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Research Article

Current Research Status of Circulating Free DNA in Clinical Application

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Abstract: Circulating free DNA (cfDNA) refers to the free DNA nucleotides in body fluids, but the dynamic response in vivo tumor load and full assessment of genetic heterogeneity in cancer patients provides a non-invasive diagnostic approach. It is one of the effective biomarkers for cancer screening and diagnosis, and also provides for the prognosis for patients with advanced cancer and forecast information. In this paper, the clinical application of cfDNA is reviewed.

Keywords: cfDNA; A malignant tumor; Treatment; Gene

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Malignant tumors are a major concern in the world, and their mortality rate is high. Early detection and early treatment of tumors greatly improves the cure rate and prolongs the survival period of patients. Therefore, cancer screening and early diagnosis are the most effective strategies to reduce mortality. Serum tumor markers are currently the main screening method for malignant tumors, but their detection sensitivity and specificity are not high; imaging and pathology are the main diagnostic methods, but imaging and pathological diagnosis account for a relatively high proportion of advanced tumors. Therefore, other simple and efficient screening and diagnosis methods need to be proposed. In recent years, a large number of studies have shown that cfDNA liquid biopsy is expected to become a non-invasive biomarker for early cancer diagnosis.

1 The Source of cfDNA and Detection Methods

1.1 The Source of Circulating Free DNA

cfDNA refers to deoxynucleotides that are circulating free in body fluids. In 1948, cfDNA was first discovered in healthy people[1]. In 1977, Leon[2] et al. found that the level of cfDNA in tumor patients was significantly higher than that of normal people. Later, some scholars confirmed this view and proposed that the detected genes in the cfDNA of cancer patients are consistent with the tumor genome. However, the source and release mechanism of cfDNA are still unclear so far. Some scholars speculate that cfDNA is derived from apoptotic cells[3]. However, another study showed that patients with lymphoma, lung, ovarian, and cervical tumors who received radiotherapy had a significant decrease in cfDNA levels, indicating that necrosis is the main route of cfDNA release[2]. In addition, some studies have shown that cfDNA is secreted from living cells[4]. In addition, many factors in the body may affect the release of cfDNA, and more studies are needed for verification in the future. Therefore, apoptosis,

necrosis, and secretion of living cells seem to be part of the causes of cfDNA production; however, the exact mechanism of cfDNA release remains elusive.

1.2 Detection Methods for cfDNA

cfDNA exists in a variety of body fluids such as blood, urine, pleural fluid, and ascites, etc., all of which are the main sources of samples. High blood extraction efficiency and more advantages for tumor mutation analysis are currently the main sources of samples. Currently, cfDNA extraction methods include digital PCR, Sanger sequencing, second-generation sequencing, and large-scale horizontal sequencing. The characteristics of each method are different.

2 Application and Development of cfDNA in Pathology

2.1 Research on cfDNA in Gastrointestinal Malignant Tumors

A large number of studies have shown that cfDNA has a suggestive significance in the diagnosis of gastrointestinal malignancies. In 2018, a study proposed that cfDNA may be an economical, minimally invasive, and effective new biomarker for the diagnosis of gastric cancer[5]. In 2020, studies from Chinese scholar Zhong[6] et al. came to the same conclusion. They monitored the cfDNA concentration of 116 patients with gastric cancer receiving chemotherapy and 40 healthy people. The plasma cfDNA concentration of gastric cancer patients was significantly increased, and its diagnostic effect was excellent. Compared with traditional tumor markers, it can be used as tumor biomarkers to monitor the therapeutic effect of gastric cancer. A large number of studies have also shown that cfDNA is also an effective marker for the diagnosis and monitoring of colorectal cancer. The studies of Marcuello[7] and Toiyama[8] have verified this view.

In 2017, Tie[9] et al. found that cfDNA can not only be used for the diagnosis of bowel cancer, but also for detecting tiny residues and predicting the recurrence of bowel cancer. This inspired a new method for post-treatment monitoring and recurrence of bowel cancer. In 2019, Liebs[10] et al. detected common point mutations in the KRAS and BRAF oncogenes in the cfDNA of 65 cancer patients, and compared them with mutations in tumor tissues. They found that cfDNA can be used for mutation monitoring as a personalized treatment, but sensitivity limited its early application. In the same year, a US study[11] conducted a prospective study on 42 patients with gastrointestinal cancer and patients with acquired targeted therapy resistance, and found that in the case of acquired resistance, cfDNA can be effectively detect a variety of drug resistance changes simultaneously, but single-lesion tumor biopsy often fails to identify the existence of multiple clinically relevant drug resistance mechanisms. This discovery is of great significance for guiding clinical treatment. However, there is no clear conclusion on whether cfDNA concentration is related to gender, age, and pathological type. More scientific research is still needed.

2.2 Research on cfDNA in Gynecological Tumors

Breast cancer and ovarian cancer are common gynecological malignancies that threaten women's health. A large number of studies have proved that cfDNA is a biomarker for the diagnosis and prognosis of gynecological malignancies[12]. Yang[13] et al. studied selected breast cancer, benign breast tumors patients and healthy people, and concluded that cfDNA detection can be used for early diagnosis of breast cancer. Ge[14] et al. analyzed the cfDNA levels of 61 breast cancer patients before and after neoadjuvant chemotherapy and concluded that the dynamic changes of cfDNA levels reflect the state of the disease, which can be used to monitor the treatment efficacy of patients. Zhou et al.[15] analyzed CA153, CEA and cfDNA in 62 patients with recurrent breast cancer and found that the positive rate of cfDNA was much higher than that of CA153 and CEA. cfDNA was more sensitive and specific in monitoring breast cancer recurrence.

In 2015, Shao[16] conducted a study on 36 patients with ovarian cancer, 22 patients with benign ovarian tumors and 19 healthy people, and found that the cfDNA level of the ovarian cancer group was significantly higher than the other two groups, which can be used for early diagnosis of ovarian cancer. At the same time, it was found that the cfDNA level increased after the operation and then gradually decreased, which can be used to monitor the treatment effect. In 2017, Weigelt[17] et al. found that BRCA1/2 reversal mutations in cfDNA sequencing can help breast and ovarian cancer patients undergoing PARP

inhibitory treatment. In 2018, Park[14] et al. used digital polymerase chain reaction (dPCR) to confirm that the same TP53 mutation exists in ovarian cancer tissue and cfDNA, simplifying the diagnosis of high-grade serous ovarian cancer. Currently, cfDNA has been increasingly studied in gynecological tumors, and the scope is expanding, for example: studies on the mutations in cfDNA that affect the prognosis, and the resistance mechanism of gene mutations in treatment, etc.

2.3 Research of cfDNA in Lung Cancer

In 2016, overseas scholars discovered that cfDNA can not only be used for the diagnosis of lung cancer, but its sensitivity is also higher than that of traditional tumor markers (SSC-Ag, CA125, CEA)[18]. Guo [19] et al. verified that the cfDNA detection method can detect EGFR lung cancer mutations, which can be used as a predictive marker for targeted therapy; some scholars have proposed that according to the analysis of the plasma cfDNA levels in patients with different pathological types of NSCLC, there is no significant difference in the plasma cfDNA levels in patients with adenocarcinoma and squamous cell carcinoma. This conclusion needs a lot more experimental studies to verify.

2.4 Research in Other Aspects

In 2016, Mazutek[20] et al. found that high levels of cfDNA are unique to patients with head and neck squamous cell carcinoma (HNSCC). The results proved the diagnostic potential of cfDNA testing in the early detection and monitoring of human papillomavirus positive HNSCC. In 2018, Valpione[21] conducted a prospective study on cfDNA concentration in 43 patients with metastatic melanoma, and concluded that cfDNA is a surrogate marker of tumor burden in patients with metastatic melanoma and is a prognostic indicator for predicting the overall survival time. In addition to the above-mentioned tumors, cfDNA has been studied in esophageal cancer, prostate cancer, and renal clear cell carcinoma.

In addition to malignant tumors, research on other diseases is also emerging, such as: obstetrics, neonatology, infectious diseases, and autoimmune diseases, etc. Click[22] et al. used PCR technology to detect cfDNA in tuberculosis patients, which confirmed the application of cfDNA detection in tuberculosis and proposed a new method for tuberculosis diagnosis. In 2018, a study[23] found that patients with systemic lupus erythematosus had high levels of cfDNA, and the results proved that cfDNA has great value in assessing the disease activity of patients with systemic lupus erythematosus and treatment monitoring. Related reports in 2020 pointed out that the amount of cfDNA is a key factor in the detection of trisomy 21[24], and its influence on the detection rate is very crucial.

3 The Advantages and Limitations of cfDNA Testing

3.1 The Advantages of cfDNA Testing

cfDNA has been proposed as a liquid biopsy test. It is not only minimally invasive, easy to obtain, simple to operate, yield rapid results and the possibility of repeated analysis. At the same time, it can overcome the shortcomings of the limitations of tissue biopsy and can comprehensively evaluate the genetic heterogeneity of tumors. In short, cfDNA has established a high-sensitivity and specific detection method that can be used in the diagnosis, prognosis and treatment monitoring of cancer patients or to assist the diagnosis of other diseases in future.

3.2 The Limitations of cfDNA Detection

DNA is a challenging sample type, which has the characteristics of low yield and complex fragment distribution. Currently, cfDNA testing has many problems. For example: sample collection is not uniform; extraction methods are diverse, but the efficiency varies; as well as the choice of kits and the storage time and temperature of samples. In conclusion, the research of cfDNA is still in the experimental stage, and more breakthrough research is needed in this field to solve the problems mentioned above, so that cfDNA can be better used for disease diagnosis.

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