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High-dimension single-cell analysis applied to cancer

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ABSTRACT

High-dimension single-cell technology is transforming our ability to study and understand cancer. Numerous studies and reviews have reported advances in technology development. The biological insights gleaned from single-cell technology about cancer biology are less reviewed. Here we focus on research studies that illustrate novel aspects of cancer biology that bulk analysis could not achieve, and discuss the fresh insights gained from the application of single-cell technology across basic and clinical cancer studies.

1. Introduction

The concept of analyzing heterogeneous cell populations at single-cell resolution has long held great interest for investigators across diverse fields. Methods that characterize single cells, such as flow cytometry and immunohistochemistry, have been workhorses of biological research for decades. However, what has changed over the last few years is the dramatic increase in the number of diverse approaches that address high-dimensional analysis of single cells. These approaches include single-cell transcriptome and genome sequencing, as well as high throughput qPCR and mass cytometry for multiplex detection of proteins. High dimension also refers to the number of cells being analyzed. Depending on the technique, this can be hundreds to millions of single cells. Thus, high-dimension single-cell analysis involves not only high number of targets, but also a high number of cells.

It is thought that cancer starts with changes in a single cell. Shaped by selective forces exerted by the microenvironment, the immune system, and exposure to a wide variety of environmental insults, additional changes accumulate until a tumor is formed that escapes immune surveillance and grows progressively. Indeed, each malignancy is its own experiment in evolution, leading to heterogeneity among cancer cells within one patient and heterogeneity amongst patients with the same disease. One of the hallmarks of high-dimension single-cell analysis is its unparalleled ability to characterize cell-to-cell heterogeneity. Thus, using high-dimension single-cell analysis to study cancer is a natural fit, explaining why this approach is increasingly adopted by cancer researchers. As summarized in Fig. 1 and reviewed below, single-cell technology has transformed our understanding of tumor heterogeneity, including intrinsic and extrinsic factors that could co-drive disease initiation, progression, relapse, and metastasis. With the advancement of technology, it is now feasible to collect genome-wide profiles of DNA, RNA, histone modifications, chromatin accessibility, DNA methylation, nuclear lamina interactions and chromosomal contacts, as well as the protein signatures of single cells. This has prompted many reviews and perspectives on applying single-cell technology to cancer (extensively reviewed in Navin, 2015b [see Fig. 1 for timeline] and others: Van Loo and Voet, 2014; Navin, 2015a; Sun et al., 2015; Saadatpour et al., 2015; Wills and Mead, 2015; Mato Prado et al., 2016; Schmidt and Efferth, 2016; Tellez-Gabriel et al., 2016; Ye et al., 2016; Zhang et al., 2016b; Zhu et al., 2017; Muller and Diaz, 2017). Rather than focusing on technology, this review addresses how single-cell analysis improves our understanding of tumor heterogeneity at multiple layers (genetic/epigenetic, transcriptomic, proteomic, multiomic).

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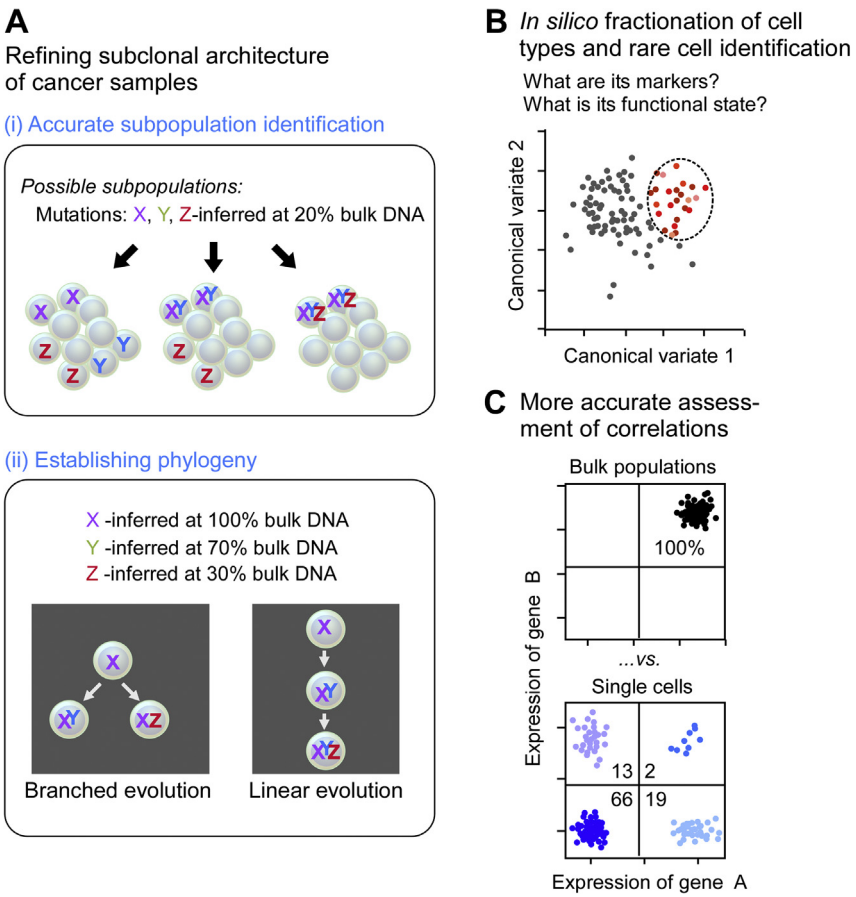

Fig. 1. Key uses of high-dimension single-cell analysis in studying cancer.

2. Overview of single-cell technology

Different aspects of single-cell technology will be briefly summarized. Each section will begin with a list of recent reviews that provide more in-depth descriptions of the topic being discussed. These reviews should be consulted for citations to the primary references. Fig. 2 presents the overall workflow for single-cell analysis. After the isolation of single cells, high-dimension technology is applied either to discover heterogeneity (typically in a small set of patient samples) or to validate aspects of heterogeneity (typically in greater numbers of patient samples).

2.1. Single cell isolation

Most single-cell analysis requires isolation of single cells, previously reviewed by Bheda and Schneider (2014) and Hu et al. (2016a). Table 1 in Wang and Navin (2015) effectively summarizes the methods used to isolate both abundant and rare cells. For abundant cells, these methods include serial dilution, mouth pipetting, flow sorting, robotic micromanipulation, and microfluidic platforms. The use of microfluidics is particularly attractive because it reduces the cost and labor required to process hundreds to thousands of single cells. As summarized by Prakadan et al. (2017) the most commonly used microfluidic methods include: (1) valve-based devices, which provide precise control of cells and reagents, and are best suited for implementing complex, integrated workflows; (2) droplets, which provide dramatic advantages in scale and speed, enabling very high throughput (thousands to tens of thousands of cells); and (3) nanowells (devices with nanoliter-sized wells), which provide operational simplicity and lower the barriers to adoption and the development of new protocols. One difficulty often encountered with microfluidics is the disconnect between the availability of microfluidic equipment and the timing/location of sample collection. Flow sorting into conventional microwells (typically 96- or 384-well plates) enables archiving of single-cell lysates, which provides flexibility in the timing and location of sample collection. For example, single-cell lysates from multiple locations can be shipped on dry ice for processing at a central site. In addition, flow sorting enables precise capture of rare populations that are often depleted or lost using other methods. Thus, as shown in Fig. 2A, flow sorting and microfluidics are now the predominant methods used for isolating single cells when the cells being analyzed are relatively abundant.

2.2. Genome analysis

Recent reviews of single-cell DNA sequencing (scDNA-seq) include Macaulay and Voet (2014), Wang and Navin (2015), Sun et al. (2015), Szulwach and Livak (2016), and Gawad et al. (2016). The common first step in analyzing the genome of a single cell is whole genome amplification (WGA). Three main types of WGA have been developed (Fig. 2 in Gawad et al., 2016): (1) isothermal multiple displacement amplification (MDA); (2) PCR methods such as degenerate oligonucleotide primed PCR (DOP-PCR); and (3) hybrid methods such as PicoPLEX and multiple annealing and looping based amplification cycles (MALBAC) that have a short isothermal amplification step followed by PCR amplification. MDA has greater genomic coverage and a lower error rate, but the other

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