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MOLECULAR TESTING AND DIAGNOSTICS (J KHOURY, SECTION EDITOR)

Circulating Tumor Cells: State-of-the-art Update on Technologies and Clinical Applications

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Abstract

Purpose of Review Circulating tumor cells represent rare events in the peripheral blood of patients with cancer that can provide insight into tumor biology. CTC enumeration, isolation, and analysis represent liquid biopsy approaches whose role in the management of patients with cancer continues to evolve in the era of precision medicine. This review presents an overview of technologies central to studying CTCs.

Recent Findings Technologies for CTC isolation can be divided into two categories: label-dependent and label-independent. Label-dependent techniques utilize biological properties such as cell surface proteins, while label-independent techniques utilize distinctive physical properties such as cell size, density, and plasticity. Advances in microfluidics designs as well as hybrid combinations of label-dependent and label-independent techniques have resulted in unprecedented improvements in CTC isolation, permitting not only the detection and enumeration of these rare events but also providing the means for studying them and exploring them as a new dimension of cancer biomarkers.

Summary With advances in tools for isolating and studying CTCs in hand, questions regarding the clinical utility of CTC enumeration in peripheral blood, detection of CTC-associated biomarkers, and analysis of dynamic changes in CTCs during the course of cancer therapy represent exciting new opportunities for cancer research.

Keywords Circulating tumor cells · Biomarker · Cancer

Introduction

Circulating tumor cells (CTCs) are defined as neoplastic cells that have broken off from the primary tumor and are circulating in the blood and/or lymph [1]. This review will focus on CTCs present in the peripheral blood. It is believed that CTCs may be involved in the process of tumor metastasis—a major cause of cancer mortality—by virtue of their ability to break off from the primary tumor, enter the bloodstream (intravasation), travel through the circulatory system, evade the immune system, exit the bloodstream (extravasation) within a distant organ, and form a secondary tumor [2]. Several questions related to CTC biology remain unanswered or incompletely answered. For instance, why cells break off from the tumor, how they evaded the immune system, and how they attach themselves to a distant organ and establish metastasis. One of the main challenges of isolating CTCs is their characteristic scarcity in peripheral blood. It is estimated that there is typically 1 CTC in the peripheral circulation for every billion blood cells in patients with metastatic carcinoma. Several technologies have been developed to isolate CTCs from blood. These technologies are capable of capturing and isolating CTCs for further downstream studies. However, the diversity of CTC isolation technique is presenting new challenges that preclude optimal comparison across platforms [3]. Areas that could benefit from standardization include definition of

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CTCs, sample types, and patient subsets who could benefit from CTC enumeration and analysis [4]. These variables pose challenges to implementing standards for isolation techniques that will define the accuracy, specificity, and sensitivity of assays for detecting CTCs. The purpose of this review is to introduce the main CTC isolating systems available currently and provide an overview of the strengths and limitations of each system.

Clinical Utility of CTC Detection

CTCs have been shown to be a valuable prognostic marker in several solid tumors including breast, prostate, and colon cancers. Analysis of CTCs can potentially present new information regarding the primary tumor from which they were shed. Moreover, since CTCs are known to share genetic similarities with the primary tumor, detection of CTCs and interrogation of specific characteristics can be used to monitor and track disease progression as well as study heterogeneity and clonal evolution of a tumor [2, 5]. Additionally, being able to analyze CTCs can permit more precise prognostication and more effective therapy selection [5, 6••, 7, 8]. For example, CTC analysis may permit the detection of early signs of dissemination, providing opportunities for intervention before full-fledged metastasis or relapse [7].

Intratumoral and intertumoral clonal heterogeneity is highly pervasive in most cancer types [9]. Genomic instability in proliferating cancer cells generates subclonal mutations [10–14]. It has also been shown that secondary tumors will develop heterogeneity relative to the corresponding primary tumor, suggesting that the secondary tumor can potentially present a completely different genomic profile from the primary tumor—also known as intertumoral heterogeneity. Cells obtained from a single biopsy site may thus provide limited representation of a tumor's complexity [15]. While multiple biopsies can be taken to maximize precision, such an approach might not always be feasible and generally increases the morbidity risks associated with the biopsy procedure. These factors have increased the attractiveness of developing liquid biopsy techniques that could provide a collated overview of a patient's tumor burden at a particular point in time.

Several studies have demonstrated a correlation between the number of CTCs present in the peripheral blood and progression-free survival (PFS) and/or overall survive (OS) in patients with various types of metastatic cancers, namely breast, prostate, and colorectal carcinoma. Namely, patients with higher CTCs have shorter PFS and OS relative to patients with lower counts of CTCs [6••, 16••, 17••]. Patients with metastatic disease who had < 5 CTCs per 7.5 ml of blood prior to starting a new therapy were more likely to have a better clinical response than similar patients who had > 5 CTCs per 7.5 ml of blood [18].

Main CTC Detection Approaches

CTC isolation techniques can be split into two categories: label-dependent and label-independent. Label-dependent techniques utilize biological properties to isolate CTCs, while label-independent techniques utilize distinctive physical properties to isolate CTCs [7]. Biological properties used for label-dependent techniques include cell surface proteins, DNA, RNA, and other classes of biomarkers. Physical properties of a tumor cell that are utilized in label-independent techniques include size, density, and plasticity.

The most common form of label-dependent techniques is the use of antibodies against epithelial cell adhesion molecule (EpCAM) to detect CTCs. EpCAM is a transmembrane glycoprotein found almost exclusively in benign and neoplastic epithelial cells. EpCAM is uniformly expressed in carcinomas. In one approach, anti-EpCAM is conjugated to magnetic beads to perform immunomagnetic separation. Another approach entails the use of anti-EpCAM antibodies to capture epithelial cells by employing microfluidic chips. These chips have built-in conduits for unidirectional blood flow lined by anti-EpCAM antibodies bound to their walls. As the blood flows through the chip, the CTCs adhere to the antibodies and are captured for analysis. The principle of utilizing the EpCAM as a way of identifying and isolating CTCs is referred to as *positive selection*, since EpCAM is expressed exclusively on CTCs and not on peripheral blood cells [7]. Conversely, the principle of utilizing CD45 (common leukocyte antigen), an antigen that is expressed on peripheral blood cells but not CTCs, to identify and isolate the latter is referred to as *negative selection*. The basis behind negative selection is the removal of any leukocytes within a sample leaving behind an isolate that is highly enriched for CTCs.

Label-independent techniques commonly rely on differentiating physical properties of cells such as size, density, and plasticity. Size-based isolation techniques use a microfluidic chip without antibody-based positive or negative selection; the microfluidic design consists of a chip causes larger cells, such as CTCs, to be trapped within the chip while allowing smaller peripheral blood cells to pass through. Once the sample completely passes through, a backflow wash is applied to dislodge the trapped CTCs and collect them for analysis [3]. Density-based techniques are the simplest to conduct, since they only require the use of a centrifuge; they rely on the principle that CTCs are typically less dense than red blood cells. A number of CTC isolation platforms represent hybrids of multiple techniques combining label-dependent and label-independent principles [7]. One uses both positive and negative selection to isolate CTCs and eliminate leukocytes within the same sample for a better purity. Another uses size-based filtration technique for better recovery then uses positive and/or negative selection for better purity.

CellSearch

Probably one of the most commonly used systems for isolating CTCs is the CellSearch System (Menarini Silicon Biosystems). The CellSearch System is currently the only FDA-approved CTC isolating system for clinical use in patients with breast, prostate, and colon cancer. CellSearch uses both positive and negative selection techniques, meaning it uses anti-EpCAM antibodies to identify CTCs and anti-CD45 antibodies to identify leukocytes. In addition, the system also utilizes a variant of immunomagnetic separation to isolate cells. CellSearch uses Ferrofluid, which is a black magnetic liquid that is added onto the antibodies during the preparation stage instead of the magnetic beads engineered onto the antibodies. The developers classify CTCs as EpCAM+/CD45−/cytokeratin+/DAPI+ [19]. The CellSearch system is a combination of kits. The CellSave Preservative tubes used to store blood samples from donors. The packaging claims that the tube is filled with EDTA and other cellular preservatives that can store samples at room temperature for up to 96 h. The CellTracks AutoPrep system is an automated machine that will help to prepare samples for isolation and analysis. The reagents used in the CellTracks are provided in the CellSearch Circulating Tumor Cell Kit. Finally, the CellTracks Analyzer II is used to image and count the sample using its built-in fluorescence microscope. When using the CellSearch system as a diagnostic tool, researchers use a cutoff value of 5 CTCs per 7.5 ml [4, 6••, 17••, 20]. Patients who have a CTC count < 5 CTCs per 7.5 ml of blood before receiving treatment are generally predicted to have significantly longer PFS and OS.

AdnaTest

The AdnaTest (Qiagen; Hilden, Germany) is another commonly used system for CTC isolation and detection. Like the CellSearch system, AdnaTest is a label-dependent technique that uses positive selection with anti-EpCAM antibodies coupled with immunomagnetic separation for isolation. Though AdnaTest is not FDA approved, it is CE-IVD approved. Unlike the CellSearch system, AdnaTest does not use negative selection for anti-CD45 antibodies,coupled with uses magnetic beads instead of Ferrofluid on their antibodies. In addition, AdnaTest tests for other tumor markers such as MUC-1, HER2, and GA733-2, to name a few [20–22]. The AdnaTest also comes in its own kits developed by AdnaGen. Instead of having one kit dedicated to isolating CTCs in general, AdnaTest has developed multiple kits specifically for isolating CTCs from colon, breast, prostate, ovarian, and lung cancer. There are two parts for each of the cancer kits mentioned: A Select kit and a Detect kit. Blood samples are collected and stored in the AdnaTube which contains EDTA and other cell preservatives. Immunomagnetic separation is performed on the samples using the AdnaTest Select kit for the specific cancer being tested. After the cells have been lysed, the AdnaTest Detect kit may be used to isolate mRNAs from the separated CTC, and reverse transcribing the mRNAs for RT-PCR. When compared with the CellSearch system, the results show that there are varying degrees of differences ranging from higher to lower detection values or simply no significant difference between the two systems [20–22].

CellCollector

The CellCollector (GILUPI; Postdam, Germany) is a label-dependent isolating system that is also CE approved. The CellCollector system is unique in that, unlike the systems listed in this review, the CellCollector system is an in vivo technique. The techniques listed previously are all in vitro. The CellCollector system utilizes a medical wire to isolate CTCs. To accomplish this, the medical wire has a golden tip that is coated with an anti-EpCAM antibody-infused hydrogel. First, an indwelling catheter is inserted into the patient's arm vein. Next, the medical wire is inserted through the catheter and left in the patient's vein for 30 min. After 30 min, the medical wire is removed and is ready for downstream processing. The CellCollector medical wires come in two models: Detektor CANCER01 (DC01) or Detektor CANCER02 (DC02). DC01 is the first model created by GILUPI. It is 16-cm long with a 2-cm rounded golden tip. The DC02 is the newer model. It is longer than the DC01 (18 cm) with a 4-cm twisted golden tip. GILUPI states that the newer model with its longer and twisted tip provides better hemodynamic structure for unobstructed blood flow, and allows customization of each tip with different antibodies besides anti-EpCAM antibodies.

Microfluidic Chips (Positive Selection)

Microfluidic chips are devices about the size of a microscope slide, or smaller, engineered with built-in microfluidics channels. The channel can either be a straight channel or a complex network of capillaries. On either side of the chip is an input or output hole that allows for a sample to flow through the channels with ease. One of the main features of the microfluidic chip is the control of flow rate. This allows researchers to choose whether they want a faster flow rate for a shorter experimental time, or a slower flow rate for a more precise measurement. The adjustment of flow rate also allows for the control of shear stress within the channels. Some cells within a sample may be more fragile than others, so a slower flow rate is employed to prevent more fragile cells from being destroyed. A microfluidic chip can either be a label-dependent or label-independent technique depending on the type of chip. Some examples of a label-dependent microfluidic chips are the CTC-chip and the herringbone chip. The CTC-chip is a microfluidic chip with a straight channel

that has within it an array of microposts. The microposts within the chip are coated with anti-EpCAM antibodies to allow the capturing of CTCs. An apparatus is required to house the microfluidic chip and to apply a flow through the chip. This apparatus can either be purchased or self-made depending on preference. Regardless of how the apparatus was acquired, it needs to be able to house the microfluidic chip and the sample, and have a method to apply a steady flow through the chip. Another label-dependent microfluidic chip is the herringbone chip. Instead of having an array of microposts, the herringbone chip has indented groves in the shape of a herringbone design on the roof of the chip. Additionally, the surfaces of the channels are coated with anti-EpCAM antibodies. The purpose of these groves is to generate microvortexes within the chip to increase the probability that CTCs will come in contact with the surfaces of the channel. This will in theory increase the amount of CTCs captured.

Parsortix (Label-Independent Microfluidics)

Parsortix is a label-independent isolating system (Angle, Plc; Guildford, United Kingdom). As stated before, this means that it does not rely on the use of antibodies to isolate CTCs. Instead, the Parsortix system relies on cell size and deformability as critical characteristics for isolation. Parsortix also utilizes a microfluidic chip to isolate CTCs. Within the microfluidic chip is a pathway that allow for blood to flow from one end to the other. As the blood flows through the chip, it encounters a staircase-like structure. This structure is used to gradually decrease the aperture size of the pathway. This allows larger and less deformable cells to be trapped, while smaller cells with preserved physiologic deformability are typically washed off. Since CTCs (> 8 μm) are generally larger than red blood cells (7 μm), CTCs will be trapped when passing through the smaller opening while red blood cells can flow through without being trapped. After the entire blood sample has passed through the chip, the automated device will flush the chip by sending a backflow through the channel to dislodge trapped cells into a collecting tube.

Strengths and Limitations

All of the systems listed in this review are capable of isolating CTCs. The decision on which technique to use is dependent on personal preference, clinical applications, laboratory considerations, and budget. Like all laboratory systems, there are strengths and limitations to each system. Label-dependent techniques rely on the same factor: overexpression of a known cell-surface antigen, usually EpCAM. However, CTCs can lose expression of EpCAM entirely due to epithelial-to-mesenchymal transition (EMT) [23]. This poses a problem for label-dependent techniques that heavily depend on EpCAM expression. Without expression of EpCAM, no anti-EpCAM antibodies will bind to these CTCs, making isolation impossible and label-dependent techniques suboptimal [5, 24, 25]. Label-independent techniques rely on the size, density, and plasticity (deformability) of CTCs as isolation parameters. Parsortix has microfluidic chips with various channel sizes ranging from 10 to 4 μm. This range allows for the isolation of CTCs with minimal contamination of red blood cells. Additionally, label-independent techniques do not rely on the expression of EpCAM, so they can isolate CTCs even after they have undergone EMT.

Conclusion

CTCs have the potential to be used as a real-time indicator of the tumors they are derived from and can be very useful in non-invasive treatment planning. It is important to note that there is a difference between circulating tumor cells and circulating tumor DNA (ctDNA). Currently, the term liquid biopsy and ctDNA are often times used synonymously due to the increasing popularity in ctDNA research. Both CTCs and ctDNA have high potentials to provide insight into cancer biology. In thoery, ctDNA provides more genomic insight into a tumor's mutation status, whereas CTC provides a more comprehensive real-time representation of the tumor it came from, including RNA and protein expression profile [26–28]. CTCs are whole cells that have broken off from an existing tumor undergoing metastasis, whereas ctDNA is the DNA of cells that have been broken down as a result of other factors. With the growing number of research and development put into this area of cancer research, newer isolating techniques are being invented to better utilize CTCs to their fullest potential.

Compliance with Ethical Standards

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References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Plaks V, Koopman CD, Werb Z. Cancer. Circulating tumor cells. Science. 2013;341:1186–8.
2. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science. 2011;331:1559–64.
3. Yu M, Stott S, Toner M, Maheswaran S, Haber DA. Circulating tumor cells: approaches to isolation and characterization. J Cell Biol. 2011;192:373–82.
4. Riethdorf S, Fritsche H, Muller V, Rau T, Schindlbeck C, Rack B, et al. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. Clin Cancer Res. 2007;13:920–8.
5. Krebs MG, Hou JM, Ward TH, Blackhall FH, Dive C. Circulating tumour cells: their utility in cancer management and predicting outcomes. Ther Adv Med Oncol. 2010;2:351–65.
6.•• de Bono JS, Scher HI, Montgomery RB, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2008;14:6302–9 **Landmark paper describing the prognostic utility of circulating tumor cells in patients with metastatic prostate cancer.**
7. Maheswaran S, Haber DA. Circulating tumor cells: a window into cancer biology and metastasis. Curr Opin Genet Dev. 2010;20:96–9.
8. Joosse SA, Gorges TM, Pantel K. Biology, detection, and clinical implications of circulating tumor cells. EMBO Mol Med. 2015;7:1–11.
9. Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature. 2013;501:338–45.
10. Parsons DW, Jones S, Zhang X, Lin JCH, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321:1807–12.
11. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366:883–92.
12. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol. 2012;30:413–21.
13. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell. 2013;152:714–26.
14. Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469:356–61.
15. Crowley E, Di Nicolantonio F, Loupakis F, et al. Liquid biopsy: monitoring cancer-genetics in the blood. Nat Rev Clin Oncol. 2013;10:472–84.
16.•• Cohen SJ, Punt CJ, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26:3213–21 **Landmark paper describing the prognostic utility of circulating tumor cells in patients with metastatic colorectal cancer.**
17.•• Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med*. 2004;351:781–91 **Landmark paper describing the prognostic utility of circulating tumor cells in patients with metastatic breast cancer.**
18. Toss A, Mu Z, Fernandez S, et al. CTC enumeration and characterization: moving toward personalized medicine. Ann Transl Med. 2014;2:108.
19. Wang L, Balasubramanian P, Chen AP, Kummar S, Evrard YA, Kinders RJ. Promise and limits of the CellSearch platform for evaluating pharmacodynamics in circulating tumor cells. Semin Oncol. 2016;43:464–75.
20. Andreopoulou E, Yang LY, Rangel KM, Reuben JM, Hsu L, Krishnamurthy S, et al. Comparison of assay methods for detection of circulating tumor cells in metastatic breast cancer: AdnaGen AdnaTest BreastCancer Select/Detect versus Veridex CellSearch system. Int J Cancer. 2012;130:1590–7.
21. Muller V, Riethdorf S, Rack B, et al. Prognostic impact of circulating tumor cells assessed with the CellSearch System and AdnaTest Breast in metastatic breast cancer patients: the DETECT study. Breast Cancer Res. 2012;14:R118.
22. Van der Auwera I, Peeters D, Benoy IH, et al. Circulating tumour cell detection: a direct comparison between the CellSearch System, the AdnaTest and CK-19/mammaglobin RT-PCR in patients with metastatic breast cancer. Br J Cancer. 2010;102:276–84.
23. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8.
24. Hyun KA, Koo GB, Han H, Sohn J, Choi W, Kim SI, et al. Epithelial-to-mesenchymal transition leads to loss of EpCAM and different physical properties in circulating tumor cells from metastatic breast cancer. Oncotarget. 2016;7:24677–87.
25. Xu L, Mao X, Imrali A, Syed F, Mutsvangwa K, Berney D, et al. Optimization and evaluation of a novel size based circulating tumor cell isolation system. PLoS One. 2015;10:e0138032.
26. Bidard FC, Weigelt B, Reis-Filho JS. Going with the flow: from circulating tumor cells to DNA. *Sci Transl Med*. 2013;5:207ps214.
27. Ma M, Zhu H, Zhang C, et al. "Liquid biopsy"-ctDNA detection with great potential and challenges. Ann Transl Med. 2015;3:235.
28. Chan F, Goodman O, Fink L, Vogelzang NJ, Pomerantz D, Khoury JD. Dramatically elevated circulating tumor cell numbers in a patient with small cell neuroendocrine carcinoma of the prostate. Arch Pathol Lab Med. 2010;134:120–3.