# Circulating tumor DNA: a resuscitative gold mine?

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Tumor tissue specimens obtained by surgical or biopsy procedures remain to be the only source of the tumor DNA required for the molecular and genomic analysis of human cancers. However, such tumor tissue sampling has several clinical limitations: there are invasive procedures; a single tumor biopsy cannot reflect the intratumoral heterogeneity or the difference between primary tumor and metastasis, and it is difficult to sample frequently. Alternatively, blood-based biomarkers could overcome these limitations. However, only several clinically proven protein markers are commonly used in the diagnosis and prognosis of human cancer. In addition, these protein markers are often secreted not only by tumor cells but also by normal or benign tumor cells.

Circulating cell-free DNA (cfDNA) is an alternative blood-based biomarker, and has been under intense investigation as a non-invasive biomarker for the real-time monitoring of tumor patients in the past decades. cfDNA was first reported in human plasma by Mandel and Metais in 1948 (1). However, their study attracted little attention at that time. Until 1977, Leon *et al.* found increased cfDNA levels in the serum of cancer patients (2). It took about 10 years before Stroun *et al.* proved the presence of neoplastic characteristics plasma DNA (3), and two other studies demonstrated that tumors can shed DNA into the circulation (4,5). Many subsequent meritorious studies revealed the quantitative or qualitative changes of cfDNA in cancer patients and suggested cfDNA as a promising novel biomarker for tumor diagnosis and prognosis. For example, concentration (6,7), stability (8,9), or tumor-specific changes (such as mutation of oncogenes and tumor suppressors, DNA methylation and microsatellite instability) of cfDNA have been reported as promising cancer biomarkers (10-14).

Circulating tumor DNA (ctDNA) was originated from apoptotic or necrotic tumor cells or actively secreted by tumor cells, which reflected tumor-specific changes in the primary or metastatic tumor tissues. Because all types of cancers harbor somatic genetic alterations, ctDNA is a candidate surrogate for the entire cancer genome. ctDNA attracts extensive attention for its promising application in tumor diagnosis, prognosis, and monitoring therapy response. The potential clinical application of ctDNA is often referred to as a "liquid biopsy" that was used to define circulating tumor cells (CTCs) (15,16).

A recent study conducted by Bettegowda and colleagues detected ctDNA in 640 patients with different types of cancers (17). Their results showed that ctDNA was detectable in more than 75% of patients with certain types of cancers (advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers), whereas ctDNA was negative in more than half of patients with primary brain, renal, prostate, or thyroid cancers. In addition, their work also confirmed the value of ctDNA in the tumor targeted therapy using colorectal cancer (CRC) as a model. As we know, it is the first report that systemically evaluates the utility of ctDNA in a large cohort of patients with different types of cancers.

Their work provides solid supporting for the future utility of ctDNA in cancer described as followed.

## ctDNA as a diagnostic marker

The basic consideration of ctDNA is its promising application as an invasive diagnostic marker. Although many groups have evaluated the potential diagnostic value

of ctDNA in different types of cancers, it is difficult to compare these results for varied methods applied in these studies. Bettegowda *et al.* try to solve it. Their aim was to compare the potential value of ctDNA for different types of cancers using relative standardized and comparable methodologies. Firstly, they have enrolled 136 metastatic tumors from 14 different tissues, as well as 41 patients with primary brain tumors. Except that more than 50% of patients with medulloblastomas, metastatic kidney, prostate or thyroid cancers and more than 90% of gliomas patients, showed undetectable ctDNA, most patients with other types of cancer harbored detectable ctDNA levels. Notably, although ctDNA was detectable in most patients with metastatic cancers, the concentrations of ctDNA varied among patients even with the same tumor type. They then evaluated ctDNA in patients with localized cancers of all types, and revealed that ctDNA was found in 55% (122/223) of the patients. In addition, they compared the ctDNA in four representative cancers (breast, colon, pancreas, and gastroesophageal cancers), and showed that ctDNA were detectable in 49% to 78% of patients with localized tumors and in 86% to 100% of patients with metastatic tumors of these four types.

These results were obtained by first identifying a mutation in a tumor and then determining whether that same mutation was detectable in the plasma. The sensitivity and specificity of the ctDNA criteria were evaluated detailed using CRC as a model. A specificity of 99.2% and a sensitivity of 87.2% were obtained, suggesting the high agreement between the plasma and tumor tissue (17). In addition to point mutations, we should not neglect other genomic alterations that include amplifications, deletions, aneuploidy, and translocations, which represent some of the most clinically useful genomic alterations in cancer. Bettegowda *et al.* (17) compared the quantity of two different types of genetically altered DNA fragments (rearrangements and single-base substitutions) in the plasma of 19 patients and revealed that the absolute number of circulating DNA fragments with point mutations versus rearrangements was highly correlated. As the authors mentioned, although ctDNA-based early detection strategies are promising, some formidable obstacles need to be overcome before their clinical application. In a screening setting, it is not easy to determine the number of genomic alterations assessed in most types of cancers. In addition, other issues, including the relationship between mutation type and the tumor type, false-positive findings, and the "overdiagnosis" of benign tumors or precancerous lesions, also need to be considered extensively.

## ctDNA as a prognostic biomarker

Bettegowda's data show that ctDNA analysis can also provide important prognostic information in patients with metastatic disease (17). For example, they revealed that the concentration of ctDNA in metastatic CRC patients reflected the tumor burdens and was negatively associated with survival time. Actually, several previous studies have reported that the presence of ctDNA in plasma seems to be a relevant prognostic marker for CRC patients and may be used to identify patients with a high risk of recurrence (18,19). Similar data were also reported for breast cancer (16). In addition, recent studies indicated that ctDNA appeared to be a better prognostic marker than CTC count (20,21).

## ctDNA as a predictive marker

The analysis of ctDNA is also promising to monitor response of targeted therapy, which provides an early warning of relapse and the potential information about the genetic basis of resistance. Bettegowda *et al.* (17) evaluated the utility of this approach in monitoring the response to EGFR targeted therapy in CRC patients. They identified 70 somatic mutations that appeared after therapy was initiated but were not found in the tumor tissue or in the plasma before EGFR blockade. About half of these mutations occurred in KRAS codon 12, which are reported to cause resistance to EGFR blockade, and have been observed to arise after EGFR blockade *in vitro* as well as *in vivo*. A recent study also got similar results and observed some potentially clinically relevant mutations that are not detected in archival CRC tissue (22), suggesting the better representative of "liquid biopsy" over tissue biopsy. The results highlight the value of ctDNA as a potential noninvasive "real time" tool for tumor characterization. Many studies have confirmed the clinical potential of ctDNA in monitoring therapy response (23,24).

## Methodological considerations

In the past decade, the resuscitation of ctDNA was driven mainly by the rapid technical advances, and methodological issue was the key of ctDNA study.

Unlike other cancer biomarkers, the specificity of somatic alterations is based on the fact that these changes are present in the genome of cancer cells but not in that of normal cells. The rapid advances on the cancer genomics have revealed extensive information of the genetic alterations that are responsible for the cancer initiation and progression (25), providing the possibility to improve cancer diagnosis and therapy, whereas technical advances in digital genomics have opened the door for reliably detecting rare events in complex mixtures of tumor-specific and wild-type DNA .

Owing to the low concentration and high degree of fragmentation of cfDNA, it is not easy to establish an efficient detection assay with enough sensitivity for meaningful clinical application. Lacking harmonization and comparability of analysis methods for ctDNA made it difficult to evaluate different ctDNA studies. First, preanalytical procedures need to be standardized, and difference in blood processing, sample types (serum or plasma), and DNA isolation among different studies should be taken into account when evaluating these studies. Second, the sensitivity and specificity of different ctDNA assays should be evaluated in detail.

Generally, there are two kinds of methods for the analysis of ctDNA, targeted and untargeted approaches (26). The former includes the analysis of known genetic alterations identified in the tumor tissues that have implications for therapy decisions, such as mutations in K-RAS, C-KIT or EGFR. The second one does not require prior knowledge of any specific changes present in the primary tumor. Genome-wide analysis of ctDNA (whole genome or exome sequencing) can be used to reveal tumor-specific alterations. At the present stage, targeted approaches may be more attractive than untargeted approaches for its relative low cost/labor and common technologies in clinical utility. However, untargeted approaches show promising future for their high-throughput, rapidly decreased cost and increased fidelity.

Nevertheless, the identification of mutations at low allele frequencies across sizeable genomic regions or in a few nanograms of fragmented cfDNA template has been challenging. Owing to rapid technical advances, the sensitivity of ctDNA detection has been improved dramatically in the past decade and many new methods, including amplification refractory mutation system (ARMS) (27), digital PCR (dPCR) (17,28), beads, emulsions, amplification, and magnetics (BEAMing) (19), sequence-specific synchronous coefficient of drag alteration (SCODA) (29), and Cancer Personalized Profiling by deep Sequencing (CAPP-Seq) (30), enable the detection of mutant alleles at very low frequencies. The CAPP-Seq method correctly identified all mutations with allelic fractions >0.10% and yielded a specificity of 99% according to Newman's data (30). Recently, Forshew *et al.* reported a low-cost, high-throughput method, tagged-amplicon deep sequencing (TAm-Seq), which could identify cancer-specific mutations at low allele frequencies as low as 2%; screening of known mutations in EGFR and TP53 could achieve a detection limit of approximately 0.2%, suggesting the promising application of the method in the ctDNA-based liquid biopsy for personalized cancer genomics (31). In contrast, Bettegowda *et al.* (17) used different technologies, including BEAMing, PCR/ligation method, dPCR, and Safe-Sequencing System (Safe-SeqS) exomic sequencing, to check different target sites in different type of cancer or for different aims (17). For example, the dPCR and Safe-SeqS method used in their study could detect one mutant template in the cfDNA from 5 mL plasma.

However, mutational hotspots in most tumor-related genes are not so common except several types of cancers (such as pancreatic, colorectal and lung cancer) harboring mutational hotspots in several genes (such as K-RAS, N-RAS, PIK3CA, BRAF, TP53, and APC). To increase the detection sensitivity of ctDNA for cancer, as many as possible genetic alterations should be analyzed using a high cost-effective approach. A successful ctDNA-based assay has to consider the balance between its cost/labor and diagnostic efficiency. Martinez and his colleagues (32) analyzed the data sets from The Cancer Genome Atlas (TCGA) and revealed that 76% of 10 cancer types harbour at least one mutation in a panel of 25 genes, with high sensitivity. Newman *et al.* (30) described a novel cancer profiling method, CAPP-Seq, to quantify ctDNA in non-small cell lung cancer (NSCLC), which could evaluate specific regions of interest with a significant cost reduction and improve sensitivity over whole-exome- and whole-genome-based approaches. A targeted 139 gene panel designed for several types of somatic alterations (point mutations, translocations, etc.) was checked in plasma ctDNA, and could detect 50% patients at stage I and 100% patients at stages II to IV in a small NSCLC cohort, which were similar to the results of Bettegowda's work (17).

Due to the extensive genetic heterogeneity among different types of cancers, now it seems difficult to establish an universal detection approach of ctDNA for detecting different types of cancers. At the present stage, it may be

 

more feasible to develop several type- or group-specific ctDNA assays based on the profiles of genomic alterations in different cancers. However, due to continuously dropping sequencing costs and evolving technologies, it is just a matter of time that genome-wide approaches with high resolution to be used as a routine tool for ctDNA-based utility.

In summary, the study by Bettegowda *et al.* demonstrates that ctDNA can be used as a feasible biomarker for a variety of cancer types and clinical indications. Although the clinical utility of ctDNA still need to be evaluated through longitudinal studies of ctDNA in appropriate populations of patients, the study lay the groundwork for such future studies. It is just a matter of time that ctDNA become a commonly used clinical assay for cancer patients.

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## Footnote

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

## References

1. Mandel P, Metais P. Les acides nucleiques du plasma sanguin chez l' homme. C R Acad Sci Paris 1948;142:241-3.
2. Leon SA, Shapiro B, Sklaroff DM, et al. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646-50.
3. Stroun M, Anker P, Maurice P, et al. Neoplastic characteristics of the DNA found in the plasma of cancer patients. Oncology 1989;46:318-22.
4. Vasioukhin V, Anker P, Maurice P, et al. Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Br J Haematol 1994;86:774-9.
5. Sorenson GD, Pribish DM, Valone FH, et al. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev 1994;3:67-71.
6. Sozzi G, Conte D, Mariani L, et al. Analysis of circulating tumor DNA in plasma at diagnosis and during follow-up of lung cancer patients. Cancer Res 2001;61:4675-8.
7. Huang ZH, Li LH, Hua D. Quantitative analysis of plasma circulating DNA at diagnosis and during follow-up of breast cancer patients. Cancer Lett 2006;243:64-70.
8. Giacona MB, Ruben GC, Iczkowski KA, et al. Cell-free DNA in human blood plasma: length measurements in patients with pancreatic cancer and healthy controls. Pancreas 1998;17:89-97.
9. Wang BG, Huang HY, Chen YC, et al. Increased plasma DNA integrity in cancer patients. Cancer Res 2003;63:3966-8.
10. Ito T, Kaneko K, Makino R, et al. Clinical significance in molecular detection of p53 mutation in serum of patients with colorectal carcinoma. Oncol Rep 2003;10:1937-42.
11. Shaw JA, Smith BM, Walsh T, et al. Microsatellite alterations plasma DNA of primary breast cancer patients. Clin Cancer Res 2000;6:1119-24.
12. Wong IH, Lo YM, Zhang J, et al. Detection of aberrant p16 methylation in the plasma and serum of liver cancer patients. Cancer Res 1999;59:71-3.
13. Anker P, Lefort F, Vasioukhin V, et al. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997;112:1114-20.
14. Pinzani P, Salvianti F, Orlando C, et al. Circulating cell-free DNA in cancer. Methods Mol Biol 2014;1160:133-45.
15. Alix-Panabières C, Pantel K. Circulating tumor cells: liquid biopsy of cancer. Clin Chem 2013;59:110-8.
16. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199-209.
17. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014;6:224ra24.
18. Lecomte T, Berger A, Zinzindohoué F, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer 2002;100:542-8.
19. Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008;14:985-90.
20. Bidard FC, Madic J, Mariani P, et al. Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. Int J Cancer 2014;134:1207-13.
21. Punnoose EA, Atwal S, Liu W, et al. Evaluation of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer: association with clinical endpoints

 

in a phase II clinical trial of pertuzumab and erlotinib. Clin Cancer Res 2012;18:2391-401.
22. Tabernero J, Lenz HJ, Siena S, et al. Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. Lancet Oncol 2015;16:937-48.
23. Tie J, Kinde I, Wang Y, et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. Ann Oncol 2015;26:1715-22.
24. Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. Ann Oncol 2015;26:731-6.
25. Stratton MR. Exploring the genomes of cancer cells: progress and promise. Science 2011;331:1553-8.
26. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. Clin Chem 2015;61:112-23.
27. Spindler KL, Pallisgaard N, Vogelius I, et al. Quantitative cell-free DNA, KRAS, and BRAF mutations in plasma from patients with metastatic colorectal cancer during treatment with cetuximab and irinotecan. Clin Cancer Res 2012;18:1177-85.
28. Vogelstein B, Kinzler KW. Digital PCR. Proc Natl Acad Sci U S A 1999;96:9236-41.
29. Kidess E, Heirich K, Wiggin M, et al. Mutation profiling of tumor DNA from plasma and tumor tissue of colorectal cancer patients with a novel, high-sensitivity multiplexed mutation detection platform. Oncotarget 2015;6:2549-61.
30. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014;20:548-54.
31. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 2012;4:136ra68.
32. Martinez P, McGranahan N, Birkbak NJ, et al. Computational optimisation of targeted DNA sequencing for cancer detection. Sci Rep 2013;3:3309.

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