

Liquid Biopsy and Non-invasive Biomarkers

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Abstract. In the context of global aging and the prevalence of various diseases, traditional invasive diagnostic techniques have their limitations. Liquid biopsy, with its non-invasive and convenient features, has become a research hotspot. This article reviews its development process, from the qualitative detection of cfDNA discovered at the end of the 20th century, to the quantitative detection facilitated by NGS. And then to the clinical applications. The system comprehensively analyzes the mechanism and detection methods of key biomarkers such as cfDNA, exosomes, and CTCs. Elaborate on the innovative directions of biomarker enrichment (such as nanomaterial strategies) and multi-omics analysis algorithms (such as CNN models). At the same time, the challenges in terms of technology (stability, cost), clinical (validation data, scenario definition), and regulatory and ethical aspects of transformation were also pointed out. Introduce the research progress of ultra-high sensitivity and multi-modal integration technology. We explore cutting-edge directions such as single-cell detection and AI integration, aiming to provide theoretical references for the clinical application of liquid biopsy.

Keywords: Liquid Biopsy; Non-invasive Biomarkers; Development.

1. Introduction

In today's society, the global aging population is intensifying, and the incidence of chronic diseases (such as cancer, sepsis, hypertension, etc.) and infectious diseases (such as COVID-19, tuberculosis) is continuously increasing. Organ biopsies, surgical sampling, etc., all fall under traditional invasive diagnostic techniques. They involve high operational risks and poor patient compliance, which place greater demands on clinical treatment. Moreover, the detection rate in the early stage of the disease is relatively low, which often results in patients missing the best treatment opportunity and increasing the medical burden and social costs. As the medical system becomes increasingly urgent in pursuing precision medicine, liquid biopsy, with its advantages such as non-invasiveness, convenience, and repeatable testing, can achieve disease diagnosis and monitoring through body fluid samples such as blood, urine, and cerebrospinal fluid. It meets the current social demand for efficient and safe medical technologies.

In 1996, scientists first detected circulating tumor DNA in the blood of cancer patients, laying the theoretical foundation for liquid biopsy. At the same time, some new types of non-coding RNAs (such as circRNAs and lncRNAs), protein complexes and other biomarkers were also discovered. It demonstrates high specificity in the diagnosis of early-stage cancers and neurodegenerative diseases (such as Alzheimer's disease). Meanwhile, as research continues to deepen, liquid biopsy has expanded from disease diagnosis to treatment monitoring and prognosis assessment, providing clinicians with immediate and comprehensive information for making decisions.

However, at present, there is a lack of systematic exploration and analysis of the mechanisms and current status of key biomarkers in liquid biopsy. Therefore, this article intends to systematically introduce and discuss the detection mechanisms and current clinical application status of key biomarkers (such as cfDNA, exosomes, etc.), and thoroughly explore the core challenges faced by this technology. The paper focuses on analyzing the feasible paths for optimizing biomarker enrichment techniques and innovating multi-omics analysis algorithms, with the aim of providing more theoretical reference bases and solutions for the clinical transformation and promotion of liquid biopsy technology.

2. Liquid Biopsy Development History

From the end of the 20th century to the beginning of the 21st century, this research focused on the discovery and initial application of cell-free DNA (cfDNA). In 1996, scientists first detected circulating tumor DNA in the blood of cancer patients, laying the theoretical foundation for liquid biopsy. At this stage, the technology is limited to qualitative detection, with relatively low sensitivity, and can only be used for the diagnosis of advanced diseases.

At this stage, the technology was limited to qualitative detection with low sensitivity, and could only be applied to the diagnosis of advanced diseases. From 2010 to 2018, the popularization of next-generation sequencing (NGS) technology pushed liquid biopsy into the era of quantitative detection. The sensitivity of cfDNA testing has been enhanced, enabling precise detection of target substances such as tumor gene mutations and pathogen nucleic acids. The research on new biomarkers such as exosomes and circulating tumor cells (CTCs) has gradually expanded, broadening the application scope of liquid biopsy. For instance, the cancer diagnosis technology based on exosomal microRNAs has entered the clinical validation stage.

Since 2019, liquid biopsy technology has gradually entered clinical application. The US FDA has approved several tumor companion diagnostic reagents based on cfDNA. Reagents used for detecting EGFR gene mutations in non-small cell lung cancer [1]. In the field of infectious diseases, the rapid detection technology for pathogenic bacteria of sepsis based on cfDNA can complete the test within 6 hours, significantly reducing the time compared to traditional culture methods. Multimodal analysis (genomics, proteomics, metabolomics) has become a research hotspot, further enhancing the diagnostic accuracy and disease classification capabilities [2].

3. The Mechanism of Action of Key Biomarkers in Liquid Biopsy

Liquid biopsy, with its advantages of being non-invasive and convenient, has become an important means for disease diagnosis and monitoring. Its core relies on the precise analysis of the action mechanisms of multiple key biomarkers. The research on related non-invasive detection technologies has provided important support for the application of biomarkers [3].

3.1. Generation and Mechanism of Circulating Cell-Free DNA (cfDNA)

cfDNA mainly originates from the nucleic acid fragments released during cell apoptosis or necrosis. Under normal physiological conditions, the body can degrade cfDNA through nucleases, thereby maintaining a low concentration level of cfDNA in the blood. When the body is in a state of diseases such as tumors, infections, or tissue damage, the number of abnormal cell deaths increases significantly, resulting in a substantial rise in the release of cfDNA. Moreover, these cfDNA carry disease-specific molecular characteristics. For instance, such as genetic mutations in tumor cells, specific nucleic acid sequences of pathogens, etc. [3].

In terms of the detection mechanism, liquid biopsy based on cfDNA captures the cfDNA fragments present in the blood that carry information about diseases. By using sequencing technology (NGS) or nucleic acid amplification technology (dPCR) to analyze its molecular characteristics, the diagnosis and monitoring of the disease can be achieved. For instance, in the diagnosis of sepsis, by detecting the specific cfDNA sequences of pathogens in the blood, the type of pathogenic bacteria can be quickly identified, providing a basis for the precise use of antibiotics. In tumor diagnosis, by analyzing the genetic mutations (such as EGFR and KRAS mutations) in circulating tumor DNA (ctDNA), early screening and classification of cancer can be achieved, which is highly consistent with the goal of non-invasive diagnosis of early detection and precise identification of targets [3].

3.2. Biosynthesis and Mechanism of Action of Exosomes

The biosynthesis process of exosomes has distinct structural characteristics. It is a vesicle structure released into the extracellular fluid after the fusion of intracellular multivesicular bodies with the cell membrane, with a diameter typically ranging from 30 to 150 nanometers. And they carry various

biological molecules such as proteins, nucleic acids (mRNA, microRNA), lipids, etc. When the body is in a diseased state, the exosomes secreted by cells will undergo significant changes in quantity and content. For instance, in the exosomes secreted by tumor cells, the expression level of specific microRNAs (such as miR-21) is significantly increased. This characteristic makes it an important biomarker for cancer diagnosis.

The detection of exosomes mainly involves two key steps: separation and purification, as well as content analysis. Common separation methods include ultracentrifugation, immunomagnetic bead method, microfluidic chip method, etc. During the content analysis stage, by extracting nucleic acids or proteins from exosomes and combining techniques such as PCR, Western blot, and mass spectrometry analysis, it is possible to detect disease-specific molecular markers. Take the diagnosis of liver diseases as an example. By detecting the mRNA of alpha-fetoprotein (AFP) carried by exosomes in the blood, liver cell carcinoma can be diagnosed at an early stage. Moreover, its sensitivity is superior to that of the traditional serum AFP test, fully demonstrating the advantages of non-invasive detection techniques in improving diagnostic efficiency [3].

3.3. Mechanisms of Other Key Biomarkers

Circulating tumor cells (CTCs) are tumor cells that detach from the primary or metastatic site of a tumor and enter the bloodstream. Their core advantage lies in carrying the complete genomic information of the tumor, and their quantity is closely related to the tumor stage and prognosis. In terms of the detection mechanism, after separating CTCs through immunocapture techniques (such as antibody capture based on epithelial cell adhesion molecule EpCAM), further analysis can be conducted using cytological methods or genetic sequencing technologies. It can achieve the diagnosis of tumors and the detection of drug resistance, which provides direct cellular-level evidence for precise tumor diagnosis and treatment [4].

Circular RNAs (including circRNAs, lncRNAs, etc.) have a unique stability maintenance mechanism in the blood, which is achieved by binding to proteins or being encapsulated in exosomes to maintain stability, and their expression levels show significant tissue specificity and disease specificity. In terms of the detection mechanism, the expression levels of circulating RNA in the blood can be measured through reverse transcription PCR (RT-PCR) or NGS technology, which can help to explore its diagnostic value. For instance, the expression of circRNA HECTD1 is significantly downregulated in the blood of patients with colorectal cancer. This characteristic makes it a potential biomarker for the early diagnosis of colorectal cancer [5].

4. Innovative Strategies of Liquid Biopsy Technology

The innovative technology of liquid biopsy focuses on the enrichment of biomarkers and multi-omics analysis algorithms to enhance the efficiency and accuracy of the detection [6].

In terms of the optimization of biomarker enrichment technology, in the nanomaterial strategy, nanomagnetic beads (such as magnetic mesoporous silica nanospheres) are modified with specific antibodies to efficiently capture cfDNA, etc., and the enrichment efficiency is 3-5 times higher than that of the traditional centrifugation method. Nanofiltration separates based on size (such as exosomes, which range from 30 to 150 nanometers). Complete within 30 minutes and remain active. Innovations in immunity and molecular recognition. Multiple immune capture (such as microfluidic chip multi-target antibody array) enables one-stop detection of multiple biomarkers. It can be completed within one hour; the nucleic acid aptamer has high stability and low cost, and its recognition efficiency and sensitivity are better [7].

In the innovation of multi-omics analysis algorithms, data integration algorithms (such as CNN and Transformer models) integrate multi-dimensional data. The accuracy rate of cancer diagnosis exceeds 95%, which is higher than 85% of the results from a single omics approach; noise reduction (such as PCA) and feature selection (such as random forest) algorithms enhance efficiency and accuracy. In clinical prediction models, dynamic monitoring models (such as ctDNA mutation

abundance analysis) predict the response rate of tumor-targeted drugs to be as high as 90%. Risk stratification models (such as for tuberculosis) combine cfDNA with clinical information to facilitate resource allocation [8].

5. The Clinical Transformation Challenges of Liquid Biopsy Technology

5.1. Technical Barriers to Transformation

Issues regarding the stability and repeatability of performance testing: The current liquid biopsy techniques show that the test results among different laboratories and operators vary. For instance, the repeatability of cfDNA testing in some grassroots laboratories is only 75%. Far below the clinical requirement ($\geq 90\%$). The main reason lies in the lack of standardization in the sample processing procedures (such as the centrifugation speed of blood samples and storage temperature). The quality control system for the detection instruments and reagents is not perfect [9].

Excessive testing costs: The cost of a single test using liquid biopsy technology based on NGS is usually between 1,000 and 3,000 US dollars. It is difficult to be widely adopted in primary medical care and large-scale population screening; although the costs of dPCR and CRISPR-based technologies are relatively low, the detection throughput is limited and cannot meet the demand for simultaneous detection of multiple targets [9].

5.2. Clinical-level Transformation Barriers

Lack of large-scale clinical validation data: Most liquid biopsy techniques are still in the stage of small-scale clinical studies (with sample sizes less than 1,000 cases). The lack of multi-center and large-sample (sample size $> 10,000$ cases) clinical verification data makes it difficult to prove its diagnostic effectiveness in different populations and at different disease stages. For instance, the pancreatic cancer diagnostic technology based on exosomal microRNAs demonstrated good performance in small-scale studies, but in multi-center validation, the diagnostic sensitivity dropped from 92% to 78%, indicating that the clinical applicability of the technology still requires further verification [10].

The clinical application scenarios are not clearly defined: The clinical positioning of some liquid biopsy technologies is unclear. For instance, in the early screening of tumors, the existing technologies are unable to distinguish between "benign lesions" and "early malignant tumors". This leads to a relatively high false positive rate (the false positive rate of some techniques is over 15%), which may result in excessive medical treatment; at the same time, the complementary relationship between liquid biopsy and traditional diagnostic techniques (such as tissue biopsy) is not yet clear, and clinicians lack a unified understanding of the acceptance and usage norms of the technology [10].

5.3. Transformation Obstacles at the Regulatory and Ethical Levels

The regulatory approval system is not well-established: The approval standards for liquid biopsy technology vary among different countries and regions. For instance, the US FDA adopts the "Breakthrough Devices" channel to expedite the approval process, while some developing countries have not yet established a dedicated approval procedure for liquid biopsy, which has hindered the global promotion and application of this technology. Meanwhile, the liquid biopsy technology is advancing rapidly, but the regulatory approval process is lengthy (typically 2 - 3 years), making it difficult to keep up with the pace of technological development.

Ethics and Privacy Protection Issues: Liquid biopsy technology involves a large amount of molecular genetic information of patients, and there is a risk of information leakage. In prenatal diagnosis of fetuses (such as non-invasive prenatal testing NIPT based on maternal peripheral blood cfDNA), it may give rise to ethical controversies such as fetal gender selection and gene editing. Furthermore, in large-scale population screening, how to strike a balance between "early detection of diseases" and "avoiding excessive anxiety" remains a core ethical issue that needs to be addressed.

6. Development of New Technologies

6.1. Research and Development of Ultra-High Sensitivity Detection Technology

Single-molecule detection technology: Develop single-molecule detection technology based on quantum dot labeling and surface-enhanced Raman scattering (SERS), enabling precise detection of extremely low-concentration biological markers (such as pg-level cfDNA, single CTCs) in body fluids; for example, quantum dot-labeled cfDNA detection technology can detect a single mutated cfDNA molecule in 10 μ L of blood sample, with a sensitivity that is 10 to 100 times higher than existing technologies.

Real-time dynamic detection technology: Develop implantable or wearable liquid biopsy devices that can continuously monitor the dynamic changes of biomarkers in body fluids, achieving real-time early warning of diseases; for example, wearable skin sensors that detect exosomal microRNAs in sweat to monitor the blood sugar control of diabetic patients in real time, providing real-time basis for insulin dosage adjustment.

6.2. Development of Multimodal Integration Technology

Detection - Analysis Integrated Equipment: Develop an integrated device that combines sample pre-treatment, biomarker capture, and detection analysis. For example, a "liquid biopsy chip laboratory (Lab-on-a-Chip)" based on microfluidic chips can complete the entire process of blood sample filtration, exosome enrichment, cfDNA sequencing, etc. on the chip, reducing the detection time to within 30 minutes and being compact (like the size of a mobile phone), suitable for on-site testing and emergency rescue scenarios.

Multi-biomarker Combined Detection Technology: Develop multimodal detection technology that can simultaneously detect multiple biomarkers (cfDNA, exosomes, CTCs, proteins). For example, a technology platform combining flow cytometry and NGS can complete CTCs counting, cfDNA mutation analysis, and exosome protein detection in a single test, providing multi-dimensional evidence for disease diagnosis.

7. Research Frontiers and Unsolved Problems

7.1. Research Frontiers Direction

Single-cell level liquid biopsy: Explore liquid biopsy methods based on single-cell sequencing technology, by separating individual CTCs or circulating endothelial cells from the blood, to analyze molecular heterogeneity at the cellular level, providing a new perspective for precise cancer typing and research on drug resistance mechanisms; for example, single-cell RNA sequencing technology can reveal the gene expression differences of different subgroups in CTCs, helping to identify the key cell subgroups for tumor recurrence.

Cross-species liquid biopsy: Expand the application of liquid biopsy technology in veterinary fields and food safety areas, such as detecting cfDNA in animal blood for early diagnosis of livestock and poultry infectious diseases (such as African swine fever); detecting cfDNA of pathogens in food samples to achieve rapid traceability of foodborne diseases.

Deep integration of artificial intelligence (AI) and liquid biopsy: Utilize AI technology to optimize the entire process of liquid biopsy, including sample processing parameter optimization, detection data interpretation, and clinical decision support; for example, an AI-based sample processing system can automatically adjust parameters such as centrifugation speed and antibody concentration to achieve efficient enrichment of biomarkers; AI-assisted clinical decision systems can combine liquid biopsy results with patient clinical information to provide personalized treatment recommendations for doctors.

7.2. Unsolved Issues

Source and functional mechanism of biomarkers: The tissue origin and physiological function of many new biomarkers (such as circRNAs, exosomal proteins) remain unclear, making it difficult to explain their association with diseases, which limits the reliability of diagnostic models; for example, an increase in a certain circRNA in the blood may originate from multiple tissues, making it impossible to precisely locate the lesion site.

Universality of technology and individual differences: The applicability of existing liquid biopsy technologies varies among different populations (such as different ages, races, and patients with underlying diseases); for instance, the background concentration of cfDNA in the blood of the elderly is relatively high, which may interfere with the test results; how to adjust according to individual differences is also a key area for future research. Conclusion

Liquid biopsy, as an important means of precision medicine, has advanced from theoretical exploration to clinical application. By analyzing markers such as cfDNA and exosomes, combined with technological innovation, it significantly improves the efficacy of disease diagnosis and monitoring. However, issues such as insufficient technical stability, high costs, lack of large-scale validation data in clinical practice, ambiguous application scenarios, and regulatory and ethical controversies still restrict its widespread promotion. In the future, it is necessary to further clarify the origin and functional mechanism of biomarkers, optimize the universality of the technology to cope with individual differences. With the development of ultra-high sensitivity and multimodal integration technologies and the deep integration of AI, liquid biopsy is expected to break through the existing bottlenecks and play a greater role in early disease screening and precise treatment, providing key support for reducing medical burdens and improving patient prognosis.

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