CLINICAL APPLICATIONS OF CIRCULATING TUMOR DNA (CTDNA) IN CANCER DIAGNOSIS AND PROGNOSIS

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ABSTRACT
Circulating tumor DNA (ctDNA) is an emerging and valuable biomarker today and is increasingly used in precision oncology. A liquid biopsy is used to identify ctDNA, which are small fragments of DNA released into the bloodstream by a tumour. The primary advantage of ctDNA testing is that it is minimally invasive and can be used to monitor cancer in real time, as opposed to tissue biopsy. The review explores the biological mechanism of ctDNA, technologies for its detection, and the clinical uses of ctDNA in cancer diagnosis and prognosis. The use of ctDNA enables early detection of cancer, provides genetic information related to cancer's genetic mutations and can be used for information to choose personalized treatment. It is also helpful for assessing response to treatment, for the detection of minimal residual disease (MRD) and to predict cancer relapse and survival. In other cancers such as colorectal, breast and lung cancer these have been established to be clinically useful. While there are challenges associated with sensitivity, standardization, cost, and accessibility, current progress in sequencing technologies, artificial intelligence and multi-omics is promising the enhanced use of these in the clinic. In summary, ctDNA is a promising strategy that will significantly contribute to the personalization of cancer diagnosis, prognosis and management.
Keywords: *Circulating Tumor DNA, ctDNA, Liquid Biopsy, Cancer Diagnosis, Cancer Prognosis, Minimal Residual Disease, Precision Oncology.*
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1. Introduction

Cancer is a major cause of mortality and public health problems throughout the world. A growing number of cancers has made it evident that precision medicine is needed to deliver customised care based on the molecular nature of each tumour. Diagnosis of cancer has always been done using tissue biopsies, but these biopsies are invasive and can only use a part of the genetic diversity of the cancerous mass. Therefore, liquid biopsy offers a new hope for overcoming these restrictions. A liquid biopsy can use materials derived from the tumor in the blood, other body fluids, to provide a less invasive evaluation of cancer. Circulating tumor DNA (ctDNA) is a special type of biomarker that has attracted a lot of interest in liquid biopsy. ctDNA is small fragments of DNA shed into the blood from tumor cells and contain altered (mutated) DNA found in cancer. The clinical significance of ctDNA is that it can aid in early detection, molecular characterization, disease monitoring and prognosis. For instance, for one disease, ctDNA can carry out identification of actionable mutations and early detection of recurrence before it is detectable via images. Accordingly, the use of ctDNA is emerging as a valuable tool in modern personalized cancer management. This article reviews aspects of the biological primers, the technologies for getting to these ctDNAs, and the applications of these technologies in cancer diagnosis and prognosis.

2. Biological Basis and Detection Technologies of Circulating Tumor DNA

2.1. Biological Characteristics and Origin of ctDNA

Circulating tumor DNA (ctDNA) is a small amount of DNA released by tumor cells into the bloodstream. It is a small portion of the quantity of cell free DNA (cfDNA) in blood that incorporates changes in the genome of the tumor, including alterations in its DNA sequence, like DNA alterations, insertions or deletions, or copy number alterations (Saha et al., 2022). The molecular information carried by ctDNA has made its way into cancer diagnosis and monitoring and it has become an important marker. Tumor cells release ctDNA by various biological mechanisms, such as active secretion by living tumor cells, necrosis (cell injury and death), and apoptosis (programmed death). When cancer cells grow and die, the bits of DNA become detectable in the blood and can be found in blood samples (1). Because large tumors release more DNA into the blood, a higher level of ctDNA is often seen in patients whose cancer is in its late stages. The mixture of circulating and cell-free DNA is referred to as the ctDNA, but it is not the same as the cfDNA. Cell-free DNA from healthy cells as well as those from diseased cells, whereas the ctDNA from tumor cells contains cancer-related genetic alterations

(2). Various biological parameters can affect the level of ctDNA, such as tumour size, tumour stage, tumour location, tumour vascularization and treatment efficacy. Consequently the levels of ctDNA in patients can be markedly different.

2.2. Technologies for ctDNA Detection and Analysis

The forensics of ctDNA detection and characterization has developed a number of high-tech methods. Digital Polymerase Chain Reaction (dPCR) is a very powerful technique that allows the detection of specific mutations even from very low levels of ctDNA. A more recent mutation detection and treatment monitoring method, Droplet Digital PCR (ddPCR), divides patient's illustrated samples into thousands of droplets for increased accuracy and sensitivity when identifying the mutation (3). Another widely-adopted technology is called Next Generation Sequencing (NGS), which can analyze multiple genes and mutations at the same time. NGS differs from PCR-based approaches, which look at specific changes, by offering a complete profile of the genome of any tumor. This is useful in order to distinguish mutations representing "actionable change" and track the progression of tumors over time (4). Polymerase chain reaction (PCR) based technologies have relatively low cost and high sensitivity but are only applicable to known mutations. Meanwhile, NGS affords more widespread clinical information, is more expensive, more specialized, and more data intensive.

Technology	Main Application	Advantages	Limitations
dPCR	Detection of specific mutations	High sensitivity, rapid analysis	Limited mutation coverage
ddPCR	Monitoring low-frequency mutations	Very high accuracy and sensitivity	Targets known mutations only
NGS	Comprehensive genomic profiling	Detects multiple genetic alterations simultaneously	Higher cost and complex analysis

Table 1: Comparison of Major Technologies Used for ctDNA Detection

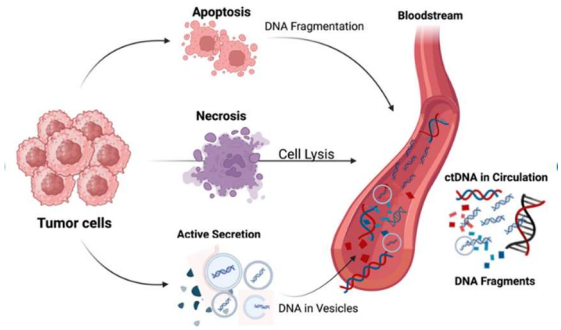

Figure 1: Biology and Release Mechanisms of Circulating Tumor DNA (ctDNA)
(Source: https://www.researchgate.net)

3. Clinical Applications of ctDNA in Cancer Diagnosis

3.1. ctDNA for Early Cancer Detection and Screening

The most promising use of circulating tumor DNA (ctDNA) would be in early detection and screening of cancer. During early stages of tumour growth, DNA fragments are released into the blood, and can be used as a potential biomarker in the bloodstream prior to clinical symptoms. Blood samples can be analyzed for genetic alterations that are exclusive to tumors and linked to the various forms of cancer, including mutations, amplifications, and methylation changes. Detection of these molecular changes at an early stage can help in the correct diagnosis and improve the chances of successful treatment. Studies have been conducted that show the ability of ctDNA to detect mutations in colorectal, lung and breast cancer before tumors are recognized by traditional imaging techniques (5). It is important to be able to make this early diagnosis, so that early intervention can be provided and survival outcomes optimized. The low invasiveness of the test is also an important benefit to ctDNA-based screening. Tissue biopsies are traditional methods and involve surgery and can cause discomfort and complications for the patient. Re-testing is easier and safer, however, as only a blood sample is needed for ctDNA. Consequently, the use of liquid biopsy is becoming an inspiring method to implement for cancer population screening and early diagnosis.

3.2. Tumor Genotyping and Molecular Profiling Using ctDNA

The use of ctDNA for tumor genotyping and molecular profiling has become important. Tumour genetics information from ctDNA can be used to determine actionable mutations that matter for therapy decisions. Actionable mutations are changes in the genes that may be specifically treated to make treatment work more effectively. In non-small cell lung cancer, for instance, ctDNA testing can identify

the target EGFR mutations so that the doctors can prescribe EGFR-targeted drugs like osimertinib to the patient (6). Likewise, mutations in the genes KRAS, BRAF, PIK3CA can be detected with ctDNA testing and can be harnessed to develop a personalized treatment plan.

This allows better precision oncology strategy to match treatment with patient to best treatment probably to benefit the patient. There are several benefits of ctDNA testing over the conventional tissue biopsy. Sometimes tissue samples are hard to get and especially in POCD and inaccessible areas (7). In addition, the tumor's genetic makeup may not be uniform across all cells, so a single sample may not be a true picture of the whole genetic makeup. The less invasive alternative is available with ctDNA and it can support the simultaneous inclusion of genetic information from several tumor locations.

3.3. Monitoring Tumor Heterogeneity and Disease Evolution

Cancer is a dynamic illness which keeps changing over time. Patients may experience a form of genetic heterogeneity called "spatial" in the same tumor. Furthermore, the tumors may also develop mutations throughout treatment that can cause temporal heterogeneity and therapeutic resistance. The use of ctDNA would be a good way to follow these changes over time. The ctDNA potentially represents the overall picture of tumor genetics because it originates from various locations in the body. The regular checking of blood can be used to monitor the molecular changes while helping detect potential resistance mutations and monitor disease progression (8). In particular, resistance mutations may be identified months before clinical disease progression is seen on imaging months after drug resistance develops when patients commence targeted therapy. This real-time molecular monitoring helps to make timely treatment changes and contributes to clinician's personalised treatment plans(9).

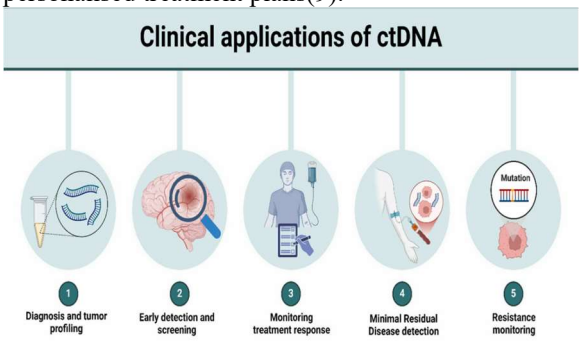

Figure 2: Clinical Applications of Circulating Tumor DNA (ctDNA) in Cancer Diagnosis
(Source: https://ars.els-cdn.com)

4. Clinical Applications of ctDNA in Cancer Prognosis

4.1. Detection of Minimal Residual Disease (MRD)

The minimal residual disease (MRD) represents the very few cancer cells remaining from the treatment, undetected with conventional imaging, and not seen with usual clinical examinations. These surviving cells may, therefore, form new tumors later. Therefore, the accurate detection of MRD is crucial for the outcome of treatment and to define the patient's risk of future relapse. Circulating tumor DNA (ctDNA) is a new very sensitive biomarker for MRD detection (10). In patients who have had surgery, chemotherapy or radiotherapy, ctDNA tests may detect circulating DNA from the tumor even if imaging tests look normal. This is useful to clinicians for learning whether there is any remaining disease on a molecular level.

It has been established in several studies that patients who have detectable ctDNA after treatment have a much higher challenge of relapse than patients who do not have detectable ctDNA. When it comes to colorectal cancer, the presence of ctDNA after surgery was strongly linked to future relapse. This means that ctDNA tests can be used to distinguish a higher risk patient who can benefit from further treatment, from a lower risk patient who may have no further therapy needed. Hence, ctDNA-based MRD assessment is a key improvement in clinical cancer management and prognosis.

4.2. Monitoring Therapeutic Response Through ctDNA Dynamics

Changes in ctDNA levels over the course of the treatment give important clues to the patient's response to therapy. Since ctDNA corresponds to tumor burden, a reduction in ctDNA can be a sign of successful therapy and tumor reduction. On the other hand, rising or constant levels of ctDNA may indicate poor response to treatment or disease progression. There are several benefits to ctDNA monitoring, including its ability to give up-to-the-minute information. Even though the outcomes of treatment can take weeks or months to be seen using traditional imaging techniques, changes to the ctDNA can be observed much earlier (11). This helps the doctor to have a quicker assessment of the success of the therapy and take a decision on changes to the therapy.

The dynamics and variations of ctDNA can also be employed to detect therapeutic resistance. Over time the targeted therapies or other medications may lose their effectiveness as cancer cells acquire new genetic changes. These mutations which are associated with resistance might be detectable in ctDNA prior to the development of clinical and/or radiologic signs of progression (12). For example, in lung cancer patients with targeted therapy, resistance mutations to the

EGFR gene can be identified using the ctDNA analysis. If caught early, such changes can help healthcare providers adapt to new treatment approaches while the disease is still in a beginning stage. Thus, the use of ctDNA monitoring allows for more advanced and tailored cancer management.

4.3. Prediction of Cancer Recurrence and Survival Outcomes

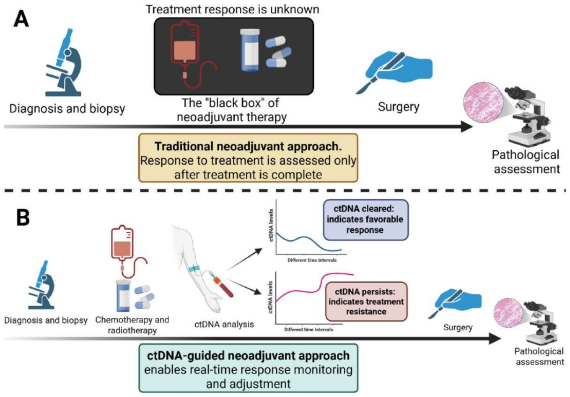

Figure 3: ctDNA-Guided Monitoring of Treatment Response and Therapeutic Resistance
(Source: https://media.springernature.com)

The prediction of cancer recurrence and outcomes of survival are one of the most significant prognostic uses of ctDNA. Many studies have shown that ctDNA positivity is correlated with a higher risk of recurrence. People whose ctDNA levels remain detectable are probably at higher risk for recurrence than people who test negative for ctDNA. Recurrence prediction is closely related to DFS, as is the ctDNA status. Disease-free survival is the length of time after treatment when the patient hasn't had any signs or symptoms of cancer (13). Patients with undetectable ctDNA have been shown to have better disease-free survival than patients who remain positive at cyclomark.Patients with an undetected cyclomark have been shown to have better disease-free survival than patients with detected cyclomark.

The use of this information can guide clinicians to closer surveillance and provide more therapeutic intervention for patients. The presence of ctDNA also gives insights into overall survival (OS). As ctDNA levels continue to rise or stay elevated, there are often poorer clinical outcomes and decreased mortality. Conversely, patient outcomes tend to be improved when ctDNA is completely cleared following treatment (14). As such, the use of ctDNA has emerged as a valuable prognostic marker and is actively utilized for risk stratification and tailored follow-up treatment and management in various cancer indications.

Cancer Type	Prognostic Role of ctDNA	Clinical Significance
Colorectal Cancer	Detection of MRD and recurrence risk	Guides adjuvant therapy decisions
Breast Cancer	Monitoring treatment response and relapse	Supports personalized follow-up
Lung Cancer	Detection of resistance mutations and progression	Predicts survival outcomes
Pancreatic Cancer	Assessment of disease burden	Identifies high-risk patients

Table 2: Prognostic Significance of ctDNA Across Different Cancer Types

5. Clinical Applications of ctDNA in Major Cancer Types

5.1. Clinical Utility of ctDNA in Colorectal Cancer

The use of circulating tumor DNA (ctDNA) in colorectal cancer treatment has emerged as a valuable biomarker. It may help detect cancer early by spotting genetic changes characteristic of a tumour in blood before cancer symptoms appear, before they are advanced. Minimal residual disease (MRD) detection following surgery is also a common application of ctDNA. Patients who are positive on a post-treatment ctDNA test are more likely to have some cancer remaining than those who are negative either (15). In addition, through regular ctDNA monitoring, relapses can be detected months before they are discernible on imaging tests and clinical intervention and treatment planning can be done earlier.

5.2. Clinical Utility of ctDNA in Breast Cancer

The DNA of a cell or cells derived from the tumor can be used to perform a molecular test to inform about the molecular profile and treatment options, which is called concurrent tumor DNA or ctDNA. The blood based ctDNA test can identify important genetic alterations, including some mutations that lead to treatment resistance. One benefit is that if a series of ctDNA tests is ordered, the response to treatment can be tracked throughout the treatment. The amount of ctDNA can also be used to calculate the burden of the tumour and the response to treatment (16). In addition to serving as a prognostic marker, however, ctDNA positivity for several months following therapy has also been associated with increased risk of recurrence and poor clinical survival.

5.3. Clinical Utility of ctDNA in Lung Cancer

There is a great clinical potential for ctDNA in lung cancer, especially for non-small cell lung cancer

(NSCLC). It is one of the uses most relevant, in determining the options for targeted therapies because EGFR mutations can be identified. When tissue biopsy is not possible, ctDNA testing can be used as an alternative (17). It is also used to track disease progression as well as get to know mutations which could develop during treatment that are resistant to the drug. The early diagnosis of these molecular alterations enables prompt changes in treatment and better patient management.

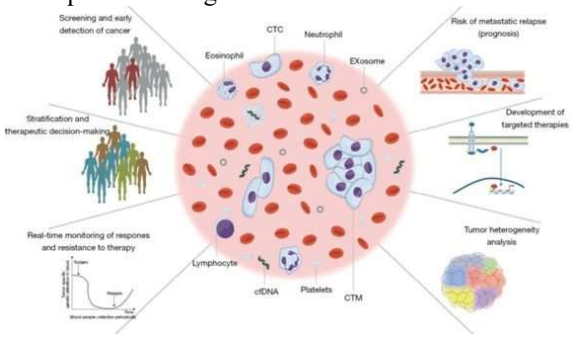

Figure 4: Major Clinical Applications of ctDNA in Cancer Diagnosis, Prognosis, and Treatment Monitoring

(Source: https://www.biochain.com)

6. Challenges and Future Perspectives of ctDNA-Based Oncology

6.1. Current Challenges in Clinical Implementation

Several barriers have prevented the frequent application of circulating tumor DNA (ctDNA) in clinical practice. A big problem is that of analytical sensitivity. If the tumor burden is low and/or early stage, the amount of ctDNA may be very low, thus making it difficult to detect. This can result in misdiagnosis and impact on clinical decisions. A second significant consideration is that there is no standardization with regard to the test for ctDNA (18). There may be variations in results due to differing methods in sample collection, testing platforms, and methods of data analysis between different laboratories. This creates a challenge when findings are to be compared between studies and between health care contexts.

The cost and availability are also barriers. There are also advanced technologies available, including Next-Generation Sequencing (NGS) which needs advanced equipment, expertise and many resources. Thus, the availability of ctDNA testing might be restricted in low-income health care systems (19). Furthermore, regulatory authorities are still developing procedures for ctDNA diagnostic clearance and validation/quality control, which causes a clinical validation and regulatory challenge.

6.2. Future Perspectives and Emerging Developments

There is great potential for the future of ctDNA-based oncology as a technique, given the advances being made in both technology and science. The use of this artificial intelligence (AI) is growing in the field of ctDNA analysis to enhance detection of mutations, data interpretation and prediction of treatment response. The clinical decision making process could benefit from the use of AI models, which can help improve the accuracy and efficiency of the diagnosis. Another new trend is the merging of ctDNA into omics technologies like genomics, transcriptomics and proteomics (20). These data sources can be integrated to offer greater insight in the biology of a tumor. Additionally, ctDNA is projected to be at the centre of personalized oncology, aiding in the decision-making process for personalized treatment approaches. There will continue to be clinical trials that use ctDNA as a method to guide treatments, a method which will be evaluated until ctDNA is demonstrated in a broader clinical setting in many cancer types.

7. Conclusion

The use of circulating tumor DNA (ctDNA) as a biomarker has become a crucial advancement in contemporary treatment strategies for cancer, particularly within precision oncology. This review addressed the biological origin of ctDNA, the principle technologies employed for its detection and the emerging clinical uses in diagnosis and prognosis of cancer. In addition to early cancer detection, molecular profiling, and real-time monitoring tumor heterogeneity, ctDNA has the potential to provide information that can't be obtained from standard tissue biopsies.

The importance of ctDNA for prognosis lies in its use to detect minimal residual disease (MRD), to monitor treatment response, and to predict cancer recurrence and survival. It has proven to be clinically useful in multiple cancer types, including colorectal cancer, breast cancer and lung cancer. There are specific issues with sensitivity, standardization, cost, and regulation; however, continuous advances in sequencing technologies, AI, and multi-omics methods can help make it more clinically implementable. Hence ctDNA will play a progressively more significant role in the future aspect of personalized diagnosis, prognosis and treatment decision making within the care of a cancer patient.

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