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Article

Advances in the effects of circulating tumor DNA in lung cancer on the physiological and mechanical properties of lung tissue

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Abstract: Lung cancer is one of the most common and lethal cancers in the world, and early diagnosis and precise treatment are crucial for improving patient survival. The physiological and mechanical properties of the lung tissue play a crucial role in the progression and diagnosis of lung cancer. Compared with traditional tissue biopsy, liquid biopsy technology provides a new perspective for lung cancer diagnosis and treatment monitoring. Circulating tumor DNA (ctDNA), as an important component of liquid biopsy, has attracted much attention due to its non-invasiveness and ability to dynamically reflect changes in the tumor genome. ctDNA is a fragment of DNA released by tumor cells into the bloodstream, carrying the genetic characteristics of the tumor cells, and may also be influenced by the mechanical microenvironment of the tumor tissue, which the mechanical stress and strain within the lung tissue due to breathing mechanics, as well as the mechanical forces exerted by the growing tumor mass, could potentially affect the release and fragmentation patterns of ctDNA. Therefore, ctDNA testing reflects tumor burden and genomic characteristics. In recent years, with the development of highly sensitive detection technologies, such as digital polymerase chain reaction (PCR) and high-throughput sequencing, research on the application of ctDNA in lung cancer has made significant progress. In lung cancer management, the application of ctDNA focuses on the following aspects: firstly, ctDNA can be used for early screening and diagnosis to help detect tiny tumor loads. Abnormal biomechanical changes in the lung may precede the appearance of detectable tumors, and analyzing ctDNA in relation to these mechanical alterations might help in detecting nascent tumor at an earlier stage. Second, during treatment, ctDNA can be used for dynamic monitoring of treatment response and detection of drug resistance mutations. Finally, ctDNA can also be used as a biomarker for prognostic assessment to help predict patient survival and risk of recurrence. Although the application of ctDNA in lung cancer shows great potential, its clinical application still faces some challenges, such as detection sensitivity, lack of standardised processes and complexity of bioinformatics analysis. Therefore, further research and clinical validation are necessary to promote the widespread use of ctDNA in lung cancer management. In this paper, we review the current status and prospects of circulating tumor DNA in lung cancer.

Keywords: lung cancer; biomechanics; circulating tumor DNA; mechanics; liquid biopsy

1. Introduction

Lung cancer is the leading cause of cancer-related deaths in China. In 2022, there were approximately 4.8247 million new cancer cases in China, with 1.0606 million new lung cancer cases, accounting for 22.0% of all malignant tumor cases, with an incidence rate of 75.13 per 100,000 people [1]. These statistics highlight a significant public health challenge, as lung cancer not only poses a threat to individual patients but also places a substantial burden on the healthcare system. The high incidence rate

indicates that early detection and effective treatment strategies are urgently needed to mitigate this growing health crisis. Lung cancer often has subtle symptoms in its early stages, and these are easily overlooked by patients [2]. Common early symptoms, such as a persistent cough or slight chest discomfort, can be mistaken for less serious conditions, leading to delays in diagnosis. This underscores the importance of public awareness campaigns to educate individuals about potential warning signs and the need for regular screenings, especially for high-risk populations. While imaging techniques have improved early lung cancer diagnosis rates, they come with limitations such as high false-positive rates, overdiagnosis, and radiation exposure. These limitations can lead to unnecessary anxiety and invasive procedures for patients, as well as increased healthcare costs. Consequently, there is a pressing need for alternative diagnostic methods that can provide accurate results without the associated risks of traditional imaging. This means many patients are diagnosed at advanced stages, significantly affecting their prognosis. In recent years, treatment options for lung cancer have evolved dramatically. In addition to traditional surgery and radiochemotherapy, targeted therapies for mutations in genes such as epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1, receptor tyrosine kinase (ROS1), and neurotrophic tyrosine receptor kinase (NTRK), as well as immune checkpoint inhibitors (ICIs), have improved the efficacy of treatments for lung cancer patients to some extent [3–6]. These advancements represent a significant shift towards personalized medicine, where treatment plans can be tailored based on the genetic profile of the tumor. This approach not only enhances treatment effectiveness but also minimizes unnecessary side effects associated with conventional therapies.

However, as treatments progress, drug resistance often limits the long-term efficacy of therapies [7], leading to poor treatment outcomes. Resistance mechanisms can arise from various factors, including genetic mutations and the tumor microenvironment, complicating treatment strategies. Understanding these mechanisms is crucial for developing next-generation therapies that can overcome resistance and improve patient survival rates. Overcoming resistance, selecting appropriate biomarkers, and reducing immune-related adverse reactions are urgent issues that need to be addressed. The complexity of lung cancer biology necessitates a multifaceted approach to treatment, integrating both pharmacological and non-pharmacological strategies to enhance overall patient outcomes. Therefore, finding a sensitive and specific biomarker to accurately identify tumor baselines and predict tumor progression and genetic changes is crucial for patient management.

Circulating tumor DNA (ctDNA) is free DNA that is actively released by tumor cells or released into bodily fluids after tumor cell necrosis or lysis [8]. This unique characteristic of ctDNA makes it an invaluable tool in cancer diagnostics, as it can provide real-time insights into tumor dynamics without the need for invasive procedures. As a major bomarker molecule for liquid biopsy technology, ctDNA has attracted increasing attention in recent years. Compared with traditional tissue biopsies, ctDNA testing is non-invasive, minimally invasive, and easily repeatable, reducing patient suffering and risks [9]. The ability to perform multiple tests over time allows for continuous monitoring of tumor evolution, enabling clinicians to make timely adjustments to treatment plans based on the patient's current condition. ctDNA

testing reflects the spatial heterogeneity of tumors, helping to understand their complexity and treatment response [10]. This heterogeneity can significantly impact treatment efficacy, as different regions of a tumor may respond differently to therapy. Therefore, ctDNA analysis can provide a more comprehensive view of the tumor's biological behavior, informing more effective and personalized treatment strategies. This article aims to review the biological characteristics of ctDNA and its clinical applications in early diagnosis, drug efficacy prediction, and prognosis assessment in lung cancer, as well as to discuss its future prospects. By exploring the potential of ctDNA in these areas, we hope to highlight its role in transforming lung cancer management and improving patient outcomes.

2. Biological characteristics and detection methods of ctDNA

2.1. Biological characteristics of ctDNA

ctDNA mainly originates from necrotic or apoptotic tumor cells and circulating tumor cells. In addition to passive release, tumor cells can also actively secrete ctDNA through exosomes and microvesicles. The release of ctDNA is influenced by various factors, including the characteristics of the tumor itself, the tumor microenvironment, mechanisms of cell death, inflammation, and immune responses. Studies have shown that hypoxia, metabolic stress, and other factors can induce cell apoptosis or necrosis, affecting ctDNA release, while the biological characteristics of different tumor cells (such as chromosomal instability and cell metabolic state) can also influence the quantity and characteristics of ctDNA release. In normal individuals, ctDNA concentration is low, averaging only 13 ng/mL, whereas in cancer patients, the average concentration of ctDNA in plasma is 180 ng/mL. Its half-life is relatively short, usually less than 2 h [11]. ctDNA contains various tumor-related information, including gene mutations, deletions, insertions, rearrangements, copy number variations, and methylation. Compared to conventional tumor markers, ctDNA can dynamically reflect changes in tumor load during treatment and can detect residual tumor lesions earlier [12]. This suggests that ctDNA testing has high sensitivity and specificity in tumor detection. Moreover, due to the short half-life of ctDNA, it can quickly reflect the current tumor status, which is helpful for real-time tumor monitoring.

2.2. Detection methods of ctDNA

ctDNA concentration in blood is low, requiring highly sensitive tools for detection. Studies have shown that ctDNA concentration in serum is 3–24 times higher than in plasma. However, white blood cells rupture during blood clotting, releasing genomic DNA that may interfere with ctDNA concentration and purity. Additionally, ctDNA is unstable in blood due to the presence of DNA enzymes, making it more prone to degradation. Therefore, plasma ctDNA is purer and more suitable for ctDNA detection [13].

Current ctDNA detection methods are diverse, including Bead-Emulsion-Amplification (BEAMing), digital polymerase chain reaction (PCR), Amplification Refractory Mutation System PCR (ARMS-PCR), Tagged-Amplicon Deep Sequencing (TAM-Seq), Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq), and

Next-Generation Sequencing (NGS), among others. BEAMing is a high-sensitivity detection technique that combines digital PCR and flow cytometry. It enables the detection of low-concentration ctDNA in blood, aiding in the identification of genetic mutations in early-stage tumors. ARMS-PCR is a PCR-based technology that relies on allele-specific amplification to detect known gene point mutations. Its core principle involves leveraging the complementarity between the primer's 3'-end nucleotide and the template to distinguish wild-type and mutant alleles. ARMS-PCR and digital PCR each have distinct advantages suited to different applications. ARMS-PCR is simple to operate, cost-effective, and ideal for detecting known high-abundance mutations. In contrast, digital PCR offers superior sensitivity and precise quantification, making it particularly effective for detecting low-abundance ctDNA mutations, especially in early cancer screening and liquid biopsy applications. TAM-Seq, CAPP-Seq, and NGS are all based on high-throughput sequencing principles. Through optimized techniques (primer design, library construction, bioinformatics analysis), these methods achieve high sensitivity and specificity. TAM-Seq is well-suited for high-throughput, low-cost detection scenarios, particularly excelling in monitoring advanced-stage tumors. CAPP-Seq offers advantages in sensitivity and specificity, making it ideal for early diagnosis and personalized treatment monitoring. NGS provides comprehensive genetic variant detection capabilities and can overcome tumor heterogeneity, making it applicable to diverse clinical scenarios. However, its sensitivity is limited by sequencing error rates, background noise, and biases introduced during sample preparation. The complexity of data processing further complicates the detection of low-frequency mutations. Despite these challenges, emerging technologies like CODEC offer novel solutions to enhance detection sensitivity and accuracy [14]. These techniques can perform both qualitative and quantitative analyses of ctDNA. Qualitative analysis can detect gene mutations, deletions, insertions, fusions, rearrangements, copy number variations, methylation, microsatellite instability (MSI), and loss of heterozygosity (LOH), while quantitative analysis can calculate the real-time ctDNA concentration in blood. As a non-invasive detection method, ctDNA has important implications in cancer screening, prognosis, and treatment. It is considered an almost ideal next-generation tumor biomarker due to its minimal harm, high sensitivity and specificity, and ability to be repeatedly tested.

3. Applications of ctDNA in early diagnosis of lung cancer

Early-stage lung cancer often has no obvious symptoms or only very mild symptoms, which makes it easy to confuse with other common respiratory issues, thus delaying diagnosis and treatment. Although imaging examinations are considered the gold standard for diagnosing some cancers, early lung cancers are often small and slow-growing, making it difficult to detect small, low-density early lesions using common chest X-rays. CT scans have issues such as high false-positive rates, overdiagnosis, and radiation exposure. Furthermore, they are not highly sensitive for detecting FDG-negative nodules smaller than 5 mm. While CT is relatively inexpensive in China, it remains costly in many other countries, making it impractical to rely solely on imaging for lung cancer screening. Additionally, imaging examinations have inherent delays and cannot accurately reflect the evolution of the

tumor inside the body [15]. Traditional tumor markers also have low sensitivity and specificity in detecting early lung cancer. For example, carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), cytokeratin fragment 19 (CYFRA21-1), pro-gastrin-releasing peptide (Pro-GRP), and squamous cell carcinoma antigen (SCC) show poor sensitivity in early lung cancer detection. ctDNA testing uses highly sensitive molecular biology techniques to isolate and analyze these fragments from blood samples in order to obtain the genetic characteristics of the tumor. With its non-invasive nature, high sensitivity, and real-time monitoring capability, it provides an important tool for early tumor screening, personalized treatment, and recurrence monitoring. Therefore, ctDNA, as a novel liquid biopsy biomarker for lung cancer, holds great promise in early diagnosis.

Wang [16] developed an economical and effective ctDNA methylation detection method for lung cancer, called LunaCAM, based on multiplex qPCR technology, and evaluated its value in early lung cancer detection and differential diagnosis. Through DNA methylation analysis of 429 plasma samples (including 209 lung cancer samples, 123 benign disease samples, and 97 healthy participants), they identified optimal markers. The results showed that the LunaCAM-S model achieved an AUC of 0.90 between lung cancer and healthy individuals, while the LunaCAM-D model achieved an AUC of 0.81 in distinguishing lung cancer from benign diseases. In subsequent validation, LunaCAM-S identified 58 lung cancer patients (90.6% sensitivity), and LunaCAM-D excluded 20 patients without cancer evidence (83.3% specificity). The study found that LunaCAM-D performed significantly better than CEA blood tests in predicting lung cancer.

Cohen [17] developed the CancerSEEK test, a multi-analyte blood test that detects mutations in circulating proteins and free DNA to diagnose eight common cancers. This test is crucial for early cancer detection. Researchers used CancerSEEK in 1005 non-metastatic patients with specific organ cancers, including ovarian, liver, stomach, pancreatic, esophageal, colorectal, lung, and breast cancers. The median sensitivity of the test was 70%, with sensitivities ranging from 69% to 98% for five cancers that lack routine screening methods. In addition, Chen [18] recruited resected non-small cell lung cancer (NSCLC) patients at different stages, including early-stage (Stage I and II) and advanced-stage (Stage III and IV) lung cancer patients, as well as a healthy control group. They used high-throughput sequencing technology to analyze ctDNA in blood samples from the patients. The researchers focused on tumor-specific mutations, such as EGFR, KRAS, TP53, and other genetic mutations, and compared these results with traditional imaging techniques (such as CT scans) and tissue biopsy results to evaluate the sensitivity, specificity, and accuracy of ctDNA detection. The study found that in the early stages of NSCLC, the sensitivity of ctDNA detection for tumor-related mutations ranged from 40% to 60%, which was significantly higher than the sensitivity of traditional imaging methods, especially in cases with lower tumor burden. This research suggests that, compared to traditional imaging, ctDNA detection provides more sensitive early detection capabilities, particularly in patients with small tumors or lower tumor burden. Although ctDNA detection is more prominent in advanced-stage cancer patients, it can still serve as an indicator of disease presence to some extent in the early stages.

These findings suggest that liquid biopsy technologies offer high sensitivity and can detect abnormalities early in the tumor process, often identifying disease progression earlier than traditional imaging exams [19]. Therefore, liquid biopsy technologies can assist in early tumor screening and warning for healthy and high-risk populations. In addition, combining ctDNA testing with traditional tumor marker tests can compensate for the limitations of using a single method. ctDNA testing provides genetic information about the tumor, while traditional markers reflect the overall metabolic status of the tumor. By combining ctDNA testing with traditional marker tests, a more comprehensive tumor diagnosis and monitoring can be achieved. In postoperative monitoring, ctDNA can be used to detect minimal residual disease (MRD), while traditional markers can assess overall tumor burden. The high sensitivity of ctDNA testing can detect early tumor signals that are difficult to identify with traditional methods, while traditional markers can be used to validate and complement ctDNA results. However, at present, ctDNA still faces challenges in detecting low concentrations of ctDNA, laboratory standardization, and cost control. More research is needed to confirm its effectiveness in diagnosing early lung cancer through ctDNA testing.

4. Advances in ctDNA applications in lung cancer drug treatment

ctDNA provides a comprehensive reflection of the same mutational genome information as tumor tissue, capturing both spatial and temporal tumor heterogeneity more accurately than tissue sequencing. Studies have applied ctDNA to guide targeted and immunotherapy treatments, particularly in detecting drug resistance [20].

Qiu [21] used ultra-deep targeted NGS to evaluate the clinical utility of ctDNA in predicting the risk of dynamic relapse and the benefits of adjuvant chemotherapy (ACT) in NSCLC patients. The study found that ctDNA positivity after surgery and ACT was significantly associated with poorer relapse-free survival (RFS). In stage II-III patients, ctDNA positivity after surgery predicted a benefit from ACT, while ctDNA-negative patients had low relapse risks, regardless of ACT. ctDNA positivity during disease monitoring preceded radiologic relapse by a median of 88 days. This study emphasizes the importance of ctDNA analysis as a reliable biomarker for predicting disease relapse.

Another study [22] recruited 62 NSCLC patients receiving first-line pembrolizumab chemotherapy and analyzed changes in plasma ctDNA. The results showed that early changes in ctDNA allele frequencies after treatment initiation were associated with radiographic response and long-term clinical prognosis. At the first follow-up 21 days after treatment, the median change in ctDNA allele frequency was −90.1% in patients with radiologic response, −19.9% in stable patients, and +28.8% in progressing patients. These findings suggest that the rapid decline in ctDNA correlates with clinical benefits, and ctDNA may serve as an early biomarker for predicting drug response or resistance to immunotherapy.

Moding [23] investigated the use of ctDNA to predict the outcome of non-small cell patients treated with immune checkpoint inhibitor (ICI) consolidation after radiotherapy. The investigators analyzed blood and tissue samples from patients who received and did not receive ICI consolidation therapy, respectively, and found that

circulating tumor DNA was detectable prior to treatment in 78% of patients who did not receive ICI consolidation therapy and 75% of patients who received ICI consolidation therapy, respectively, and that all of these patients who had detectable circulating tumor DNA had undergone 24 months of radiotherapy treatment after progression, whereas those patients who did not have detectable circulating tumor DNA prior to treatment did not have any progression after 24 months of radiotherapy treatment, confirming that ctDNA is a biomarker for predicting disease progression after a patient has received radiotherapy. Of the eight patients who progressed, all had circulating tumor DNA detected from their blood and tissue samples prior to or at the time of disease progression, 4.1 months earlier than the imaging detection of disease progression, making the detection of circulating tumor DNA prior to, or early in the course of, a patient's consolidation therapy a strong predictor of disease progression. In addition, those patients with undetectable circulating tumor DNA after radiotherapy had a good prognosis regardless of whether they received consolidation therapy, suggesting that these patients with negative circulating tumor DNA may not have increased patient benefit even if they received consolidation therapy. In contrast, among patients with detectable circulating tumor DNA after radiotherapy, those who received consolidation therapy had a better prognosis than those who did not. The results of this study suggest that the detection of circulating tumor DNA may provide guidance on whether to receive consolidation therapy in patients with NSCLC after radiotherapy and make predictions about treatment outcomes. In addition, Raja [24] investigated the use of circulating tumor DNA to predict survival in patients treated with anti-PD-L1 therapy, and an early reduction in ctDNA was associated with improved survival in patients with lung and bladder cancers treated with duvarizumab. The study found that changes in variant allele frequency (VAF) of somatic mutations preceded imaging responses, and that patients with reduced VAF at 6 weeks had significantly smaller tumor volumes and longer progression-free and overall survival. This suggests that reduction in ctDNA VAF early in treatment may be a valuable predictor of long-term immunotherapy survival benefit.

Studies have also shown that ctDNA can guide therapy decisions and predict treatment efficacy, including resistance mutations and monitoring the evolution of resistance [25,26]. A report from the Tisch Cancer Institute at Mount Sinai in the United States, led by Mack et al., points out that the detection and analysis of ctDNA can identify actionable driver gene mutations and resistance mutations for targeted therapies. The study used ctDNA technology to conduct gene testing on plasma samples from 8388 advanced NSCLC patients, focusing on mutations in driver genes and resistance genes. The results showed that the mutation rate of actionable oncogenes was 48%, including EGFR (26.4%), mesenchymal-epithelial transition factor (MET) (6.1%), and b-raf proto-oncogene, serine/threonine kinase (BRAF) (2.8%) gene alterations, as well as gene fusions (ALK, RET, and ROS1; 2.3%). This suggests that therapy-induced resistance mutations are common, including both driver gene-dependent and non-driver gene-dependent changes. Among patients who experienced disease progression while on targeted EGFR therapy, the rate of resistance mutations in plasma was 64%. Additionally, compared with tissue tests at diagnosis, ctDNA testing increased the identification rate of driver gene mutations by 65%.

Bettegowda [27] used digital PCR technology to assess the ability of ctDNA to detect tumors in 640 patients with different cancer types, and to investigate whether ctDNA could provide clues to the mechanisms of resistance to EGFR inhibitors. Among 24 patients who had an objective response to treatment but later relapsed, 96% developed one or more gene mutations involving the mitogen-activated protein kinase (MAPK) pathway. This assists clinicians in detecting treatment responses based on resistance. Therefore, ctDNA can be used for early cancer diagnosis, monitoring tumor development, prognosis assessment, and personalized drug guidance, showing broad potential for application.

5. ctDNA in predicting prognosis in lung cancer patients

ctDNA testing is a promising research area due to its high sensitivity [28]. Studies have found that the ctDNA level in many late-stage cancer patients can be used to predict prognosis [29,30].

Zhang [31] found that pre-treatment ctDNA VAF was significantly negatively correlated with overall survival (OS). They proposed that pre-treatment VAF could serve as a surrogate marker for tumor burden and other prognostic variables, including ECOG performance status, baseline liver metastasis status, baseline lymph node metastasis status, smoking status, and tumor PD-L1 status. Additionally, the integration of pre-treatment and treatment VAFs to define a "molecular response" framework was shown to have a stronger correlation with response evaluation criteria in solid tumors (RECIST) compared to treatment VAF alone.

Song [32] evaluated ctDNA as a prognostic marker in advanced NSCLC disease monitoring. They found that high plasma ctDNA levels at baseline were associated with liver and bone metastases and shorter OS. Moreover, patients with complete ctDNA clearance had a significantly reduced risk of disease progression or death compared to those without ctDNA clearance, with extended progression-free survival and OS.

ctDNA plays a crucial role in the prognostic assessment of lung cancer patients. Studies have shown that ctDNA levels are closely associated with tumor burden, stage, treatment response, and relapse risk. High ctDNA levels typically indicate a higher tumor burden and more aggressive tumor characteristics, which are linked to poorer prognosis. In particular, ctDNA concentrations are often elevated in advanced lung cancer patients and can be used to evaluate treatment efficacy and disease progression. For instance, when treatment is effective, ctDNA levels usually decrease, while they may rise again if treatment fails or resistance develops. Furthermore, ctDNA testing can monitor potential resistance mutations, such as EGFR T790M, providing insight for personalized treatment. By dynamically monitoring ctDNA levels, doctors can detect early signs of tumor relapse and intervene promptly. Therefore, ctDNA, as a non-invasive and real-time biomarker that reflects the tumor's status, offers significant support for the prognostic assessment and personalized treatment of lung cancer.

6. Future outlook on ctDNA detection

In conclusion, ctDNA detection plays a crucial role in the early detection, diagnosis, treatment guidance, and prognosis prediction for lung cancer patients.

Therefore, research that uses liquid biopsy-based technologies to dynamically monitor ctDNA levels in patients with treated lung cancer, in order to assess the risk of cancer recurrence, holds significant importance. By monitoring ctDNA levels in lung cancer patients, it is possible to detect the potential for tumor recurrence in a timely manner and intervene in the patient's treatment, thereby extending the patient's survival while also reducing treatment costs, which has good economic benefits. However, there are still some shortcomings in current research on ctDNA. In terms of ctDNA testing, due to the low tumor burden in the early stages, the amount of ctDNA released may be very small, which significantly reduces the sensitivity of ctDNA testing. This is particularly true for patients with small tumors or localized lesions, where ctDNA may be insufficient to detect, leading to false-negative results. Moreover, the ctDNA release patterns may vary between different patients' tumors, and even within different regions of the same tumor, different types of ctDNA might be released. As a result, ctDNA may not cover all mutations or genetic features, which affects sensitivity. In terms of the detection process, the lack of standardized testing procedures may lead to variations in the sensitivity of ctDNA detection. Differences in sample collection quality for ctDNA may also affect the sensitivity of the test. On the other hand, the complexity of biological information analysis can affect both the specificity and sensitivity of ctDNA diagnosis. Therefore, further research and clinical validation are needed to advance the application of ctDNA in lung cancer. In terms of ctDNA research in lung cancer samples, most of the existing studies on early lung cancer diagnosis are small-sample retrospective analyses, which face issues such as insufficient sample size, poor data integrity, selection bias, confounding factors, and result extrapolation. Therefore, it is recommended to conduct multi-center, large-scale prospective studies, recruiting lung cancer high-risk populations from different regions, ethnicities, and age groups. By detecting ctDNA, these studies could further confirm the superiority of ctDNA in the early diagnosis of lung cancer. In terms of lung cancer types, most current studies focus on common subtypes (such as non-small cell lung cancer), with limited research on rare subtypes (such as large cell neuroendocrine carcinoma and adenoid cystic carcinoma). Given the unique biological behaviors and treatment needs of rare lung cancer subtypes, relevant studies should be conducted to further explore the characteristics of ctDNA, optimize detection methods, and expand clinical applications. This could provide more accurate diagnosis and treatment plans for patients with rare lung cancer subtypes, improving their prognosis and quality of life. In summary, the combination of ctDNA detection with imaging and traditional tumor markers offers a more comprehensive tool for the early diagnosis, treatment guidance, and prognosis assessment of lung cancer. Based on the ctDNA levels and mutation characteristics in lung cancer patients, future research could further integrate ctDNA with transcriptomics, proteomics, and other multi-omics data to build more comprehensive and accurate prognostic models. Through deep learning and multi-omics data integration, the accuracy and clinical applicability of prognostic models could be significantly improved, providing strong support for personalized treatment and precision medicine in lung cancer patients.

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