



## Circulating tumor DNA in liquid biopsy: Current diagnostic limitation

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### Abstract

With the rapid development of science and technology, cell-free DNA (cfDNA) is rapidly becoming an important biomarker for tumor diagnosis, monitoring and prognosis, and this cfDNA-based liquid biopsy technology has great potential to become an important part of precision medicine. cfDNA is the total amount of free DNA in the systemic circulation, including DNA fragments derived from tumor cells and all other somatic cells. Tumor cells release fragments of DNA into the bloodstream, and this source of cfDNA is called circulating tumor DNA (ctDNA). cfDNA detection has become a major focus in the field of tumor research in recent years, which provides a new opportunity for non-invasive diagnosis and prognosis of cancer. In this paper, we discuss the limitations of the study on the origin and dynamics analysis of ctDNA, and how to solve these problems in the future. Although the future faces major challenges, it also contains great potential.

**Key Words:** Cell-free DNA; Circulating tumor DNA; Liquid biopsy; Cancer; Diagnosis; Prognosis

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**Core Tip:** Tumor liquid biopsy based on cell-free DNA detection has become a major hotspot in the field of tumor research in recent years. Circulating tumor DNA (ctDNA) is a DNA fragment that breaks down from cells in primary tumors or even new tumors formed by metastasis, and enters the peripheral circulation. ctDNA analysis provides a non-invasive method for cancer detection and monitoring, which is important for the management of clinical patients.

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## TO THE EDITOR

We read with interest a basic study by Terasawa *et al*[1], who assessed the origin of circulating tumor DNA (ctDNA), elucidated the dynamics of ctDNA levels, assessed ctDNA levels using xenografted mice after treatment, and determined whether tumor size and tumor invasion correlated with ctDNA levels. ctDNA is the cell-free DNA (cfDNA) of tumor origin. We congratulate the authors on their work and contributions in this field. At present, it is not clear what factors (*e.g.*, tumor size and tumor invasion) affect the levels of ctDNA, and there are always questions about the origin of ctDNA. It is crucial to address these issues in order to make ctDNA an effective and practical biomarker for liquid biopsy in clinical practice, and to fully tap into its potential. In this regard, Terasawa *et al*[1] explored the origin and dynamics of ctDNA with potential contributions to this issue.

Using BALB/c-nu/nu mice inoculated with the TE11 cell line, the authors established tumor xenotransplantation. In order to perform ctDNA analysis, several groups of mice were killed at appropriate time points after xenotransplantation. The findings shed light on the origin and dynamics of ctDNA, suggesting that tumor size is an important factor. Moreover, the results of this study showed that when the tumor was completely removed, the ctDNA disappeared after  $\geq 1$  d. Although the results were satisfactory, in future studies, researchers need to further understand the biological characteristics of ctDNA (such as ctDNA release and clearance mechanisms) and improve the sensitivity of ctDNA detection. The authors also pointed out in their discussion that, with respect to residual tumors, although all mice underwent pathological autopsies in this study, it was not possible to completely identify residual tumors, which is essentially a sensitivity issue of ctDNA detection.

In the early stage of cancer, the content of ctDNA in plasma is low, and the number of somatic mutations based on a certain mutation is even lower[2]. The detection and analysis of ctDNA brings many obstacles to clinical cancer detection, especially early detection. Previous research has shown that only when the ctDNA content in cfDNA is  $\geq 10\%$ , accurate information of tumor can be obtained[3]. However, with the exception of some patients with advanced tumors who have high amounts of ctDNA in plasma, the ctDNA content in most patients with tumors does not meet this standard[4]. This makes detecting ctDNA difficult, especially in the early stages of cancer. At present, increasing the depth of sequencing is mainly used to improve the sensitivity and accuracy of ctDNA detection, but increasing the depth of sequencing may bring false positive results, because cfDNA of non-tumor origin may also carry various tumor-associated mutations[5]. These problems have always limited the clinical application of ctDNA liquid biopsy.

Due to these shortcomings, in addition to detecting tumor mutations, the field of liquid cancer biopsy is actively exploring non-invasive detection methods based on other characteristics of plasma cfDNA, such as fragment size[6,7], methylation[8-11], end coordinates[12], and chromatin accessibility of cfDNA[13], which are currently being studied. Renaud *et al*[14] used fragment length characteristics of cfDNA to diagnose metastatic castration-resistant prostate cancer. Heeke *et al*[15] used cfDNA methylation data to classify small cell lung cancer (SCLC) and discovered SCLC subgroups based on DNA methylation data, which can serve as potential biomarkers to guide patient classification and clinical precision treatment. The United States Food and Drug Administration has approved the first blood-based colorectal cancer (CRC) screening product, *SEPT9* gene testing[16,17]. The study by Jiang *et al*[12] showed that cancer-related end coordinates in plasma cfDNA can be applied to liver cancer early detection. Using plasma cfDNA and protein markers, Cohen *et al*[18] developed CancerSEEK, which can detect eight common cancers of the lung, colorectum, liver, stomach, esophagus, pancreas, breast, or ovary. In my previous studies, cfDNA chromatin open state was used to distinguish patients with esophageal cancer from individuals without cancer[13]. Li *et al*[19] developed a multimodal epigenetic sequencing analysis (MESA) method based on cfDNA, MESA, for the detection of CRC, which can capture and integrate various epigenetic features in cfDNA, such as cfDNA methylation and nucleosome occupancy. These studies open up new ideas for cfDNA-based liquid biopsy in non-invasive diagnosis. Many studies have shown that the total level of cfDNA is correlated with tumor staging[20,21], suggesting that cfDNA has prognostic potential. In addition, cfDNA can serve as a real-time indicator of therapeutic efficacy, allowing for earlier observation of therapeutic effects than clinical trials, thanks to its short half-life[22-24].

At present, research on improving the sensitivity of ctDNA detection mainly focuses on *in vitro* sequencing and analysis, such as detecting multi somatic mutations and integrating DNA methylation or fragmentation patterns[25,26]. An inherent challenge faced by all these methods is the low amount of ctDNA in the collected blood samples, which limits sensitivity. Increasing the volume of the blood sample can improve sensitivity of the detection. However, for patients who are weak or ill, it is impractical. In addition, some have proposed methods that are closer to tumor sampling or increase tumor DNA loss. These methods also have certain limitations, such as requiring prior knowledge of tumor location, being limited to specific primary tumors, requiring invasive surgery, and being costly. Recently, a research team has developed two intravenous inducers to improve the recovery of ctDNA in blood collection, which can temporarily delay the clearance of cfDNA in the body[27]. Uptake by liver-resident macrophages and degradation by circulating nucleases are two natural mechanisms by which cfDNA is cleared. In this study, the authors developed two intravenous inducers that can act on these mechanisms and improve the recovery rate of ctDNA when used 1-2 h before blood withdrawal. Although this strategy can significantly improve the sensitivity of ctDNA detection in preclinical models, the

safety and tolerability of the formulation, as well as whether this effect can be translated into human patients, remain to be determined.

With the rapid development of science and technology, cfDNA is rapidly becoming an important biomarker for tumor diagnosis, monitoring and prognosis, and this cfDNA-based liquid biopsy technology has great potential to become an important tool of precision medicine. Despite its enormous potential, there is still a long way to go. Research on blood sample collection, cfDNA isolation, and Next-Generation Sequencing data analysis is currently limited and needs to be focused on in the future. In addition, the biological characteristics of ctDNA are also of great research value. Meanwhile, it is necessary to confirm the effectiveness and practicability of cfDNA as a diagnosis/prognosis marker to further promote the clinical application of cfDNA-based liquid biopsy.

## FOOTNOTES

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