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Commentary

Mutation-based circulating tumor DNA detection approach to monitoring the therapy response in breast cancer☆

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Breast cancer is responsible for the highest number of cancer-related mortalities in females.[1] For breast cancer patients who need systematic chemotherapy, especially with locally-advanced breast cancer, neoadjuvant chemotherapy (NAC) is pivotal in optimizing subsequent treatment strategies and is essential for enhancing overall survival rates.[2] Thus, monitoring NAC response is crucial to identify the patients achieving a pathologic complete response (pCR) and the non-responders early to de-escalate or change therapy, respectively. However, current monitoring methods, such as imaging technologies (e.g., ultrasound and magnetic resonance imaging [MRI]), traditional serum biomarkers (e.g., CA15-3 and CEA), and core-needle biopsy with Ki67 assay during the NAC, cannot meet the need to measure the dynamic response in NAC accurately.[3]

The circulating tumor DNA (ctDNA) dynamic has been demonstrated to be associated with tumor burden in multiple cancer types, while its utility in monitoring the NAC response was still unclear. Recently, Magbanua *et al.* (2023)[4] investigated the clinical significance of the ctDNA analysis in epidermal growth factor receptor 2 (HER2)-negative breast cancer patients receiving NAC in the ongoing I-SPY2 trial (NCT01042379).[2,5] For each participant, ctDNA was analyzed before, during, and after NAC. The bespoke multiplex polymerase chain reaction (PCR) next-generation sequencing-based assay was applied to cell-free DNA (cfDNA) to detect patient-specific somatic mutations previously selected by whole-exome sequencing of the pretreatment biopsy samples.[4]

In this mutation-based approach, ctDNA positivity rates were 91% for triple-negative breast cancer (TNBC) and 69% for the hormone receptor (HR)-positive/HER2-negative breast cancer before NAC. The higher pretreatment ctDNA positivity and concentration were associated with aggressive clinicopathologic variables and mRNA profiling features of cell cycle and immune-associated signaling. Additionally, the ctDNA positivity rates decreased during the NAC in both subtypes; and the ctDNA negativity after NAC correlated with a better prognosis, even in non-pCR patients. However, early clearance of ctDNA three weeks after treatment was only related to good NAC response in TNBC, but not in HR-positive/HER2-negative breast cancer. In a substudy of the NeoALTTO trial (2019),[6] which analyzed the ctDNA in breast cancer patients receiving neoadjuvant anti-HER2 therapy, ctDNA positivity rates decreased during the NAC as well. Among them, the patients with undetectable ctDNA before NAC had the highest pCR rates.

Due to the absence of common mutations in breast cancer, the whole-exome or panel-based sequencing of the pretreatment tumor sample is routinely needed for mutation-based ctDNA monitoring in patients with breast cancers. Moreover, somatic mutations only exist in a subgroup of tumor cells. At the same time, this approach fails to detect the newly-developed treatment-resistance mutations generated during or after the NAC. In comparison, unique cfDNA methylation and fragmentomics patterns are universally present in particular cancer types.[7] Thus, there has been a growing interest in detecting ctDNA using the methylation and fragmentomics features for monitoring therapeutic response, which does not rely on patient-specific somatic mutation information.

As a pilot study, Takahashi *et al.* (2017)[8] used one-step methylation-specific PCR to detect the methylation status in the promoter region of the *RASSF1A* gene in cfDNA (met-ctDNA). The met-ctDNA showed higher sensitivity than the serum protein biomarkers, as the positivity rate of met-ctDNA before NAC (23.0%) was significantly higher than that of CEA and CA15-3 (7.4–8.6%). Similarly, met-ctDNA significantly decreased after NAC in responders but not non-responders. Moss *et al.* (2020)[9] detected and quantified the breast-derived cfDNA in breast cancer patients before and throughout NAC using three breast-specific differential methylation signatures. Before NAC, breast-derived cfDNA was detected in 80% of patients at 97% specificity. After NAC, non-pCR patients had elevated levels of breast-derived cfDNA. Additionally, levels of breast-derived cfDNA during the last month of NAC were significantly lower than those before NAC for the pCR patients but not non-pCR patients. Namely, the methylation-based ctDNA detection approach could predict the NAC response and the presence of residual disease in a universal manner.

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Furthermore, ctDNA has been demonstrated to shed in a particular fragment pattern. Recently there was one piece of evidence suggesting that the cfDNA fragmentomics profile can also predict pathological response after NAC. Wang *et al.* (2023) demonstrated that combining the cfDNA 5′-end motif profile with the MRI imaging could successfully predict the post-NAC pCR in patients with locally advanced rectal cancer, yielding areas under the curve (AUCs) of 0.92–0.96.[10] In our current study (2023) on 33 breast cancer patients who received NAC, the cfDNA fragmentomics features exhibited excellent discriminative power in distinguishing 9 patients with pCR and 24 patients with non-pCR (AUC = 0.82, 95% confidence interval [CI], 0.68–0.97; unpublished results), which provided an alternative approach for the NAC response prediction for breast cancer patients. Meanwhile, the pCR patients still showed more breast cancer-related cfDNA fragmentomics features than patients with benign breast nodules (Wilcoxon, $P = 3.6 \times 10^{-3}$; unpublished results).

In conclusion, ctDNA has been recently demonstrated as a predictor for NAC response and survival through mutation-based, methylation-based, and fragment-based approaches. Considering the different advantages of these three ctDNA detection approaches, real-world studies investigating the clinical significance of combing these approaches in NAC are still needed.

Declaration of completing interest

The authors have no conflicts of interest to declare.

Author contributions

J.L., Y.H., and X.W. wrote the manuscript. All the authors have read and approved the final manuscript for publication.

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