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LETTER TO THE EDITOR

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 ≥ 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 ≥ 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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REFERENCES

1 **Terasawa H**, Kinugasa H, Nouso K, Yamamoto S, Hirai M, Tanaka T, Takaki A, Okada H. Circulating tumor DNA dynamics analysis in a xenograft mouse model with esophageal squamous cell carcinoma. *World J Gastroenterol* 2021; **27**: 7134-7143 [PMID: 34887633 DOI: 10.3748/wjg.v27.i41.7134]

2 **Siravegna G**, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol* 2017; **14**: 531-548 [PMID: 28252003 DOI: 10.1038/nrclinonc.2017.14]

3 **Murtaza M**, Dawson SJ, Tsui DW, Gale D, Forshew T, Piskorz AM, Parkinson C, Chin SF, Kingsbury Z, Wong AS, Marass F, Humphray S, Hadfield J, Bentley D, Chin TM, Brenton JD, Caldas C, Rosenfeld N. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 2013; **497**: 108-112 [PMID: 23563269 DOI: 10.1038/nature12065]

4 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA Jr. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

5 **Aravanis AM**, Lee M, Klausner RD. Next-Generation Sequencing of Circulating Tumor DNA for Early Cancer Detection. *Cell* 2017; **168**: 571-574 [PMID: 28187279 DOI: 10.1016/j.cell.2017.01.030]

6 **Mouliere F**, Chandrananda D, Piskorz AM, Moore EK, Morris J, Ahlborn LB, Mair R, Goranova T, Marass F, Heider K, Wan JCM, Supernat A, Hudecova I, Gounaris I, Ros S, Jimenez-Linan M, Garcia-Corbacho J, Patel K, Østrup O, Murphy S, Eldridge MD, Gale D, Stewart GD, Burge J, Cooper WN, van der Heijden MS, Massie CE, Watts C, Corrie P, Pacey S, Brindle KM, Baird RD, Mau-Sørensen M, Parkinson CA, Smith CG, Brenton JD, Rosenfeld N. Enhanced detection of circulating tumor DNA by fragment size analysis. *Sci Transl Med* 2018; **10** [PMID: 30404863 DOI: 10.1126/scitranslmed.aat4921]

7 **Underhill HR**, Kitzman JO, Hellwig S, Welker NC, Daza R, Baker DN, Gligorich KM, Rostomily RC, Bronner MP, Shendure J. Fragment Length of Circulating Tumor DNA. *PLoS Genet* 2016; **12**: e1006162 [PMID: 27428049 DOI: 10.1371/journal.pgen.1006162]

8 **Gao Q**, Lin YP, Li BS, Wang GQ, Dong LQ, Shen BY, Lou WH, Wu WC, Ge D, Zhu QL, Xu Y, Xu JM, Chang WJ, Lan P, Zhou PH, He MJ, Qiao GB, Chuai SK, Zang RY, Shi TY, Tan LJ, Yin J, Zeng Q, Su XF, Wang ZD, Zhao XQ, Nian WQ, Zhang S, Zhou J, Cai SL, Zhang ZH, Fan J. Unintrusive multi-cancer detection by circulating cell-free DNA methylation sequencing (THUNDER): development and independent validation studies. *Ann Oncol* 2023; **34**: 486-495 [PMID: 36849097 DOI: 10.1016/j.annonc.2023.02.010]

9 **Zhou X**, Cheng Z, Dong M, Liu Q, Yang W, Liu M, Tian J, Cheng W. Tumor fractions deciphered from circulating cell-free DNA methylation for cancer early diagnosis. *Nat Commun* 2022; **13**: 7694 [PMID: 36509772 DOI: 10.1038/s41467-022-35320-3]

10 **Luo H**, Wei W, Ye Z, Zheng J, Xu RH. Liquid Biopsy of Methylation Biomarkers in Cell-Free DNA. *Trends Mol Med* 2021; **27**: 482-500 [PMID: 33500194 DOI: 10.1016/j.molmed.2020.12.011]

11 **Luo H**, Zhao Q, Wei W, Zheng L, Yi S, Li G, Wang W, Sheng H, Pu H, Mo H, Zuo Z, Liu Z, Li C, Xie C, Zeng Z, Li W, Hao X, Liu Y, Cao S, Liu W, Gibson S, Zhang K, Xu G, Xu RH. Circulating tumor DNA methylation profiles enable early diagnosis, prognosis prediction, and screening for colorectal cancer. *Sci Transl Med* 2020; **12** [PMID: 31894106 DOI: 10.1126/scitranslmed.aax7533]

12 **Jiang P**, Sun K, Tong YK, Cheng SH, Cheng THT, Heung MMS, Wong J, Wong VWS, Chan HLY, Chan KCA, Lo YMD, Chiu RWK. Preferred end coordinates and somatic variants as signatures of circulating tumor DNA associated with hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2018; **115**: E10925-E10933 [PMID: 30373822 DOI: 10.1073/pnas.1814616115]

13 **Liu S**, Wu J, Xia Q, Liu H, Li W, Xia X, Wang J. Finding new cancer epigenetic and genetic biomarkers from cell-free DNA by combining SALP-seq and machine learning. *Comput Struct Biotechnol J* 2020; **18**: 1891-1903 [PMID: 32774784 DOI: 10.1016/j.csbj.2020.06.042]

14 **Renaud G**, Nørgaard M, Lindberg J, Grönberg H, De Laere B, Jensen JB, Borre M, Andersen CL, Sørensen KD, Maretty L, Besenbacher S. Unsupervised detection of fragment length signatures of circulating tumor DNA using non-negative matrix factorization. *Elife* 2022; **11** [PMID: 35894300 DOI: 10.7554/eLife.71569]

15 **Heeke S**, Gay CM, Estecio MR, Tran H, Morris BB, Zhang B, Tang X, Raso MG, Rocha P, Lai S, Arriola E, Hofman P, Hofman V, Kopparapu P, Lovly CM, Concannon K, De Sousa LG, Lewis WE, Kondo K, Hu X, Tanimoto A, Vokes NI, Nilsson MB, Stewart A, Jansen M, Horváth I, Gaga M, Panagoulias V, Raviv Y, Frumkin D, Wasserstrom A, Shuali A, Schnabel CA, Xi Y, Diao L, Wang Q, Zhang J, Van Loo P, Wang J, Wistuba II, Byers LA, Heymach JV. Tumor- and circulating-free DNA methylation identifies clinically relevant small cell lung cancer subtypes. *Cancer Cell* 2024; **42**: 225-237.e5 [PMID: 38278149 DOI: 10.1016/j.ccell.2024.01.001]

16 **Song L**, Li Y. SEPT9: A Specific Circulating Biomarker for Colorectal Cancer. *Adv Clin Chem* 2015; **72**: 171-204 [PMID: 26471083 DOI: 10.1016/bs.acc.2015.07.004]

17 **Church TR**, Wandell M, Lofton-Day C, Mongin SJ, Burger M, Payne SR, Castaños-Vélez E, Blumenstein BA, Rösch T, Osborn N, Snover D, Day RW, Ransohoff DF; PRESEPT Clinical Study Steering Committee, Investigators and Study Team. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 2014; **63**: 317-325 [PMID: 23408352 DOI: 10.1136/gutjnl-2012-304149]

18 **Cohen JD**, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, Hruban RH, Wolfgang CL, Goggins MG, Dal Molin M, Wang TL, Roden R, Klein AP, Ptak J, Dobbyn L, Schaefer J, Silliman N, Popoli M, Vogelstein JT, Browne JD, Schoen RE, Brand RE, Tie J, Gibbs P, Wong HL, Mansfield AS, Jen J, Hanash SM, Falconi M, Allen PJ, Zhou S, Bettegowda C, Diaz LA Jr, Tomasetti C, Kinzler KW, Vogelstein B, Lennon AM, Papadopoulos N. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018; **359**: 926-930 [PMID: 29348365 DOI: 10.1126/science.aar3247]

19 **Li Y**, Xu J, Chen C, Lu Z, Wan D, Li D, Li JS, Sorg AJ, Roberts CC, Mahajan S, Gallant MA, Pinkoviezky I, Cui Y, Taggart DJ, Li W. Multimodal epigenetic sequencing analysis (MESA) of cell-free DNA for non-invasive colorectal cancer detection. *Genome Med* 2024; **16**: 9 [PMID: 38225592 DOI: 10.1186/s13073-023-01280-6]

20 **Picardo F**, Romanelli A, Muinelo-Romay L, Mazza T, Fusilli C, Parrella P, Barbazán J, Lopez-López R, Barbano R, De Robertis M, Taffon C, Bordoni V, Agrati C, Costantini M, Ricci F, Graziano P, Maiello E, Muscarella LA, Fazio VM, Poeta ML. Diagnostic and Prognostic Value of B4GALT1 Hypermethylation and Its Clinical Significance as a Novel Circulating Cell-Free DNA Biomarker in Colorectal Cancer. *Cancers (Basel)* 2019; **11** [PMID: 31635093 DOI: 10.3390/cancers11101598]

21 **Fu R**, Huang J, Tian X, Liang C, Xiong Y, Zhang JT, Jiang B, Dong S, Gong Y, Gao W, Li F, Shi Y, Liu Z, Gao X, Chen R, Zhong W, Zhang Y. Postoperative circulating tumor DNA can refine risk stratification in resectable lung cancer: results from a multicenter study. *Mol Oncol* 2023; **17**: 825-838 [PMID: 36732646 DOI: 10.1002/1878-0261.13387]

22 **Stejskal P**, Goodarzi H, Srovnal J, Hajdúch M, van 't Veer LJ, Magbanua MJM. Circulating tumor nucleic acids: biology, release mechanisms, and clinical relevance. *Mol Cancer* 2023; **22**: 15 [PMID: 36681803 DOI: 10.1186/s12943-022-01710-w]

23 **Ko JMY**, Ng HY, Lam KO, Chiu KWH, Kwong DLW, Lo AWI, Wong JC, Lin RCW, Fong HCH, Li JYK, Dai W, Law S, Lung ML. Liquid Biopsy Serial Monitoring of Treatment Responses and Relapse in Advanced Esophageal Squamous Cell Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 32466419 DOI: 10.3390/cancers12061352]

24 **Boonstra PA**, Wind TT, van Kruchten M, Schuuring E, Hospers GAP, van der Wekken AJ, de Groot DJ, Schröder CP, Fehrmann RSN, Reyners AKL. Clinical utility of circulating tumor DNA as a response and follow-up marker in cancer therapy. *Cancer Metastasis Rev* 2020; **39**: 999-1013 [PMID: 32367253 DOI: 10.1007/s10555-020-09876-9]

25 **Liu S**, Wang J. Current and Future Perspectives of Cell-Free DNA in Liquid Biopsy. *Curr Issues Mol Biol* 2022; **44**: 2695-2709 [PMID: 35735625 DOI: 10.3390/cimb44060184]

26 **Roviello G**, Lavacchi D, Antonuzzo L, Catalano M, Mini E. Liquid biopsy in colorectal cancer: No longer young, but not yet old. *World J Gastroenterol* 2022; **28**: 1503-1507 [PMID: 35582130 DOI: 10.3748/wjg.v28.i15.1503]

27 **Martin-Alonso C**, Tabrizi S, Xiong K, Blewett T, Sridhar S, Crnjac A, Patel S, An Z, Bekdemir A, Shea D, Wang ST, Rodriguez-Aponte S, Naranjo CA, Rhoades J, Kirkpatrick JD, Fleming HE, Amini AP, Golub TR, Love JC, Bhatia SN, Adalsteinsson VA. Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies. *Science* 2024; **383**: eadf2341 [PMID: 38236959 DOI: 10.1126/science.adf2341]

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