REVIEW ARTICLE

Circulating tumor cells as "liquid biopsies" to understand cancer metastasis

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Circulating tumor cells (CTCs) are a subset of cancer cells that are shed from the primary or metastatic tumors into the bloodstream. CTCs are responsible for the establishment of blood-borne distant metastases but their rarity, estimated at one CTC per billion blood cells, presents the biggest technical barrier to their functional studies. Recent advances in CTC isolation technology have allowed for the reliable capture of CTCs from the whole blood of cancer patients. The ability to derive clinically relevant information from CTCs isolated through a blood draw allows for the monitoring of active disease, avoiding the invasiveness inherent to traditional biopsy techniques. This review will summarize recent developments in CTC isolation technology; the development of CTC-derived models; the unique molecular characteristics of CTCs at the transcriptomic, genomic, and proteomic levels; and how these characteristics have been correlated to prognosis and therapeutic efficacy. Finally, we will summarize the recent findings on several signaling pathways in CTCs and metastasis. The study of CTCs is central to understanding cancer biology and promises a "liquid biopsy" that can monitor disease status and guide therapeutic management in real time. (Translational Research 2018; ■■:■■-■■)

Abbreviations: ANGPTL4 = angiopoietin-like 4; CDX = CTC-derived xenograft; CK = Cytokeratin; CNA = copy number alteration; CTC = circulating tumor cell; DAXX = death-associated protein 6; EMT = epithelial-mesenchymal transition; EpCAM = epithelial adhesion molecules; INDEL = insertion/deletion; JNK = c-Jun N-terminal kinases; MET = mesenchymal-epithelial transition; NSCLC = nonsmall cell lung cancer; PDX = patient-derived xenograft; PSA = prostate specific antigen; RNA-seq = RNA sequencing; SCLC = small cell lung cancer; SNV = single nucleotide variation; TGF-β = transforming growth factor beta

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INTRODUCTION

As a complex multistep event that enables the primary tumor to spread and colonize other parts of the body, metastasis contributes up to 90% of cancer-related mortality.[1] Metastasis requires the primary tumor cells to invade surrounding tissue, intravasate, transit while surviving anoikis, extravasate into tissues different from the original site, and adapt to the new microenvironment in a way that enables the formation of a macroscopic tumor.[2-4] Circulating tumor cells (CTCs) are directly involved in the metastatic process, presenting a unique opportunity to study cancer metastasis and progression. Additionally, clinicians can access CTCs through a blood draw, raising the possibility of utilizing CTCs as a "liquid biopsy" to determine

prognosis or monitor therapeutic response.[5-7] However, the prevalence of CTCs is estimated to be as low as 0−10 cells per 10 mL of blood in patients with metastatic disease, making their rarity the biggest challenge to isolation.[8] Despite this, novel experimental models to amplify these rare cells and newly refined analytic techniques have begun to shed light on the unique properties of CTCs. These measurable properties must be correlated with clinical outcomes before CTCs can be used as a "liquid biopsy" to monitor disease status and inform clinical management (Fig 1). This review will focus on recent insights into CTC biology that inform our understanding of the metastatic process and the unique role CTCs play in cancer progression.

CTC ISOLATION

The greatest technical challenge in capturing and studying CTCs is their rarity: it is estimated to be approximately 1 CTC per billion blood cells. CTC capture technologies have overcome this by exploiting the properties of CTCs that are distinct from other blood cells. Currently, the most commonly used technology is the CellSearch platform by Veridex, which is an immunoaffinity-based assay that isolates CTCs by positively selecting for an epithelial cell surface marker—specifically, epithelial cell adhesion marker (EpCAM). Captured cells can then be immunostained to confirm epithelial origin, verifying that they are positive for the epithelial marker cytokeratin (CK) and negative for the leukocyte marker CD45. The majority of competing CTC isolation technologies also relies on EpCAM and CK staining. Because of these techniques, the majority of CTC research exclusively involves EpCAM-positive cells.

EpCAM is prevalent in CTCs with epithelial characteristics but it is not a universal CTC biomarker. CTCs that have undergone epithelial-to-mesenchymal transition and CTCs of mesenchymal origin can be EpCAM-negative.[9] Additionally, cancer cell lines and CTCs isolated from patients have shown a wide range of EpCAM expression, with a portion of these cells being EpCAM-negative.[10-12] Some technologies have tried to avoid biasing capture toward EpCAM-positive CTCs by removing CD45+ leukocytes, a process of negative selection, but these technologies suffer from low purity. Other negative selection technologies have relied on the unique mechanical properties of CTCs conferred by their greater nucleus-to-cytoplasm ratio, larger size, and abnormal nuclear morphology. These technologies have isolated CTCs based on cytoskeletal stiffness (which is potentially important in extravasation), density gradient centrifugation, porous microfiltration (which differentiates cells by size), and dielectrophoresis (which separates cells based on polarizability).[9] However, these technologies lack specificity, which is particularly problematic when

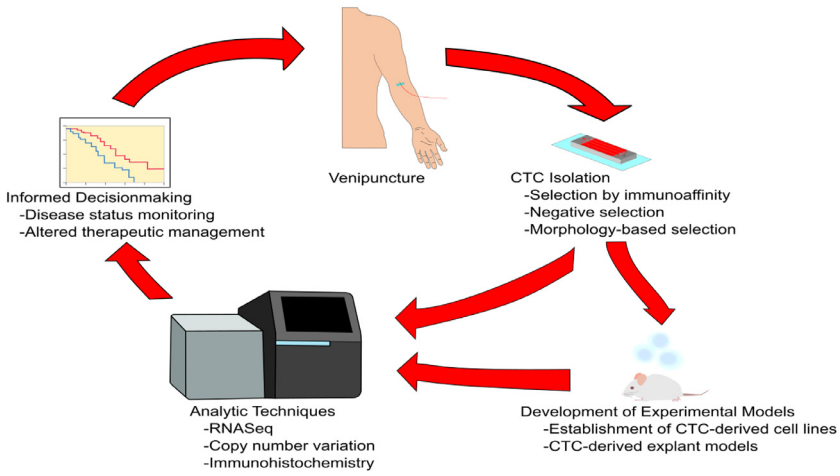

Fig 1. Illustration of circulating tumor cells (CTCs) as a "liquid biopsy" for cancer patients. CTCs obtained from venipuncture are purified through 1 or several isolation techniques. These cells can be further amplified through the development of experimental models or analyzed directly through techniques requiring very little input material. Properties of CTCs at the genomic, transcriptome, or proteomic level can then be correlated to clinical outcomes either through overall survival or predicting therapeutic prognosis. This information can alter medical management with the process repeated at a future point in time. The current ability to do this is limited by the lack of understanding of the mechanistic role of CTCs in cancer progression. However, using serial CTC isolations as a "liquid biopsy" is predicted to become increasingly prevalent in guiding patient care and management.