACS analysis assessing the expression of a specific protein to the most sophisticated techniques that allow the analysis of single-cell genome, transcriptome and proteome. The breakthrough of single-cell analysis, and specifically single-cell–omics, was an important milestone in some areas such as cancer, stem cells, epigenetics or immunology.

In the immunology field, single-cell technologies were critical for the discovery of new gene networks and novel cell subpopulations, elucidating relationships between cell clonality and their functional phenotypes, and in the discovery of new cell states or cell types within the immune system (Vieira Braga et al., 2016). For example, Stubbington et al., using a new computational method comparing the paired T cell receptor (TCR) sequences from lymphocyte single-cell RNA sequence data, were able to directly correlate T cell clonal origin with the functional phenotype in a mouse Salmonella infection model (Stubbington et al., 2016). Buetner and colleagues developed a single-cell latent variable model (scLVM) allowing identification of otherwise undetectable subpopulations of cells that correspond to different stages during the differentiation of naive T cells into T helper type 2 (Th2) cells (Buettner et al., 2015). In another study, analysis of single-cell messenger RNA sequencing revealed rare intestinal cell types (Grun et al., 2015). Also, single-cell analysis of CD4$^+$ T-cell differentiation characterized three major different cell states during Th2 polarization, from the intermediate activated cell state to the mature cytokine-secreting effector state (Proserpio et al., 2016).

In the cancer field, single-cell studies have allowed significant progress in understanding carcinogenesis, progression, metastases and drug resistance (Qian et al., 2016). Single-cell RNA-seq led for example to the identification of distinct tumor subpopulations in lung adenocarcinoma (Min et al., 2015), showed that there was subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells (Kim et al., 2015b), and also led to the identification of distinct gene expression patterns, including candidate biomarkers for melanoma circulating tumor cells (Ramskold et al., 2012)

Single-cell genome, epigenome and transcriptome sequencing led as well to considerable advancement in the stem-cell field, improving the knowledge in both pluripotent and tissue-specific stem cells (Wen and Tang, 2016). Single-cell RNA-seq provided the opportunity to decipher gene expression dynamics during mammalian pre-implantation development, by analyzing transcriptome profiles from both human and mouse cells undergoing pre-implantation (Deng et al., 2014; Yan et al., 2013). Based on single-cell RNA-seq studies, it was also possible to identify new stem cells types (Treutlein et al., 2014) and dissect cell heterogeneity among a stem cell population (reviewed in Wen and Tang, 2016).

Single-cell technology can also be a great asset for the virology field. Viruses are dependent on the host cell to replicate and therefore, heterogeneity in the host cell population will be reflected in viral infection outcome (Fig. 1). In many cases, with many cell types, achieving 100% of infected cells is difficult. This result can be due (i) to the heterogeneity of virus particles, *i.e.* defective viruses or

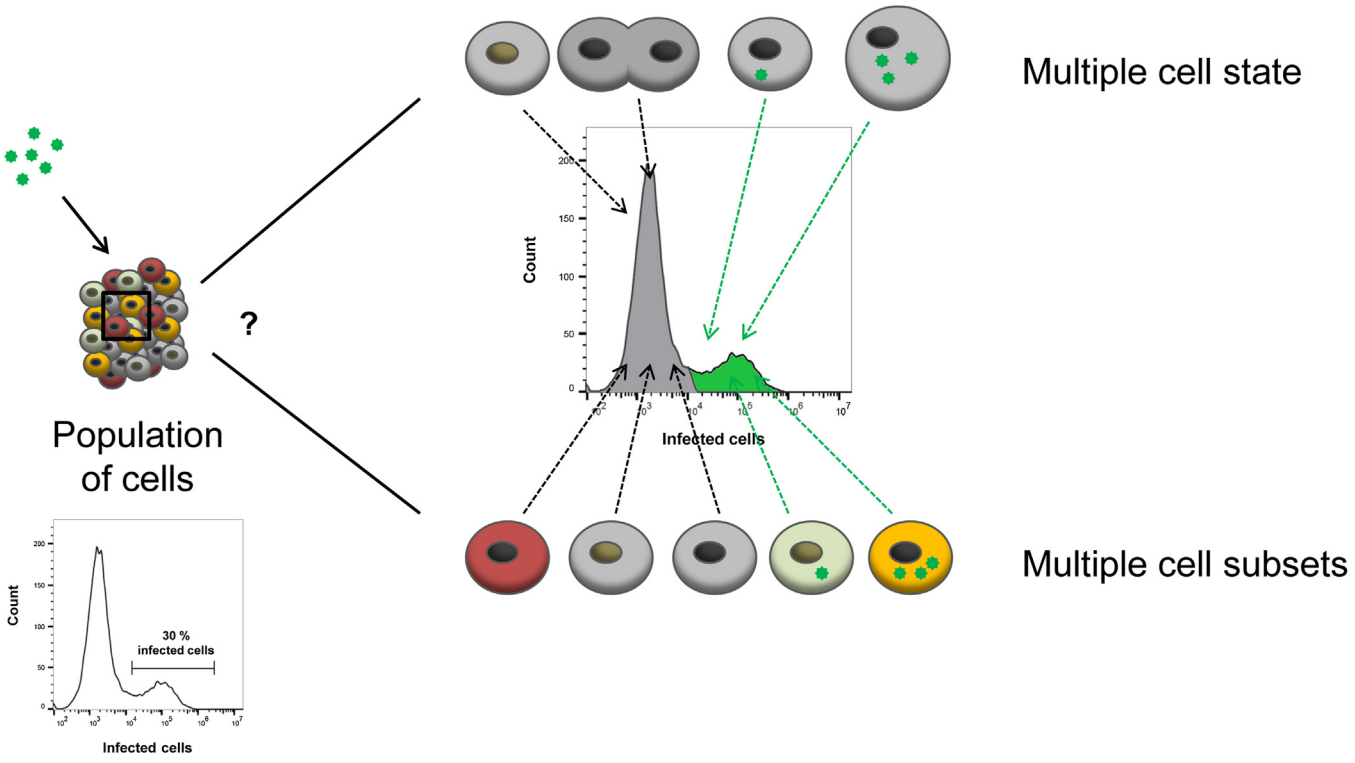

Fig. 1. Impact of cellular heterogeneity on viral infection. The percentage of infected cells in a population can reflect the cellular heterogeneity in response to viral exposure. The source of this heterogeneity can be due to different cellular states (activation stage or cell cycle) or different cellular subsets of the same cell type (*e.g.* CD4$^+$ T cells subsets). Indeed, specific cell states or cell subsets may be permissive to viral infection while other cell states and other cell subsets may be resistant to viral infection. The proportion of infected cells at the population mixed level would mirror the proportion of permissive cells within the total population.