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Mini Review

Advances in Research and Application of Liquid Biopsy in Lymphoma

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Abstract

Liquid biopsy as a new rising non-invasive testing method plays an important role in assist of the diagnosis, evaluation of the efficacy and prognosis, monitoring of early recurrence and drug resistance. It has been widely applied in some type of solid tumors. Lymphoma is a group of heterogeneous hematological malignancies which most subtypes without specific tumor markers and evaluation of the efficacy and prognostic is mainly depends on and limited to biopsy and imaging. Compared to other solid tumors, lymphoma tumor cells were characterized with access to peripheral easier and immunoglobulin molecules diversity can be monitored as marker, which is more applicable to liquid biopsy. With advances in the detection technology, research and application of liquid biopsy in lymphoma has attracted more attentions.

Keywords: Liquid Biopsy, Circulating Tumor DNA, Lymphoma

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Introduction

Evaluation of the efficacy and prognosis has an important clinical significance in the management of cancer, which has a various ways and methods. High sensitivity of PCR molecular detection technology has been routinely applied to the diagnosis and treatment of leukemia, such as detection of BCR-ABL1 fusion gene in patients with chronic myeloid leukemia by quantitative real-time PCR, the sensitivity is about 10^{-5}. However, for solid tumors such as lymphoma, the current diagnosis and evaluation of the efficacy and prognosis mainly depends on biopsy and imaging. But biopsy is an invasive testing method, repeated sampling is not convenient, and focal sampling cannot fully reflect the heterogeneity of tumor samples. Imaging is limited by its radioactivity and the increased risk of secondary tumors, furthermore the detection sensitivity is low and cannot monitor of early disease progression and recurrence. Liquid biopsy as a new rising non-invasive testing method provides a beneficial way for the diagnosis, evaluation of the efficacy and prognosis, monitoring of early recurrence and drug resistance. It has been widely applied in some type of solid tumors such as lymphoma.

The Concept of Liquid Biopsy

Liquid biopsy is a technique for the diagnosis and evaluation of disease by detecting circulating tumor cell (CTC), circulating tumor DNA (ctDNA), or other markers (such as exosomes) which are released into the blood from the primary or metastatic sites of the tumor. At present, it is mainly analyzed by detecting the sequence of tumor-marked DNA or the altered expression of tumor-related RNA.

CTC represents free tumor cells released from the primary or metastatic sites of the tumor into the peripheral blood circulation. It has a complete cell structure containing DNA, RNA and protein, which can provide richer tumor-related information than ctDNA.[1] Cell free DNA (cfDNA) represents the sum of the free DNA in plasma or serum released from the healthy, inflammatory and tumor tissue cells after apoptosis or necrosis, mostly in the form of double-stranded DNA fragments of about 200 bp. The latest study findings can help identify the origin of cfDNA by analyzing the cfDNA fragments, constructing the maps of genome-wide nucleosome and comparing with the death and physiological conditions of various cells.[2] The ctDNA is part of cfDNA that is derived from the cfDNA of tumor cells. The cfDNA or ctDNA exist in various forms in plasma, urine and other humor such as free DNA fragments, nucleosomes, exosomes, virtosomes, etc.[3] Exosomes are a type of vesicle-like body secreted by cells actively. Many researches have shown that exosomes are involved in the progression and metastasis of tumor.[4]

The Basis and Application of Liquid Biopsy

Liquid biopsy is an important technological advance in solid tumor research and clinical application in recent years. Early studies found that the average level of CTC or ctDNA in tumor especially metastatic tumor patients was higher than in healthy controls.[5,6] The average number of CTC in peripheral blood of metastatic solid tumors was 60 ± 6.93 cells/7.5 mL.[6] The levels of CTC or ctDNA are different in different types and stages of tumors.[7] Later studies found that CTC or ctDNA carry the same molecular abnormalities as the primary tumor tissue which provide a commendable tumor marker for the monitoring of solid tumors. However, it has been limited by the isolation of microscale CTC cells and the detection of low proportion gene mutations of ctDNA for a long time. The study of solid tumors still mainly depends on biopsy and imaging. With advances in high-throughput sequencing technology and the cancer genome, more and more gene mutations associated with diagnosis and treatment have been identified, which making it possible to analyze the whole genome sequencing or tumor related gene mutations of peripheral blood cfDNA.

Many studies have shown that liquid biopsy has potential application values in genotyping, evaluation of the efficacy and prognosis, monitoring of recurrence and drug resistance of many solid tumors. Previous studies made it clear that the number of CTC in peripheral blood associated with progression-free survival and overall survival in patients with metastatic breast, colorectal, and prostate cancer.[8] The corresponding CTC detection system, CellSearch, has also been approved by the Food and Drug Administration (FDA) for the prognostic evaluation of these three solid tumors. Bettegowda et al reported that the sensitivity and specificity of ctDNA for detecting *KRAS* gene mutations in patients with metastatic colon cancer were 87.2% and 99.2%, respectively, and drug resistance mutations in ctDNA were detected in 96% of patients who failed EGFR inhibitor treatment.[7]

Research and Application of Liquid Biopsy in Lymphoma

Lymphoma is a group of heterogeneous hematological malignancies which originated from peripheral lymphoid tissue. Lymphoma could be divided into various subtypes according to the origin of cell types and pathological changes in the organizational structure, and which with diverse prognosis due to different cell origin, pathological types and invasiveness. Although lymphoma is a type of solid tumor with organization structure, it is more likely to infiltrate the bone marrow. Compared to other solid tumors, lymphoma tumor cells were characterized with access to peripheral easier. Theoretically most B or T cell lymphomas have characteristic immunoglobulin (IG) or T cell receptor (TCR) gene rearrangement sequence in addition to tumor-specific gene mutations which can be used as molecular markers, while most lymphoma subtypes do not have specific tumor markers currently. Therefore, liquid biopsy has distinctive characteristics and advantages in the application of auxiliary diagnosis and MRD monitoring in lymphoma.

The Content of cfDNA in Lymphoma

One study found that cfDNA in different lymphoma subtypes are quite variable, the concentrations of cfDNA in untreated diffuse large B cell lymphoma (DLBCL), Hodgkin's lymphoma (HL) and mantle cell lymphoma were significantly higher than healthy controls (12.1 ng/mL), which were 26.9 ng/mL, 25.7 ng/mL and 23.1 ng/mL, respectively, whereas the concentration was lower in follicular lymphoma (14.7 ng/mL).[9] In another study of pediatric de novo lymphoma, cfDNA in various lymphoma (46 ng/mL) was also significantly higher than healthy controls (1.6 ng/mL). Furthermore, cfDNA in different staging lymphoma patients has significant differences. Roschewski et al reported that the median concentration of cfDNA in early stage DLBCL patients was significantly lower than that in progression.[10]

The Value of Liquid Biopsy in Clinical Application of Lymphoma

Auxiliary Diagnosis and Genotyping

With widely application of the next-generation sequencing (NGS) technology, genetic variation of related genes and clonal rearrangement sequence of IG/TCR gene by detecting ctDNA can be used in genotyping of lymphoma patients. Scherer et al detected ctDNA by NGS to sequence 268 related genes in untreated DLBCL patients (CAPP-Seq[11]), the results showed that the detection specificity for mutation in primary biopsy was 99.3%, and the median repeatability greater than 85% for single nucleotide variation and fusion gene in 97% (n=37) ctDNA.[12] Rasi et al detected ctDNA by ultra-deep NGS to sequence 59 related genes in untreated DLBCL patients, the results showed that the detection specificity for mutation (VAF>15%) in primary biopsy was 92%.[13] In some other lymphoma subtypes except DLBCL, ctDNA also has a good role of genotyping.[14,15]

Each B cell has a specific IG rearrangement sequence, and each T cell has a specific TCR rearrangement sequence. Based on this principle, the clonal rearrangement sequence of IG/TCR gene can partly guide judgment of B/T cell clonality. Earlier studies have found that clonal rearrangement of IG gene can be detected in plasma or serum DNA of B-cell neoplasia.[16] Armand et al detected ctDNA in patients with untreated DLBCL and mediastinal large B-cell lymphoma, 69% (11/16) of which showed gene sequence is consistent with the clonotype of primary tumor tissue.[17] Kurtz et al found that gene sequence in 82% untreated DLBCL patients and 100% relapsed DLBCL patients are identical to the clonotype of primary tumor tissue by detecting cfDNA.[18] Generally, ctDNA is an IG gene clonal rearrangement marker that can monitor the clonotype of primary tumor, which can be applied in the diagnosis and MRD monitoring in lymphoma by combining NGS technology.

Although liquid biopsy has potential value in the genotyping of lymphoma, detection sensitivity of different mutation types and application in different lymphoma subtypes still need further study and verification.

Evaluation of the Efficacy and Prognosis

Several studies have found that cfDNA correlates with tumor burden, IPI score, LDH, clinical progression in patients with DLBCL.[9,10,18] By dynamically monitoring of ctDNA, Kurtz

et al found that changes in ctDNA correlate with response to treatment and are consistent with changes in imaging, which can predict treatment failure earlier.[10,19,20] One study of correlation between cfDNA and prognosis found that prognostic significance was distinctive in different lymphoma subtypes. A high level of cfDNA associated with poor prognosis in patients with untreated HL, DLBCL and follicular lymphoma.[9,21,22] However, there was no significant difference in overall survival between primary central nervous system lymphoma (PCNSL) patients with and without mutations detected in cfDNA.[14]

Prediction of Recurrence

Imaging is the primary method in remission lymphoma monitoring but cannot effectively monitor of early progression and recurrence which limited by its radioactivity and detection sensitivity. Compared with PET/CT, ctDNA has a higher specificity for predicting recurrence.[18] By monitoring ctDNA in complete remission DLBCL patients, one study found that who with positive tumor-specific IG gene rearrangement has a higher risk of disease progression than negative ones.[10] Roschewski et al reported that dynamic monitoring of ctDNA in remission DLBCL patients enabled to predict recurrence a median of 3.5 months in advance.[10] Scherer et al reported that ctDNA could predict recurrence in 90% (9/10) patients with relapsed DLBCL a median of 162 days in advance.[12]

Conclusion

As a non-invasive testing method, liquid biopsy has advantages in convenient operation and repeatable sampling, meanwhile, it can reflect the heterogeneity of tumors more comprehensively and guide the individualized treatment more precisely. To explore the role of liquid biopsy in genotyping and prognostic evaluation of lymphoma is of great value in clinical application and guidance. Combined with traditional biopsy, imaging and liquid biopsy will help to significantly improve the treatment and prognosis in lymphoma.

Authors' Contributions

All authors contributed equally to this research.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

Ethical Approval

Not applicable.

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