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Evaluation of circulating tumor DNA as a biomarker for gynecological tumors

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Circulating tumor DNA (ctDNA) is a DNA fragment released into the peripheral blood by apoptotic or necrotic tumor cells, which carry the basic genetic information after gene mutation. The detection and analysis of ctDNA can provide genomic information on the size and development of tumor; therefore, it is considered as an emerging and promising tumor biomarker for cancer progression, reoccurrence, and routine monitoring after surgery. Albeit the isolation method is relatively simple due to the stability of ctDNA, the abundance of ctDNA is extremely low, and result from the high content of background cell free DNA (cfDNA), the large difference between individuals, and the need of predicted detection sites, it is necessary to analyze ctDNA in a comprehensive way. At present, the main methods that meet the needs of ctDNA detection with sensitivity and specificity are amplification refractory mutation system PCR (ARMS-PCR), digital-PCR (dPCR) and high-throughput sequencing. Among them, dPCR has advantages in practicality, which can realize absolute quantification of single-molecule DNA, detect and trace DNA molecules and conduct quantitative analysis, providing a reliable basis for clinical monitoring of tumor recurrence and minimal residual diseases. The study reviews the detection and potential value of ctDNA in gynecologic tumors.

The clinical symptom of breast cancer at early stage lacks specificity. In 2015, Olsson *et al*[1] conducted ctDNA testing on blood samples of 20 postoperative breast cancer patients, which showed that ctDNA had a specificity of 100% and a sensitivity of 93% for predicting disease recurrence. In 86% of breast cancer patients, ctDNA predicted breast cancer recurrence averagely 11 months earlier compared to the imaging predictions. Saliou *et al*[2] used phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) as a common mutated gene in breast cancer. They found that tumor could be detected earlier than surgery, with 93% consistency with preoperative plasma samples through ctDNA detection of this mutated gene in plasma. Riva *et al*[3] also used droplet digital PCR (ddPCR) to track the mutations of TP53 during neoadjuvant chemotherapy and found deceased ctDNA level in plasma of patients with significant clinical efficacy during the course of diagnosis and treatment. Furthermore, no ctDNA was detected after surgery, and patients with poor prognosis had elevated ctDNA levels. Another study came to the same conclusion by measuring methylated circulating tumor DNA (met-ctDNA) levels during neoadjuvant chemotherapy. So, ctDNA detection is feasible to predict the curative effect of neoadjuvant chemotherapy. At present, it is believed that the difficulty in the treatment of metastatic breast cancer may be related to the lag clinical, and early detection of micro-lesions. Therefore, ctDNA is a good biomarker for the prediction of recurrence and metastasis and evaluation of prognosis for high-risk breast cancer patients.

Triple negative breast cancer (TNBC) is a highly invasive disease with a high recurrence rate and a low overall survival rate. In a study of 50 patients with TNBC after neoadjuvant therapy (NAT),[4] the frequently mutated gene TP53 in their plasma ctDNA was analyzed by next-generation sequencing (NGS) and by ddPCR in the biopsy tissue to dynamically monitor the development of the tumor. In another study, 40 patients with early non-metastatic TNBC with TP53 mutation were enrolled, results showed that increased ctDNA level was associated with tumor progression during neoadjuvant chemotherapy, indicating poor prognosis. Therefore, the detection of plasma ctDNA in TNBC patients during NAT can help

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timely understand the mutation of tumor gene, dynamically monitor the development of tumor, and predict the early recurrence of TNBC patients after NAT.[5]

Early detection is important to improve the survival rate of ovarian cancer patients, with the 5-year survival rate of early treatment up to 90%; however, only about 20% when in the late stage. For ovarian cancer diagnosis, ctDNA has high specificity and sensitivity in the early stage. Bettegowda *et al*[6] studied 640 ovarian cancer patients, and found 96% of the patients had mutations in one or more genes involved in the mitogen-activated protein kinase pathway.[6] These findings suggest that ctDNA methylation or mutation testing may be useful in the diagnosis of ovarian cancer. Genetic mutations may indicate the need for improved chemotherapy regimens or new sensitive chemotherapy drugs in patients undergoing second-stage chemotherapy. ctDNA mutation detection can also be achieved in the early diagnosis of tumors. Phallen *et al*[7] detected somatic mutation in 68% of early-stage ovarian cancer tissues, which was highly consistent with ctDNA detection. Studies have used the cfDNA integrity index (the ratio of long cfDNA fragments to short cfDNA fragments) for tumor risk factor assessment and stratification. Zhang *et al*[8] studied the *Alu* repeat sequence of plasma samples from 48 ovarian cancer cases, ovarian cyst and healthy women. The integrity index, *Alu* fragment and integrity index of ovarian cancer group were significantly higher than that of control group. ctDNA concentrations are higher in aggressive and metastatic tumor types, such as high grade serous ovarian cancer (HGSC). To determine whether ctDNA is useful in localized ovarian cancer, ctDNA-specific mutations in adult ovarian granulosa cell tumors (AGCTs) have been examined. Farkkila *et al*[9] tested the plasma Forkhead Box L2 (FOXL2) gene in 33 AGCTs patients, and found that 36% of the patients had positive mutation, which preliminarily proved that ctDNA could be used for the diagnosis of AGCTs.

Breast cancer susceptibility gene 1/2 (*Brca1/2*) and *TP53* mutations are common mutations in ovarian cancer. *Brca1/2* mutation is associated with chemotherapy resistance of ovarian cancer; therefore, it is used to monitor and evaluate the prognosis of patients. Ratajska *et al* detected the tumor tissues of 121 ovarian cancer patients, and simultaneously detected the *Brca1/2* somatic mutation of ctDNA, both of which were consistent.[10] ctDNA detection can cover all *Brca1/2* mutations in tumor tissues, and progression free survival (PFS) is significantly longer in BRCA reverse mutation-negative patients than in mutation-positive patients. Kim *et al*[11] detected HGSC tissue and plasma TP53 mutations, and the coincidence rate of both was 100%. The allele count (TP53MAC) of the mutant gene showed that the mutation content of ctDNA TP53 was positively correlated with the degree of surgical tumor reduction of ovarian cancer. ctDNA was extracted again 3 months after the last chemotherapy, divided into the high TP53MAC group ($\geq$0.2 copy/L) and the low TP53MAC group ($<$0.2 copy /L) according to the ctDNA count. The time to disease progression (TTP) of patients in the high TP53MAC group was lower, and the TP53MAC group was significantly shortened. TP53MAC was correlated with TTP.

At present, cervical cancer is one of the most common gynecological malignant tumors. In 2019, Liao *et al*[12] investigated the relationship between ctDNA in plasma and clinicopathology, efficacy and prognosis of cervical cancer. They included 188 cervical cancer patients and 200 controls. They found ctDNA content of the cervical cancer and control group were 15.76 ± 3.18 ng/mL and 7.82 ± 1.63 ng/mL, respectively. ctDNA content was significantly different in histological grade, infiltration depth, lymphatic metastasis and Federation of Gynecology and Obstetrics (FIGO) stage.

ctDNA in endometrial cancer and ovarian cancer has been comprehensively studied. Huang and Zhang[13] suggested that ctDNA is similar to cancer antigen 125 in sensitivity to the diagnosis of endometrial cancer and ovarian cancer, but with higher specificity. By comparing the levels of ctDNA before and after surgery, the researchers observed a significant correlation between the loss of ctDNA after surgery and patient prognosis (overall survival and PFS). Among the 10 patients with follow-up data, the average ctDNA level of the four patients was $>$10 copy/ml, and all of them died of disease. The ctDNA of five patients could not be detected after surgery, and all survived by the end of the study, and two of them had survived for more than 5 years. But the authors also suggest that preoperative ctDNA levels were not associated with patient survival. The results suggest that accurate ctDNA testing can predict the prognosis of patients, and can be used as an indicator of postoperative follow-up monitoring to guide early detection of recurrence and metastasis.

In conclusion, ctDNA detection and analysis can provide more approaches for diagnosis, prognosis and treatment of gynecological tumor. As ctDNA testing is still in the scientific research stage and lacks standardized management, the clinical effectiveness and practicability of ctDNA testing still need to be further evaluated before it can be converted into a standard clinical tool. It is believed that with the continuous progress of new detection methods, ctDNA will be used as a new tumor marker for clinical use, promoting the implementation of personalized therapy and precision medicine.

Conflicts of interest

None.

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