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Looking back, to the future of circulating tumor cells

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ABSTRACT

Detection and analysis of circulating tumor cells (CTCs) from patients with metastatic malignancies have become active areas of research in recent years. CTC enumeration has already proven useful in establishing prognosis for patients with metastatic breast, colon, and prostate cancer. More recently, studies are going beyond enumeration, exploring the CTCs as a means to better understand the mechanisms of tumorigenesis, invasion, and metastasis and the value of CTC characterization for prognosis and tailoring of treatment. Analysis of CTC subpopulations, for example, is highlighting the importance of the epithelial to mesenchymal transition (EMT), a process which may be crucial for allowing tumors to invade into and grow at sites distant from the original tumor site. Similarly, the detection of CTCs expressing markers of stemness may also have important implications for treatment resistance. Genomic analysis of CTC and CTC subpopulations may allow for selection of novel therapeutic targets to combat treatment resistance. CTCs become a particularly valuable biospecimen resource when tissue biopsies are unavailable or not feasible and liquid biopsies allow for serial monitoring. Lastly, cultures of patient-derived CTCs may allow for an evaluation of therapeutic strategies performed ex vivo and in real time. This review article will focus on these developments, starting with the CTC pathogenesis, going on to discuss the different platforms available for CTC isolation and their use to date in these arenas, then will explore multiple topics including the existing data concerning CTC subpopulations and their clinical relevance, genomic characterization, and lastly, avenues for future research.

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1. Introduction

Isolation and analysis of circulating tumor cells (CTCs) from the bloodstream of patients with metastatic malignancies have emerged as ways to better understand tumorigenesis as well as the processes of invasion and metastasis. Although epithelial CTCs were first described well over 100 years ago (Ashworth, 1869), only recently has CTC enumeration been shown to be clinically useful as a prognostic biomarker in epithelial malignancies including breast (Cristofanilli et al., 2004), colon (Cohen et al., 2008), and prostate cancer (De Bono et al., 2008). In comparison to traditional metastatic tissue biopsy, isolation of CTCs offers numerous

Abbreviations: ALDH, Aldehyde dehydrogenase; AR, Androgen receptor; CAM, Collagen adhesion matrix; CK, Cytokeratin; CTC(s), Circulating tumor cell(s); depFFF, Dielectrophoretic field-flow fractionation; ELISPOT, Enzyme-linked immunosorbent spot assay; EMT, Epithelial to mesenchymal transition; EpCAM, Epithelial cell adhesion molecule; ER, Estrogen receptor; FAST, Fibre-optic array scanning technology; MACS, Immunomagentic cell separation; MCC, Microfluidic cell concentrator; MET, Mesenchymal to epithelial transition; mCRPC, Metastatic castration-resistant prostate cancer; PR, Progesterone receptor; PSA, Prostate-specific antigen; PSMA, Prostate specific membrane antigen; VEGF, Vascular endothelial growth factor.

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advantages: collection of peripheral blood is easy to perform, relatively inexpensive, presents significantly less risk to patients, is less morbid, and is easily repeatable over time. The idea of 'real-time' serial monitoring of CTCs is attractive as it allows for an estimate of circulating disease burden over time, can permit researchers to track and discover novel changes in cancer cells (Murtaza et al., 2013), and can shed light on some of the fundamental processes that lead to an aggressive phenotype, metastasis, and resistance to current therapies.

In order to best understand how CTCs are currently being deployed in the clinical arena, and their utility in understanding cancer cell biology, we will first review the fundamental processes that lead to the release of tumor cells into the bloodstream, then focus on currently available platforms for CTC detection/isolation and clinical uses of CTCs, then discuss what is known about CTC heterogeneity and subpopulations including CTCs expressing mesenchymal markers and/or stemness, and finally discuss the potential for ex vivo CTC expansion and the possibility of using CTCs to select targets for therapy.

2. Pathogenesis of circulating tumor cells and the role of the epithelial to mesenchymal transition

Despite the understanding that tumor cells must travel through the bloodstream or lymphatics in order to establish metastases, a singular, precise definition of a CTC remains elusive. It is widely hypothesized that CTCs originate from cells within the primary or metastatic tumor that acquire the ability to detach from the basement membrane, invade through the tissue stroma, and enter into the blood vessels (Nowell, 1976). The acquisition of this invasive phenotype is hypothesized to occur in response to the increasing tissue hypoxia that develops as tumors grow and compete for resources (Semenza, 2012), which in turn leads to neovascularization and lymphangiogenesis (Cao et al., 2013). A critical concept that has emerged to be relevant to CTCs is the epithelial to mesenchymal transition (EMT), a process first observed in embryonic development (Shook & Keller, 2003) (Fig. 1). EMT enables epithelial cells to lose their apical–basal polarity, detach from neighboring cells, acquire a fibroblast-like morphology, invade through the surrounding stroma, and become more resistant to apoptosis (Vincent-Salomon & Thiery, 2003). During this process, tumor cells lose expression of specific epithelial markers including E-cadherin (Comijn et al., 2001), EpCAM (Went et al., 2004), and cytokeratin (Savagner, 2010), gain expression of mesenchymal cytoskeletal and adhesion proteins such as vimentin and N-cadherin (Kokkinos et al., 2007), and upregulate kinases and growth factors including c-MET (Giordano et al., 2000), TGF-β (Oft et al., 1996), Wnt (Chen et al., 2012), and FOXC1 (Xia et al., 2013). EMT is thought to be controlled by a family of genes including, among others, Snail, Twist, Zeb, and E47 (Berx et al., 2007).

Whether nascent CTCs undergo EMT in order to escape the primary or metastatic tumor and enter the bloodstream, or whether epithelial-like CTCs are simply 'shed' from a tumor that has eroded into a blood vessel and undergo EMT while in transition, is still a matter of debate. CTCs derived from epithelial tumors can be detected using multiple different epithelial markers (Table 1). While the presence of epithelial CTCs in circulation would seem to support the notion that CTCs are simply shed by large tumors, it is also possible that primary tumor cells that have undergone EMT in order to enter into and survive in the circulation can subsequently undergo a reverse process termed mesenchymal to epithelial transition (MET), whereby cells revert back to their epithelial state. This concept is supported by the finding of CTCs expressing mesenchymal markers including vimentin (Kallergi et al., 2011), N-cadherin (Balasubramanian et al., 2012), CD44 (Theodoropoulos et al., 2010), as well as reports of CTCs expressing markers of both epithelial and mesenchymal lineage (Armstrong et al., 2011), implying the presence of a transition state between epithelial and mesenchymal. Similar support comes from the clinical observation that epithelial tumors with mesenchymal differentiation (for example, sarcomatoid renal cell cancers) are more aggressive and prone to early dissemination (Cangiano et al., 1999). The concept of a dynamic flux between epithelial and mesenchymal states may also better explain the process of metastasis, which allows for individual or small groups of mesenchymal-like tumor cells to invade and colonize distant sites, then revert back to an epithelial state once a suitable niche is found. It should be stressed that to date no one single model of metastasis encompasses the entirety of all observational findings and a number of other factors, including the site of origin of the tumor cell and the degree of cell heterogeneity within a tumor, may come into play. Nevertheless, the EMT/MET model of dissemination has advantages in explaining the wide variety and plasticity of CTCs observed to date, and has important implications for the direction of future research.

3. Methods of circulating tumor cell isolation

CTCs occur at very low frequency in the bloodstream, generally estimated at fewer than 1 CTC per million leukocytes. Nucleated non-

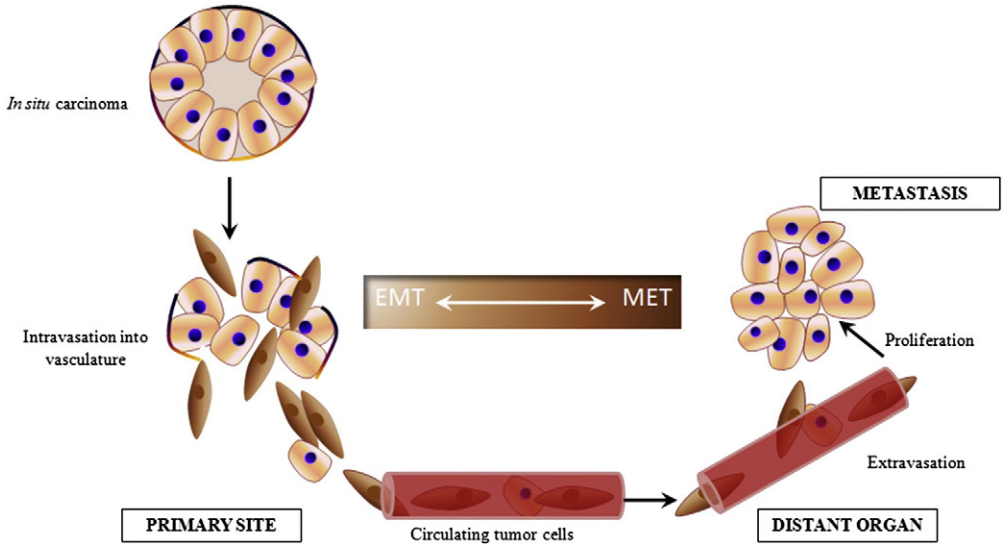

Fig. 1. CTCs, EMT, and cancer progression: A schematic of the reversible EMT model for cancer progression. Invasion involves epithelial cells losing their polarity and detaching themselves from the basement membrane through EMT activation. Metastasizing cells then directly enter the circulation. Circulating tumor cells then travel to distant sites and exit the bloodstream. Once they extravasate they may proliferate and reverse back to an epithelial phenotype to form macrometastases.