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Review

Potential Management of Circulating Tumor DNA as a Biomarker in Triple-Negative Breast Cancer

Mao Shang [1,2], Chunxiao Chang [3], Yanqing Pei [4], Yin Guan [5], Jin Chang [6], HuiHui Li [3]✉

1. School of Medicine and Life Sciences, University of Jinan Shandong Academy of Medical Sciences, Jinan, Shandong, China
2. Department of Radiation Oncology, Shandong Cancer Hospital Affiliated to Shandong University, Shandong Academy of Medical Sciences, Jinan, Shandong Province, China. 250117
3. Department of Medical Oncology, Shandong Cancer Hospital Affiliated to Shandong University, Shandong Academy of Medical Sciences, Jinan, Shandong Province, China. 250117
4. Department of Quality Management Office, Shandong Cancer Hospital Affiliated to Shandong University, Shandong Academy of Medical Sciences, Jinan, Shandong Province, China. 250117
5. Department of Medical Oncology, Beijing Chao-Yang Hospital, Beijing, China.
6. Oncology department, Affiliated Hospital of Taishan Medical university.

✉ Corresponding author: Huihui Li, MD, PhD, Department of Medical Oncology, Shandong Cancer Hospital and Institute, Shandong Cancer Hospital affiliated to Shandong University, 440, Jinyan Road, Jinan, Shandong, China. Tel: 15553103209; Email address: 15553103209@163.com

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Abstract

As a specific subtype of breast cancer, Triple-negative breast cancer (TNBC) is associated with worse prognosis and higher tumor aggressiveness than HER2-amplified or hormone receptor positive breast cancers. Circulating tumor DNA (ctDNA), as a non-invasive "liquid biopsy", is an emerging original blood-based biomarker for early breast cancer diagnosis, monitoring treatment response, and determining prognosis. In TNBC patients, ctDNA has an inherent tendency to characterize tumor heterogeneity and metastasis-specific mutations providing a key alternative to tumor tissue profiling. Several studies have already demonstrated the potential of ctDNA in TNBC patients from early to advanced stages of the disease including diagnosis, therapy decisions and assessment of prognosis. This review provides a critical brief summary of the evidence that gives credence to the utility of ctDNA as a biomarker for its role into clinical management in TNBC.

Key words: triple-negative breast cancer, circulating tumor DNA, liquid biopsy

Introduction

Patients with triple-negative breast cancer (TNBC), which is negative for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, account for 15–20% of all breast cancer patients [1-3] and are generally considered to have poor prognosis [4, 5]. Early diagnosis and appropriate treatment can improve the prognosis. Given that TNBC patients are not respondent to endocrine therapy and HER2 targeted therapy, chemotherapy is the mainstay treatment in both the early and advanced-stages [6, 7].However, patients without complete response make up approximately 80% of TNBC [8], and there is a rapid recurrence and metastasis [9]. Therefore, it is essential to excavate novel biomarkers to guide the treatment and improve the clinical management of TNBC. Histopathological examination of tumor biopsy specimen was considered as the gold standard for diagnosing TNBC. However, this approach has several limitations. First, owing to tumor heterogeneity, a biopsy specimen may not be representative of the entire tumor, let alone distant metastasis [10]. In addition, tissue processing, fixatives, and storage may diminish DNA quality and affect later mutation detection [11]. In the end, biopsy is a very invasive method and not always feasible in clinical practice. Currently, TNBC patients have no unanimous biomarkers that would aid in diagnosing, treating, and determining the prognosis of TNBC [12]. Novel predictive biomarkers commited to patients

suffering from TNBC should be urgently needed. Plasma circulating tumor DNA (ctDNA), including genetic information about not only the primary tumor but also metastatic disease [13-15], could be used as a non-invasive practice and might be a good surrogate to the current solid biopsy and help in TNBC management.

In contrast to tissue biopsy, "liquid biopsy" is performed on cancer-derived material captured in a blood sample. Because it acts as a repository of genetic material from throughout the body, circulating blood may provide material for more comprehensive mutation profiling. Furthermore, "liquid biopsy" provides a non-invasive means of obtaining timely, comprehensive information and could provide the foundation for real-time tumor monitoring. Finally, research shows that tissue and liquid biopsies have excellent concordance in terms of gene mutation status by using BEAMing that could be theoretically conducted in lieu of a biopsy [16]. Recently, Garcia-Murillas et al. [17] and Olsson et al. [18] showed that using ctDNA-based liquid biopsy for real-time disease monitoring may improve the clinical management of breast cancer. These studies have indicated that sufficient sensitivity and specificity were eventually confirmed in terms of predicting an early recurrence with ctDNA, which might make it a monitoring tool for early tumor detection, therapy modification, and prognostic evaluation. Several small clinical trials have investigated the potential use of ctDNA as a biomarker for early breast cancer screening, monitoring treatment response, and determining prognosis [17, 19-21]. In this review, we will highlight the latest research of ctDNA as a "liquid biopsy" in TNBC, and what role ctDNA plays in improving the clinical management of TNBC.

Detection of ctDNA

Malignant tumors can release significant amounts of DNA, including fragments as large as 180–200bp, into the bloodstream via cell death–associated mechanisms such as necrosis and apoptosis [22-25]. Researchers can derive mutation information from such ctDNA by measuring its concentration and determining its nucleotide sequence [26]. Although highly sensitive and specific methods have been developed to detect ctDNA, ultrasensitive technologies that can detect the minimum amount of ctDNA in a sea of normal ctDNA are urgently needed for early cancer detection because of its very low levels of ctDNA. Such detection requires technology standardization coupled with accurate criteria to determine sample adequacy [27, 28].

Screening

Previous studies have indicated that an increase in ctDNA reflects an increase in tumor burden [29] and patients with early-stage breast cancer, therefore, may have only low concentrations of ctDNA. Owing to little information about genomic mutations it provided, ctDNA is rarely used as a biomarker for early diagnosis. In terms of early-stage TNBC, biomarkers as a means of screening disease was mainly investigated at the tissue DNA in TNBC [30, 31].For example, Elena et al. analysis showed that the acquisition of TP53 mutations may lead to increased genetic instability and increase the likelihood of an microglandular adenosis (MGA) and/or atypical MGA (AMGA) to progress to TNBC [31]. In this context, they collected two pure MGAs and eight cases of MGA and/or AMGA associated with in situ or invasive TNBC and subjected to massively parallel sequencing. They found that TNBCs are often clonally associated with synchronously diagnosed ipsilateral MGA and/or AMGA, providing circumstantial evidence to indicate that MGA may constitutes a non-obligate precursor of TNBC.

Although limited data are available for TNBC specifically, the validity of using ctDNA for the early diagnosis of breast cancer in general has already been studied. For example, Phallen et al. used massively parallel sequencing to detect ctDNA based on targeted error correction sequencing [21]. They assessed plasma samples from 200 patients with stage I or II colorectal, breast, lung, or ovarian cancer for somatic mutations. Thereinto, the authors found high concordance between ctDNA and breast tumor samples in terms of mutational status, which suggests that ctDNA analysis may provide a noninvasive means for early breast cancer screening. Similarly, a recent study demonstrated the possible implementtation of plasma tumor DNA detection in patients with early-stage breast cancer [32]. The authors assessed the feasibility of detecting PIK3CA mutations in the plasma of 29 patients before surgery and found 93% concordance between confirmed mutations in tumor tissues and mutations in pre-surgery plasma samples. The high level of sensitivity (93.3%) and specificity (100%) of the ddPCR in this study provides a proof of concept and confirms that ctDNA analysis is feasible for patients with early-stage breast cancer. Another study showed that quantitative analysis of the methylation pattern of plasma cell-free DNA (cfDNA) in breast cancer patients might be a valuable non-invasive tool for early detection [33]. Due to limited data, as a novel biomarker for the early detection of breast cancer, ctDNA needs further development and validation.

Monitoring treatment response

Conventional chemotherapy with anthracyclines, taxanes, and/or platinum agents is the current standard of care for TNBC patients [34]. Unfortunately, because of disease heterogeneity, not all TNBC patients have a good response to chemotherapy. Currently, whether treatment should be personalized for patients with different TNBC subtypes remains unclear [35, 36]. Moreover, there are no guidelines or regulatory approvals for the use of ctDNA-based liquid biopsies to monitor treatment response in TNBC patients. Therefore, ctDNA concentration and sequencing analysis requires extensive study before it can be used with confidence to make treatment decisions for TNBC patients.

Early stage

CtDNA can be detected in the plasma and serum of patients with advanced cancer [37] and thus may be a noninvasive source to characterize the somatic genetic features of their tumors [38-40]. Data about whether ctDNA analyses would be applicable to early-stage cancer are limited in part because the low tumor burden of early-stage disease makes the detection of ctDNA challenging [32, 41], as very low levels of plasma ctDNA are usually not discoverable [42, 43]. Nevertheless, in the setting of primary breast cancer, the clinical utility of ctDNA-based liquid biopsy remains unclear, as the percutaneous biopsy of breast tumors is preferred.

However, using ctDNA for the dynamic detection of treatment response can establish the rationale for using ctDNA-based liquid biopsy to detect early metastasis, inform timely therapy modification, and avoid overtreatment in patients with early-stage breast cancer. Kim et al. [44] using gene expression profiling and ER protein assays, found that the low-level expression of ESR1 is correlated with tamoxifen resistance in ER-positive primary breast cancer and is associated with treatment outcome. In another study designed to monitor therapy response in patients with early-stage breast cancer. Olsson et al. [18] demonstrated that tumor-specific chromosomal rearrangements in ctDNA could be detected on average 11 months before the clinical observation of metastases.

One study showed a remarkable correlation of methylated ctDNA with the primary breast tumor's response to neoadjuvant chemotherapy [45]. The authors concluded that methylated ctDNA is more sensitive than carcinoembryonic antigen or cancer antigen CA15-3 as a marker of sensitivity to neoadjuvant chemotherapy. In clinical practice, TNBC typically display larger tumor size and higher proliferation at the time of diagnosis, and are often treated by neoadjuvant chemotherapy (NCT) before surgery. However, only 20–30% of patients with TNBC achieve pathological complete response (pCR) following NCT, meanwhile the response to NCT was highly divergent [46, 47]. Previous evidence supporting the association between O6-Methylguanine-DNA methyltransferase (MGMT) expression status and achievement of pCR following NCT in basal-like breast cancers (BLBCs) comes from Katsuya et al., who showed that attenuated expression of MGMT is predictive of a pCR [48]. In a study of 36 TNBC patients, Riva.F et al. using ddPCR, detected TP53 mutations in all patients' plasma before NCT, after 1 cycle of neoadjuvant chemotherapy, before surgery, and after surgery to assess tumor response to NCT [49]. Notably, 27 of 36 patients (75%) had TP53 mutations at baseline. During the treatment, all patients' ctDNA levels declined, and no patients had detectable ctDNA after surgery. One patient with rising ctDNA levels experienced tumor progression during NCT. In this study, Riva.F et al. demonstrated that the detection and quantification of ctDNA is a very promising tool for assessing response to NCT. Another study drew similar conclusions. Chen, Y. H. et al. [50] followed 38 early-stage triple-negative breast cancers and utilized next-generation in matched tumor, blood and plasma to detect recurrence and metastasis as early as possible. Of those 33 patients who had primary tumor mutation, 4 had ctDNA mutations. For those patients where ctDNA mutation was detected, recurrence was rapid. However, in next few studies, the results have been disappointing. Of the 84 TNBC patients treated with alkylating agents in the NCT setting, 58.3% have MGMT methylation in addition to the status of unmethylated (27.4%) or indeterminate (14.3%). Confirming these data, Caterina and colleagues found that although high methylation levels in FFPE samples were association with clinical response to therapy, no association between MGMT methylation status and pCR was found [51]. The association between gene mutations and NCT response was also seen in other primary tumor studies. Using next generation sequencing, 1,977 genes were analyzed from 56 pre-treatment TNBC biopsies before treatment. Lips et al. [52] found that TP53 mutations are the most frequent clonal event (55%), followed by TTN mutations (14%) and PIK3CA mutations (9%). However, few mutations were common among the samples, and they were unable to find any recurrent mutations associated with NCT response. Further, they observed no difference in the mutation rates between responders and non-responders. A similar finding was reported from a subsequent analysis

between TP53 mutations and the response to NCT in biopsy tissue from TNBC [53]. In this study, Silvia et al. [53] indicated that TP53 mutations did not predict the NCT response in TNBC patients. Furthermore, analysis of the predictive of PIK3CA mutation on TNBC agents was performed in a biomarker analysis of 81 patients with residual disease after NCT, with finding of PIK3CA mutation not associated with pCR [54].

Advanced stage

Measuring treatment response in patients with metastatic breast cancer is usually done by serial clinical evaluation, the assessment of the serum CA15-3 levels, and serial radiographic imaging. However, serial radiographic imaging is expensive, often inconclusive, and may not detect changes in tumor burden. CA15-3 assessment has low sensitivity, and changes in CA15-3 levels do not necessarily reflect tumor response or progression [55]. Tampellini et al. [56] revealed that CA15-3 alone is not usable as a biomarker for monitoring therapy response in patients with advanced breast cancer.

Because no marker for monitoring therapy response in patients with metastatic breast cancer has yet reached wide clinical use, researchers hope to use ctDNA mutations as biomarkers for dynamically detecting treatment response. Dawson et al. [29] suggested that ctDNA analysis has great potential for the real-time monitoring of tumor burden and may be a better measure of treatment effectiveness in patients with metastatic disease. Several recent studies have investigated the ctDNA mutations associated with targeted therapy response in patients with HER2-positive breast cancer and endocrine therapy response in patients with ER-positive metastatic breast cancer [57-59]. For example, the ESR1 mutation has attracted special attention as a mechanism of endocrine therapy resistance in metastatic breast cancer. In a study evaluating the use of NGS- and ddPCR-based techniques to detect ESR1 mutations in the cfDNA of 48 patients with ER-positive breast cancer, Guttery et al. [57] demonstrated that ESR1 mutations mainly arise following the treatment of metastatic disease and can predict resistance to aromatase inhibitor–based therapy. Similarly, the detection of cfDNA nucleotide alterations to assess response to anti–HER2-targeted therapies has been investigated. In one study using NGS, 46 genes were detected from an assessment of 486 single-nucleotide variants [59]. Notably, only 7 genes considered relevant to targeted therapy resistance were detected in the treatment-resistant group. In addition, two patients in whom HER2 S855I mutations were detected derived sustained benefit from anti-HER2 therapy.

Overall, patients with TNBC do not benefit from endocrine or targeted therapy. Furthermore, TNBC, especially metastatic TNBC, treated with chemotherapy can progress without warning. Thus, a new sensitive hematological tumor biomarker of chemotherapy response in patients with metastatic TNBC is urgently needed. CtDNA analysis can represent an alternative to metastatic biopsies in molecular screening programs [60, 61]. Currently, ctDNA may be used to identify molecular alterations of immunotherapy effect in patients of metastatic TNBC. Results have reported that ctDNA detected the response of anti-PD1 immunotherapy of patients with nonsmall cell lung cancer, metastatic melanoma and other malignancies [62-66]. The programmed death receptor 1 (PD-1) is an inhibitory immune checkpoint receptor that can limit autoimmunity, regulate the activity of effector T cells in the periphery in response to an inflammatory stimulus [67, 68]. Research shows that programmed death 1 ligand 1 (PD-L1) is expressed in approximately 20% of TNBC, suggesting anti-PD-L1/anti-PD1 therapy may play a potential role in this patient population [69]. Furthermore, the above studies have shown a correlation between serial analysis of ctDNA and tumor response to anti-PD-1 therapy. It therefore appears that ctDNA may be a useful test for predicting response to immunotherapy in metastatic TNBC. However, no studies have ever published in this field.

Assessing prognosis

Many studies have investigated the prognostic value of ctDNA in multiple cancers, including medullary thyroid carcinoma [70], pancreatic cancer [71], lung adenocarcinoma [72], gastric cancer [73], and hepatocellular carcinoma [74]. Although detectable levels of unmutated ctDNA in breast cancer patients have been related to prognosis, studies investigating the prognostic value of mutated cfDNA levels in breast cancer patients have provided mixed results. Visvanathan et al. found that a high cumulative methylation index in cfDNA was associated with shorter median PFS and overall survival (OS) of breast cancer patients. They also found that high cumulative methylation index levels at week 4 were related to worse PFS [75]. In a meta-analysis involving 3,915 breast cancer patients, Sheng et al. [76] found that ESR1 and PITX2 promoter methylation were linked to worse OS. In contrast, Huang et al. [77] found no association between plasma ctDNA levels and clinicopathological parameters. Another study also yielded ambiguous results. Iqbal et al. [19] found no significant difference in the OS durations of breast cancer patients based on

their baseline levels of ALU115, ALU247, and DNA integrity.

With the rapid development of NGS technologies, the obstacle of identifying highly diluted ctDNA among normal germline cfDNA has been circumvented [78]. Recent evidence has shown that ctDNA sequencing may have prognostic value in predicting residual disease after neoadjuvant chemotherapy with high specificity [50]. Several novel biomarkers of TNBC, some of which remain controversial for assessing prognosis, must be elucidated. In early-stage TNBC, a previous subgroup analysis of a retrospective study in patients with TNBC who received adjuvant chemotherapy after surgery, low mRNA expression in patients with TP53 missense mutation was associated with poor prognosis [79]. In this study, Ji-Yeon Kim et al. concluded that TP53 mutation and its expression were a potential prognostic marker of TNBC. An in-depth study of ctDNA mutation was performed by Takeshita et al. [80], who used ddPCR of cfDNA to determine the PIK3CA mutation status of 49 patients with early-stage TNBC. Of these 49 patients, 12 (24%) had PIK3CA mutations and were followed for a median time of 54.4 months. Takeshita et al. confirmed that PIK3CA mutations are correlated with relapse-free survival and breast cancer–specific survival and demonstrated that PIK3CA mutations in TNBC are related to PI3K pathway–dependent androgen receptor phosphorylation, which is considered to be an independent prognostic factor for TNBC. In metastatic TNBC, the prognostic value of ctDNA was contradictory. Madic et al. [81] found that the baseline ctDNA levels of patients with metastatic TNBC were not predictive of radiological tumor response and were not correlated with time to progression or OS duration. They noted that because the prognostic value of ctDNA is still under evaluation, further exploration in TNBC is urgently needed. However, recent findings of a retrospective cohort study assessing the prognostic effect of ctDNA to characterize somatic copy number alterations (SCNAs) and quantify tumor fraction (TFx) in metastatic TNBC.Using low-coverage (0.13) whole-genome sequencing (WGS), Stover et al. [82] profile SCNAs and TFx, they found that certain SCNAs including chromosomal gains in drivers NOTCH2, AKT2, and AKT3 were more frequent in metastatic TNBC versus primary TNBC. Moreover, gain or amplification at 18q11 and 19p13 identified a subset of TNBC with poor prognosis in the metastatic setting. In addition to evaluate SCNA differences in primary versus metastatic TNBCs, they also identified that patients with TFx≥10% had significantly inferior survival and remained significant independent of clinicopathologic factors. In view of the above results, although it is essential to continue a further study including larger numbers of specimens, we have abundant reasons to believe that ctDNA mutations can serve as a prognostic information for TNBC.

Conclusion

A highly sensitive ctDNA-based system is urgently needed in further studies for early detection and management of TNBC patients. Patients with TNBC have increased risk of relapse and inferior outcomes. Despite the aggressive biology and poor prognosis associated with TNBC, currently, there are no acknowledged ctDNA-based targeted treatment regimens available for patients with this disease subtype. This lack of targeted therapy and detrimental biology highlight the urgent need for a novel biomarker to facilitate a more tailored treatment approach to improve outcomes for patients with TNBC. Several studies have characterized genes and pathways in TNBC that may be targeted therapeutically [83-87], and these drug targets, coupled with improved ctDNA detection at all stages of disease, will improve the management of TNBC patients considerably.

Currently, despite the great progress made in exploiting ctDNA for determining prognosis and assessing NCT response in patients of TNBC, ctDNA is rarely used for early disease screening and the detection of the response to treatment of advanced stage. The hope that the early detection of TNBC, when the disease is most amenable to cure, can afford opportunities for treatment while sparing patients from overtreatment has not yet been realized. Several studies with small samples have already demonstrated the use of ctDNA in the management of TNBC, but the extent to which the detection of ctDNA influences treatment decisions and affects survival outcomes remains unclear. Moreover, the amount of ctDNA can greatly vary as time goes on, demanding abundant molecular detection technologies able to detect them even when present at low levels. In the meantime, there is a great heterogeneity among the ctDNA data in different studies, technical platforms and patient populations. A standardization of cfDNA analyses in terms of sample collection, processing, and molecular techniques is needed. Finally, there is currently no industry standard or widespread clinical acceptance for the use of ctDNA, nor a uniformly agreed upon platform in TNBC. In the TNBC research setting, there are many barriers to universal clinical implementation of detection in ctDNA, including the need for plasma samples, cost of testing, lack of submitting an expense account, and poor understanding of the clinical implications associated

with tumor mutations. In order to advance our knowledge of this complicated and everchanging field, we desperately need large, international, multi-institutional cooperative trials that will allow us to enroll patients with less common germline mutations to accomplish the process of TNBC diagnosis to treatment to evaluate prognosis.

Abbreviations

AMGA: atypical microglandular adenosis; BLBCs: basal-like breast cancers; cfDNA: cell-free DNA; ctDNA: circulating tumor DNA; ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; MGA: microglandular adenosis; MGMT: O6-Methylguanine-DNA methyltransferase; NCT: neoadjuvant chemotherapy; OS: overall survival; pCR: pathological complete response; PD-1: programmed death receptor 1; PD-L1: programmed death 1 ligand 1; PFS: progression-free survival; PR: progesterone receptor; SCNAs: somatic copy number alterations; TFx: tumor fraction; TNBC: triple-negative breast cancer; WGS: whole-genome sequencing.

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Competing Interests

The authors have declared that no competing interest exists.

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