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REVIEW

Latest Research Progress of Liquid Biopsy in Tumor—A Narrative Review

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Abstract: Human life expectancy is significantly impacted by cancer, with liquid biopsy emerging as an advantageous method for cancer detection because of its noninvasive nature, high accuracy, ease of sampling, and cost-effectiveness compared with conventional tissue biopsy techniques. Liquid biopsy shows promise in early cancer detection, real-time monitoring, and personalized treatment for various cancers, including lung, cervical, and prostate cancers, and offers innovative approaches for cancer diagnosis and management. By utilizing circulating tumor DNA, circulating tumor cells, and exosomes as biomarkers, liquid biopsy enables the tracking of cancer progression. Various techniques commonly used in life sciences research, such as polymerase chain reaction (PCR), next-generation sequencing (NGS), and droplet digital PCR, are employed to assess cancer progression on the basis of different indicators. This review examines the latest advancements in liquid biopsy markers—circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes—for cancer diagnosis over the past three years, with a focus on their detection methodologies and clinical applications. It encapsulates the pivotal aims of liquid biopsy, including early detection, therapy response prediction, treatment monitoring, prognostication, and its relevance in minimal residual disease, while also addressing the challenges facing routine clinical adoption. By combining the latest research advancements and practical clinical experiences, this work focuses on discussing the clinical significance of DNA methylation biomarkers and their applications in tumor screening, auxiliary diagnosis, companion diagnosis, and recurrence monitoring. These discussions may help enhance the application of liquid biopsy throughout the entire process of tumor diagnosis and treatment, thereby providing patients with more precise and effective treatment plans.

Keywords: liquid biopsy, circulating tumor DNA, circulating tumor cells, exosomes, DNA methylation

Introduction

The latest report titled "Global cancer burden growing, amidst mounting need for services" was released by the International Agency for Research on Cancer (IARC) of the World Health Organization on February 2, 2024, highlighting the increasing global cancer burden, which requires urgent attention worldwide.[1] Based on current data, the IARC has projected and expressed new concerns that the global cancer burden is anticipated to escalate further by 2050. The data suggest that by 2050, approximately 25 years from now, the number of new cancer cases is expected to surpass 35 million, representing a 77% increase from 20 million cases in 2022. Concomitantly, by 2050, the mortality rate from cancer is likely to double.

Liquid biopsy is a noninvasive biomarker detection technology that was defined as an early cancer detection method in 2013.[2] It is commonly used for the detection of early-stage cancer, offering the advantage of detecting cancer before symptoms appear. Liquid biopsy is also used in late-stage cancer treatment decisions. Compared with tissue biopsy, liquid biopsy offers notable benefits, including noninvasiveness, high precision, minimal sampling risk, fewer sampling constraints, and straightforward sample preparation. In contrast to conventional cancer detection approaches, liquid biopsy has been extensively used in noninvasive diagnostic procedures and molecular profiling. The samples used for liquid biopsy include body fluids containing tumor-related biomarkers, such as blood, urine, saliva, bronchoalveolar lavage fluid (BALF)/sputum, cyst fluid, or cerebrospinal fluid, with blood being the most common sample[3] (Figure 1). In liquid biopsy, prevalent biomarkers include ctDNA, CTCs, exosomes, and circulating tumor RNA (ctRNA). Interestingly, the latest research has revealed that the "TEPs (tumor-educated platelets, TEPs) phenotype" can also be utilized in liquid

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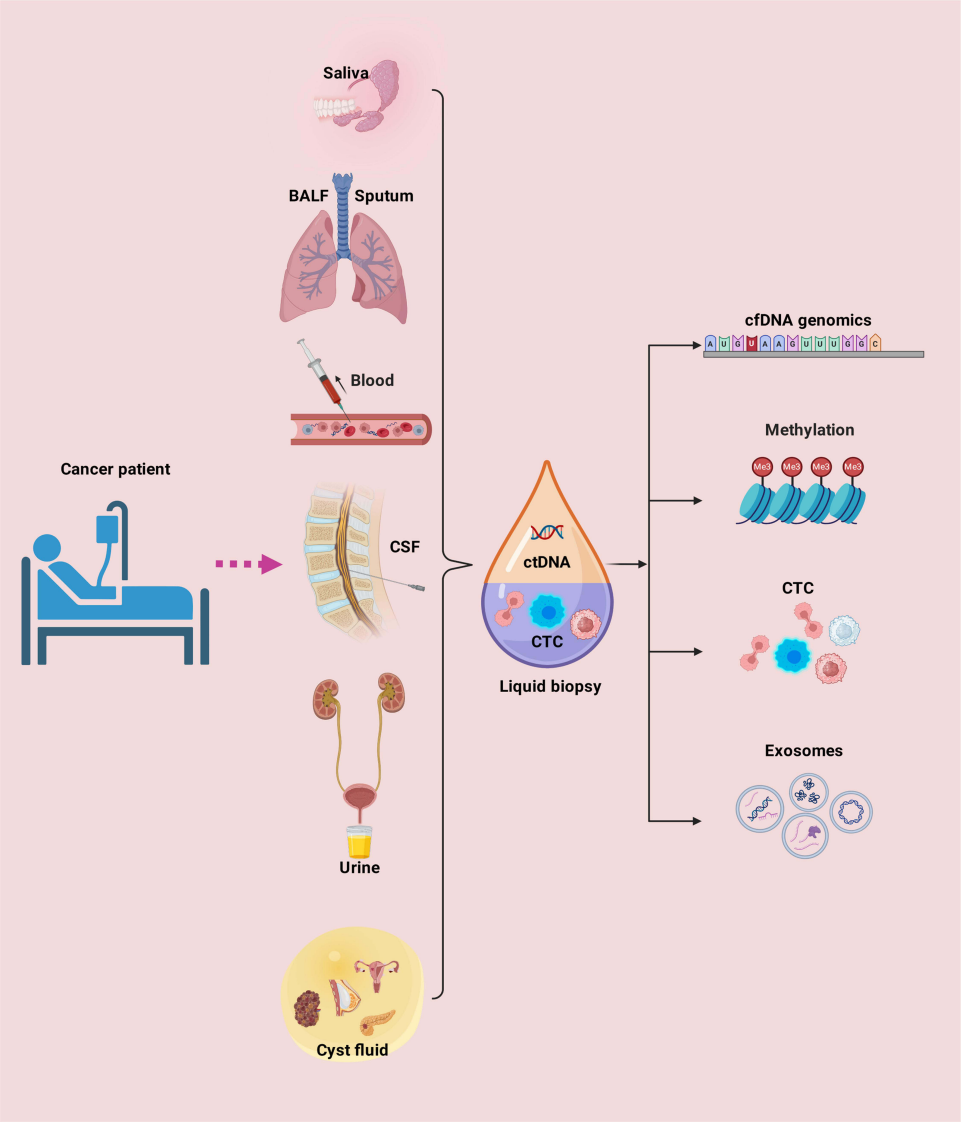

Figure 1 The bodily fluids of cancer patients can be used for liquid biopsy. The bodily fluid samples that can be used for liquid biopsy include the patient's saliva, BALF / sputum, blood, urine, CSF, and cyst fluid from cystic lesions. These samples can be used for the analysis of CTCs, ctDNA, exosomes, and other tumor markers. BALF bronchoalveolar lavage fluid; CSF cerebrospinal fluid; ctDNA circulating tumor DNA; CTC circulating tumor cells; cfDNA cell-free DNA.

biopsy. TEPs engage in communication with CTCs, the TME (tumor microenvironment), and the tumor itself through multiple mechanisms and at multiple levels, playing crucial roles in malignant tumors and ultimately promoting cancer metastasis.[4] Cancer cells discharge genetic substances, including nucleic acids and proteins, into the bloodstream through mechanisms such as apoptosis, necrosis, or metastasis. Identifying these biomarkers allows for the surveillance of tumor initiation or recurrence, supports diagnostic categorization, and facilitates the refinement of therapeutic approaches. This method provides benefits such as its non-invasive nature, high patient adherence, and the capability for continuous monitoring. Additionally, liquid biopsy can depict the genetic makeup of the tumor, which is crucial for enhancing the accuracy of cancer diagnosis and treatment. Currently, the Food and Drug Administration (FDA) in the United States has sanctioned clinical employment solely for ctDNA and CTC. Exosomes, although relatively new to the field, have attracted significant interest due to their high prevalence, diverse sources, and extended half-life when compared to CTCs and ctDNA, positioning them as a favored subject in liquid biopsy studies. In contrast to ctDNA, which is found in limited amounts, has ambiguous tissue origins, and is susceptible to degradation, non-coding RNAs (ncRNAs) have recently become a focal point as biomarkers for liquid biopsy due to their abundance and comparative stability. This

article will offer a concise summary of the biological properties, main detection techniques, and clinical uses of the aforementioned liquid biopsy markers, to provide clarity on their clinical efficacy and practical application. This review holds importance for informing both foundational experimental research and clinical application in the realm of liquid biopsy.

Overview of Liquid Biopsy Biomarkers and Detection Techniques

Circulating Tumor Cells (CTCs)

CTCs are predominantly present in the peripheral circulatory system and play a crucial role in tumor metastasis. The formation of CTCs involves two mechanisms: active shedding, characterized by epithelial cells undergoing epithelial-mesenchymal transition (EMT),[5] and passive shedding from the primary tumor site.[6] CTCs detected in the blood can be either viable or apoptotic, with only viable CTCs being meaningful for assessing cancer metastasis.[7] With a half-life spanning from 1.0 to 3 hours, CTCs possess the ability to provide real-time reflections, rendering them suitable for dynamic monitoring purposes.[8] On the basis of this characteristic, fragmented or apoptotic CTCs can also be detected in peripheral blood, leading to background noise in the detection results of certain CTC markers.[9] In clinical applications of CTC detection via liquid biopsy, in addition to evaluating cancer through the enumeration of individual CTCs, CTCs can also be molecularly characterized, including the identification of therapeutic targets[10] and resistance mechanisms.[11]

Detecting CTCs presents a notable challenge since most of them are identified and eliminated by the immune system upon entry into the peripheral blood, leaving only a minuscule surviving fraction, resulting in extremely low concentrations of CTCs in the blood (1–100 per mL of whole blood).[12] To analyze CTCs effectively, it is imperative to employ capture, enrichment, and separation techniques that are highly efficient, specific, and sensitive and that preserve cell viability. The detection technology for CTCs must meet several requirements: ① the ability to isolate highly specific CTCs; ② the capability of handling small quantities of CTCs (less than 100 target cells per 10^9 blood cells); ③ demonstrating high performance for whole blood samples and validated through clinical trials; ④ the capability of categorizing heterogeneous CTC subpopulations to differentiate cancer cells with different metastatic potentials; and ⑤ allowing the release of viable cells for downstream analysis.[13]

Physical methods leverage the unique physical and biological properties of different blood cells. Techniques include microfiltration on the basis of cell size,[14,15] density gradient centrifugation on the basis of cell density,[16] and gel electrophoresis on the basis of charge.[17] Biological methods rely on the specificity of antigen-antibody reactions and utilize different surface markers and adhesion molecules on cells. Targeted screening is conducted based on techniques such as immunomagnetic beads and microfluidic chip[18] technology, aiming to selectively enrich tumor cells by screening for positive enrichment and removing nontumor cells for negative enrichment. Representative technological platforms include CellSearchTM,[19] MagSweeperTM,[20] CTC-Chip,[21] nanotube-CTC-chip,[21] and CellCollectorTM.[22,23] Authorized by the US FDA in 2004, the CellSearch system is employed for diagnosing and treating breast, prostate, and colorectal cancers. The CellCollector system, introduced as an innovative in vivo CTC capture method, obtained CE certification in 2012 and CFDA Class III certification in 2017 and is applicable to lung, prostate, breast, and other cancers.

After enrichment, CTCs can be analyzed via fluorescence labeling combined with immunological and nucleic acid testing techniques such as immunofluorescence, fluorescence in situ hybridization (FISH), flow cytometry, real-time quantitative PCR, and emerging technologies such as droplet digital PCR (dd-PCR) and NGS for cellular morphology, functional subtyping, proteomics, and genomic analysis.[24–27]

Recent research has revealed that the combination of single-cell RNA sequencing with CTC analysis enables the detection of somatic mutations and alterations in oncogene copy numbers. This integration offers deeper insight into CTC heterogeneity and aids in pinpointing different cancer subtypes. As a result, it enhances the comprehension of the molecular pathways involved in cancer advancement and prognosis, allowing for forecasts of changes in tumor characteristics and the development of drug resistance. These advancements hold significant promise for guiding truly personalized therapeutic strategies tailored to individual patients, thereby optimizing treatment outcomes and enhancing patient care.[28–30] The origin of CTCs allows researchers to explore therapeutic targets and drug resistance mechanisms corresponding to cancer cells. The relevant studies can be categorized into three categories based on the molecular

characteristics of the target analytes: protein, RNA, and genes. Mutations in genes encoding therapeutic target sites or downstream signaling proteins within CTCs have the potential to impact the effectiveness of targeted medications. For example, mutations in genes such as EGFR (epidermal growth factor receptor, EGFR) in lung cancer,[31] AR (androgen receptor, AR) in prostate cancer,[32] and PIK3CA in breast cancer[33] can lead to the evasion of targeted therapy by cancer cells.

ctDNA

Fragmented DNA, known as cell-free DNA (cfDNA), is present in the cell-free portion of blood. Research indicates that circulating cfDNA exists in normal cellular states but significantly increases during cell damage or necrosis.[34] The formation of cfDNA is associated with cell apoptosis, producing DNA fragments ranging from 166 to 1000 bp in length, with a predominant length of 166 bp (representing the length of DNA wrapping around a nucleosome), which are released through cellular phagocytosis or cell death.[35,36] cfDNA detection is widely used in prenatal medicine for applications such as sex identification,[37] single-gene hereditary disease identification,[38] and noninvasive prenatal testing for conditions such as Down syndrome.[39] The term ctDNA is used to describe cfDNA that originates from tumor cells. The length of ctDNA identified in the plasma of cancer patients typically falls between 90 and 150 base pairs, which is shorter than conventional cfDNA fragments.[40] ctDNA is one of the most commonly used blood-derived markers in liquid biopsy, providing genomic information on tumor cells from patients. Like most cfDNA, ctDNA has a short half-life, ranging from 16 minutes to 2.5 hours, due to rapid clearance by the kidneys, liver, and spleen once released into bodily fluids.[41] The genetic information carried by ctDNA is consistent with that of its parent cells. By detecting ctDNA point mutations, deletions/insertions, methylations, and copy number abnormalities, one can observe parental tumor gene defects and epigenetic changes,[42] providing important information for tumor diagnosis, minimal residual disease (MRD), prognosis analysis and metastasis monitoring. In various studies, ctDNA has long been considered to dynamically reflect the progression of cancer in patients.[43,44]

Currently, common detection methods for ctDNA are categorized on the basis of technical principles into PCR-based detection technologies and NGS-based detection technologies.[45,46] Owing to its increased analytical sensitivity and specificity, digital PCR (D-PCR) is a frequently utilized method for ctDNA detection. This technology allows for targeted gene amplification, even in the presence of a substantial background of wild-type alleles, facilitating high-throughput analysis.[47] For absolute quantification, D–PCR enables measurement without relying on a calibration curve of known quantity samples. However, initial sample mixture preparation through quantitative PCR (Q-PCR) is necessary. Notable techniques frequently employed for absolute quantification through D-PCR include BEAMing technology (an acronym for bead, emulsion, amplification, and magnetic separation) and ddPCR.[47] ddPCR offers low detection limits, allowing for the quantitative measurement of minimal amounts of ctDNA.[48]

PCR-based detection technologies offer the advantages of high sensitivity, rapid speed, and cost-effectiveness but are limited in the total number of target points that can be detected.[49] NGS is a proficient technology for DNA sequencing and genetic data acquisition, allowing for the simultaneous analysis of numerous short DNA sequences, followed by alignment or realignment with a reference genome. NGS methods utilized in ctDNA detection include whole-genome sequencing (WGS), whole-exome sequencing (WES), deep sequencing, TAm-Seq, Safe-SeqS, FASTSeqS, and CAPP-Seq.[50] The use of WGS on cfDNA has proven effective in revealing chromosomal irregularities linked to malignancies,[51] focal amplifications, and gene rearrangements.[52] Hybrid capture sequencing has been introduced as a noninvasive approach to analyze the evolutionary genomic profile of whole-exome mutations in cancer.

NGS-based technologies generally have lower sensitivity than PCR-based technologies do, with sensitivity inversely proportional to the number of analyzed sites, where WES has the lowest sensitivity.[53] Challenges in the detection of ctDNA in liquid biopsy include occasionally extremely low levels of ctDNA, selecting the correct cancer-specific genomic alterations, and accurately quantifying the number of mutated fragments in samples.[54,55]

Exosomes

Exosomes display a concave hemispherical shape and are categorized as vesicular structures featuring a lipid bilayer membrane configuration with a diameter ranging from approximately 40 to 100 nanometers.[56] They are derived from

a wide range of sources and are released by the majority of cell types, both immune and nonimmune, such as platelets, leukocytes, mast cells and tumor cells. Extracellular vesicles known as exosomes are ubiquitously found in multiple biofluids and are enveloped by a double-layered lipid membrane that encapsulates vital molecular cargo reflective of their cell of origin, encompassing proteins, lipids, and genetic material (both DNA and RNA). These nanocarriers actively participate in myriad health-sustaining and disease-associated mechanisms.[57,58] An increasing number of studies suggest that exosomes significantly contribute to promoting cancer progression, dissemination, and the formation of new blood vessels. They also act as mediators of tumor immune responses[59] and have been implicated in eliciting chemoresistance.[60]

Compared with ctDNA and CTCs, exosome analysis encounters limitations in terms of isolation, analytical sensitivity, and stability; however, exosomes offer numerous potential advantages. Exosomes, which are abundant in bodily fluids such as blood, urine, and cerebrospinal fluid, are widely distributed and exhibit good stability, making them more accessible at desired detection levels than are ctDNA and CTCs.[61] As valuable sources of both quantitative and qualitative data, exosomes can signal the existence of malignancy and tumor burden.[62] Extensive research has shown that exosomes derived from cancer cells contain specific nucleic acids and proteins that mirror their cellular origin. Like CTCs, exosomes, which carry specific proteins and RNA, are commonly used in diagnostic strategies that involve a combination of multiple markers[63] and RT–PCR technology for RNA amplification and detection,[64] representing primary methods for identifying cancer-specific exosomes.

In clinical settings, exosome cargo, particularly miRNAs, circRNAs, and other noncoding RNAs, has shown significant promise in cancer diagnosis and prognosis.[65] A study involving 108 non-small cell lung cancer (NSCLC) patients and 54 healthy controls revealed that elevated serum levels of miR-141 were observed in NSCLC patients, effectively distinguishing between lymph nodes and distant metastases. Subsequent subtype analysis demonstrated a correlation between miR-141 expression and overall survival rates, specifically in lung adenocarcinoma rather than squamous cell carcinoma.[66] Additionally, specific miRNAs found in sputum, including miR-21-5p, miR-210-3p, and miR-486-5p, have proven valuable for early non-small cell lung cancer detection.[67] In a separate study, the expression levels of the lncRNA XLOC_009167 were evaluated in whole blood samples from 61 lung cancer patients and 57 healthy individuals. The results revealed significantly higher lncRNA XLOC_009167 expression in lung cancer patients than in healthy controls ($P < 0.0001$), with an AUC of 0.7398, sensitivity of 78.7%, and specificity of 61.8%. Compared with the conventional biomarkers CYFRA21-1, CEA72-4, and NSE, the lncRNA XLOC_009167 showed superior diagnostic efficacy.[68] Research by Sun et al[69] highlighted the potential of exosomal hsa_circ_0004001, hsa_circ_0004123, and hsa_circ_0075792 for hepatocellular carcinoma diagnosis, achieving an AUC of 0.89 when combined, with a sensitivity of 90.5% and specificity of 78.1%. These findings emphasize the unique value of exosomes in early cancer diagnosis and prognosis.

Advances in Liquid Biopsy Clinical Applications

Tumor Early Warning and Diagnosis

Liquid biopsy has progressed notably in clinical practice, primarily owing to its heightened technical sensitivity. The biomarkers utilized in liquid biopsy often display irregularities in the initial phases of tumor formation, preceding any detectable alterations seen in imaging modalities or the rise of conventional tumor indicators. This characteristic makes liquid biopsy a valuable tool for the early detection and screening of tumors in individuals, including those with elevated risk factors. By identifying molecular signatures of cancer in easily accessible bodily fluids, liquid biopsy facilitates a less invasive approach to detecting tumors much earlier than conventional methods do, thereby contributing to more timely interventions and improved patient outcomes. It paves the way for proactive monitoring and tailored interventions, enhancing the ability to detect cancer in its initial stages even before clinical symptoms emerge or imaging becomes indicative, benefiting both the general population and those considered at high risk (Figure 2).

Cohen et al[70] outlined a method for detecting eight prevalent types of malignant tumors (lung, breast, ovarian, gastric, liver, pancreatic, colorectal, and esophageal cancers) via a blood test named CancerSEEK. This method integrates the analysis of eight protein biomarkers with ctDNA evaluation of mutations in 16 genes. In a study involving 1005 patients

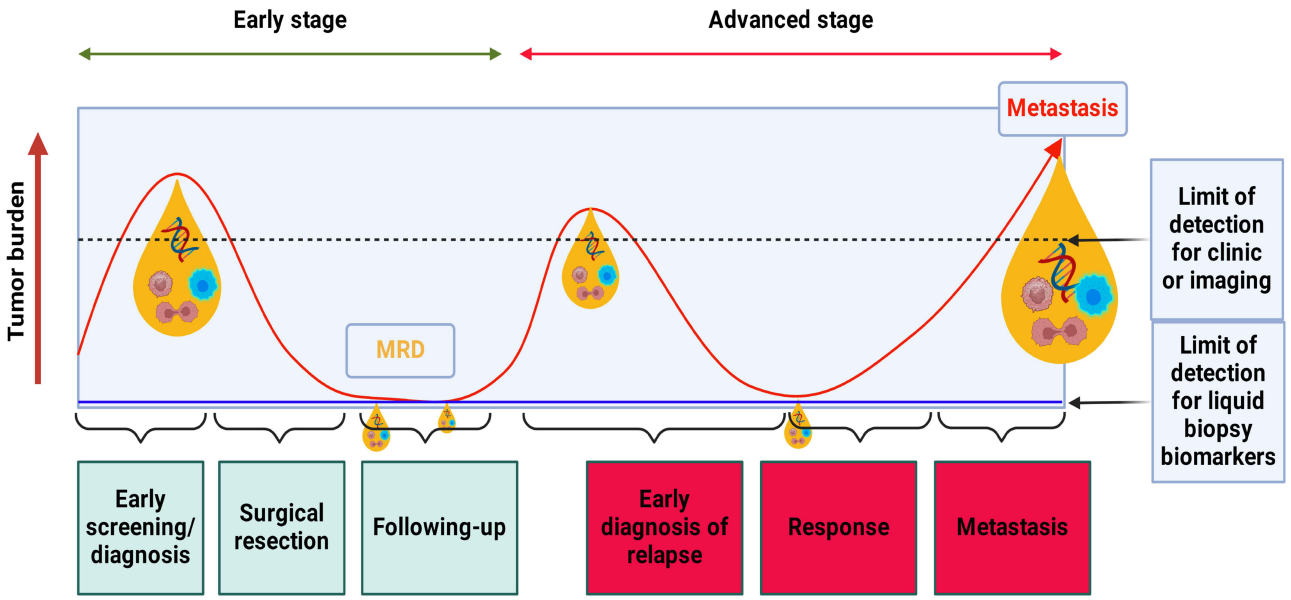

Figure 2 Liquid biopsy for cancer patients. The diagram illustrates the applications of liquid biopsy in the early diagnosis of cancer in the early stages of the disease, postoperative MRD monitoring, and in the advanced stages for early postoperative recurrence, treatment response, and metastasis. Different sizes of droplets represent the amount of detectable biomarkers they contain. MRD minimal residual disease.

clinically diagnosed with these eight nonmetastatic cancers, the specificity exceeded 99%, and the aggregate median detection rate was approximately 70%, underscoring the potential of ctDNA as an early cancer diagnostic biomarker. In a study focused on females diagnosed with breast carcinoma, benign breast diseases, and healthy controls, Shi et al[71] used qRT-PCR methodology to examine the differentiation antagonist nonprotein coding RNA (DANCR). By contrasting the concentrations of the serum exosome-encapsulated long noncoding RNA DANCR, they identified this biomarker as an independent prognostic determinant for breast cancer. Furthermore, the integration of the conventional markers CA153 and CEA led to a notable increase in diagnostic precision. In a multicenter prospective study by Li et al[72] esophageal squamous cell carcinoma (ESCC) patients and healthy volunteers were included. Saliva exosomes were subjected to RNA sequencing to identify tRNA-derived small RNAs (tsRNAs). Research revealed that a paired biomarker consisting of tRNA-GlyGCC-5 and a newly discovered microRNA, sRESE, obtained from exosomal fractions in the saliva of ESCC patients, excelled in differentiating ESCC patients from healthy individuals and exhibited remarkable sensitivity (90.50%) and specificity (94.20%). These findings underscore the tangible value and promising application of exosome-derived molecules as diagnostic indicators for oncological purposes.

In recent years, the use of peripheral blood for cfDNA methylation testing has become an important noninvasive diagnostic method for early cancer detection. Research as early as 2008 confirmed varying degrees of methylation in the SEPTIN9 gene promoter in colon cancer tissues and proposed that peripheral blood SEPTIN9 methylation could be used for early screening of colorectal cancer.[73] In 2024, the New England Journal of Medicine published two consecutive studies on noninvasive colorectal cancer screening.[74,75] One of the studies introduced Shield, a colorectal cancer early screening tool based on blood cfDNA genomic mutations, DNA methylation status, and fragmentome patterns. In the context of the ECLIPSE study, the Shield assay demonstrated diagnostic capabilities with a sensitivity of 83.1% (95% CI: 72.2% to 90.3%) and a specificity of 89.6% (95% CI: 88.8% to 90.3%) for colorectal cancer.[74] In the BLUE-C study focused on colorectal cancer, the methylation screening of the genes LASS4, LRRC4, PPP2R5C, and ZDHHC1 revealed a detection sensitivity of 93.9% (95% CI: 87.1–97.7%) and a sensitivity of 43.4% (95% CI: 41.3–45.6%) for identifying late-stage precancerous lesions. It also exhibited a specificity of 92.7% (95% CI: 92.2–93.1%) for nontumor findings or negative colonoscopies.[75] A multicenter prospective study involving 1381 patients confirmed that RNF180 and SEPTIN9 gene methylation are ideal markers for gastric adenocarcinoma, with a much greater sensitivity for early gastric cancer

(stage I, stage II) than traditional protein tumor markers such as CEA (carcinoembryonic antigen, CEA), CA199 (carbohydrate antigen 19–9, CA199), and CA125 (carbohydrate antigen, CA125).[76]

In addition to their application in screening for individual types of cancer, DNA methylation biomarkers have also been widely researched for screening across multiple types of cancer. The pan-cancer biomarker PCDHGB7 is highly methylated not only in 17 cancer types in the TCGA database but also in 13 clinical tumor samples.[77] The SIX6 gene shows widespread high methylation in 678 clinical samples from 10 common cancer types. Elevated methylation levels of the SIX6 gene yield receiver operating characteristic (ROC) curve area under the curve (AUC) scores of 0.99, 0.94, and 0.93, corresponding to the diagnostic accuracies for cervical carcinoma, endometrial malignancy, and urothelial cancer, respectively. These findings suggest that elevated SIX6 methylation occurs early in tumor progression and can be utilized in early clinical cancer screening. A study on the application of cfDNA sequencing technology in early cancer screening revealed that cfDNA methylation is the best biomarker for predicting cancer signals, with methylation classifiers having the highest sensitivity for tumor detection.[78] With its emphasis on the early identification of diverse cancer forms, the THUNDER investigation revealed that a targeted examination of methylation patterns in cfDNA extracted from blood samples attained a detection sensitivity of 69.1% and a specificity rate of 98.9% for pinpointing six distinct cancer varieties, including colorectal, esophageal, hepatic, pulmonary, ovarian, and pancreatic cancers.[79] Crafted by a Tsinghua University research consortium, the SRFD-Bayes diagnostic framework is grounded in the epigenetic traits of circulating free DNA methylation patterns, and its efficacy has been substantiated across a spectrum of malignancies encompassing breast carcinoma, colorectal adenocarcinoma, pulmonary squamous cell carcinoma, lung adenocarcinoma, hepatocellular cancer, and prostate malignancy. This innovative model has an aggregated precision of 76.9% in pinpointing the tumor's origin, an excellent sensitivity rate of 86.1% in the early detection of tumors, and attains a specificity of 94.7%, highlighting its robust discriminatory capacity.[80]

Recently, as artificial intelligence (AI) technologies have rapidly advanced, their utilization has expanded across various sectors of society. Specifically, in the medical field, researchers have shown that the development of AI-driven personalized diagnostic models can significantly enhance the early detection of Alzheimer's disease. Through optimization, a model achieved an impressive diagnostic accuracy of 91.59% in a three-class differentiation test set, with high areas under the curve (AUCs) for distinguishing cognitively normal (CON), mild cognitive impairment (MCI), and Alzheimer's disease (AD) states of 0.9524, 0.9651, and 0.9807, respectively.[81] Hou and colleagues developed a diagnostic system that combines AI with noninvasive liquid biopsy techniques to detect glomerular diseases. Their research revealed sensitivities of 95.0%, 97.0%, 97.0%, and 94.0% for diagnosing IgA nephropathy, membranous nephropathy, diabetic nephropathy, and minimal change disease, respectively. The system accurately demonstrated specificities of 98.8%, 98.5%, 98.5%, and 98.5% in distinguishing IgA nephropathy, membranous nephropathy, diabetic nephropathy, and minimal change disease, respectively. Overall, the diagnostic system achieved an outstanding accuracy rate of 96.0% across all disease categories.[82] Gottardo et al[83] reported that the integrated application of AI and multiomics can enhance the diagnosis and prognosis of lung cancer through liquid biopsy. These achievements highlight the promising role of AI in oncological diagnostics, paving the way for innovative strategies in cancer detection and therapy.

Tumor MRD and Recurrence Monitoring

A small number of cancerous cells are present in the body after treatment. These residual cancer cells have the potential to cause tumor recurrence and metastasis, yet they are not detectable through imaging or conventional clinical assessments. Only laboratory methodologies with high levels of sensitivity have the ability to discern these malignant cells among a substantial multitude of noncancerous cells. The advancement of sequencing technologies and biosensors has demonstrated encouraging outcomes of liquid biopsy for MRD detection, enabling its use in monitoring MRD, assessing treatment efficacy, and tracking recurrence. As indicated in a study conducted by Gristina and et al,[84] there is an increasing focus on incorporating liquid biopsy techniques into the context of NSCLC. This is especially relevant in postoperative situations, where monitoring MRD enables swift detection of cancer recurrence after radical treatment, facilitating timely and personalized interventions. Chaudhuri et al[85] conducted personalized tests via a detailed sequencing method (CAPP-seq) on samples taken from 40 individuals diagnosed with locally advanced lung cancer stages I to

III. They also analyzed 54 ctDNA samples from healthy individuals, resulting in a total of 255 samples for the study. In evaluable recurrent patients, ctDNA could be detected in up to 94% of patients at follow-up, with adverse outcomes observed in patients where ctDNA was detectable posttreatment, preceding radiographic progression. Hellmann et al[86] analyzed ctDNA through CAPP-Seq at monitoring time points during and after programmed cell death 1 ligand 1 (PD-L1) inhibitor therapy in 31 advanced NSCLC patients who experienced long-term benefit (progression-free survival ≥12 months), revealing that almost all patients whose ctDNA was not detected during monitoring maintained nonprogression, whereas all patients whose ctDNA was detectable eventually progressed. Garcia-Murillas et al[87] executed a prospective study incorporating 55 patients in the early phases of breast cancer, all of whom were undergoing preliminary chemotherapy prior to surgical intervention. Following the completion of curative treatment, plasma ctDNA was consistently monitored at a specific postoperative time or during follow-up to evaluate the risk of metastatic recurrence. This study confirmed that ctDNA analysis could define MRD and provide more precise prediction of recurrence. Tie et al[88] carried out a forward-looking investigation on a cohort of 230 patients diagnosed with stage II colon cancer, all of whom had undergone surgical excision. The primary postsurgical surveillance through ctDNA analysis revealed a 3-year recurrence-free survival ratio of 90% among those with undetectable ctDNA, in stark contrast to the 0% rate observed in the cohort with positive ctDNA findings. In their recent 2021 study, Nakano et al[89] also confirmed the efficacy of ctDNA in identifying MRD after radical surgery in patients with upper tract urothelial carcinoma.

When DNA methylation biomarkers are used for MRD analysis, characteristic mutation information from the initial malignant site is not needed. Its sensitivity is not dependent on the quantity of high-frequency mutations in patients, showing great potential for MRD detection.[90] On the basis of the results of the LUNAR-1 study,[91] which utilized ctDNA mutations combined with ctDNA methylation for the identification of MRD and surveillance of recurrence in individuals with colorectal malignancy, the integrated analysis of ctDNA methylation improved the sensitivity by 25% to 36% compared with the use of ctDNA mutations alone. In a prospective Phase II–III clinical trial[92] (registration number: NCT00958737) involving 805 colorectal cancer patients, pre- and postoperative plasma samples were analyzed for ctDNA methylation biomarkers (WIF1 and NPY) to assess the relationship between MRD and tumor recurrence. Compared with the ctDNA-positive group, the ctDNA-negative group presented a significantly greater 2-year disease-free survival rate (82% vs 64%).[92] In another retrospective study involving 87 stage II–III breast cancer patients, after neoadjuvant chemotherapy, the level of hypermethylation of ctDNA based on the level of methylation in the RASSF1A promoter region (met-ctDNA) detected in plasma samples from patients was found to be strongly associated with the remaining amount of tumor tissue (P=0.008). Additionally, within 1 year postsurgery, 3 out of 7 patients who were positive for met-ctDNA experienced tumor recurrence, whereas no recurrence was observed in met-ctDNA-negative patients.[93] In colorectal cancer, a scoring system combining cfDNA methylation biomarkers with serum CEA can predict recurrence before clinical or imaging detection of recurrence, with a median lead time of 106 days (range: 90–232 days).[94] Among prostate cancer patients, those who did not show serum GSTP1 methylation after two cycles of docetaxel treatment had longer survival times, suggesting that serum free GSTP1 methylation may be a potential clinical trial surrogate endpoint and clinical decision-making tool.[95]

Discussion

Tissue biopsy is presently considered the benchmark for tumor diagnosis. However, traditional biopsies involve extracting tissue samples from the tumor site, which poses risks of infection and internal bleeding and can lead to a negative patient experience. Liquid biopsy, as an improved diagnostic and monitoring approach, extracts biomarkers from patient blood and other bodily fluids in a noninvasive or minimally invasive manner, providing specific, accurate molecular information sensitively and conveniently. Therefore, it is gradually gaining more attention.

Biomarkers from liquid biopsy can provide continuous genetic mutation information, better reflecting the heterogeneity of a tumor in time and space, including its aggressiveness and overall molecular landscape. They can be utilized for tumor diagnosis, prognosis, MRD, and metastasis monitoring. Early intervention and timely treatment adjustments can improve the overall survival rate of cancer patients.

Although liquid biopsy has shown unparalleled superiority in early cancer diagnosis, prognosis, and monitoring, serving as a promising diagnostic and monitoring approach, large-scale clinical applications still face numerous

challenges. CTCs exhibit low abundance, diverse morphology, and significant heterogeneity in the peripheral blood. Current single capture technologies have specific limitations, while dual-mode capture technologies are still under investigation. ctDNA is present at very low levels in peripheral blood, indicating tumor burden without specifying tumor staging or localization. The extraction of exosomes lacks standardized, rapid, and feasible methods, with each approach conferring unique strengths and limitations, and methods ensuring both quantity and quality simultaneously are lacking. Highly sensitive detection methods may introduce bias and lead to false positives, thereby limiting the clinical application of liquid biopsy.

Conclusion

As technology progresses, including advancements in artificial intelligence that promise to refine data interpretation and prediction models, our comprehension of cancer development has improved. Standardized techniques for liquid biopsy processing, extraction, and analysis are being established, which address the challenges of cost and heterogeneity associated with these innovative methods. Coupled with extensive multicenter and prospective clinical trials, the clinical utility and feasibility of liquid biopsy will continue to gain recognition, offering significant benefits in terms of personalized medicine and patient care.

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Disclosure

The author reports no conflicts of interest in this work.

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