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Research review paper

Circulating tumor cell isolation, culture, and downstream molecular analysis

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ABSTRACT

Circulating tumor cells (CTCs) are a major contributor of cancer metastases and hold a promising prognostic significance in cancer detection. Performing functional and molecular characterization of CTCs provides an in-depth knowledge about this lethal disease. Researchers are making efforts to design devices and develop assays for enumeration of CTCs with a high capture and detection efficiency from whole blood of cancer patients. The existing and on-going research on CTC isolation methods has revealed cell characteristics which are helpful in cancer monitoring and designing of targeted cancer treatments. In this review paper, a brief summary of existing CTC isolation methods is presented. We also discuss methods of detaching CTC from functionalized surfaces (functional assays/devices) and their further use for ex-vivo culturing that aid in studies regarding molecular properties that encourage metastatic seeding. In the clinical applications section, we discuss a number of cases that CTCs can play a key role for monitoring metastases, drug treatment response, and heterogeneity profiling regarding biomarkers and gene expression studies that bring treatment design further towards personalized medicine.

1. Introduction

According to the Centers for Disease and Control Prevention (CDC), cancer records the second highest reason of death worldwide, with > 90% of deaths being caused by cancer cell metastasis (Hong and Zu, 2013; Wicha and Hayes, 2011). This is largely due to the fact that malignant tumors have the ability to shed tumor cells that invade surrounding tissue and enter the lymphatic and circulatory systems (Asghar et al., 2012b). These cells, known as circulating tumor cells (CTCs), ultimately establish new metastasis at other tissue and organ sites throughout the body (Chaffer and Weinberg, 2011; Harouaka et al., 2014; Maheswaran and Haber, 2010; Wan et al., 2011).

CTCs were first discovered more than hundred years ago by Thomas Asworth (Ashworth, 1869). Since then, many studies have focused on discovering efficient CTC detection and isolation techniques, with the prospects of using CTCs as a 'liquid biopsy' for peripheral blood analyses and an early biomarker for response to systemic therapies (Alix-Panabières and Pantel, 2013; Diaz and Bardelli, 2014; Lianidou, 2014a,b; Wan et al., 2012; Wang et al., 2013). However, a number of challenges are associated with CTC isolation, detection, and downstream analysis. CTCs are sparse, approximately 1–100 cells can be found per milliliter of blood, along with 10^6–10^7 red blood cells (Asghar et al., 2013; Hafeez et al., 2012; Ilyas et al., 2014a; Ilyas et al., 2014b; Miller et al., 2009). Increasing blood sample volumes is a possible resolution that provides more accurate measurements, but comes with its own time constraints and patient care challenges. CTC heterogeneity is another major obstacle, as various groups of CTCs have significant variations in surface expression of biomarkers (Attard and de Bono, 2011; Ignatiadis and Dawson, 2014). Currently, CellSearch, the exclusively US-FDA (Food and Drug Administration) cleared device for CTC detection is a prognostic indicator for breast, prostate and colorectal cancer (Krebs et al., 2011; Sieuwerts et al., 2009). A great clinical need still exists for low-cost, non-invasive, and efficient CTC detection and isolation devices. Herein, we review the most promising CTC isolation methods and apply more focus on future directions of CTC technology (Fig. 1). The different isolation methods are categorized on the basis of specific CTC characteristics such as physical properties (size, elasticity, surface charge) (Asghar et al., 2012b; Gascoyne et al.,

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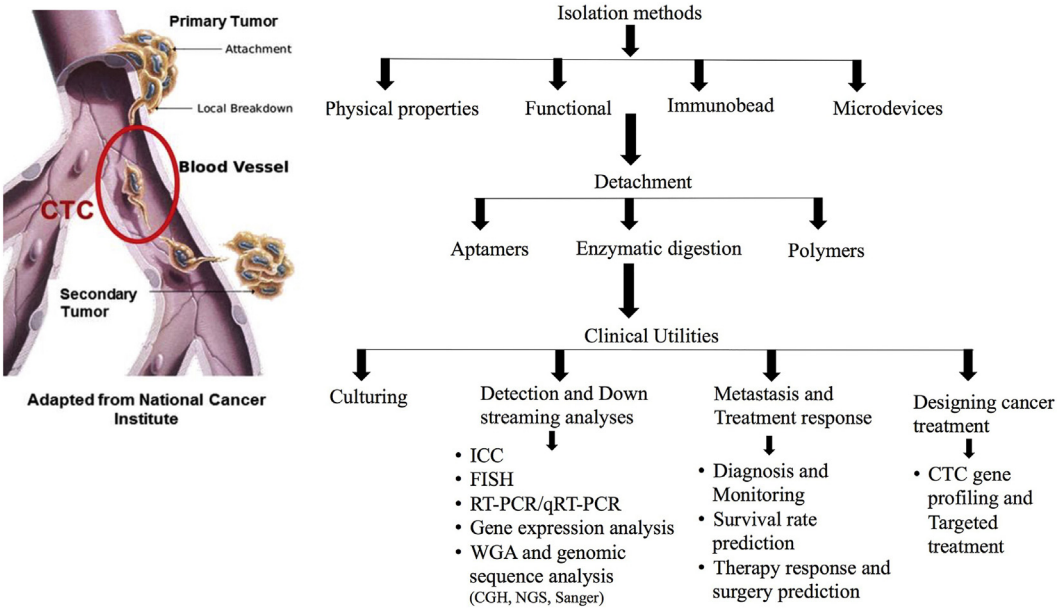

Fig. 1. Outline of existing isolation, detection and characterization techniques and promising future clinical utilities.

2009; Moon et al., 2011; Müller et al., 2005; Vona et al., 2000; Zheng et al., 2011), biological characteristics such as cellular function (Alix-Panabières, 2012; Lu et al., 2010) and the expression of tumor-specific surface proteins (Allard et al., 2004; Helzer et al., 2009; Lu et al., 2013; McKeown and Sarosi, 2013; Riethdorf et al., 2007; Stott et al., 2010; Talasaz et al., 2009). After isolating CTCs from patient samples, releasing CTCs from the capturing substrate presents with challenges. The successful detachment of CTCs is an important step for establishing ex-vivo CTC cultures and obtaining morphological information. In this paper, we review downstream processing steps, describing CTC release from substrate with the use of various enzymatic actions, aptamers, and polymers. Protocols and success rates for culturing CTCs from cancer patients demonstrating heterogeneous CTC morphological properties are also discussed, and a description of ex-vivo CTC culturing under various cell culture conditions for disease model development is provided. Moreover, the clinical aspects of CTCs are described, and examples of how CTCs can participate in monitoring metastasis and drug therapy responses are discussed.

2. CTC isolation methods

Since the discovery of CTCs, several isolation techniques have been developed. However, these techniques are often limited by the presence of extremely low number of CTCs in patient blood (1–100 cells/mL), as well as their fragile and heterogeneous nature (Alix-Panabières and Pantel, 2013; Zheng et al., 2013). CTC fragility becomes a concern when the cells need to be detached from the various chips and membranes that are used to isolate them. We discuss detachment after introducing the major CTC isolation methods developed thus far. Most of the existing technologies consist of a two-step process of cell enrichment and subsequent detection. Cell enrichment involves capturing CTCs based on their physical properties, including size, elasticity, density, and charge (Gascoyne et al., 2009; Moon et al., 2011; Müller et al., 2005; Vona et al., 2000; Zheng et al., 2011), and various biological characteristics, such as cellular functions (Alix-Panabières, 2012) and tumor-specific surface proteins (Allard et al., 2004; Helzer et al., 2009; Lu et al., 2013; McKeown and Sarosi, 2013; Riethdorf et al., 2007; Stott et al., 2010; Talasaz et al., 2009). Detection methods then allow for single-cell level specificity when counting CTCs and further separating them from normal blood cells. These detection methods include visual microscopy, immunostaining, biomechanical discrimination and polymerase chain reaction (PCR) (Alix-Panabières and Pantel, 2013).

2.1. Physical property-based assays

Enrichment via physical properties, such as size and membrane capacitance, allows one to isolate CTCs quickly without labeling (Kim et al., 2016). Unfortunately, these techniques present certain limitations, as current technologies lack specificity and yield less pure results than functional assays due to cell heterogeneity (Hong and Zu, 2013; Wang et al., 2013). Dielectrophoretic field-flow fractionation (DEP-FFF) employs separation by size and polarizability using membrane capacitance and can process 30 million cells within 30 min with high recovery rates. However, it requires very specific parameters such as cell type and electric field frequency (Gascoyne et al., 2009; Zieglschmid et al., 2005). Metacell filtration device, isolation by size of epithelial tumor cells (ISET), ScreenCell Cyto, and dead flow fractionation techniques all use size to select for CTCs (De Giorgi et al., 2010; Dolfus et al., 2015; Hou et al., 2013; Vona et al., 2004; Wang et al., 2013). With the exception of Metacell, these size-based techniques quickly isolate CTCs, which are usually larger in size than other blood cells, but fail to enrich smaller CTCs and those with similar deformability to leukocytes (Dolfus et al., 2015; Joosse et al., 2015; Zheng et al., 2011). It is also difficult to release the captured CTCs from porous membranes for downstream analyses. To overcome this challenge, a Parsotrix method is developed which is a size-based selection method that involves a cassette device for collecting CTCs that are readily available for subsequent studies, overcoming the detachment limitation (Joosse et al., 2015). In summary, size-based CTC isolation methods provide high throughput, however these methods find limited applicability in clinical settings due to heterogeneity of CTCs in term of their size.

2.2. Functional assays

Functional assays to detect only viable CTCs may overcome some of the limitations of physical heterogeneity. However, current CTC methods based on cell functional properties face issues regarding product purity. These include analyzing CD45 protein levels and collagen adhesion matrix (CAM) removal and uptake, using the EPISPOT assay (Epithelial Immunospot) (Alix-Panabières, 2012) and CAM assay (Vita Assay) (Lu et al., 2010), respectively. The CAM assay measures CTC