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Current Research in Pharmaceutical Sciences

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crps online CRPS CURRENT RESEARCH IN PHARMACEUTICAL SCIENCES

ISSN: 2250 – 2688

Received: 19/12/2021
Revised: 28/12/2021
Accepted: 31/12/2021
Published: 08/01/2022

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DOI: 10.24092/CRPS.2021.110403

Website: www.crpsonline.com

Quick Response Code:

Evaluating Clinical Applications of Liquid Biopsy by Combining Circulating Tumor DNA and Tumor cells

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ABSTRACT

"Liquid biopsy" concentrates on the identification of 'Circulating Tumor Cells (CTC) as well as 'Cell-free Tumor DNA' (ctDNA) in the systemic circulation of individuals suffering from cancer, has received a lot of interest due to its evident clinical implications for individualized therapy. CTC and ctDNA studies have created fresh diagnostic approaches and are now the foundations of liquid biopsy. The existing study concentrates on cancer diagnoses, prognosis prediction in people having treatable diseases, observing systemic therapy, and patient categorization depending on the identification of aimed therapeutics or resistance mechanisms. Although the use of CTCs and ctDNA for initial cancer diagnosis is gaining popularity, existing techniques experience major challenges in terms of particularity and sensitivity. Prognosis projection in people with the treatable disease so far realized in some cancer entities, especially in breast cancer. Sequential assessments of CTCs or ctDNA can also be used to monitor the progress or non-success of systemic medicines (such as hormone and chemotherapy as well as various targeted therapies).To integrate liquid diagnostic tests into personalized medicine, interventional studies on therapy stratification based on CTC and ctDNA analyses are required.

Key words: Tumor cells, DNA, Circulating cells, Chemotherapy, Liquid biopsy.

1. INTRODUCTION

Initially, in the development and progression of an original tumor, cells are released into the systemic circulation (e.g., prostate, colorectal, breast, or lung cancer). Different methods that take advantage of their physical and biological characteristics can be used to enrich and detect these circulating tumor cells (CTC). For cancer patients, CTC studies are referred to as present time "liquid biopsy".[1] CTC study is an immensely moving field, with more than 17,000 publications in PubMed until December 2015. The "liquid biopsy" was recently used to describe the examination of circulating cell-free tumor DNA (ctDNA) discharged through necrotic or apoptotic cancer cells. [2] Researchers have been able to examine ctDNA within blood plasma for cancer-specific abnormalities thanks to the development of sophisticated molecular tests; as a result, CTC and ctDNA techniques had grown into competitive biological markers. [3] As a result, we believe that the knowledge gained from CTCs and ctDNA is distinct, flattering, and based on circumstance use. [4] The current study focused on the clinical approaches of ctDNA and CTCs in cancer patients as a liquid biopsy after a brief explanation of the biology and detection methods. Tumor cells are discharged into the systemic circulation by the original tumor and metastatic locations. CTCs have a short half-life in the systemic circulation (which is 1 to 4 hours). [5]

It's still a subject of debate in case of the CTC discharge into the systemic circulation is an arbitrary procedure or preordained by a particular biological program. Nonetheless, epithelial tumor cells face tough circumstances in the circulation, and CTCs are likely to experience a tough selecting procedure. [6] These are congruous by the surveillance that programmed cell death CTCs or segmented CTCs are repeatedly seen within the peripheral blood of malignancy patients. [7] The authorization of existing CTCs occurs via eructation towards secondary organs. For instance, a relative examination of CTCs in the peripheral and mesenteric veins in patients with colorectal cancer revealed that the liver apprehends tumor cells discharged from the original malignancy, that also constant with the affluence of animal information on the topic. Since the spreading of cancer cells to distant metastasis sites requires a faithless excursion via vasculature, which is promoted by close interrelation with activated macrophages and platelets. [8,9] As a result, tumor cells form heteroaggregates that assist in endothelial adhesion and hence contribute to metastasis.[10] Furthermore, the migration of metastatic cells in the bloodstream is frequently reliant on chemokine gradients, such as CCR9, CCR7, CCR4, and CXCR4, which guide cancer cells along the vasculature. [12] The CTC identification in patients after some time (months or years) after initial tumor removal suggests that cancer cells can circulate again into the systemic circulation from secondary metastatic locations. [5-12] It is uncertain in what way these CTCs subscribe to metastatic dissemination and development. Preceding research outcome in a mice model indicates that cancer cells could be returning to the originating location, this procedure is known as 'Tumor self-seeding. [13] From the finest information of ours, studies found no clear evidence of this occurring in cancer patients; even so, it is fascinating to record the identification of disseminated tumor cells (DTC) in the bone marrow of breast cancer patients is interconnected not only to metastatic recurrence but also to locoregional recurrence with potentially extra aggressive metastatic variants. [14] More information may be gained from upcoming genetic investigations of DTCs and CTCs as well as metastatic and primary lesions of the same patients.

2. ESSENTIAL IN ctDNA DETECTION

2.1 CTCs

The development of numerous technologies to enrich and identify CTCs has progressed recently, along with the identification and documentation of novel CTC markers. [15] It is crucial to emphasize that the CTC research concentrated on the biology of tumor propagation, in certain on EMT influencing tumor cells with possible stem cell-like features. [16] As a result, various groups have established novel technologies to choose and recognize CTCs that have undergone EMT. [17] CTC tests often begin with an enrichment phase, which boosts the focus of CTCs through many log units and makes single tumor cells easier to detect. [18] CTC can then be detected in a variety of methods. In theory, CTCs can be enriched favorably or pessimistic based on biologic qualities (e.g., expression of protein markers) or based on physical properties (i.e., deformability, density, size, or electric charges). A combination of physical and biological features in the same device can also be used to generate positive or negative CTC enrichment. Immunologic, molecular, and functional tests can then be used to identify CTCs. [19] Many research groups previously concentrated on functional assessments employing CTC cultures/cell lines and xenografts. [20] Drug susceptibility may be tested using these *in vitro* and *in vivo* models.

2.2 ctDNA

Extremely sensitive and particular techniques for detecting ctDNA, such as TamSeq, BEAMing Safe-SeqS, and digital PCR to identify single-nucleotide mutations in ctDNA or whole-genome sequencing to determine copy-number modifications, have been developed. In general, innovations could be separated into aimed perspectives that target to recognize mutations in a preconceived arrangement of genes (for example, KRAS in the context of EGFR inhibited by antibodies) and untargeted strategies that intend to screen the genome and discover fresh genomic abnormalities, such as those which confer resistance to a particular target therapy. [21] The limitations and strengths of certain technologies have lately been discussed. [22] Despite concerted attempts to raise detection limits, targeted techniques show higher analytic sensitivity than untargeted procedures. [23] Recently, ultrasensitive technologies have emerged that can identify the tiniest quantities of ctDNA in the 'sea' of typical cfDNA, which is necessary for the initial identification of tumor or insignificant residual illness. [24]

3. SCREENING AND EARLY DETECTION OF CANCER

In most cancer screening studies, patients with cancer are compared to healthy controls (healthy individuals or patients with benign diseases). Cohort studies are inconvenient because they require large research groups and long follow-up periods. To expedite the validation process, focusing on individuals with a greater possibility of growing tumors [for example, COPD patients are a good approach].

3.1 CTCs

According to Ilie and colleagues, CTCs might be observed in individuals with COPD who did not have clinically evident lung cancer. [25] In this research, there were 168 patients with COPD (68.6%) and 77 persons without COPD (31.4%) in the trial, including 42 control smokers and 35 nonsmoking healthy people. Low-dose spiral CT was used to monitor COPD patients once a

year. CTCs were discovered in 3% of COPD patients (5 of 168 patients). The yearly CT-scan screening of CTC-positive COPD patients found lung nodules 1 to 4 years after CTC diagnosis, resulting in rapid surgical resection and histopathologic diagnosis of initial-stage lung cancer, although no CTCs were found in the controlled smoking and nonsmoking healthy persons. Curiously, CTCs found in COPD patients displayed a heterogeneous expression of epithelial and mesenchymal markers. These early findings must be validated in larger cohorts, and the source which might lead to unspecific observations in non-cancer patients, such as the discharge of epithelial cells into the systemic circulation of patients with inflammatory bowel diseases, [26] must be discovered.

3.2 ctDNA

The diagnosis of cancer by monitoring ctDNA has gotten a lot of attention. [27,28] The most challenging technical problem is detecting extremely small levels of ctDNA in blood samples with varying amounts of cfDNA, as well as selecting the appropriate panel of cancer-specific genetic abnormalities. The Johns Hopkins researchers recently evaluated the potential of ctDNA to detect tumors in 640 individuals with diverse cancer types using digital polymerase chain reaction-based technologies.[29] The ctDNA was detected in Only 48 percent to 73 percent of individuals with localized cancers, such as breast adenocarcinoma, gastroesophageal cancer, colorectal cancer, and pancreatic cancer. Even though, these identification values are insufficient for initial cancer diagnosis. The ctDNA was often found in individuals who did not have recognizable CTCs. [30] CTCs, on the other hand, had not improved, but rather resolved in platelets with a large influence of leukocytes, a method with a low sensitivity that is rarely utilized in current CTC diagnoses. Many teams are competing for much more sensitive ctDNA technologies at present. For instance, the group of Maximilian Diehn's has created a novel method known as "cancer personalized profiling by deep sequencing"(CAPP-Seq). [31-34] CAPP-Seq was carried out for non–small cell lung cancer (NSCLC) using a design that covered many classes of somatic changes and found mutations in >95 percent of tumors with 96 percent accuracy for mutant allele fractions as low to around 0.02 percent. Although ctDNA was found in most of the patients with stage II-IV cancer, hardly 50% of individuals with stage I NSCLC was diagnosed. The amount of ctDNA in the cfDNA fraction was found to be roughly 10-fold lower in patients with stage I malignancies than in patients with more advanced disease. This variation is not unexpected and it could be seen in other tumor types as well. Thus, furthermore, technological advancements, such as the capacity to analyze greater blood volumes, are necessary to achieve acceptable sensitivity for initial cancer diagnosis. [35-40]

4. CONCLUSION

The CTC and ctDNA analyses have built the latest diagnostic routes and are currently the pillars of liquid biopsy diagnostics. In the future, to what extent may they replace tumor biopsies is a subject that remains conjecture. A liquid biopsy might provide an alternative for the primary diagnosis of malignancies that are difficult to biopsy, such as lung cancer, as well as restaging/molecular analysis of metastatic lesions. Furthermore, liquid biopsy diagnostics may aid in focusing the existing cancer screening techniques on individuals at higher risk, which would be reducing health consequences (for example radiation in mammography) and healthcare expenditures. Despite few promising initial results and tremendous interest from the public press and diagnostic organizations (cancer diagnosis with a drop of blood), initial cancer detection confronts major sensitivity and specificity challenges. On the other hand observing, ctDNA as well as CTCs throughout systemic therapy in patients with cancer, is an approach that may be easy to accomplish and bring closer to clinical practice. Several studies have shown that analyzing ctDNA and CTCs for mutations conferring responsiveness or resistance to targeted therapy is conceivable in cancer patients with the final stage. Various testing for mutations in genes encoding therapeutic targets and the equivalent resistance genes will be conducted in the upcoming future. NSCLC, in particular, is a fascinating tumor category for this application since many mutations leading to specific targeted therapy in small cohorts of responding individuals have recently been revealed, and biopsies are difficult to obtain in a significant number of patients.

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