Pharmacology & Therapeutics 141 (2014) 209–221

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

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

Associate editor: B. Teicher

Circulating tumor cells: Advances in isolation and analysis, and challenges for clinical applications

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ARTICLE INFO

Keywords:
Circulating tumor cells
CTC
Cancer biomarker
Prognostic biomarker
Predictive biomarker

ABSTRACT

Circulating tumor cells (CTCs) are rare cancer cells released from tumors into the bloodstream that are thought to have a key role in cancer metastasis. The presence of CTCs has been associated with worse prognosis in several major cancer types, including breast, prostate and colorectal cancer. There is considerable interest in CTC research and technologies for their potential use as cancer biomarkers that may enhance cancer diagnosis and prognosis, facilitate drug development, and improve the treatment of cancer patients. This review provides an update on recent progress in CTC isolation and molecular characterization technologies. Furthermore, the review covers significant advances and limitations in the clinical applications of CTC-based assays for cancer prognosis, response to anti-cancer therapies, and exploratory studies in biomarkers predictive of sensitivity and resistance to cancer therapies.

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Contents

1. Introduction

The process of cancer metastasis by which tumor cells detach from a primary site, spread through the circulatory system, and form distant secondary tumors is responsible for the majority of cancer deaths (Hanahan & Weinberg, 2011). Since they were first described by Thomas Ashworth in 1869, the presence of circulating tumor cells (CTCs) has been suggested to be associated with cancer by various early studies (Ashworth, 1869; Carey et al., 1976; Myerowitz et al., 1977; Gallivan & Lokich, 1984). Through a proposed process known as the epithelial–mesenchymal transition (EMT), epithelial cells of solid tumors undergo cellular changes that enable them to escape their structural confines via increased mobility and invasiveness, to enter into the bloodstream, and to adhere and develop into distant metastases (Thiery, 2002; Steeg, 2003). Thus, it is very attractive to isolate and characterize CTCs, as they may represent both the phenotypic and genetic compositions of the primary tumors and potentially serve as a "liquid biopsy" for any metastatic tumors.

A validated CTC enrichment and enumeration technology have been established in which CTC counts above a known threshold are a prognostic marker and predictor of patient outcome in metastatic breast (Hayes et al., 2006), prostate (Danila et al., 2007), and colon cancers

Abbreviations: AR, androgen receptor; CK, cytokeratin; CTC, circulating tumor cell; DEP, dielectrophoresis; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; EpCAM, epithelial cell adhesion molecule; ER, estrogen receptor; FDA, US Food and Drug Administration; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IF, immunofluorescence; ISET, isolation by size of epithelial tumor cells technique; PR, progesterone receptor; PSA, prostate specific antigen; PSMA, prostate-specific membrane antigen; ptDNA, plasma tumor DNA; RT-PCR, reverse transcription polymerase chain reaction.

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0163-7258/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.pharmthera.2013.10.004

(Cohen et al., 2008). Based on these clinical trials, the US Food and Drug Administration (FDA) cleared the CellSearch® technology (Veridex, LLC, Raritan, NJ, USA) for CTC enrichment and enumeration for the above indicated cancers. The success of CellSearch® proves that enumeration of CTCs is indeed a surrogate for active disease and that increased CTC numbers are predictive of worse prognosis. Also, by demonstrating the successful isolation of clinically relevant cells from the blood of cancer patients, it revealed the potential for further analysis of CTCs beyond enumeration.

There is great interest in obtaining molecular information from CTCs, as they may constitute a read-out for both primary and metastatic tumors. Success in CTC-based analysis has the potential to provide real-time and non-invasive surrogates for diagnosis and prognosis, predictive biomarkers for making treatment decisions, and samples for monitoring drug resistance. The majority of conventional cancer treatments have had limited success in curing metastatic disease. As tumors evolve, even an effective response to therapy is typically short lived, and patients often relapse within 12–24 months of therapeutic intervention (Lacy et al., 1998; Cristofanilli et al., 2005; Ushijima, 2009). CTCs may provide a source for longitudinal molecular analysis of tumors during the clinical management of patients that could facilitate both clinical investigations and cancer patient care.

The fact that CTCs occur at extremely low levels in the circulation and are obscured by billions of peripheral blood cells has hindered their isolation and molecular characterization (Alix-Panabières & Pantel, 2013). There have been numerous efforts, and many technologies developed to enrich and analyze CTCs, many of which have been explored and evaluated with samples from cancer patients. This review will mainly focus on CTC enrichment technologies, studies, and applications that have been successfully tested or evaluated with clinical samples. We will review the recent advances that have been made towards applying CTC assays to clinical practice, discuss the substantial challenges facing the field, and elaborate on future prospects.

2. Isolation of circulating tumor cells current advances

CTC isolation techniques must be sensitive enough to capture the rare and heterogeneous population of CTCs, while also being sufficiently specific for substantial enrichment against blood cells. It is also important for the isolation to be repeatable, reliable, rapid, cost-effective, capable of processing clinically-relevant volumes of blood, and compatible with process automation and downstream CTC analysis. Further, it is desirable for some analyses that isolated cells will maintain their viability and that they experience minimal disturbance caused by the isolation process that might alter their status or phenotype.

Various approaches that have been developed for CTC isolation from blood are discussed below. The technologies are grouped by their principle of CTC enrichment as illustrated in Fig. 1, and summarized in Table 1. These technologies are typically evaluated using cell line model systems for multiple performance parameters (i.e. capture efficiency/recovery, enrichment against leukocytes, cell viability, processing speed, blood sample capacity) and then validated through testing with clinical samples. The optimal isolation approach may require a compromise among performance parameters, and is likely to depend on the intended downstream application.

Due to differences in the underlying principles of isolation, the cells acquired by different methods are likely to be overlapping CTC subpopulations. Thus, it is important to fully characterize isolated CTCs and to establish clinical correlation and usability as in the case of the CellSearch® clinical trials. It is also important to compare various CTC isolation approaches to fully appreciate the benefits and drawbacks of each method. Practically, this may be achieved through blind comparison with the CellSearch® instrument using duplicate clinical samples.

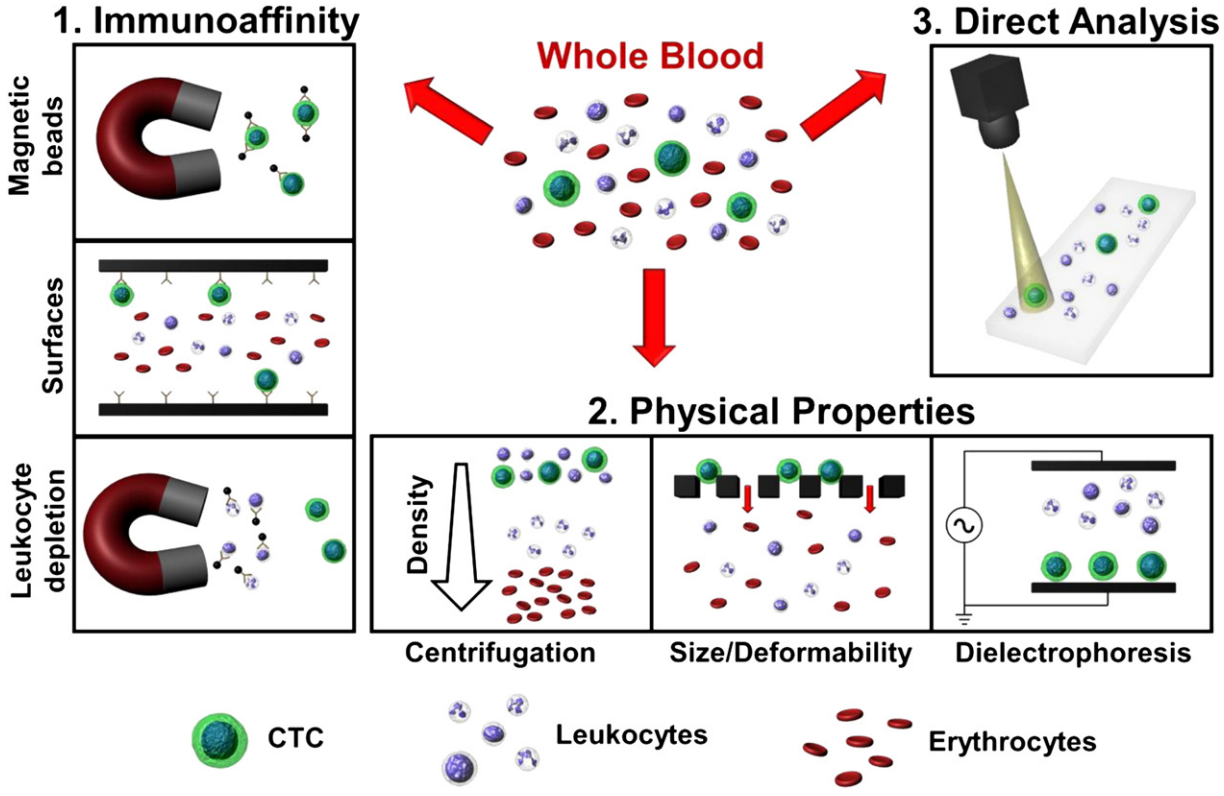

Fig. 1. Approaches for CTC isolation from whole blood. 1: Immunoaffinity based techniques target specific markers to selectively enrich CTCs or deplete leukocytes. 2: Physical properties may be exploited to separate CTCs from blood cells based on differences in density, size, deformability and electrical properties. 3: Direct analysis is achieved by high throughput assaying of all cells in blood after erythrocyte lysis.