

Tumor-Educated Platelet RNA and Circulating Free RNA: Emerging Liquid Biopsy Markers for Different Tumor Types

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Abstract

The incidence and mortality from malignant tumors continue to rise each year. Consequently, early diagnosis and intervention are vital for improving patient prognosis and survival. The traditional pathological tissue biopsy is currently considered the gold standard for cancer diagnosis. However, it suffers from several limitations including invasiveness, sometimes not repeatable or unsuitable, and the inability to capture the dynamic nature of tumors in terms of space and time. Consequently, these limit the application of tissue biopsies for the diagnosis of early-stage tumors and have redirected the research focus towards liquid biopsies. Blood-based liquid biopsies have thus emerged as a promising option for non-invasive assessment of tumor-specific biomarkers. These minimally invasive, easily accessible, and reproducible tests offer several advantages, such as being mostly complication-free and efficient at monitoring tumor progression and tracing drug resistance. Liquid biopsies show great potential for cancer prediction, diagnosis, and prognostic assessment. Circulating tumor-educated platelets (TEPs) possess the unique ability to absorb nucleic acids from the bloodstream and to modify transcripts derived from megakaryocytes in response to external signals. In addition, circulating free RNA (cfRNA) constitutes a significant portion of the biomolecules present in the bloodstream. This paper aims to provide a comprehensive overview of the current research status regarding TEP RNA and cfRNA in liquid biopsies from various tumor types. Our analysis includes cancers of the lung, liver, pancreas, breast, nasopharynx, ovary and colon, as well as multiple myeloma and sarcoma. By synthesizing this information, we intend to establish a solid theoretical foundation for exploring potential applications of circulating RNA as a reliable biomarker for tumor diagnosis and monitoring.

Keywords: cancer; liquid biopsy; tumor-educated platelets; plasma; serum; biomarker; RNA

1. Introduction

Cancer, or malignant neoplasm, is associated with high global incidence and mortality rates. There has been a worrisome surge in cancer incidence over the past 6 years, with approximately 4 million new cases and 1.4 million deaths [1,2]. Traditional tissue biopsy serves as the gold standard for tumor diagnosis. However, it is limited by its invasiveness, high risk, potential complications, and lack of reproducibility and applicability in certain cases [3–6]. Tissue biopsies are also unable to capture dynamic information on tumor development and epigenetic characteristics [7], thereby restricting their overall applicability. The limitations inherent with tissue biopsy have led the oncology field to shift its research focus towards the assessment of circulating components in the blood [8]. This approach, known as blood-based “liquid biopsies”, offers numerous advantages including being minimally invasive, easily accessible, reproducible, and free from potential complications. Moreover, liquid biopsies are efficient at monitoring disease progression and tracking tumor drug resistance. They have been extensively studied and found to be a viable alternative for the noninvasive assessment of tumor-specific biomarkers [9].

As a minimally invasive method for cancer detection and monitoring, liquid biopsy has the potential to revolutionize cancer diagnosis. It allows for comprehensive and precise analysis of tumors and their microenvironment at multiple levels. The careful application of liquid biopsies enables earlier detection of cancer development, prognostic evaluation of tumors at various stages, and the identification of novel targets for personalized treatment. Furthermore, blood tests can be utilized for pre-treatment tumor classification, thereby allowing enabling personalized treatment, early intervention, treatment response monitoring, regular assessment of treatment efficacy, and the follow-up and early detection of disease recurrence. Currently, researchers are investigating the different components of the blood in various cancer types (Fig. 1). Amongst these, significant attention has been focused on biomolecules such as circulating tumor DNA/RNA (ctDNA/ctRNA) and circulating cell-free DNA/RNA (cfDNA/cfRNA), as well as circulating tumor cells (CTCs), extracellular vesicles (including exosomes and tumor vesicles), and tumor-educated platelets (TEPs). These hold immense value for early tumor diagnosis and for the assessment of treatment efficacy [10].



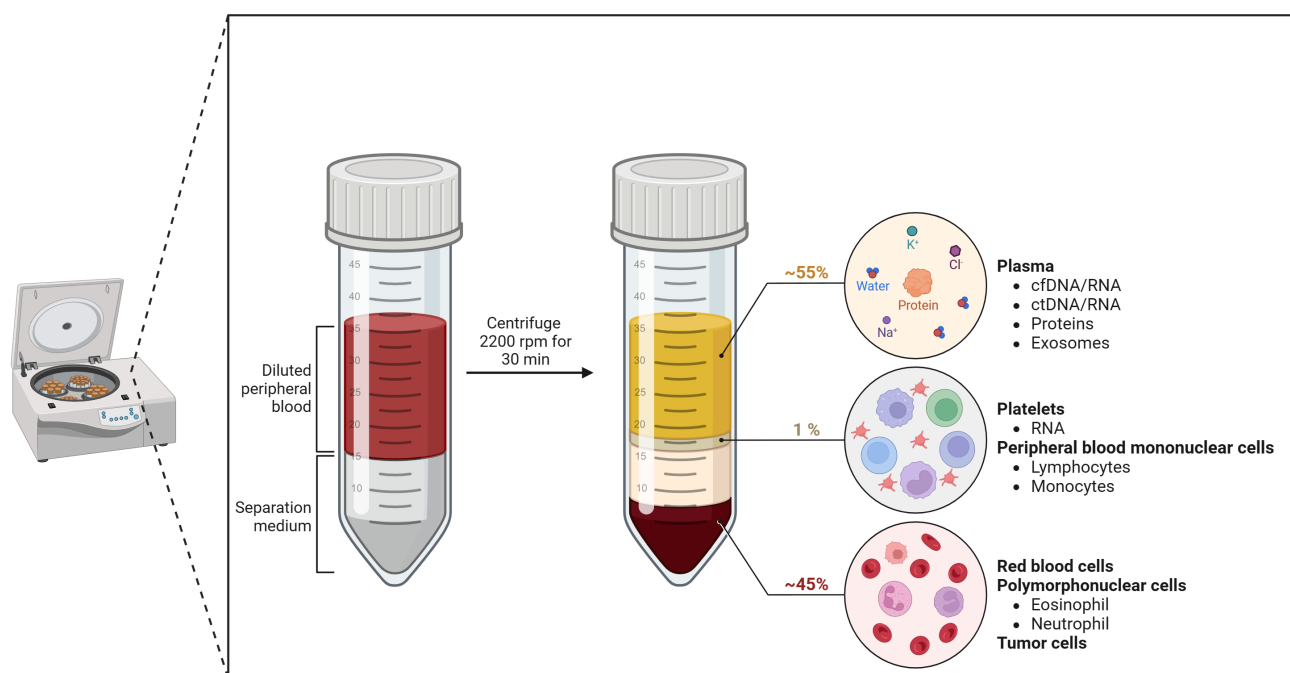


Fig. 1. Peripheral Blood Cells Isolation by Density Gradient Centrifugation. ctDNA/RNA, circulating tumor DNA/RNA; cfDNA/RNA, circulating cell-free DNA/RNA.

Platelets are small cell fragments shed from the cytoplasm of mature megakaryocytes resident in the bone marrow, and comprise an important component of the blood [11]. In addition to their well-known functions in thrombosis and hemostasis regulation, platelets also play a vital role in the immune system. They are actively involved in both innate and adaptive immune responses, and contribute to various processes such as atherosclerosis, angiogenesis, and lymphatic vessel development [12]. The interaction between tumor cells and circulating platelets has been implicated in tumorigenesis, angiogenesis, tumor spread, and metastasis [13]. Platelets can influence the development and progression of cancer through several mechanisms [14–16]. Firstly, they can aggregate around tumors, thereby promoting tumor growth and evading immune elimination. Secondly, they facilitate the adhesion of tumor cells, allowing them to evade killing by the immune response. Thirdly, the activation of platelets promotes tumor cell invasion and metastasis through various processes, including the synthesis of lipid products [17], release of proteins from alpha granules [18], induction of epithelial-mesenchymal transition (EMT) [19], angiogenesis, and facilitating the resistance and extravasation of tumor cells [20]. On the other hand, platelet activation functions rely on tumor cells to induce changes in the transcriptome profile of platelets [21]. This can occur directly through the transfer of tumor-derived RNA [22], or indirectly through the release of signals that regulate platelet mRNA processing [23]. In the presence of tumor cells and the tumor microenvironment (TME), these changes lead to the conversion of immature platelet mRNAs into mature mRNAs that are subsequently

translated into functional proteins, ultimately resulting in the generation of tumorigenic platelets. Sequencing analysis of mRNA from TEPs has revealed a remarkable potential to differentially diagnose limited and metastatic tumors with an accuracy of 96%. Additionally, sequencing of mRNA from TEPs could identify the primary tumor location of six distinct tumor types with an accuracy of 71% [24]. Of note, miRNAs are the most widely studied of the various RNA types. These small non-coding RNA molecules are typically 19 to 24 bases in length and play a crucial role in the post-transcriptional regulation of gene expression. They can sometimes behave as either oncogenes or tumor suppressor genes, and exert their regulatory influence over various cellular pathways with remarkable stability [25]. This stability may be due to the protective effects of miRNA within exosomes and/or protein complexes. It is worth noting that miRNAs are not only the most abundant RNA species in peripheral blood, but also the predominant RNA species found in TEP-derived RNA [21–26].

In summary, platelets are involved in cancer development and progression, making them a promising source of biomarkers. In this paper, we review the current research status on TEP-RNAs in various cancer types. Additionally, we explore potential applications of TEP RNA in early cancer diagnosis, prognosis, and treatment, and offer insights into its future prospects as a biomarker. The investigation of cfRNA derived from plasma or serum has also attracted significant attention in recent years. The extraction and isolation methods for cfRNA and platelets are similar [27] and will be comprehensively reviewed in this paper (Supplementary Table 1). The application of platelets and

cfRNA in cancer research is therefore very promising, and more in-depth studies should reveal their full potential in cancer diagnosis, treatment and prognosis.

2. Advances in the Study of TEP RNA and cfRNA in Various Cancer Types

TEP RNA and cfRNA have recently received considerable attention in the liquid biopsy field. They are considered to be potential tumor biomarkers due to their high stability and detectability. The continuous development of experimental techniques such as microarray sequencing and quantitative real-time polymerase chain reaction (qRT-PCR) has enabled in depth analysis of circulating RNA, including investigation of its potential roles in tumorigenesis, progression and prognostic assessment. Currently, this field of oncology research is undergoing rapid development. The current paper synthesizes the latest scientific findings and summarizes the current status of research on TEP RNA and cfRNA in different tumor types including lung, liver, pancreatic, breast, nasopharyngeal, ovarian, colorectal, multiple myeloma and sarcoma. Our aim is to provide an essential reference that allows a deeper understanding of circulating RNA and facilitates its application as a potential tumor biomarker.

2.1 Advances in the Study of TEP RNA and cfRNA in Lung Cancer

Lung cancer (LC) is one of the most common malignancies worldwide, with rising incidence and mortality rates in recent years. According to the World Health Organization (WHO), there were approximately 18.1 million new cancer cases and 9.6 million cancer-related deaths worldwide in 2018. Of these, 2.09 million (11.5%) of all new cancers were LC, while 1.76 million (18.3%) of all cancer-related deaths were from LC [28]. Approximately 934,700 new cases of LC were reported in China in 2016, representing an incidence of 73.48 per 100,000 individuals. Additionally, there were approximately 440,500 deaths, resulting in a death rate of 56.82 per 100,000 individuals [29]. LC is now the leading cause of cancer-related death in men worldwide, and the second leading cause in women [30]. The histopathological subtypes of LC are classified as small cell LC and non-small cell LC (NSCLC). The latter accounts for the majority (85%) of cases and includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma [31]. Early-stage LC may show mild or even no typical clinical manifestations. Consequently, the majority of patients are diagnosed at an advanced stage, with the presence of lymph node or distant metastases. This adversely affects both the treatment outcomes and prognosis of LC [32]. Therefore, establishing early diagnosis and implementing timely interventions has major significance for disease progression and prognosis. At present, LC diagnosis relies primarily on a combination of clinical manifestations, diagnostic imaging, biochemical tests, and histopathological analysis. The biomarkers carcinoembryonic antigen

(CEA), cancer antigen 125 (CA125) and cytokeratin 19 fragment (CYFRA21-1) are widely used in the detection and diagnosis of LC, but are more accurate for the diagnosis of advanced LC rather than early LC [33]. Meanwhile, molecular diagnostics is gradually improving and showing increasingly advantageous features [27]. Among these methods, the molecular expression profile of cancer samples show good potential for cancer classification. However, classical molecular diagnostic tests are limited by the availability of tissue samples and are somewhat hindered in clinical practice by time and space constraints. The development of liquid biopsies has addressed these problems very well. In the subsequent section, we provide a comprehensive review of TEP RNA and cfRNA in the context of LC research.

Li *et al.* [32] utilized lncRNA microarrays to investigate the potential diagnostic value of TEP RNA in LC. They found that the expression levels of the TEP lncRNAs GTF2H2-1 and RP3-466P17.2 in LC patients were significantly lower than in the healthy population, whereas the levels of TEP lncRNA ST8SIA4-12 was significantly higher ($p = 0.0001$). Similar differences were observed between individuals with early-stage LC and the normal population. To further investigate the accuracy of TEP RNA for LC diagnosis, the researchers constructed receiver operating characteristic (ROC) curves. TEP lnc-GTF2H2-1, RP3-466P17.2 and ST8SIA4-12 showed good performance as biomarker complexes for the diagnosis of LC, with a combined area under the curve (AUC) of 0.921, sensitivity of 82.6%, and specificity of 87.1%. Additionally, these biomarker complexes showed promising diagnostic potential for early-stage LC, with a combined AUC of 0.895, sensitivity of 93.6%, and specificity of 69.8%. Furthermore, TEP linc-GTF2H2-1 had the ability to distinguish between patients with early- and advanced-stage LC. When combined with other biomarkers such as CEA, CYFRA21-1, and neuron-specific enolase (NSE), the diagnostic performance of TEP linc-GTF2H2-1 for distinguishing between intermediate and advanced stages was significantly enhanced (AUCs of 0.799, 0.806, and 0.790, respectively). The results were significantly better than those of each biomarker alone (AUCs of 0.780, 0.797 and 0.773, respectively). Lastly, the combination of these four biomarkers resulted in a notable improvement in LC diagnosis (AUC of 0.899, sensitivity of 76.6%, and specificity of 85.0%), thus further emphasizing the potential role of TEP RNA in tumor diagnosis and assessment of disease progression. Another study used a similar approach to investigate the levels of Apoptotic Chromatin Condensation Inducer 1 (ACIN1) mRNA in platelets derived from 146 LC patients and 58 healthy individuals [33]. The level of ACIN1 mRNA was found to be significantly higher in platelets from LC patients compared to platelets from healthy controls ($p = 0.015$). Moreover, ROC curve analysis for LC detection showed a sensitivity of 82.7%, specificity of 44.8%, accuracy of 0.724, and AUC of 0.608.

The ACIN1 mRNA level was not significantly correlated with age, sex, type of pathology, or presence of metastasis ($p < 0.05$). Thus, TEP ACIN1 mRNA may be a novel, specific molecular biomarker for LC diagnosis. Another study found that it might be an m-RNA of ITGA2B and SELP that platelets overexpress rather than in their protein form in early stage of LC, then play a role in mRNA form [34]. These were significantly elevated in NSCLC ($p < 0.001$). Furthermore, they could accurately differentiate between NSCLC and controls, with an AUC of 0.922 for ITGA2B mRNA and 0.799 for SELP mRNA in the training cohort, and 0.888 and 0.716 respectively in the validation cohort. Both ITGA2B mRNA and SELP mRNA are therefore potentially useful for NSCLC diagnosis, with ITGA2B being superior. An elevated level of ITGA2B mRNA was also identified as an independent risk factor for poor overall survival (OS) in NSCLC patients. By combining ITGA2B, CEA and clinical staging, a predictive model for OS was constructed that led to substantial improvement in the prognostic assessment of NSCLC. Nilsson *et al.* [35] conducted a study that compared mRNA from platelets and plasma for the early diagnosis of LC. They evaluated the cancer-induced rearrangement of echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase (EML4-ALK) mRNA. This mRNA exhibited higher diagnostic efficacy in platelets (65% sensitivity and 100% specificity) compared to plasma (21% sensitivity and 100% specificity), suggesting that RNA derived from platelets may be a more reliable biomarker for diagnosing LC. Co-incubation experiments showed that exosomes derived from LC cell lines could transfer tumor-derived, EML4-ALK rearranged RNA into platelets. This result highlights a potential mechanism by which tumor cells can manipulate platelet behavior in the circulation. The researchers continuously monitored the platelet level of EML4-ALK rearranged RNA during the entire course of treatment in LC patients and found that it was altered in response to targeted drug administration. Moreover, alterations in platelet EML4-ALK expression were observed approximately two months before drug resistance-associated changes were detected by conventional imaging methods. This finding could help with earlier identification of LC patients showing drug resistance phenomenon after targeted therapy. The study also revealed a close correlation between EML4-ALK levels in platelets and shorter progression-free survival (PFS) of patients, thus providing clinicians with more precise and timely guidance for clinical decision-making.

Researchers have also focused on the role of cfRNA in the diagnosis and treatment of tumors, with studies showing that both quantitative and qualitative analysis of cfRNA can guide the selection of treatment options and monitor treatment efficacy [36]. Furthermore, cfRNA has broader applications compared to TEP RNA since it encapsulates a range of information, including the transcriptome of cancer cells, mutational data, and epigenetic regulatory information [37]. Hence, further in-depth studies on the application of cfRNA

for early diagnosis, prognostic assessment and monitoring treatment response in LC appear warranted.

The miR-21 microRNA has been linked to various cancer types, including NSCLC [38], liver cancer [39], and pancreatic cancer [40]. Significantly higher miR-21 levels were observed in the plasma of NSCLC patients compared to controls ($p < 0.0001$) [38]. ROC analysis yielded an AUC of 0.729 for miR-21, with a sensitivity of 61.04% and specificity of 83.33%. These findings suggest that plasma miR-21 has the potential to serve as a non-invasive biomarker for the detection of NSCLC. Both surgery ($p = 0.0002$) and chemotherapy ($p = 0.0250$) led to significant decreases in the level of miR-21, although neither intervention returned the levels to normal. Thus, plasma miR-21 may be valuable for the early diagnosis of LC, as well as for prognosis. Other studies have also reported altered miR-21 levels in other tumor types, including liver and pancreatic cancer (described separately below). Li *et al.* [41] reported that plasma miR-486 ($p = 0.008$) and miR-150 ($p = 0.0488$) levels could distinguish LC patients from healthy individuals. The AUC for miR-486 was 0.926, with a sensitivity of 90.9% and specificity of 81.8%, while the AUC for miR-150 was 0.752, with a sensitivity and specificity of 81.8% each. Subsequent investigations found that miR-486 (plasma vs. tissue $p = 0.0027$) was diagnostically superior to miR-150 (plasma vs. tissue $p = 0.0876$). Moreover, the plasma level of miR-486 before and after surgery showed predictive value for the risk of NSCLC recurrence. Another study reported that the levels of several miRNAs were significantly altered in the serum of NSCLC patients [36]. Specifically, miR-15b-5p was substantially upregulated, whereas miR-16-5p, miR-17b-5p, miR-19-3p, miR-20a-5p, and miR-92-3p were significantly downregulated. The authors constructed a combined prediction model utilizing miR-15b-5p, miR-16-5p, and miR-20a-5p. This demonstrated high sensitivity and specificity in both the training set (AUC = 0.93, sensitivity = 86.17%, specificity = 91.38%) and in the validation set (sensitivity = 94.29%, specificity = 94.23%), suggesting that it could be a useful diagnostic approach for LC.

These findings also highlight the potentially important role of serum RNA in the diagnosis and treatment of cancer, particularly for early detection and prognostic evaluation. It has been reported that serum RNA is accurate and sensitive for the detection of LC metastases, even before these are visible on imaging scans [35]. Serum RNA offers several advantages over tissue biopsy, including non-invasiveness and better visualization of tumor heterogeneity, gene alterations, and clonal evolution, as well as providing a comprehensive view of tumor dynamics [42,43]. In addition, the evaluation of serum RNA, is very convenient for the monitoring of tumor drug resistance and targeted therapy [44]. Moreover, the postoperative management of cancer patients can be optimized by monitoring tumor burden and detecting minimal residual disease [45]. Further research is needed to validate the possible application of serum RNA in cancer,

but these biomarkers have recently become an active area of interest for researchers. They should also help in gaining a better understanding of the mechanisms of cancer development, as well as providing new ideas and approaches for cancer treatment and management.

2.2 Advances in the Study of TEP RNA and cfRNA in Hepatocellular Carcinoma

Primary liver cancer is a prevalent malignancy worldwide, ranking sixth in terms of incidence and second in terms of mortality [46]. It therefore presents a significant threat to human life and well-being. There are approximately 782,000 new cases of hepatocellular carcinoma (HCC) per year worldwide, accounting for 75%–85% of all primary liver cancers [47,48]. The primary techniques utilized for early, non-invasive detection of HCC include serum alpha-fetoprotein (AFP), ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI). Among these methods, serum AFP and US are the most commonly used diagnostic approaches [49]. Nevertheless, they have a high false-negative rate ranging from 10% to 30%. Hence, there is an urgent need to discover novel indicators with high sensitivity and specificity in order to increase the diagnostic accuracy for early-stage HCC and to facilitate early treatment and improve prognosis. Next, we will review the relevant advances of TEP RNA and cfRNA in HCC research in detail.

Numerous studies have reported that miRNA-122, which is specifically expressed in the liver, is a key regulator in liver development and liver diseases. MiRNA-122 is also upregulated in the peripheral circulation of HCC patients. MiRNA-21 is one of the most prevalent miRNAs in the bloodstream and is present at high levels in nearly all types of solid cancers [38,40]. In light of these findings, researchers have conducted quantitative real-time polymerase chain reaction (qRT-PCR) assays to assess the diagnostic value of TEP miRNA-122 and TEP miRNA-21 in HCC [39]. The levels of both were significantly increased in individuals with liver cancer compared to normal subjects ($p < 0.05$). With appropriate cut-off values, the diagnostic sensitivity of TEP miRNA-122 reached 100.0% with a specificity of 93.3%, while that of TEP miRNA-21 was 80.0% with a specificity of 93.3%. In another study, TEP miR-495-3p and TEP miR-136-5p showed decreased expression levels in HCC patients compared to controls, whereas TEP miR-1293 showed an increased level [50]. The AUC for miR-1293 was 0.78, and for miR-495-3p it was 0.76. These findings suggest that TEP RNA may serve as an auxiliary diagnostic marker for liver cancer, thus complementing the existing biomolecular library utilized for HCC diagnosis. CfRNA has also been investigated in regard to HCC. Okajima *et al.* [51] found the plasma miR-224 level in HCC patients was significantly different to that of normal controls ($p < 0.0001$). They did this by mining and analyzing the NCBI database, and then validating with qRT-PCR experiments. Their findings were consistent with re-

sults obtained in liver cancer tissues ($p = 0.0011$) and cell lines ($p = 0.0150$). Moreover, the plasma assay results were consistent with corresponding tissue assay results ($p = 0.0005$). Results from a large cohort trial showed that plasma miR-224 displayed high accuracy for the diagnosis of HCC (AUC 0.908) and was also feasible for prognostic assessment ($p = 0.0058$). These findings offer a new and reliable option for the clinical diagnosis of early-stage HCC. Lin *et al.* [52] conducted a parallel study to determine if serum miR-224 has the same diagnostic value as plasma miR-224 in patients with HCC. Their findings were remarkably consistent with those of Okajima *et al.* [51]. Serum miR-224 is therefore also considered to be a potential non-invasive biomarker for the early diagnosis and prognostic assessment of HCC. However, neither of these two research groups investigated whether there were differences in the miR-224 level between serum and plasma, nor did they determine which source exhibited superior diagnostic efficacy. Another study confirmed that the serum level of miR-224 in HCC patients was positively related to the BCLC (Barcelona Clinic Liver Cancer) stage ($p = 0.005$) and was reflective of tumor status and liver injury [53]. Moreover, a notable correlation was observed between serum miR-224 and serum AFP. This further highlights the significance of serum miR-224 as an independent factor in the diagnosis and prognostic assessment of HCC patients throughout the entire clinical diagnosis and treatment process. Ratnasari *et al.* [54] reported that plasma miRNA 29c-3p derived from patients had an inhibitory effect on tumorigenesis and disease progression. This miRNA can regulate various blood parameters, including the neutrophil-to-lymphocyte ratio (NLR), platelet count, cholinesterase (ChE), and albumin. MiRNA 21-5p and miRNA 155-5p were also found to have tumor-promoting effects and to significantly impact neutrophils in blood biochemical indexes. Therefore, the combined application of clinical indicators and RNA in peripheral blood could greatly improve the accuracy of early diagnosis and prognosis in HCC patients. Another study found that serum miR-375 showed a significant negative correlation with the progression of hepatitis B [55]. The level of serum miR-375 was notably lower in patients with cirrhosis and HBV-HCC (HCC associated with hepatitis B virus) compared to the healthy population ($p < 0.05$). *In vitro* experiments demonstrated that overexpression of miR-375 significantly inhibited the proliferation and migration of HCC cells. Furthermore, the serum level of miR-375 in patients with elevated AFP and CEA was significantly lower than in individuals with normal levels of these protein biomarkers ($p < 0.05$). MiR-375 had an AUC of 0.838, a sensitivity of 73.9%, and a specificity of 93.0% in the overall cohort. In the liver cancer cohort, miR-375 showed a higher AUC (0.768) and sensitivity (93.8%) compared to AFP (AUC: 0.584, sensitivity: 75.0%). These results suggest that serum miR-375 could be a useful biomarker for the detection of HBV-HCC with relatively high accuracy. A recent study found that serum miR-106b had significant dis-

crimutory value for different stages of HCC ($p < 0.001$), with an AUC of 0.885, sensitivity of 90.0%, specificity of 66.7% [49]. Importantly, the serum miR-106b level was not associated with hepatitis, obesity, or long-term alcohol consumption. Together, these findings demonstrate the great potential of TEP RNA and cfRNA in liver cancer diagnosis, while providing new directions for individualized management and treatment of this disease.

2.3 Advances in the Study of TEP RNA and cfRNA in Pancreatic Cancer

Although great progress has been made in understanding the molecular mechanisms of pancreatic cancer (PC) development, it remains one of the deadliest cancer types. In the absence of new diagnostic methods and/or treatments, PC is expected to become the second leading cause of cancer-related deaths by the end of 2023. Surgical resection is the most effective treatment for PC, but the majority of patients (80–85%) are unable to undergo surgery due to the presence of local invasion and/or distant metastases [56]. Hence, there is an urgent need to develop new biomarkers for the early diagnosis and prognostic assessment of PC in order to enhance the clinical management of these patients [57]. Wang *et al.* [40] selected four plasma miRNAs (miR-21, miR-210, miR-155 and miR-196a) that are overexpressed in PCs in order to assess their effectiveness as biomarkers. Inhibition of miR-21 was previously found to reduce the proliferation, invasion and migration of pancreatic intraepithelial neoplasia (PanIN) cells in a mouse model, and also to delay the progression of PanIN to PC [58]. Vila-Navarro *et al.* [59] recently identified a set of 14 miRNAs in plasma that were significantly elevated in the plasma of patients with PC or intraductal papillary mucinous neoplasm (IPMN) compared to healthy controls. These (miRNAs) (let7e-5p, let-7f-5p, miR-103a-3p, miR-151a-5p, miR-151b, miR-16-5p, miR-181a-5p, miR-192-5p, miR-21-5p, miR-221-3p, miR-23a-3p, miR-320a, miR-33a-3p and miR-93-5p) have been suggested as potential biomarkers for non-invasive diagnostic procedures in PC, with the combination of miR-33a-3p, miR-320a, and Carbohydrate Antigen 19-9 (CA19-9) showing the highest diagnostic accuracy (AUC = 0.948). Moreover, miR-181b-5p and miR-548d-3p were found to be significantly increased in the plasma of PC patients, but a similar upregulation was not observed in precancerous IPMN patients. These two miRNAs were therefore considered to be potential biomarkers for monitoring the malignant transformation of IPMN, although more prospective validation studies are required before their clinical application. Cao *et al.* [60] reported that a combination of three plasma miRNAs (miR-486-5p, miR-126-3p, and miR-106b-3p) had an AUC of 0.891 for the differentiation of PC from chronic pancreatitis (CP), thereby surpassing the diagnostic accuracy of CA19-9 (AUC = 0.775). Another study found that the diagnostic value of miR-486-5p alone for differentiating PC patients, CP patients and healthy controls was equivalent to that of

CA19-9 [61]. Mazza *et al.* [62] analyzed three miRNAs (miR-1225p, miR-1273g-3p and miR-6126) overexpressed in PC patients. They found that a combination of plasma miR-1273g-3p with CA19-9 levels could more accurately differentiate PC patients from healthy individuals (AUC = 0.940) compared to miR-1273g-3p alone (AUC = 0.703) and CA19-9 (AUC = 0.906). Plasma miR-181b, miR-196a and miR-210 are reported to have similar properties, and since their levels correlate significantly with lymph node metastasis ($p = 0.0010$, 0.0008 , and 0.0013 , respectively), clinical stage ($p = 0.0048$, 0.0319 , 0.0027) and vascular invasion ($p = 0.0002$, 0.0016 , 0.0019), they could be used as prognostic markers [63]. Other investigators have reported that miR-99a-5p, miR-200c-3p, and miR-365a-3p could effectively distinguish PC patients with poor prognosis after resection and those with longer survival times [64].

2.4 Advances in the Study of TEP RNA and cfRNA in Breast Cancer

Breast cancer (BC) is one of the most common and deadly cancers in women worldwide. The incidence of BC (30%) has surpassed that of LC (13%) as the most common malignancy in women worldwide [65]. BC is also the leading cause of cancer-related deaths in the female population, with metastasis and recurrence being the main causes of mortality [66]. The cure rate for >90% when the tumor remains localized in the breast. However, if the cancer cells spread to distant sites through metastasis, the 5-year survival rate is <30%. The primary treatment approach for advanced BC involves chemotherapy-based combination therapy. However, the effectiveness of first-line chemotherapy is only 30% to 50%, while the efficacy of second-line chemotherapy is even lower [67]. Early screening and diagnosis therefore play a crucial role in improving BC prognosis and subsequently the treatment outcomes.

On the basis of previous findings, researchers have validated changes in the serum miR-155 level of BC patients [68]. This was significantly increased in BC patients ($p < 0.001$) compared to healthy controls and had good accuracy for the diagnosis of BC (AUC = 0.801, sensitivity of 65.0%, specificity of 81.8%). Tumor resection or adjuvant chemotherapy resulted in significantly lower serum miRNA-155 levels (Comparison before and after surgery: $p = 0.0010$ and patients after chemotherapy versus healthy people: $p = 0.5042$). Moreover, the levels of commonly used tumor markers such as carbohydrate antigen 15-3 (CA15-3), CEA and tissue peptide-specific antigen (TPS) did not show this trend. Although the study had the usual limitations, it seems clear that serum miR-155 could be a reliable biomarker for the early diagnosis and prognosis of BC. An in-depth study was also carried out on whether circulating lncRNA H19 could be used as a biomarker for BC diagnosis and surveillance [69]. The results showed significantly increased levels of lncRNA H19 in both BC tissues and plasma ($p < 0.05$), with a high concordance between the two. Moreover, elevated levels of lncRNA H19 exhib-

ited better diagnostic accuracy for BC (AUC = 0.81, specificity = 86.7%) compared to the conventional tumor markers CEA (AUC = 0.52, specificity = 50.0%) and CA153 (AUC = 0.66, specificity = 60.0%). When combined, the three markers showed improved diagnostic accuracy, with an AUC of 0.84 and specificity of 96.7%. A significant decrease in plasma lncRNA H19 level was observed in postoperative samples ($p = 0.0006$), indicating its potential as a biomarker for early screening and prognostic monitoring of BC. The plasma lncRNA H19 levels also correlated with postoperative residual tumor load ($p < 0.05$) and was different in different BC subtypes (HER2+, ER+, and triple-negative). This biomarker could therefore be a potential predictor for the efficacy of neoadjuvant treatment [70]. Shimomura *et al.* [71] found that a combination of 5 serum miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p and miR-6875-5p) could detect BC with 89.7% accuracy, 97.3% sensitivity and 82.9% specificity. Moreover, platelet TPM3 mRNA entered BC cells via microvesicles and enhanced their ability to infiltrate and metastasize. This observation highlights the potential involvement of platelet microvesicles in BC metastasis and offers new insights for the development of therapeutic approaches that target this process [72]. Plasma miRNA levels may also be predictive for the clinicopathological characteristics, chemotherapy efficacy and prognosis of BC patients with recurrent metastatic cancer [67]. The above findings suggest several new blood-based tumor biomarkers for the diagnosis and treatment of BC. However, further extensive validation studies are required before these findings can be applied in the clinical setting.

2.5 Advances in the Study of TEP RNA and cfRNA in Other Tumors

Nasopharyngeal carcinoma (NPC) is a common malignant tumor of the nasopharynx, with an annual incidence of 15–50 cases per 100,000 individuals in the southern region of China and Southeast Asian countries [73]. Similar to other cancer types, there are numerous challenges in the early diagnosis of NPC. The detection of DNA and antibodies for Epstein Barr virus (EBV) in the peripheral blood are commonly used diagnostic methods, but have limitations in terms of their sensitivity and specificity. Hence, the identification of novel biomarkers is urgently needed to improve the early detection of NPC. Significantly higher expression levels of TEP miR-34c-3p and TEP miR-18a-5p were reported in patients with NPC ($p < 0.001$) [74]. These also showed high accuracy, sensitivity and specificity for NPC diagnosis (TEP miR-34c-3p: AUC = 0.952, sensitivity = 94.44%, specificity = 86.11% and accuracy = 91.11%; TEP miR-18a-5p: AUC = 0.884, sensitivity = 85.19%, specificity = 86.11%, accuracy = 85.55%). The diagnostic value was improved further with a combination of these two biomarkers (AUC = 0.954, sensitivity = 92.59%, specificity = 86.11%, and accuracy = 90.00%), thus offering hope for better diagnosis and treatment of NPC. In another

study, a significant decrease in the TEP lncRNA ROR level was observed in NPC patients compared with healthy individuals ($p = 0.0019$), whereas no significant difference was found in plasma lncRNA ROR ($p = 0.064$) [75]. Consequently, the diagnostic potential of TEP lncRNA ROR for NPC is better than that of plasma lncRNA ROR, aligning with the conclusion of Nilsson *et al.* [35] and highlighting the superiority of platelets over plasma in liquid biopsy. ROC analysis of TEP lncRNA ROR revealed an AUC of 0.70, a sensitivity of 60%, specificity of 70%, and an accuracy of 63.9%. When combined with EBV DNA, the positive rate increased from 58.3% (EBV DNA alone) to 74%. These findings indicate that TEP lncRNA ROR could be a valuable complement to EBV DNA and serve as a non-invasive biomarker for the diagnosis of NPC.

Ovarian cancer (OC) is the fifth leading cause of cancer death in women and faces the same challenges of early diagnosis and poor prognosis [76]. Serum miRNAs have been found to correlate with OC diagnosis and may provide a more accurate and reliable method of early diagnosis for the benefit of patients. Among them, miR-200c and miR-141 were effective at distinguishing OC patients from healthy controls ($p < 0.001$), while also correlating with different subtypes and stages of OC ($p < 0.001$). MiR200c (AUC, sensitivity and specificity of 0.79, 72% and 70%, respectively) was superior to miR-141 (AUC, sensitivity and specificity of 0.75, 69% and 72%, respectively) for the diagnosis of OC and thus has great potential as a diagnostic marker. Moreover, the miR-200c level was reported to be prognostic for the survival of OC patients [77].

The high incidence of colorectal cancer (CRC) in developing countries and its poor prognosis [78,79] have led to an increased focus on the identification of non-invasive biomarkers for this disease. lncRNAs are non-protein-coding RNA molecules of ≥ 200 nucleotides in length [80] that play important roles in various subcellular structure [81]. A recent study [82] found that circulating lncRNAs hold great promise for the diagnosis and prognosis of CRC. The levels of lncRNA ATB ($p < 0.001$) and lncRNA CCAT1 ($p = 0.024$) were significantly increased in the plasma of CRC patients compared to healthy controls while OCC-1 was not significantly different ($p = 0.24$) [83]. Further analysis showed that lncRNA ATB had greater discriminatory ability than lncRNA CCAT1 (AUC of 0.78 vs. 0.64). In addition, TEP mRNA TIMP1 expression level showed good performance for the differential diagnosis of CRC (AUC = 0.958) [84]. Xu *et al.* [85] confirmed the potential diagnostic value of TEP RNA profiles for distinguishing early CRC from non-cancerous disease. In addition, recent studies have shown [86] that platelets activate the C5a/C5aR1 axis through the PSGL-1/JNK/STAT1 signaling pathway in tumor-associated macrophages (TAMs), thereby promoting the development of colorectal cancer. The study provides new insights into the function of platelets in regulating the tumor microenvironment, and targeting intratumoral platelets may be a therapeutic strategy

for colorectal cancer patients. Together, these findings suggest a new approach for the early diagnosis and treatment of CRC.

Multiple myeloma (MM) is an incurable malignant hematologic cancer characterized by the clonal growth of plasma cells. The traditional diagnostic method for MM is bone marrow aspiration, but the invasiveness of this procedure and its inability to identify MM heterogeneity are major clinical shortcomings. Although the correlation between TEPs and MM has not been studied in detail, a low mean platelet volume was associated with poor prognosis in MM patients and could therefore be a potential marker of progression and prognosis [87,88]. The analysis of TEPs in liquid biopsies, may also provide a new method for the diagnosis of MM [89]. Another study recently found that liquid biopsies based on TEP RNA could help in the diagnosis of sarcoma [90].

3. Limitations and Future Directions of TEP RNA and cfRNA in Oncology Research

Although the study of blood-derived biomarkers has shown tremendous progress over the past decade, most of the RNAs identified so far as potential cancer biomarkers have failed to advance from the experimental stage to clinical trials. This is largely due to the limited reproducibility of RNA in blood as a biomarker the instability and low abundance of blood samples, and DNA contamination during specimen processing [91]. Therefore, further optimization of extraction processes and standardized parameter thresholds are needed before blood-related biomarkers can be used for clinical cancer diagnosis [92]. In response to the current limitations of liquid biopsies, researchers have developed ultra-high depth sequencing [93–95]. This can lower the detection limit to as little as 0.001%, thus significantly increasing the diagnostic sensitivity for various cancer types. Selecting the size of sequenced fragments and constructing libraries based on single strands are also effective methods for increasing assay sensitivity [96]. Recent work has shown that polymer structures can improve the operational performance of biosensors by increasing sensitivity, improved binding, and avoidance of non-specific interactions thereby leading to enhanced specificity. Furthermore, polymer-based materials can greatly increase signal amplification from low-concentration targets in the sample, thereby improving the sensitivity of detection [97]. Targeted balancing of the core assessment metrics in various early cancer screening products also helps to achieve flexible application of liquid biopsy technology [98].

4. Conclusions

The use of blood-based liquid biopsy as a novel, non-invasive biomarker detection method in recent years has led to revolutionary advances in clinical diagnostic and treatment technologies. By analyzing the presence of bioactive substances and RNA in plasma or serum, liquid biopsy can provide a wealth of diagnostic and testing information

for clinical purposes, including early cancer screening, tumor diagnosis and monitoring, and assessment of treatment response. In addition, liquid biopsy technology offers a wide range of applications for the prognostic assessment of diseases and clinical screening of drugs. Some studies have shown that platelet inhibitors, such as aspirin or the P2Y12 inhibitor tegretol, can slow tumor progression and the growth and metastasis of cancer cells by blocking their interaction with platelets, thus improving the progression-free survival of patients [99,100].

Despite still being an immature field, liquid biopsy is undeniably an important and growing area of investigation. To fully exploit the enormous potential of liquid biopsy for cancer prediction, diagnosis and real-time monitoring, there is an urgent need to address several issues. These include the improvement of blood sample processing and RNA extraction processes, the development of standardized parameter thresholds, and the implementation of clinical trials. This should allow liquid biopsy to become a reliable, accurate and widely used tool for early diagnosis, individualized treatment, and better prognostic assessment of cancer patients.

Abbreviations

ACIN1, Apoptotic Chromatin Condensation Inducer 1; AFP, alpha-fetoprotein; AUC, area under the curve; BC, Breast cancer; BCLC, Barcelona Clinic Liver Cancer; CA125, Cancer Antigen 125; CA19-9, Carbohydrate Antigen 19-9; CA15-3, carbohydrate antigen 15-3; CEA, Carcinoembryonic antigen; cfDNA/cfRNA, circulating cell-free DNA/RNA; ChE, cholinesterase; CP, chronic pancreatitis; CRC, colorectal cancer; CT, computed tomography; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; CYFRA21-1(cytokeratin 19 fragment), Cytokeratin Fragment 21-1; EBV, Epstein Barr virus; EML4-ALK, echinoderm microtubule-associated protein like 4-anaplastic lymphoma kinase; EMT, epithelial-mesenchymal transition; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IPMN, intraductal papillary mucinous neoplasm; MM, Multiple myeloma; MRI, magnetic resonance imaging; NLR, Neutrophil-to-Lymphocyte Ratio; NPC, Nasopharyngeal carcinoma; NSCLC, non-small cell lung cancer; NSE, neuron-specific enolase; OC, Ovarian cancer; OS, overall survival; PC, pancreatic cancer; PanIN, pancreatic intraepithelial neoplasia; PFS, progression-free survival; qRT-PCR, quantitative real-time polymerase chain reaction; ROC, receiver operating characteristic; TEPs, tumor-educated platelets; TME, tumor microenvironment; TPS, tissue peptide-specific antigen; US, ultrasound; WHO, World Health Organization.

Author Contributions

All authors listed in this article have made substantial contributions to the conception or design of the work. HH, HS, BH, JH and HZ were involved in drafting the work and reviewing it critically for important intellectual content,

BH was involved the acquisition, analysis, or interpretation of data for the work, and JH and HZ finally approved the version to be released; and agrees to be responsible for all aspects of the work to ensure that issues related to the accuracy or completeness of any part of the work are properly investigated and resolved. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2902080>.

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