



Fragmentation patterns of circulating cell-free DNA demonstrate biomarker potential for human cancers

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Introduction

The often-dismal clinical outcomes for most cancers are due to the fact that cancer patients diagnosed at advanced stages would miss the opportunities for curative treatments or effective treatments no longer available for these patients (1). Therefore, lacking clinically proven and convenient tools for early detection of cancers has been one major contributor to the poor clinical outcomes for many human cancers. In particular, considering their non-invasiveness and clinical relevance, the past decade has witnessed significant efforts of exploring patient-derived biofluids (i.e., liquid biopsy) in cancer biomarker discovery. Of great interest to the research community has been the potential utility of circulating cell-free DNA (cfDNA) in the blood as a non-invasive biomarker target. Specifically, cfDNA in patient-derived blood contains DNA fragments released from the tumor cells or from such cellular mechanisms as apoptosis and necrosis (*Figure 1*). Importantly, patient-derived cfDNA contains genetic and epigenetic information (e.g., mutations, cancer-associated cytosine modifications) from the tumor origin, which has been exploited to identify biomarkers for various human cancers, showing tremendous promise as a non-invasive biomarker target (3-6).

In contrast to previous studies that target genetic or epigenetic markers in cfDNA, recently, Cristiano and coworkers reported a new approach that exploits the cfDNA fragmentation patterns for cancer diagnosis (2). Their approach identified altered fragmentation profiles across the genomes in 236 patients with a variety of cancers,

compared to 245 healthy individuals whose fragmentation patterns reflected nucleosomal patterns of white blood cells. Findings from Cristiano *et al.* opened up new opportunities of using cfDNA as a non-invasive tool for cancer biomarker discovery by targeting a novel molecular feature in patient-derived cfDNA, showing the promise of more innovative applications of cfDNA in precision oncology.

Liquid biopsy as a promising target for cancer biomarker discovery

For cancer biomarker discovery, tissue biopsy has been the “gold-standard”, because tissue samples are believed to reflect molecular features of tumors, e.g., genetic and epigenetic features, as well as cancer type specificity (4). However, due to the invasive nature, the procedure of obtaining tissue biopsy can be accompanied by clinical risks such as bleeding and infection (7). In addition, owing to the intra-tumor heterogeneity, a hallmark of human cancers, single site biopsies may only provide limited information of the whole tumor (8). Thus, multi-site tissue biopsies, which could be difficult to obtain because of tumor accessibility (9,10), may be required for accurate pathological confirmation and diagnosis. Furthermore, continuous monitoring of disease progression is crucial during treatment (e.g., surveillance of therapeutic response). However, it may not be always clinically feasible for routine tumor biopsies along the treatment course for patients (11).

In contrast, liquid biopsy, such as cfDNA that can be isolated from the plasma of peripheral blood, is emerging

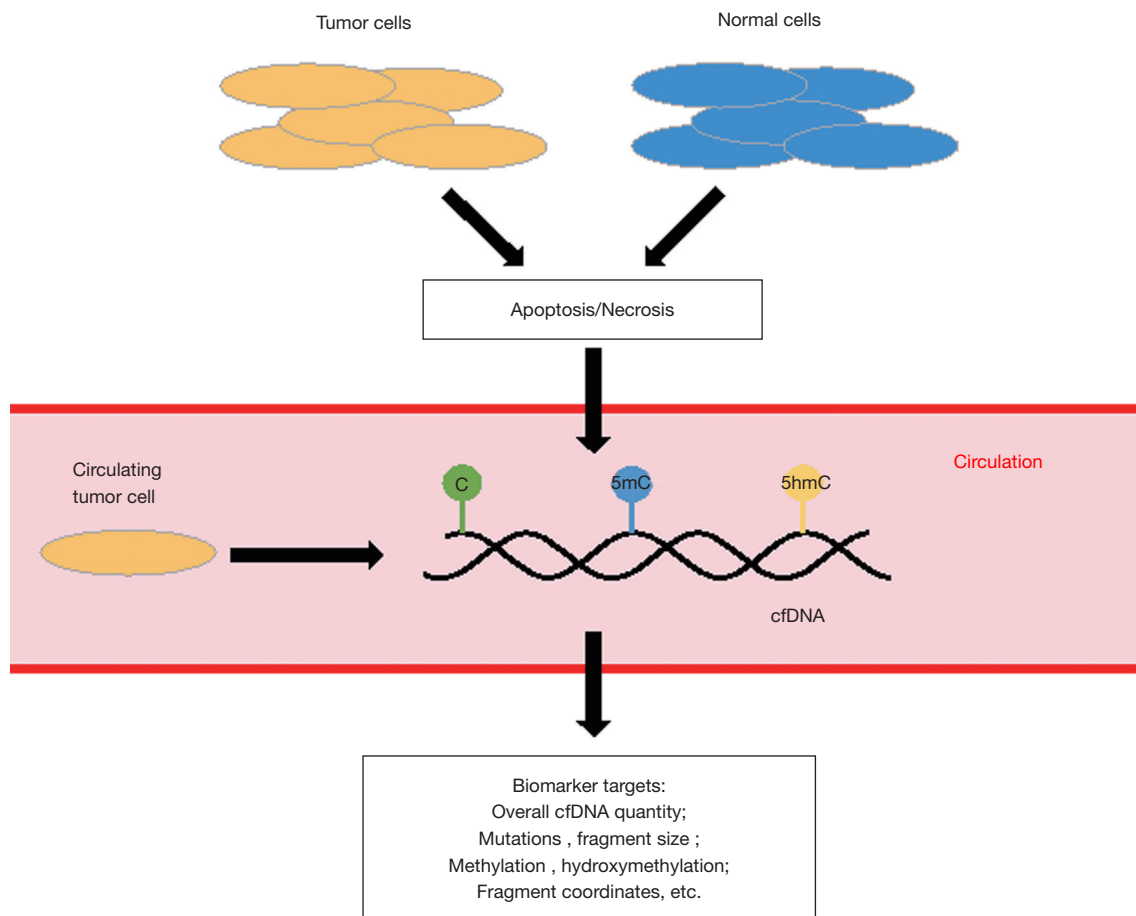


Figure 1 Biomarker targets on circulating cfDNA. Circulating cfDNA, originated from DNA fragments released from tumor cells or from apoptosis and necrosis, contains genetic and epigenetic information of the tumor origin. Cristiano *et al.* (2) exploits a new feature of the cfDNA, the fragmentation patterns, for cancer biomarker discovery. 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; cfDNA, cell-free DNA.

as a non-invasive and clinically convenient tool for cancer biomarker discovery, with potential clinical applications in early detection, diagnosis, and prognosis. The majority of previous studies have explored genetic or epigenetic markers in cfDNA for cancer biomarker discovery, because cfDNA is believed to contain genetic and epigenetic information from the tumor origin. Recent cfDNA-based studies suggested that cancer-specific genetic alterations could be used for cancer early detection and disease surveillance using this non-invasive target (12-14). For example, whole-genome sequencing (WGS) of cfDNA has been used to identify chromosomal or genetic abnormalities associated with cancer patients (15-18). However, detecting

such chromosomal alterations or single genetic alterations may be extremely challenging owing to the small number of abnormal chromosomal changes, the heterogeneity of tumors, and the small amount of tumor-derived cfDNA in the plasma (13). Based on the hypothesis that detection of a larger number of alterations in cfDNA may be more sensitive for cancer detection, a recent research by Cristiano *et al.* developed an approach called the “DNA evaluation of fragments for early interception” (DELFI), which aimed to detect the abnormalities of cfDNA fragmentation profiles at genome-wide level for various cancer patients and use this novel molecular feature of cfDNA in cancer biomarker discovery (2).

Cancer detection taking advantage of aberrant cfDNA fragmentation profiles in the blood

Technically, Cristiano *et al.*'s method was based on the low-coverage WGS of cfDNA isolated from the plasma. Coverage and size distribution of cfDNA fragments in non-overlapping 5-megabase (Mb) windows that covered the genome were examined for healthy individuals and cancer patients. The genome-wide fragmentation pattern from an individual was categorized for cancer diagnosis and tumor tissue of origin identification using machine learning. They have found that the length of cfDNA fragmentation derived from cancer patients were more variable than that from healthy individuals ($P < 0.001$). Thus, they focused on the fragmentation size of cfDNA in their analysis. Fractions of small cfDNA fragments (100–150 bp) to larger cfDNA fragments (151–220 bp) in 504 slide windows of 5 Mb across the genome in a position-dependent manner were obtained for each sample and defined as the genome-wide fragmentation profiles. Healthy individuals showed similar genome-wide profiles, which were highly correlated with lymphocyte nucleosomal DNA fragmentation profiles and nucleosome distances, suggesting that fragmentation patterns of cfDNA derived from healthy individuals were likely the result of nucleosomal DNA patterns that reflected the chromatin structure of normal blood cells. In contrast, patients with cancer showed several distinct genomic differences from healthy individuals, with variable fragment sizes at different regions. Interestingly, the fragmentation profiles in patient-derived cfDNA were significantly correlated with mutant allele fractions of the levels of *EGFR* or *ERBB2* for patients undergoing anti-*EGFR* or anti-*ERBB2* treatment, suggesting its potential as a cancer management tool.

By integrating the copy number changes from chromosomal arm features and mitochondrial copy number changes into the DELFI approach, Cristiano *et al.* obtained a score that could be used for overall cancer detection, achieving 80% sensitivity, 95% specificity, and an AUC (area under the curve) of 0.94 (95% CI: 0.92–0.96), with an AUC of at least 0.92 for each tumor stage. The AUCs for distinguishing seven cancers (e.g., pancreatic, breast, bile duct, colorectal, gastric, lung, and ovarian cancer) from healthy individuals ranged from 0.86 to 1. Additionally, the performance of the DELFI approach was further improved by combining with mutation detection in cfDNA.

Moreover, since cfDNA can be originated from tumor tissues, reflecting the genetic and epigenetic alterations of tumor tissues (19), cfDNA fragmentation profiles may

reveal regional differences between tumors. Using machine learning, Cristiano *et al.* were able to estimate the tissue of origin of circulating tumor DNA with 75% (95% CI: 69–81%) accuracy, suggesting its potential application in a pan-cancer detection algorithm.

Conclusions

Fragmentation profiling in circulating cfDNA introduced in Cristiano *et al.* represented one of the latest efforts of exploiting molecular features in cfDNA for cancer biomarker discovery and provided an alternative non-invasive diagnostic tool that could improve early detection of human cancers, thus showing its potential as a screening and management tool for precision oncology. Considering its potential clinical impact, we would recommend several directions for future studies. Firstly, extensive validation using clinical samples from patients with confounding diseases (e.g., liver cancer *vs.* liver cirrhosis) will help establish the sensitivity and clinical utility of the DELFI approach. Secondly, it will be of interest for future studies of larger sample sizes to comprehensively evaluate whether the DELFI approach is informative for tumor stages and tumor malignancy, or has prognostic value. Thirdly, by focusing on the fragmentation patterns, the DELFI approach understandably missed opportunities to utilize other important molecular features of cfDNA (e.g., epigenetic markers). For example, considering recent progress in exploiting novel epigenetic markers in cfDNA, such as 5-hydroxymethylcytosines, in biomarker discovery for human cancers (3,20,21), integrating both the fragmentation patterns and epigenetic features of cfDNA could possibly enhance our understanding of the underlying mechanisms of molecular alterations in the cancer genome and epigenome, as well as improve the detection performance. In summary, Cristiano *et al.* provided exciting new progress in this emerging area with potentially tremendous impact on clinical oncology. Though promising, applying this novel approach in a clinical setting is still challenging and has a long way to go. Extensive validation and trials will be needed for the ultimate application of the DELFI approach, possible in combination with other approaches, in the clinic. We are closer to the goal of precision oncology by another important step.

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Footnote

Conflicts of Interest: W Zhang is a shareholder of Shanghai Epican Genetech Co. Ltd., which develops epigenetic cancer biomarkers. Z Zhang has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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