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Liquid biopsy: an emerging field with new opportunities for cancer diagnosis and prognosis

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Abstract

Cancer grades among the deadliest diseases, globally causing the death of a over million people each year. Early diagnosis has been considered ideal for efficient treatment as during later stages chances of treatment become limited. However, gold standard tissue biopsy has various limitation for instance, late-stage diagnosis and its intrusive operation making it unfit for repeated sampling. Scientists are passionately looking for new technologies and techniques for cancer diagnosis and prognosis. Liquid biopsy has emerged as new diagnostic and prognostic tool for cancer, that relies on body fluids to identify biomarkers for cancer. It offers advantages like non-invasive operation, timely detection, amenable to repeated sampling, and covers the tumor heterogeneity. Wide attention has been garnered by liquid biopsy and is undergoing rapid progress in the list of target biomarkers. The most common are circulating tumor cells, circulating tumor DNA, exosomes, tumor educated platelets, and non-coding RNAs (miRNA, lncRNA etc.). Each of these biomarkers have unique advantages, making liquid biopsy indeed a technology of future for cancer diagnosis with clinical utility. In this article, we tried to provide a thorough introduction of liquid biopsy and its markers, highlighted the common biomarkers that are deployed in liquid biopsy, briefly overview their implications as indispensable diagnostic and prognostic entities for the diverse types of cancer. Moreover, discussed future prospects of this revolutionary technology in the realm of cancer diagnosis and treatment.

Key words cancer, liquid biopsy, circulating tumor cells, circulating tumor DNA, exosomes, tumor educate platelets, miRNAs, lncRNAs, circRNAs

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Introduction

Cancer is an issue of great concern worldwide for public health, ranked second in terms of number of deaths after cardiovascular diseases [1]. For positive outcomes for patients, it is very important to spot cancer as early as possible and devise a robust treatment plan. Although significant progress has been made, however, the gold standard diagnostic methods for tumors continues to be a tissue biopsy. While these biopsies provide definitive results for identifying tumors and their various types, they come with several drawbacks. The procedure's invasive nature can be detrimental to patients, this renders it inappropriate for continuous disease surveillance. Additionally, the difficulties in acquiring tissue specimens, especially from early-stage tumors, hinder effective early detection strategies [2]. A non-invasive technique known as liquid biopsy examines blood or other bodily fluids to detect cancer markers, including molecular alterations, cancer cells, or metabolic byproducts [3]. This technique serves as a valuable tool for detecting cancer in its early stages, offering advantages over traditional tissue biopsies. Blood and urine are commonly used samples for liquid biopsies, making them significantly easier to perform than conventional biopsy procedures [4].

These tests are essentially non-invasive for patients, highlighting the potential of liquid biopsies to identify and continuously track tumor development [5]. Liquid biopsy has relied on entities like circulating tumor cells (CTCs) [6], circulating tumor DNA (ctDNA) [7], tumor-derived exosomes [8], tumor-educated platelets (TEPs) [9], and non-coding RNAs (ncRNA) [10]. Modern investigations focus chiefly on pinpointing CTCs, ctDNA, exosomes, TEPs and ncRNAs as shown in **Figure 1**. In this article we specifically offer an in-depth examination of different markers with prospects for deployment in liquid biopsy. Alongside, the present-day uses of liquid biopsy across miscellaneous cancer types were also discussed.

Landmark events that led to the development of liquid biopsy

The liquid biopsy technology has experienced expeditious evolution post 2010 [11]. Some landmark events is the identification of CTCs, ctDNA, and EVs by different research groups [4]. CTCs were first spotted in the blood of deceased cancer patient that resembled those found in tumors [12]. Cf DNA was identified by Mandel and Metais as free-floating nucleic acid molecules in plasma [13]. Wolf captured the earliest electron microscope images of EVs [14]. It was posited by Stahl and Johnstone posited exosomes dissemination from cells through the fusion of multivesicular bodies (MvB) with cell membrane [15]. Moreover, cfDNA increased levels were seen in plasma of cancer patients in comparison to healthy control, inferring a correlation between levels of cfDNA in blood with presence of cancer [16].

During the age of scientific advancement, scientists successfully isolated CTCs from blood samples for the first time in 1998, demonstrating a connection to pathological staging. This discovery paved the way for CTC utilization in clinical settings [17]. Before this, scientists in 1994 leveraged PCR techniques to recognize the initial KRAS mutation cfDNA extracted from patients of pancreatic cancer, obtained results that parallels those obtained in tumor tissue samples [18]. Raposo conducted a study that provided support for the biological function of EVs. Subsequent investigations have revealed that EVs derived from cells of the immune system can serve as presenters of antigens [19]. CTCs quantity prior to treatment acts as an independent marker for both overall survival and disease-free interval in patients with advanced breast cancer [20]. The ensuing expansion of the industry led to the inclusion of diverse liquid biopsy markers in oncology guidelines and their endorsement for clinical use [21].

Commonly deployed markers in liquid biopsy

Here we provide overview of the most prominent biomarkers that are deployed in liquid biopsy [22] as shown in **Figure 2**, which also provide an overview of various characteristics of these markers.

Circulating tumor cells

A notable breakthrough in the research of CTCs happened in 1869 when Ashworth et al. discovered these cells in the blood of deceased cancer patients. CTCs, which stem from both primary and metastatic tumors, enter the circulatory or lymphatic systems of individuals with cancer and travel through their peripheral blood [23]. Despite their rarity, with only one CTC per million leukocytes and a short lifespan of 1-2.5 hours, recent research has shown a link between CTC concentrations and cancer advancement, especially in metastasis. CTCs are potent diagnostic maker of cancer and can be deployed for collecting important data for both clinical and scientific purposes [24]. Although improving the accuracy of CTC isolation and collection methods remains an ongoing issue, recent sophistication in technology have made their practical use in clinical environments possible. Technological progress has enhanced the precision in correlating CTC quantities with cancer development [25]. Escalation in the level of CTCs can be indictive of shorter progression-free survival and overall survival times. Escalated CTCs level in blood of breast cancer patient can be linked to short period of progression-free survival [26]. Consequently, CTCs detection can be key liquid biopsy marker [27].

The minuscule quantity of CTCs necessitates the use of highly sophisticated and sensitive methods for their effective isolation and detection. These techniques are constantly evolving, becoming more refined and accurate. Traditional methods leverage physical properties such as size and deformability, using techniques such as density gradient centrifugation, inertial focusing, and filtration. Some methods rely on CTCs specific markers, including epithelial cell adhesion molecules (EpCAM), vimentin, and N-cadherin [28]. Other strategies deploy marker specific immune-enrichment or immunomagnetic extraction, and used tools relying on microfluidics. To date the only FDA- authorized platform for CTCs quantification in blood is CellSearch® [29]. Despite their limitations, these methods have contributed greatly in furthering research on CTCs deployment for clinical purposes. As a minimally invasive diagnostic tool, CTCs are expected to become increasingly important in tumor early identification, progression and treatment intervention [30].

Circulating tumor DNA

Similar to CTCs, ctDNA can be isolated from blood and other bodily fluids such as ascites, pleural fluid, urine, and cerebrospinal fluid (CSF). cfDNA majorly stems from typical white blood cells and stromal cells [31]. Unlike cfDNA, ctDNA represents the existing state of the tumor. Analysis has confirmed that in cancer patients, ctDNA fragments are typically 20-50 base pairs shorter than cfDNA [32]. This characteristic renders ctDNA less vulnerable to the impact of heterogeneity within tumors. Furthermore, the brief half-life of ctDNA is crucial for its application as a real-time tumor indicator. These features provide ctDNA a marked benefit over conventional biopsy markers. In recent times, scientists have recognized the significance of ctDNA diagnostic and prognostic abilities for cancer [33]. Serum analysis of individuals with pancreatic cancer indicated increased concentrations of ctDNA, that diminished upon treatment, a marked benefit for deployment as a monitoring tool for treatment

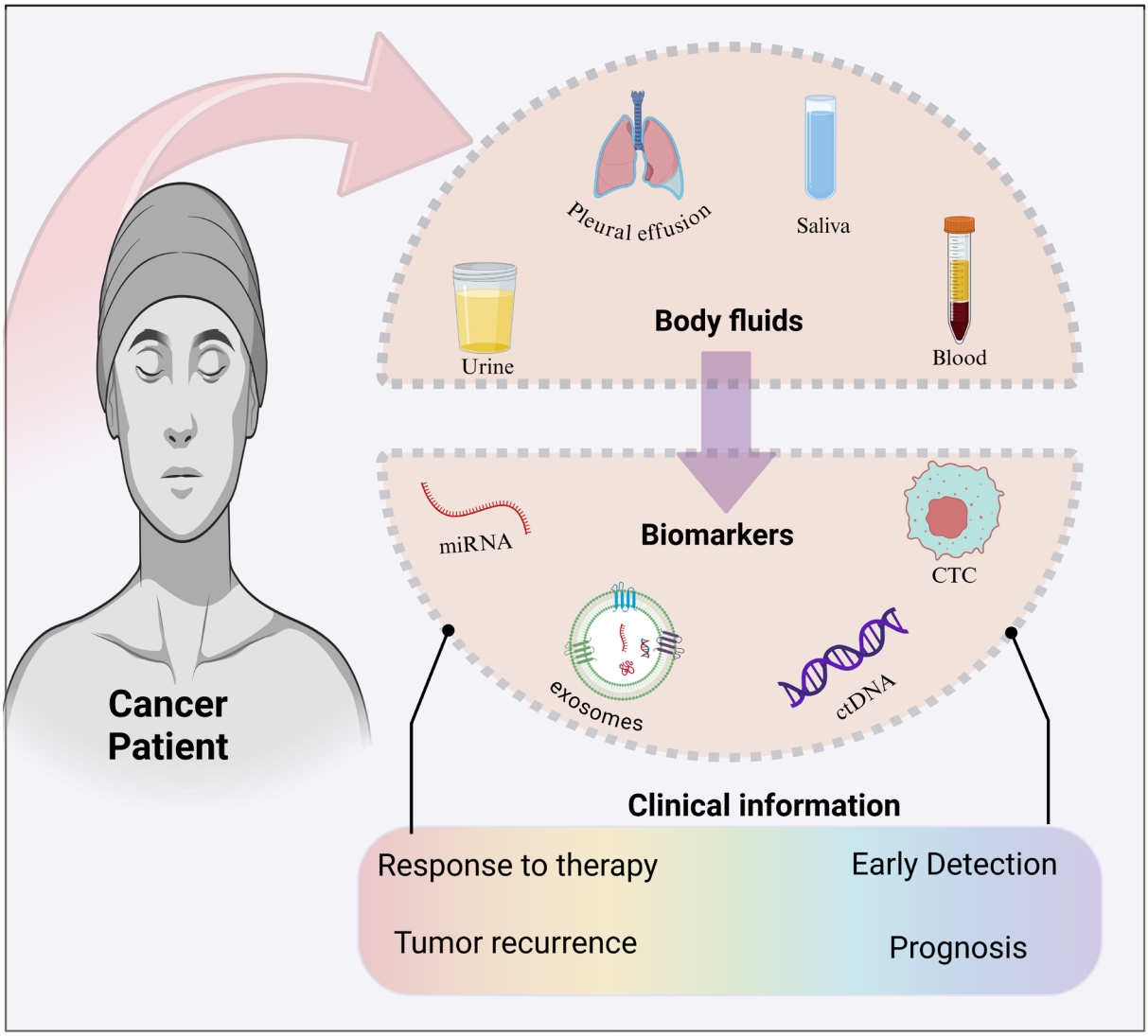

Figure 1. Schematic representation of the liquid biopsy technology. The most common body fluids and the most common markers that are deployed in liquid biopsy are represented. Meanwhile, liquid biopsy provides information that can be indispensable for clinical outcomes. Figure created with Biorender resources (Biorender.com).

response [34].

Several existing clinical applications concentrate on detecting mutations in particular genes within ctDNA. Analysis of serum ctDNA of 18 colorectal cancer patients' uncovered hotspot mutations in genes like APC, KRAS, TP53, and PIK3CA. ctDNA mutation can be indictive of patient response to treatment. Genetic modifications can disrupt the equilibrium of oncogenes, potentially leading to the onset of cancer. Therefore, detecting mutations in ctDNA is indispensable for cancer identification [35]. With the improvement is technology many robust techniques have been created for detecting ctDNA; such as RT-qPCR, ddPCR, and several standard and cutting-edge sequencing platforms [36]. It is anticipated that ctDNA tests will be extensively used in future for clinical and research purposes in the context of cancer theragnostic.

Exosomes

Exosome were first spotted in sheep reticulocytes. These structures are classified as a type of extracellular vesicles originating from endosomes. Exosomes form through membrane budding within multivesicular bodies and are released when these bodies merge with the cell membrane. Along with microvesicles and apoptotic vesicles, exosomes comprise the three main types of extracellular vesicles, primarily differentiated by their size and cellular origins [37]. Recent years have witnessed growing interest in these three subtypes. Exosomes can be detected in diverse bodily fluids, including blood, saliva, and urine. These structures fulfill various biological functions, encompassing molecular transportation, cellular communication, and immunological reactions of the body [38].

Exosomes are key player in the environment surrounding the tumor. Their impactful role in cancer progression is widely recognized [39]. The remarkable stability and comprehensive tumor cell data representation of exosomes significantly enhance liquid biopsy applications [40]. Recent cancer research has extensively explored the various exosomal components, including nucleic acids, proteins, lipids, and metabolites. Exosomal non-coding RNAs (ncRNAs) have emerged as particularly promising cancer diagnosis and treatment indicators [41]. Studies have revealed that heightened levels of specific exosomal miRNAs, [42] such as "miR-1246, miR-4644, miR-3976 and miR-4306"

can function as highly sensitive prostate cancer biomarkers [43]. Serum form the patients of bladder cancer have escalated level of "exosomal lncRNA H19" , pointing to the possibility that exosomal lncRNAs can be used as reliable biomarkers for its diagnosis [44]. The surge in research interest surrounding exosomal proteins can be attributed to their extensive diversity and high concentrations. These proteins are known to play pivotal roles in modifying the cancer microenvironment, fostering tumor expansion, and facilitating cancer dissemination [45]. Furthermore, exosomal proteins have been associated with the emergence of chemotherapy resistance in individuals battling cancer. A recent study has highlighted the role of plasma gelsolin (pGSN), a variant of the GSN protein secreted by chemotherapy-resistant ovarian cancer cells. Exosomes can facilitate the transport of this protein, which subsequently activates α5β1 integrin, resulting in elevated levels of hypoxia-inducible factor 1 subunit α. As a result, this activation enhances ovarian cancer cells prowess to withstand chemotherapy and survive [46].

The crucial role of exosomes as key markers in liquid biopsies, along with their significant clinical importance, highlights the need for robust and precise methods for their extraction and identification [47]. Recently, analytical techniques such as RT-PCR, genome sequencing, and proteomics have become more accessible for analyzing exosomal contents [48]. Commonly used methods for exosomes extraction include differential ultracentrifugation, size-based separation, immunomagnetic isolation, and microfluidic approaches. As technology advances and various scientific fields converge, it is expected that exosomes incorporated liquid biopsies would make it to clinical practice expeditiously, particularly in the realm of cancer detection [49].

Tumor educated-platelets

Originally associated primarily with blood clotting and thrombosis, platelets are now seen as important in cancer. Ranking as the second most numerous cell type in blood, platelets are vital to numerous physiological functions [50]. These encompass aiding in wound healing, involvement in development of atherosclerosis, regulating vascular growth, and affecting the process of formation of new blood vessels. A significant connection has been revealed between increased platelet levels and cancer, subsequent research reinforced this connection [51]. Studies have shown a positive correlation between platelet accumulation and mortality in cancer patients. Recently, a specific type of platelet known as TEPs have emerged as a new liquid biopsy candidate. TEPs are obtained from cancer patients and exhibit distinct RNA and protein profiles. Research suggests that TEPs assist in the growth and spread of various solid tumors [52]. In particular, spliced TEP RNA markers can yield precise details about the existence, site, and molecular characteristics of tumors, even so, the detailed mechanisms call for further examination [53]. TEPs have not yet made to clinical practice, however, several studies have indicated TEPs' diagnostic potential, anticipating their indispensable role.

Platelets and tumors exert a mutual influence on each other. Platelets continuously incorporate tumor-derived components such as proteins, nucleic acids, vesicles, and granules that alters their RNA and protein expression profiles. As components of liquid biopsies, platelets present numerous benefits; exhibit notable stability and can be easily isolated through simple centrifugation at low speeds. The genetic content found in platelets demonstrates remarkable resilience. TEP short life allows for an accurate representation of the tumor's current condition, facilitating

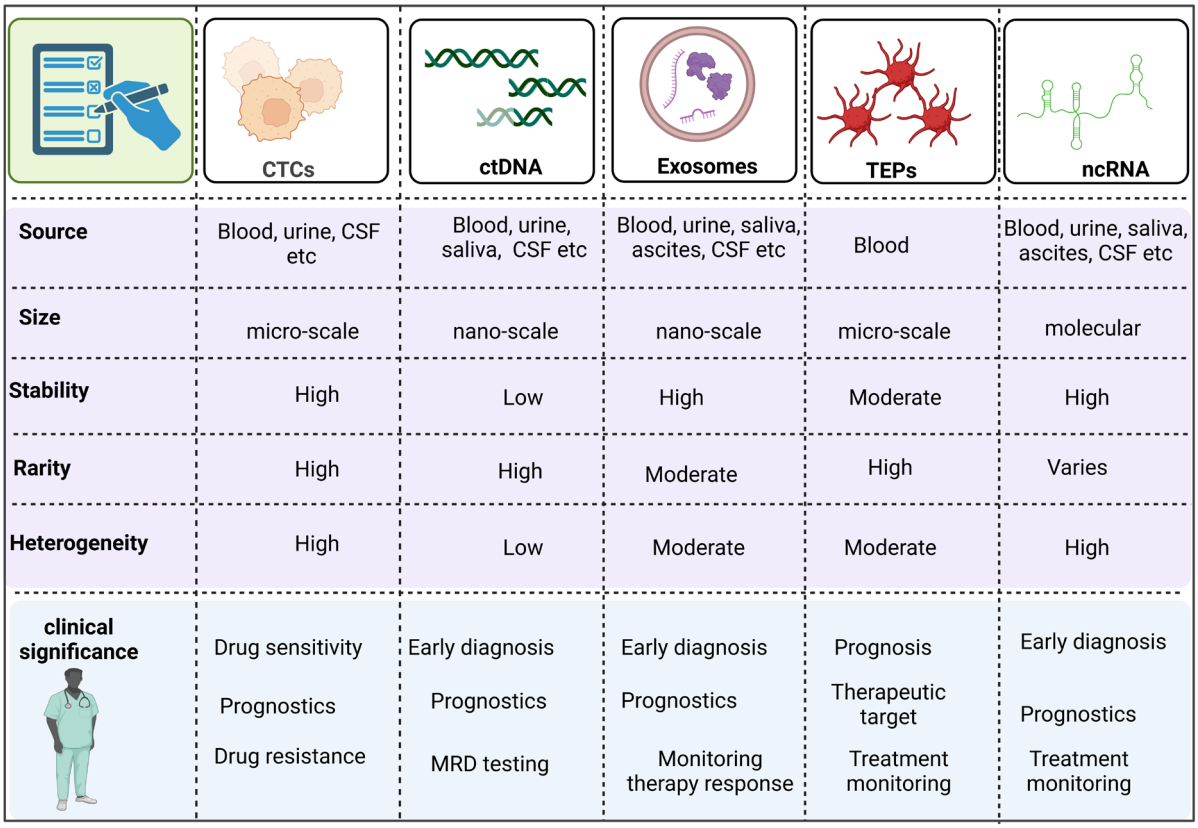

Figure 2. Comparison of the characteristics of various liquid biopsy markers. Figure created with Biorender resources (Biorendor.com).

ongoing surveillance in real time [52]. Recent research on platelets in individuals with tumors has largely focused on mRNA and lncRNAs. From the analysis of RNA sequencing data cancer patient can be successfully discerned from healthy subjects. Four specific lncRNA markers such as “LNCAROD, SNHG20, LINC00534, and TSPOAP-ASI” in platelets associated with colorectal cancer showed escalated expression both in platelets and serum samples from CRC patients, calling for lncRNAs diagnostic potential [54]. Establishment of a gene expression database specifically for platelet-based disease studies, would expedite the research progress aiming at liquid biopsies employing platelets. Nevertheless, our current grasp of the mechanisms underlying platelet RNA remains incomplete. TEPs deployment in cancer therapy is still at a conceptual stage, mandating extensive additional examination [55].

Non-coding RNAs

Unlike coding RNAs, non-coding RNAs have diverse functions within cells. Once dismissed as "junk RNA" and deemed irrelevant to cancer progression, modern research recognizes a fundamental role of non-coding RNAs in cancer onset and progression. Liquid biopsy deploying certain non-coding RNAs had shown excellent sensitivities and prospects for cancer detection [56].

miRNA and lncRNAs. MicroRNAs (miRNAs) are single-stranded RNA sequences of about “18 to 23” nucleotides, one of the categories of non-coding RNA. Have a recognized function in regulating gene expression post-transcriptionally. They attach to particular locations in the mRNA's 3′ untranslated region, diminishes the mRNA stability and inhibiting gene expression [57]. In cancer research, particularly within the realm of liquid biopsies, miRNAs are most thoroughly investigated type of non-coding RNAs. miR-21 and miR-155 escalated level are reported as indictive markers for numerous cancers, highlighting their prospective deployment as reliable liquid biopsy markers [58]. Recently, researchers have developed various miRNA detection methods, including qPCR, hybridization chain reaction, rolling circle amplification, and strand displacement amplification. These methods have played a crucial role in advancing miRNA studies, especially in elucidating its fundamental characteristics: prevalence and stability within tissues [59].

In cancer liquid biopsies, long non-coding RNAs (lncRNAs) are the second most studied non-coding RNAs. These RNA molecules, which exceed “200 nucleotides” in length and lack protein-coding capacity, perform various biological functions. They regulate gene transcription, influence miRNA control of target genes, and directly engage with proteins to influence their function and stability. Some lncRNAs also play roles in cell cycle control, impacting cellular growth and differentiation [60]. lncRNAs play critical role in cancer progression by regulating key cancer-associated transcriptional activators. There is indication of lncRNA involvement in tumor heterogeneity as there exists specific link between its expression and the type of tissue. Numerous cancer-associated lncRNAs levels are upraised in cancer patients derived serum and plasma [61]. For instance, in pancreatic ductal adenocarcinoma (PDAC) lncRNA have been shown to be useful as indictive liquid biopsy marker for the disease [62]. Upraised levels of lncRNA H19 are noticed in the plasma obtained from lung cancer patients, that heightened their possibility to be deployed as supplementary biomarker for lung cancer diagnosis [63]. As new lncRNAs are discovered expeditiously, it would be beneficial to initiate investigations focusing on explaining their specific functions and impact in cancer. New investigations are needed to evaluate lncRNAs indispensability as liquid biopsy based diagnostic tool for diverse cancer types [64].

Currently, lncRNA based diagnostic and prognostic models are also active area of research [65]. One study deployed a rm6A immune-related lncRNA to create a risk model that can be used for robust prediction about bladder cancer prognosis, immune status, and treatment response [66]. A separate study created a cuproptosis-linked lncRNA profile using intersecting lncRNAs and successfully used it for forecasting hepatocellular carcinoma outcomes and assessing the efficacy of immune checkpoint blockade (ICB) treatment [67]. The success in clinical trial is fundamental to get approval for application in clinical setting, however, it is evident that the use of miRNA and lncRNA for biomarker modeling represents a major technological intervention in the field of liquid biopsy technology [68].

Circ-RNA. Circular RNAs (circRNAs) are unique RNA molecules with a closed-loop structure, unable to encode proteins. They were first detected in 1971 during research on potato spindle tuber disease and known as self-replicating, "virus-like" RNA with a minimal molecular weight. The closed loop structure and the absence of free 5′ and 3′ ends, make these RNAs resistant to nucleases [69]. Their closed loop structure was visualized using radioactive labeling. With the advancement in research on circRNAs , it has become more evident that circRNAs are widely present and perform fundamental functions in human cells and tissues, such as acting as microRNA sponges, influencing the splicing of precursor mRNA, enhancing transcription, altering their own stability and location through interactions with RNA-binding proteins (RBPs), and producing functional proteins [70]. CircRNAs lack a typical polyA tail, preventing their direct detection using polyA tail-dependent purification methods [69]. Researchers have deployed diverse methods to detect circRNAs, including RT-PCR, RNAseq, northern hybridization, and high-throughput sequencing. These techniques involve the creation of primers that target specific reverse splice sites. CircRNAs' resistance to RNA exonuclease degradation allows their enrichment by selectively eliminating linear RNA.

Depending on the associated pathways, circRNAs can function as either proto-oncogenes or oncogenes in cancer [71]. As an example, circHIPK3 enhances the proliferation and migration of cancer cells through its activation of the miR-124/STAT3 signaling pathway. It indirectly activates STAT3, a transcription factor linked to various oncogenes and cell proliferation, by inhibiting miR-124's suppressive effect on STAT3, thereby influencing tumor cell behavior. Circ-RNA ITCH functions as an oncogene in diverse cancer types. Circ-ITCH interacts with particular microRNAs (miR-7, miR-17, and miR-214), indirectly influencing the expression of target genes [72]. These microRNAs and their targets are potentially implicated in several tumor-associated signaling pathways, including the Wnt/β-catenin and PI3K/AKT cascades. Irregular expression of circ-ITCH may facilitate tumor development by disturbing the equilibrium of these pathways. Investigations have identified circ-ITCH as a downregulated oncogene in cancers of the ovaries, prostate, brain, and stomach [73]. Ultimately, circRNAs facilitate tumor progression through multiple mechanisms. These include stimulating cellular division, circumventing growth-limiting factors, augmenting invasive and metastatic capabilities, fostering new blood vessel growth, modifying cellular energy dynamics, and eliciting inflammatory processes [74].

Conclusion and perspective

While medical technology has advanced significantly, tumor diagnosis still relies mainly on tissue biopsy. The widespread adoption of this method can be attributed to its many advantages, such as highly standardized laboratory procedures, reproducible results, long-lasting samples, and accurate measurements. Nonetheless, tissue biopsy entails numerous limitations. The

Table 1. List of studies implicating liquid biopsy biomarkers for diverse cancer type.

Cancer	Biomarker	Source	Level	Utility	Ref.
LC	ctDNA	Plasma	Up	Treatment response	[75]
	CTCs	Blood	Up	Early diagnosis	[76]
	let-7i-3p, miR-154-5p	Serum	Down	Early diagnosis	[77]
	miRNA				
HCC	CTCs	Blood	Up	Early diagnosis	[78]
	miR-221-3p, miR-223-3p, miR-10b5p, miR-21-5p	Plasma exosomes	Up	Early diagnosis	[79]
CRC	miR-203	Serum exosome	Up	Prognosis	[80]
	miR-21	Plasma exosome	Up	Recurrence & Prognosis	[81]
	ctDNA	plasma	Up	Treatment response	[82]
RCC	ctDNA	Plasma	Up	Prognosis	[83]
	miR-328-3p	Urine	Down	Prognosis	[84]
	miR-15a	Urine	Up	Early diagnosis	[85]
	has-mir-92a-1-5p	Plasma exosome	Down	Early diagnosis	[86]
UC	ctDNA	Plasma	Up	Treatment response	[87]
	miR-141	Serum	Up	Early diagnosis	[88]
	miR-151b	Serum	Up	Prognosis	[89]
MM	CTCs	Blood	Up	Early diagnosis	[90]
	ctDNA	Plasma	Up	Progression	[91]
	ctDNA mutation	Serum	Up	Progression	[92]
TC	CTCs	Blood	Up	Early diagnosis	[93]
	ctDNA	Plasma	Up	Treatment response	[94]
	ctDNA methylation	Serum	Up	Diagnoses & recurrence	[95]
	miR-29a	Serum exosome	Down	Diagnosis &prognosis	[96]

Note: "LC: Lung cancer, HCC: Hepatocellular carcinoma, CRC: Colorectal cancer, RCC: Renal cell carcinoma, UC: Urological cancer, MM: Multiple myeloma, TC: Thyroid cancer, CTCs: circulating tumor cells, ctDNA: circulating tumor DNA".

invasive approach limits sampling in high-risk zones, and the challenge of repeating procedures makes it inadequate for regular testing and observing therapeutic outcomes. Moreover, the extracted tumor data is influenced by the heterogeneous nature of the tumor and is representative of the specific location that was biopsied. Hence, it is necessary to explore alternative screening methods to enhance patient care and outcomes. Despite rapid advancements, liquid biopsy's clinical use remains restricted. This technique offers numerous advantages over traditional methods, such as minimal invasiveness, low risk, multiple sampling ability, continuous monitoring suitability, and capturing the tumor heterogeneity, as numerous studies revealed their usefulness for cancer diagnosis and prognosis (**Table 1**). However, liquid biopsy confronts challenges, including fluctuating results due to a lack of standardized practices across labs, demanding sample handling conditions, and the need for higher accuracy. A significant challenge in widespread implementation of liquid biopsy is the requirement to isolate, refine, and identify the markers. Consequently, future advancements in liquid biopsy accuracy hinge on several key factors: enhancing detection methods and analytical platforms, establishing standardized procedures, and developing unified approaches for data interpretation. These elements are essential for improving the reliability and effectiveness of liquid biopsy techniques. The incorporation of swiftly evolving artificial intelligence could enhance the efficiency of detection methods.

It is essential to realize that liquid biopsy in clinical environments is only representative of the state of that particular biomarker during the diseases, not the entire state of the diseases. As such, liquid biopsy cannot fully substitute for tissue biopsy; these techniques jointly provide a more comprehensive insight into tumor biology. For widespread deployment of liquid biopsy in healthcare settings, extensive efforts should be made in optimization and standardization of methods linked to these markers' isolation, detection and other downstream analysis techniques. The progress and adoption of liquid biopsy technology could be accelerated through refining detection strategies, integrating liquid biopsy markers, or fusing liquid biopsy with complementary diagnostic tools. Despite some knowledge gaps, liquid biopsy has become a focal point of scientific inquiry and extensive research. Although challenges remain, this innovative approach shows significant potential for integration into clinical practice.

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Ethics approval

No applicable.

Data availability

The data will be available upon request.

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Authors' contribution

DSM, SH contributed to the conception, design, writing of this review article, figures drawing and submitted the final version of the manuscript.

Competing interests

None.

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