Biotechnology Advances 35 (2017) 443–449

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Application of single-cell technology in cancer research

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ARTICLE INFO

Keywords:
Single-cell technology
Rare tumor cells
Intratumor heterogeneity
Tumor epigenetics
Individualized treatment

ABSTRACT

In this review, we have outlined the application of single-cell technology in cancer research. Single-cell technology has made encouraging progress in recent years and now provides the means to detect rare cancer cells such as circulating tumor cells and cancer stem cells. We reveal how this technology has advanced the analysis of intratumor heterogeneity and tumor epigenetics, and guided individualized treatment strategies. The future prospects now are to bring single-cell technology into the clinical arena. We believe that the clinical application of single-cell technology will be beneficial in cancer diagnostics and treatment, and ultimately improve survival in cancer patients.

1. Introduction

Incidences of cancer throughout the world are rapidly increasing, owing to the growth and aging of the population, environmental pollution, unhealthy lifestyles, and so on. Based on data from GLOBOCAN, new worldwide cancer cases and cancer deaths reached nearly 12.7 million and 7.6 million, respectively, in 2008 (Jemal et al., 2011), which increased to nearly 14.1 million and 8.2 million, respectively, in 2012 (Torre et al., 2015). Based on data from the National Central Cancer Registry (NCCR), new cancer cases and cancer deaths were estimated at nearly 3.6 million and 2.2 million, respectively, in 2012 in China (Chen et al., 2016).

Poor outcomes in cancer can be attributed to a lack of early diagnostic methods, high incidences of tumor relapse for patients with locoregional advanced diseases and ineffective treatments for patients with distant metastasis (Govindan et al., 2008; Pan et al., 2015; Subramanian et al., 2015; Wiegand et al., 2015). While, many researchers have been striving to advance laboratory breakthroughs to clinical settings, due to the complex biological characteristics in cancer cells and ubiquitous tumor heterogeneity, general progress has been slow (Braun and Pantel, 1999; Navin et al., 2010; Torres et al., 2007).

It is apparent that distinguishing tumor heterogeneity into distinct morphological and phenotypic profiles remains a challenging yet imperative goal in cancer diagnosis and treatment. Importantly, the emergence of single-cell technology in recent years has provided a powerful tool in resolving this difficulty and progressing cancer research studies. Single-cell technology can be divided into two main areas: single-cell separation and single-cell analysis. Single-cell separation is the basis of single-cell analysis, which includes flow cytometry, optical tweezers, laser capture micro-dissection (LCM) and microfluidics (Arai et al., 2005; Heenan et al., 1997; Kehr, 2003). Single-cell analysis includes genomic, transcriptomic and proteomic profiles of cancer cells. Among them, single-cell genomic analysis has shown the most encouraging progress (Kolodziejczyk et al., 2015; Winterhoff et al., 2017; Zhang et al., 2016).

Single-cell technology provides the means to detect rare cancer cells such as circulating tumor cells (CTCs) and cancer stem cells (CSCs), analyze intratumor heterogeneity (ITH), reveal the mechanism of tumor metastasis and investigate epigenetic alterations, and ultimately guide individualized treatment strategies (Fig. 1) (Chen et al., 2005; Navin and Hicks, 2011; Pezo et al., 2008; Vermeulen et al., 2008). In this review, we have outlined the application of single-cell technology in cancer research, offering our personal view regarding the future prospects of single-cell technology in this field. This review may improve understanding of the biological characteristics of malignant tumors, which is important in preventing these serious diseases and diagnosing and treating patients with them.

Abbreviations: CDXs, CTC-derived explants; CNV, copy number variation; CTCs, circulating tumor cells; CSCs, cancer stem cells; CtDNA, circulating tumor DNA; DCIS, ductal carcinoma in situ; ITH, intratumor heterogeneity; LCM, laser capture micro-dissection; NCCR, National Central Cancer Registry; P-gp, P-glycoprotein; SCATT, single-cell analysis of targeted transcriptome; SCS, single-cell sequencing; SCLC, small cell lung cancer; ScWestern, single-cell western blotting; STAR-FISH, specific-to-allele PCR-FISH

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http://dx.doi.org/10.1016/j.biotechadv.2017.04.001
Received 19 October 2016; Received in revised form 29 March 2017; Accepted 1 April 2017
Available online 05 April 2017

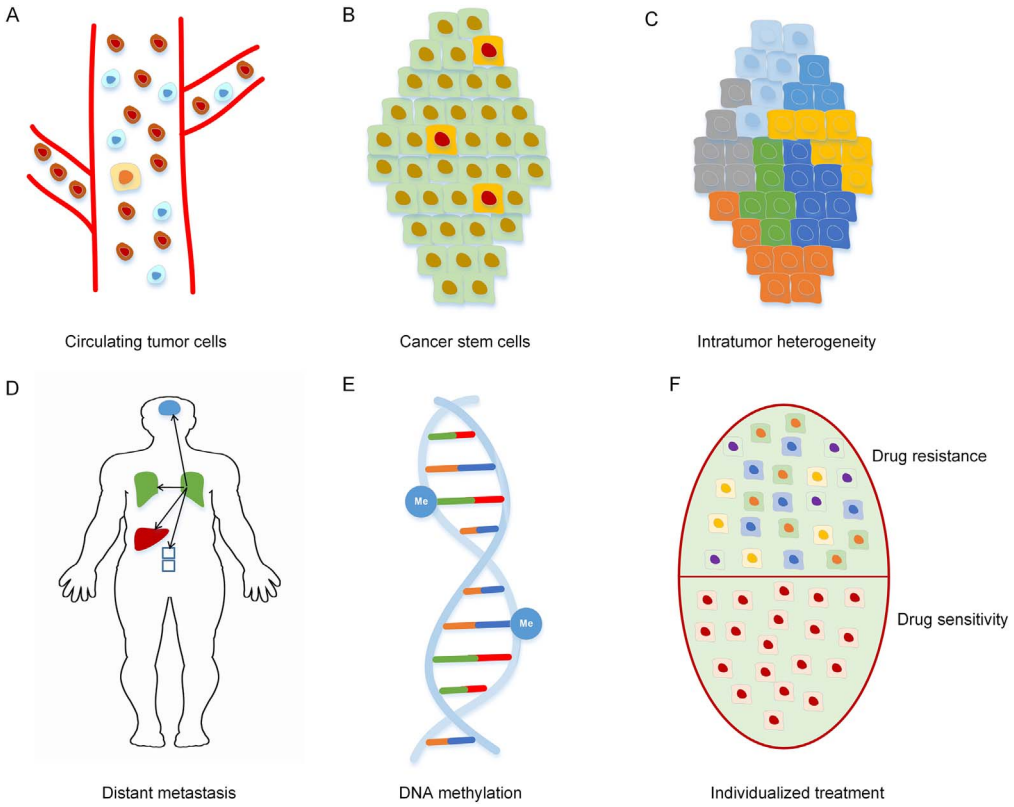

Fig. 1. The application of single-cell technology in cancer research. (A) Detecting circulating tumor cells; (B) studying cancer stem cells; (C) analyzing intratumor heterogeneity; (D) revealing the mechanism of tumor metastasis; (E) investigating epigenetic alterations; (F) guiding individualized treatment strategies.

2. Detecting rare tumor cells

2.1. Circulating tumor cells

CTCs are tumor cells released into the peripheral blood from a primary lesion or metastatic lesion by spontaneous behavior, diagnosis operation or treatment operation. The existence of CTCs means that tumor cells are not limited to the primary lesion, and instead can develop into distant metastases. Moreover, CTCs have been shown to appear in the bloodstream unexpectedly early, before metastatic lesions could be detected by histologic analysis (Rhim et al., 2012). Numerous studies have confirmed that CTCs are important in predicting disease progression and survival (Chaffer and Weinberg, 2011; Cristofanilli et al., 2004), monitoring the complex tumor genomes (Heitzer et al., 2013; Lohr et al., 2014), diagnosing tumor recurrence and metastasis (Ni et al., 2013) and guiding therapeutic management (Alix-Panabieres and Pantel, 2013; Hodgkinson et al., 2014). In this vein, CTCs lend themselves well as noninvasive biomarkers that can be easily accepted by patients.

Circulating tumor DNA (ctDNA) encodes tumor specific sequences that can be used as another form of liquid biopsy, which can be non-invasively repeated during treatment and follow-up. Several studies have shown that ctDNA can reveal genotype information of the tumor, indicating that ctDNA analysis could effectively replace tumor biopsy (Schwarzenbach et al., 2011; Thierry et al., 2014). Moreover, advances in sequencing technology has meant that ctDNA can now timely monitor tumor progression and therapeutic responses of various solid cancers (Alix-Panabieres and Pantel, 2016; Garcia-Murillas et al., 2015; Jiang et al., 2015; Tie et al., 2015). For example, researchers have shown that in metastatic breast cancer, ctDNA is more sensitive to reflect changes in tumor characteristics than CTCs and other serum markers (Dawson et al., 2013).

In 2001, the presence of CTC clusters was first detected in human colorectal cancer patients (Molnar et al., 2001). It was further verified that the abundance of CTC clusters predicted adverse outcomes in breast cancer patients (Aceto et al., 2014). Therefore, tumor-derived endothelial cell clusters provide important information of tumor vasculature at the time of diagnosis, during treatment and in follow-up monitoring appointments (Cima et al., 2016). In addition, CTC clusters have been shown to form metastatic lesions more easily than single tumor cells (Jansson et al., 2016; Mu et al., 2015) (Table 1).

2.2. Other rare tumor cells

Besides CTCs, single-cell methods can also be used to isolate and analyze other rare tumor cells such as CSCs and tumor cells in the urine or bone marrow, providing new ways for cancer diagnosis and treatment (Felthaus et al., 2011; Grun et al., 2015; Nickens et al., 2015; Wu and Tzanakakis, 2013; Yang et al., 2017). In contrast to routine histopathology and serial step section analysis, single-cell methods have an increased detection rate of early tumor dissemination (Schilling et al., 2010). For patients diagnosed with ductal carcinoma in situ (DCIS) and treated by lumpectomy alone, the five-year recurrence rate was 5%–10% (Fisher et al., 1993; Kerlikowske et al., 2003). As single-cell technology advances, CSCs and special biomarkers could be used to distinguish patients with DCIS at risk of subsequent invasive cancer. As a result, patients at lower risk of subsequent cancers could avoid excessive treatment (Kerlikowske et al., 2010).

3. ITH

3.1. ITH in solid tumors

Solid tumors are composed of malignant tumor cells and mesenchymal cells, which include endothelial cells, inflammatory corpuscle and fibroblasts. Furthermore, tumor cells often consist of multiple clonal subpopulations, even in a single lesion, which adds to the complexity of analyzing tumor samples (Heenan et al., 1997; Navin et al., 2010;