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Liquid Biopsy- A New Prospect

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Abstract

Tissue biopsy has been the mainstay in tumor diagnosis for centuries. But due to its invasiveness and the heterogeneity of tumors there was a need for an alternative or adjuvant techniques to diagnose and assess tumors. Liquid biopsy is emerging as a new technique that will open the way for the diagnosis, tumor characterization, assess disease prognosis and individualize treatment options in cancer patients.

Keywords Biopsy, CTC, ctDNA

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List of abbreviation: CTCs = Circulating tumor cells, ctDNA = Circulating tumor DNA, RBCs = Red blood cells, WBCs = White blood cells, EpCAM = Epithelial cell adhesion molecule, CK = Cytokeratin, Her2 = Human epidermal growth factor, CD = Cluster of differentiation, FDA = American food and drug administration, CFDA = Chinese national food and drug administration, SE = Subtraction enrichment, iFISH = Immunostaining fluorescence in situ hybridization

Early detection and diagnosis of cancer represents a challenge in medical practice. Tissue biopsy is the golden stone in diagnosing tumors, but it is invasive to start with and difficult to obtain more than one biopsy along the course of the disease. Nowadays, and with the advancement in technologies it is considered as part of the routine clinical practice to detect (at the molecular level) specific drivers and mutations in tumors aiming at predicting treatment response and emergence of any drug resistance. What remains as an obstacle is the heterogeneity of tumors whether of the primary site or that in the metastases, or even at different time points during the progression of the disease. Therefore, it might be very difficult to assess tumor heterogeneity with one biopsy [1,2]. Additionally, the emergence of tumor resistance to different agents is common in clinical practice. Therefore, research teams all over the world have been working on developing techniques that help in early detection and diagnosis of cancer and assessing response to treatment and prognosis [3].

Liquid biopsy (which includes circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)), gives the option of collecting more than one sample non-invasively along the course of the disease, which enables tumor detection and characterization, predicting and assessing the prognosis, response and resistance to treatment, and early detection of any relapse [3]. We will focus on the CTCs and its future prospect in cancer in this article.

CTCs detection and enrichment and biological properties

Thomas R. Ashworth, an Australian scientist, was the first to discover and describe CTCs in a blood sample collected from a patient with breast cancer in 1869 [4]. CTC is the term used to describe tumor cells that detach from the

primary tumor and circulate in the peripheral blood or lymphatics and grow in the blood, or settle and grow in the bone marrow, lymph nodes or in any other organ leading to secondary tumors [5]. These CTCs are exposed to very harsh conditions and a very small percentage of CTCs (< 0.01%) manage to survive these conditions leading to the development of metastases [6]. The important point regarding CTCs is that they can arise at any phase of tumor development, and hence (theoretically) they can be used as an early marker to diagnose the tumor and as a tool to monitor disease progression and any relapse [3].

The obstacle that delayed the use of CTCs for these purposes despite its discovery since the 19th century was the scarcity of the CTCs in peripheral blood. In the last few decades there have been giant leaps in technology that enabled researchers to develop techniques that helped in improving the separation and enrichment of CTCs [3]. This can be achieved through two main mechanisms:

a) Detection using the physical properties of CTCs:

This method uses physical properties of CTCs to separate them from the rest of cells in peripheral blood. These physical properties include the size of tumor cells to start with assuming that they are larger than other cells. The CTCs have a size of (~ 17-52 µm) that is larger than RBC (~ 6-8 µm) and WBCs (~ 7-15 µm) [7]. Another method that uses physical properties is gradient centrifugation. Ficoll density gradient (which is a hydrophilic polysaccharide with a high mass) separates CTCs from the rest of blood cells depending on the density difference assuming that tumor cells have higher amount of DNA and higher density [8]. Other physical parameters include malleability, migratory capacity and the electric charge of the CTCs beside the size and density. Counting on physical properties alone to separate and enrich CTCs falls short due to the huge variability among tumor cells in regard to their physical properties and this leads to the false detection of blood cells as CTCs. Therefore, this technique might have a high percentage of false-positive results [3].

b) Detection using the biological properties of CTCs:

This method exploits the biological properties of the CTCs and is based on taking advantage of surface markers of CTCs to detect them in an antibody-antigen binding pattern. A panel of markers can be used such as epithelial cell adhesion molecule (EpCAM), cytokeratin family members (CK 8, 18 and 19), human epidermal growth factor (Her2), N-cadherin and vimentin. This biological detection method is called immune capture method [9-11]. In principle, magnetic beads are covered by specific antibody to the target of interest and then these beads are mixed with the blood sample to allow the binding of the antibodies to their target antigens that are located on the targeted cells. Afterwards the blood sample is passed through a magnetic field that leads to isolation of targeted cells (or exclusion of the unwanted cells in certain cases) that are bound to the beads and pulled to the periphery of the tube under the effect of magnetic field enriching targeted cells. So we end up with the following pattern "Targeted cell-surface antigen-the specific antibody-magnetic beads". Immune capture can be achieved through positive and negative enrichment. Positive enrichment method uses metallic beads that are bound to antibodies targeting specific CTCs surface antigen [12].

Cell SearchTM System (CSS:Verdix LLC, NJ, USA) uses this positive enrichment method and it is the only approved system in the world for the detection of CTCs in malignant tumors by both the American Food and Drug Administration (FDA) and the Chinese National Food and Drug Administration (CFDA). This system uses EpCAM coated beads to isolate CTCs and thereafter further steps use other antibodies (CK 8 and 18 for epithelial cells, CD45 for WBCs and DAPI as a nuclear stain) to exclude

leukocytes and confirms the diagnosis [3,12,13]. Similar to all systems, this system has pros and cons. The pros are that it requires small amount of blood (7.5 ml only), and the results were considered reproducible, specific and sensitive. The cons are due to the reliance of the system on EpCAM for the detection of CTCs, then those cells that undergo epithelial-mesenchymal transition and lost their EpCAM will not be detected. Also EpCAM expression varies considerably in solid tumors due to tumor heterogeneity rendering some CTCs undetectable [14]. Additionally, antibody binding to CTCs leads to activation of some pathways and intracellular instability which affects further protein, genomic and molecular analyses of the CTCs. All those drawbacks lead researchers to develop negative enrichment method to overcome them. The negative enrichment system uses hypotonic lysis of RBCs in addition to removal of WBCs through anti-CD45 antibodies to isolate CTCs. Subtraction enrichment (SE) is another way that has been developed. It differs from usual negative enrichment but falls under the same umbrella and it uses non-hypotonic lysis method to remove the RBCs and relies on the use of multiple anti-WBCs antibodies (conjugated to beads) to remove the WBCs and allows non-disruptive detection of CTCs. To further improve the sensitivity of detecting CTCs with minimal disruption, the Cytelligen system was developed. It combines subtraction enrichment with immunostaining fluorescence in situ hybridization (iFISH) and has proven efficient in detecting CTCs from different tumors. Special probes detecting the centromere in chromosome 8 is one of the probes used in iFISH to help in the detection of CTCs taking in consideration that the large number of cancer cells have heteroploid chromosome 8. Side by side with other immunostaining the isolation and detection of CTCs becomes easier and much more clear [13,15].

The detection of CTCs is improving and developing and it is one of the hot topics that came under focus in the last decade. Researchers worldwide are still working on developing new and improving existing techniques for the detection and enrichment of CTCs. Cell Search system has been approved by FDA for the detection of CTCs in breast cancer, colorectal and prostate cancer [16]. Despite the large number of studies in the field of CTCs, they are still in the beginning of a long path before they become routine tests. One major obstacle is the high cost of the available techniques. Another drawback is the variability in the sensitivity of the detection capability among different techniques and studies. Analysis of CTCs detection in bladder cancer showed that the detection of CTCs in the peripheral blood in patients with lymph nodes metastases fell in the range of (29.1-91%). While it was higher in those with distant metastases (33-100%), and therefore the prognostic role of CTCs cannot be denied and opens the way for a larger role in the future [16].

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