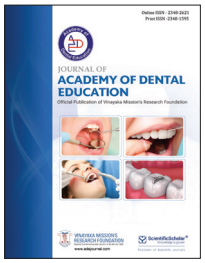
ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

https://adejournal.com/

Journal of Academy of Dental Education



Review Article

Liquid biopsy – An advance diagnostic tool for tracking of tumor cells

K. Ananya[1], Kumar Appysamy[1]

[1]Student, [2]Assistant professor, Department of Oral Medicine and Radiology, Vinayaka Mission's Sankarachariyar Dental College, Vinayaka Mission's Research Foundation (DU), Salem, Tamil Nadu, India.

***Corresponding author:**
Kumar Appysamy,
Assistant Professor, Department of Oral Medicine and Radiology, Vinayaka Mission's Sankarachariyar Dental College, Vinayaka Mission's Research Foundation (DU), Salem, Tamil Nadu, India.

drkumar@vmsdc.edu.in

Received : 26 July 2022
Accepted : 29 April 2023
Published : 14 June 2023

DOI
10.25259/JADE_33_2022

Quick Response Code:

ABSTRACT

An eminent tool is required for easy detection of disease aggressiveness, identification, and prognosis of treatment. This shows the importance of a non-invasive procedure called liquid biopsy. Liquid biopsy is a liquid biomarker that identify the tumor markers from human body fluids. The small amount of tissue obtained by needle biopsies may not represent the most aggressive subclones and some remote sites cannot be reached out by needles. Here is a review that will describe and compare and contrast sources such as circulating tumor cells, cell-free DNA, exosomes role in the liquid biopsy field.

Keywords: Liquid biopsy, Exosomes, Circulating free DNA, Circulating tumor cells

INTRODUCTION

Traditional "solid biopsy," which does not always be carried out specific circumstances or in real time, offers a non-invasive alternative in the form of liquid biopsy. Despite these many benefits, there are also a few drawbacks, including a lack of agreement on identification techniques, difficulties in analyzing the abundance of data, and a lack of evidence based in evidence-based therapy. For the diagnosis, frequent monitoring, assessment of prognosis, and treatment planning of tumor mass, circulating tumor cells (CTCs), cell-free DNA (cfDNA), and exosomes are used. We are describing in this review and comparing the source, detection technology, characteristics, and recent state of these substances. Newer technological and molecular advances, which greatly facilitate the use of microfluidics.[1]

CTC DETECTION

CTCs were identified by Ashworth in 1860 s during microscopic study of peripheral blood smear. CTCs can shed into vasculature both actively and passively by external forces or friction.

Similar to CTCs, cfDNA is also shed passively or actively released and cfDNA levels are extremely low in healthy individuals, whereas in malignant tumors, chronic inflammation it will be the reverse. CTC detection in peripheral blood is challenging because of its 8th or 7th differences in the blood cells in circulation that are both normal and pathological.[2]

Early method of CTC identification was by reverse transcription polymerase chain reaction (RT-PCR), but we cannot remove the fact that this method can also show false positive results. These studies suggest that this can be from ineffective cellular components or through the

amplification of generic genomic material and lack of direct visualization is also a drawback of RT-PCR.

At present, methods that can clearly see the target cells are used in the majority of laboratories. On the surface of CTCs, epithelial surface antigens are targeted and "picked out" from the sample of blood using positive selection techniques molecule on the epithelial cells, or Ep CAM, is one among the most often used indicators. The CELLSEARCH system is the only CTC detection method that has received U.S. approval, and numerous clinical trials have shown the predictive utility of knowing how many CTCs are present using this method.[3]

Cytokeratin is another well researched CTC identifying marker (CK) (Cytokines). CK 8, 18, 19, and 20 are the cytokeratin subfamily CTCs that are most frequently targeted. However, not all CTCs may have cytokeratin.

CTC heterogenicity leads to unbiased using multiple markers testing to detect CTC. Removal of normal cells in the blood is done to proportionally enlarge the blood sample and it is contrary to the positive selection technique which is then considered as negative depletion technique.

The CTCs are cells with many marker stains. The ability to manually identify the cells is facilitated by the appearance of a significant reduction in the cell number.

Leukocytes marked by anti-CD45 are detached with immunomagnetic separation after red blood cell lysis in the first step. Elimination of greater part of normal blood cells and diamidino-2-phenylindole labeling of the remaining ones and immunocytochemical staining.

Below is an illustration of confocal microscopy and immunostaining features of circulating tumor cells [Figure 1a].[1]

CF DNA

Cf DNA being another marker but it will be more challenging to obtain this rather than whole blood will be collected in ethylenediamine tetraacetic acid (EDTA)-coated tubes to collect CTCs because of their small number within the large amount of normal DNA tissues. After spinning to remove the cells, the tubes will be rinsed with EDTA using commercially available kits cf DNAs are extracted, the other most challenging is the identification of mutant cells and hence to avoid the drawbacks by using RT-PCR, next generation sequencing (NGS) technologies are applied that With the help of deep sequencing, safe-seq, TAM-seq, and ampli-seq, the NGS develops individualized cancer gene maps and defines them.

EXOSOMES

Exosomes which are present mostly in body fluids like plasma, urine, saliva, ascites, bronchoalveolar, and lavage fluid and they are large multivesicular bodies (MVBs) produce membrane-bound particles, which are then released into the extracellular environment by the production of MVBs with plasma membrane [Figure 1b].[1] These will remove unnecessary molecules but have a higher effect on tumor metastasis. Exosomes are excellent biomarkers.[2,4]

Diagram showing the origin of CTCs, exosomes, and cfDNA in blood. Healthy, inflammatory, or tumor tissue going through apoptosis or necrosis can all release cfDNA. In addition, tumor development or mechanical forces after surgery can actively push tumor cells into the bloodstream. Cells of the tumor can enter the circulatory system passively. Platelets can protect these tumor cells. In addition, a variety of cell types, including blood cells, normal cells, and tumor cells, can produce circulating exosomes (even platelets).[5]

Centrifugation, which is the most practical approach, is the foundation of conventional exosome separation. They can be separated using centrifugation combined with size exclusion chromatography, polymer-based precipitation, and immunoaffinity purification beads[6] with magnets to isolate highly purified exosomes ultracentrifugation-size exclusion chromatography uses a solid matrix; nevertheless, it is challenging to pull out contaminating proteins. In 2009, System Biosciences developed polymeric precipitation, which they market as Exo Quick. This technology works by catching exosomes between 60 and 150 nm in size in 30 min, producing more exosomes than ultracentrifugation. However, it is unable to prevent the mixing of non-exosomal contents with particles of a comparable size, such as apoptotic debris or other kinds of micro vesicles.

In addition, exosomes can be extracted utilizing an immunoaffinity technique for focusing on traditional exosomes, CD81+ CD63+, or CD9+, while keeping their bioactive components for further study. Only a small-volume sample can be used for the immunoaffinity technique, which isolates exosomes with certain indicators that can restrict the experimental results. Exosomes contain a wealth of RNA, it will be essential to use electron microscopy and can be analyzed by qPCR and NGS.[7]

All these were non-invasive methods, but CTCs taken from peripheral blood, cfDNAs from serum or plasma and exosomes from plasma and other body fluids.

CTCs are label dependent by CELL SEARCH and cfDNAs PCR was as exosomes by centrifugation, immunoaffinity methods, and chromatography. In both CTCs and exosomes the non-coding RNA, DNA, and protein, all could be evaluated.[8]

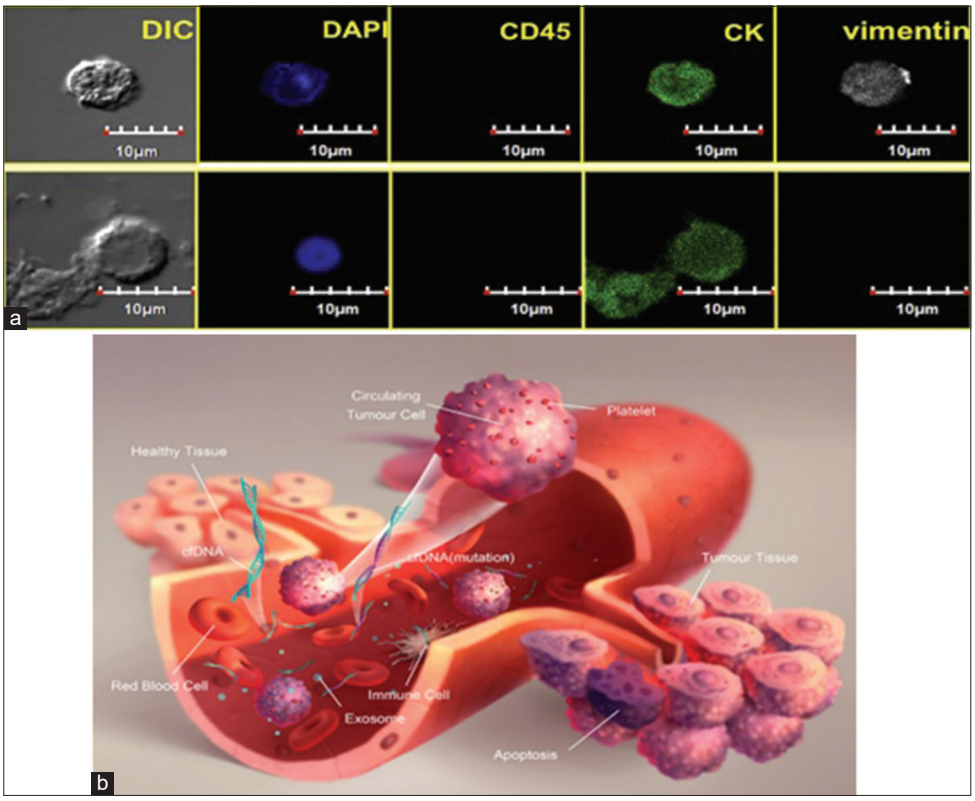


Figure 1: (a) Circulating tumor cell multi-marker staining in SCCHN. An immunocytochemical marker examination of a CTC. The cells are DAPIb (40,6-diamidino-2-phenylindol) and CD 45, respectively. Both have cytokeratin (CK) positivity, but only the first has vimentin positivity, which indicates mesenchymal tissue.[2] (b) Exosomes.[1]

An evaluation about the three liquid biopsy techniques (CTCs, cfDNA, and exosomes).

CONCLUSION

Liquid biopsy being a new methodology plays an important role in the non-invasive cancer treatment and is more precise and offers significantly more details on the prognosis and treatment improvement than conventional methods.[9] However, certain disadvantages were in case of CTCs the instability of monitoring early stage of cancer, cfDNAs where difficult to determine cancer specific markers and no RNA or protein being evaluated, exosomes show the lack of specific markers, lack of large clinical trial to validate the reliability.[10] However, the cancer screening methods such as mammogram and pap test have limited sensitivity and is applicable only to certain unique cancer type whereas liquid biopsy is a large scale cancer screening tool and more studies and research is required for the growth of this advanced diagnostic tool and more work is needed to verify and validate cfDNA biomarker in larger patient cohorts.[11]

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. McMullen KP, Chalmers JJ, Lang JC, Kumar P, Jatana KR. Circulating tumor cells in head and neck cancer. Advancing Research Evalving Science. 2016.
2. Zhang W, Xia W, Lv Z, Ni C, Xin Y, Yang L. Liquid biopsy for cancer: Circulating tumor cells, circulating free DNA or exosomes? Cell Physiol Biochem 2017;41:755-68.
3. Augustine D, Patil S. Circulating tumor cells in oral cancer. J Int Oral Health 2015;7:i-ii.
4. Gröbe A, Blessmann M, Hanken H. Prognostic relevance of

circulating tumor cells in blood and disseminated tumor cells in bone marrow of patients with squamous Cell carcinoma of the oral cavity. Clin Cancer Res 2013.
5. Kulasinghe A, Perry C, Jovanovic L. Circulating tumour cells in metastatic head and neck cancers. Int J Cancer 2014;136:2515-23.
6. Brock G, Castellanos-Rizaldos E, Hu L. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res 2015;4:280-90.
7. Chen M, Zhao H. Next-generation sequencing in liquid biopsy: Cancer screening and early detection. Hum Genom 2019;13:34.
8. Schobers G, Koeck R, Pellaers D. Liquid biopsy: State of reproductive medicine and beyond. Hum Reprod 2021;36:2824-39.
9. Ou SH, Nagasaka M, Zhu VW. Liquid biopsy to identify actionable genomic alterations. Am Soc Clin Oncol Educ Book 2018;38:978-97.
10. Nonaka T, Wong DT. Liquid biopsy in head and neck cancer: Promises and challenges. J Dent Res 2018;97:701-8.
11. Jain S, Lin SY, Song W, Su YH. Urine-based liquid biopsy for nonurological cancers. Genet Test Mol Biomarkers 2019;23:277-83.

How to cite this article: Ananya K, Appysamy K. Liquid biopsy – An advance diagnostic tool for tracking of tumor cells. J Academy Dent Educ 2023;9:31-4.