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REVIEW

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Clinical application and detection techniques of liquid biopsy in gastric cancer

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Abstract

Gastric cancer (GC) is one of the most common tumors worldwide and the leading cause of tumor-related mortality. Endoscopy and serological tumor marker testing are currently the main methods of GC screening, and treatment relies on surgical resection or chemotherapy. However, traditional examination and treatment methods are more harmful to patients and less sensitive and accurate. A minimally invasive method to respond to GC early screening, prognosis monitoring, treatment efficacy, and drug resistance situations is urgently needed. As a result, liquid biopsy techniques have received much attention in the clinical application of GC. The non-invasive liquid biopsy technique requires fewer samples, is reproducible, and can guide individualized patient treatment by monitoring patients' molecular-level changes in real-time. In this review, we introduced the clinical applications of circulating tumor cells, circulating free DNA, circulating tumor DNA, non-coding RNAs, exosomes, and proteins, which are the primary markers in liquid biopsy technology in GC. We also discuss the current limitations and future trends of liquid biopsy technology as applied to early clinical biopsy technology.

Keywords Gastric cancer, Liquid biopsy, Circulating tumor cells, Circulating tumor DNA, Non-coding RNAs, Exosomes

Introduction

Gastric cancer (GC) is the fifth most common type of cancer and the third leading cause of death worldwide [1]. The morbidity and mortality rates of GC are increasing because most GC patients are already at an advanced stage of cancer when diagnosed [2]. Because GC has a poor prognosis, few treatment options, and is prone to metastasis, recurrence, and drug resistance [3], a reliable tool for early GC screening and predicting treatment efficacy is required. Endoscopy, *Helicobacter pylori* serology, and serum pepsinogen testing are the most common clinical methods for GC screening [4]. Surgical resection, chemotherapy, and targeted therapy are the primary treatment modalities for GC [5]. Endoscopic tissue biopsy, the gold standard for GC screening, is a relatively expensive and invasive procedure with varying degrees of patient harm; a single biopsy does not typically reflect the heterogeneity of GC patients, and the sensitivity and specificity of this method are low due to tissue resection site limitations [6–8]. Furthermore, carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 199, CA724, CA125, CA242, pepsinogen, and alpha-fetoprotein are extensively used clinical markers for early GC screening. However, their specificity and sensitivity are low and lack

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GC-specific [9]. Due to the limitations of these screening methods, there is an urgent need to develop a minimally invasive method for early detection and therapeutic decision-making in GC. Therefore, a new promising screening modality, liquid biopsy, has been investigated and validated for clinical use in GC patients.

Liquid biopsy is a non-invasive technique for detecting and analyzing circulating tumor cells (CTCs), circulating free DNA (cfDNA), circulating tumor DNA (ctDNA), non-coding RNAs (ncRNAs), exosomes, and proteins in biological fluid samples (such as blood, saliva, pleural fluid, ascites, stool, urine, and cerebrospinal fluid) [10, 11]. The possible sources of liquid biopsy are illustrated in Fig. 1. Liquid biopsies have many advantages over traditional tissue biopsies. Liquid biopsies, for example, require fewer samples and can be repeated [12]. Liquid biopsy technology can be used to investigate tumor load and genetic changes in patients throughout their disease by monitoring changes at the molecular level in real-time, and it can also be used to make decisions and adjustments to subsequent treatment options [13]. Furthermore, because of the minimally invasive nature of liquid biopsy technology, it has promising clinical applications for early diagnosis and screening of GC patients, prognostic monitoring, early recurrence detection, and longitudinal monitoring of disease progression and treatment response during adjuvant and neoadjuvant therapy [14, 15]. Studies show that liquid biopsy techniques can identify GC patients in novel ways.

This review focuses on the clinical applications of liquid biopsy technology's primary markers for early diagnosis, prognosis prediction, recurrence, metastasis monitoring, chemotherapy sensitivity, and drug resistance in GC. Furthermore, we briefly describe the main liquid biopsy techniques for detecting different biomarkers in GC and summarize their clinical utility for GC patients. Finally, we discuss the limitations of liquid biopsy techniques in GC biology and speculate on their future development, which opens new avenues for GC clinical applications.

Clinical application of liquid biopsy biomarkers in GC

CTCs

In 1869, Thomas Ashworth discovered CTCs, a type of tumor cell shed into the bloodstream from primary or

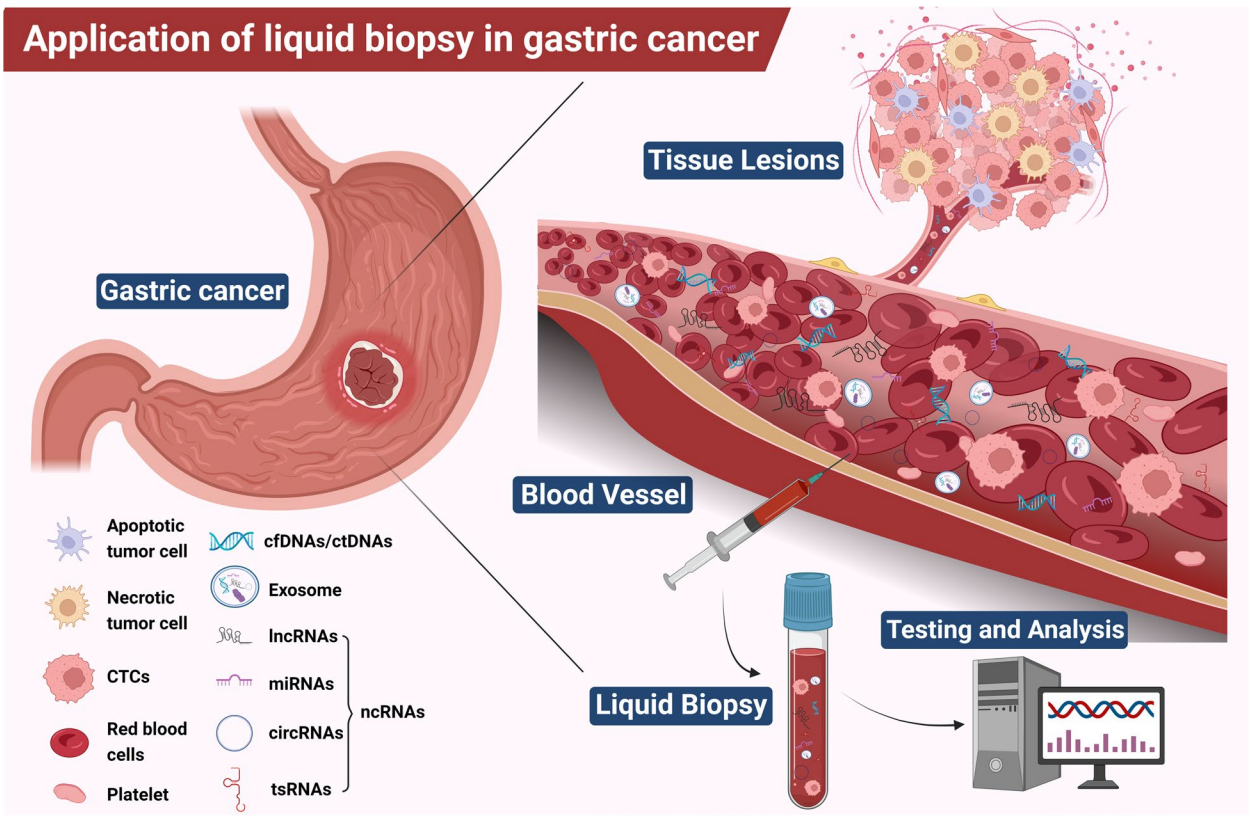

Fig. 1 Clinical application of liquid biopsy in gastric cancer (GC). Circulating tumor cells (CTCs), circulating free DNA (cfDNA), circulating tumor DNA (ctDNA), non-coding RNAs (ncRNAs), exosomes, and proteins in the blood of GC patients can be used as potential biomarkers for liquid biopsies and their expression levels can be measured to reflect the clinical status of GC. Created with BioRender.com

metastatic tumor sites [16]. CTCs are circulating nucleated cells with a diameter larger than 4 μM that can express epithelial cell adhesion molecules (EpCAM), cytokeratins (CKs) 8, 18, and/or 19, and do not use CD45 as a rejection marker for leukocytes, which are the primary basis for CTC detection [17]. CTCs are highly heterogeneous, allowing them to easily evade immune surveillance and treatment, eventually resulting in distal metastasis or tumor cell recurrence [18–20]. Furthermore, due to CTCs' short half-life (about 1–2.4 h) [21], the level of CTCs in the blood is low [22]. Despite the numerous challenges in detecting CTCs in GC, new techniques can still detect the type and number of CTCs and thus determine tumor progression [23, 24]. Liquid biopsy of CTCs has been used for decades to aid in early diagnosis, prognostic stratification, dynamic assessment, and guide treatment decisions for patients with GC [25, 26]. The clinical application of CTCs as liquid biopsy markers in GC is listed in Table 1.

Clinical application of CTCs in GC liquid biopsy

Because 80% of GC patients are asymptomatic in the early stages, early screening of patients is one of the most important challenges in GC [39]. In recent years, CTCs' role in diagnosing GC has received increased attention. Kang et al. [27] detected CTCs in 90.5% (105/116) of GC patients and identified GC patients with a CTC ≥ 2/7.5 mL of blood threshold. Their sensitivity and specificity in distinguishing GC patients from healthy controls are 85.3% and 90.3%, respectively. Their

Table 1 Clinical application of CTCs in GC

Study type	Threshold	Sample size	Sensitivity (%)	Specificity (%)	AUC	Clinical significance	References
Retrospective study	2 CTCs	116 patients with GC	85.3	90.3	0.928	Distinguish between GC patients and healthy controls and provide clinical thresholds	[27]
	Number of CTCs	20 studies	42	99	0.97	Differentiate between GC patients and healthy controls	[28]
	Single CTCs	24 patients with metastatic GC receiving chemotherapy	96	[a]	[a]	Detection of metastasis and drug resistance in GC	[29]
	CTCs/DTCs	26 studies	[a]	[a]	[a]	As the basis for GC staging	[30]
	CSV + PD-L1 + CTCs	70 patients with GC	71	[a]	[a]	Predicts treatment response and prognosis in GC patients	[31]
	CTC-PD-L1	32 patients with progressive GC	[a]	[a]	[a]	Monitor prognosis and guide future individualized immunotherapy	[32]
Prospective study	CTCs and TWIST	32 patients with metastatic GC	80.6	[a]	[a]	As a prognostic marker	[33]
	4 CTCs	52 patients with progressive GC	[a]	[a]	[a]	As a surrogate marker for the efficacy of treatment with S-1 or paclitaxel in AGC patients	[34]
	5 CTCs	65 treatment[a]negative gastric adenocarcinomas	[a]	[a]	[a]	Monitoring the prognosis and recurrence of GC	[35]
	2 CTCs	44 patients with gastrointestinal tumors	69.9	[a]	[a]	Determining the prognosis of metastatic GC	[36]
	FR + CTCs	132 patients with GC	77.8	54.5	0.68	Preoperative testing of FR + CTC levels helps predict PM and early recurrence in GC patients	[37]
	CTCs/cfDNA	45 patients with progressive GC	95.6	[a]	[a]	Predicting the efficacy and prognosis of neoadjuvant chemotherapy for progressive GC	[38]

[a] indicates that this data was not presented in the study

conclusion points out that although CTCs were not associated with the T or N stages, the detection rate of CTCs in patients with T1 and N0 stages GC was more than 80%. Similarly, Tang et al. [28] found that the sensitivity of using CTCs to detect patients with advanced GC was higher than that of detecting patients with early GC, but the specificity was almost the same. Because of the low sensitivity of the detection, they suggested that CTCs could not be used for separate screening of GC, which also suggested that CTCs should be combined with markers with higher sensitivity for better results.

In GC, CTCs have been linked to metastasis, prognosis, recurrence, and chemotherapy [40]. Hiraiwa et al., the first to investigate the clinical significance of CTCs in GC patients using the CellSearch system, found that the detection rate of two CTCs was 69.9%, two or more CTCs were significantly related to advanced tumor stage in GC patients, and the patients with distant organ metastases from GC have significantly higher numbers of CTCs than healthy controls and non-metastatic patients [36]. Similarly, Jhi et al. [33] and Negishi et al. [29] found CTCs in 80.6% (25/31) and 96% (26/27) of patients with metastatic GC, respectively, and the number of CTCs in the blood correlates with overall survival (OS). The above findings are also consistent with Ito et al.'s [35] finding that patients with CTCs > 5/7.5 mL of blood have a lower OS. In this study, the authors also found that the number of CTCs in stage III GC patients was higher than that in stage I GC patients and that the overall survival rate of patients with more than 5 CTCs was lower. Dan Zeng et al. [37] used ligand-targeted polymerase chain reaction to detect the levels of folate receptor-positive CTCs in blood samples from GC patients and found that preoperative CTCs levels have a diagnostic value in predicting peritoneal metastases in GC. Furthermore, Huang et al. [30] found CTCs in 10.8% of the resected group with a high recurrence rate and 60.2% of the unresectable group, but not in the healthy control group. After meta-analysis, they discovered that the incidence of CTCs in stage I/II GC was lower than in stage III/IV GC. In addition, Matsusaka et al. [34] and Yu et al. [38] identified CTCs in patients with progressive GC treated with neoadjuvant chemotherapy and surgery, and the OS and progression-free survival (PFS) were significantly shorter in patients treated with GC chemotherapy with a high number of CTCs. Therefore, all the preceding studies suggest that evaluating CTCs may be useful for predicting tumor progression and prognosis in GC patients. Monitoring the dynamics of CTCs in response to therapy may be a useful alternative method for assessing treatment resistance in GC patients.

Immune checkpoint blockers have rapidly gained popularity in the clinic as a novel antitumor treatment strategy in recent years [13]. Among these, programmed cell death protein 1 (PD-L1) has been extensively studied in tumor progression and metastasis and has received considerable attention [41, 42]. Meanwhile, the analysis of PD-L1 expression levels in CTCs is becoming more popular in oncology (including GC) [43, 44]. For example, Liu et al. [31] found that cell-surface vimentin (CSV) + PD-L1 + CTCs in patients with GC are associated with advanced disease and adverse effects. Cells with PD-L1 overexpression in the CSV + CTC cell population have a worse prognosis. Similarly, Cheng et al. [32] used CanPatrol CTCs enrichment technology on blood samples from 32 GC patients and found that the number and type of CTCs and CTCs-PD-L1 correlate with the clinical outcome of checkpoint blockade therapy. This evidence supports CTCs-PD-L1 expression as a prognostic factor for the efficacy of immune checkpoint blockade therapy.

Detection methods of CTCs

Since CTCs are difficult to detect in blood [21, 22, 45], establishing standardized detection methods for CTCs and investigating innovative techniques would increase the sensitivity and accuracy of diagnosing early malignant tumors (Fig. 2A) [46]. CTCs detection consists of three steps: enrichment, detection, and analysis. CTCs enrichment techniques include physical and biological enrichment. Physical enrichment does not require immunological labeling of CTCs and solely depends on their physical properties (including size, density, charge, and other biological properties) [47–49]. Bioenrichment relies on immunological antibodies, allowing for the specific capture of CTCs. This approach includes positive selection with antibodies against tumor-associated antigens such as EpCAM, CKs, mucin-1, human epidermal growth factor receptor 2, or epithelial growth factor receptor [50–52] and negative selection with antibodies against the common leukocyte antigen CD45 [53]. The CellSearch system (Veridex) is the most widely used antibody-based isolation technique and the only one approved by the Food and Drug Administration (FDA) for the detection of CTCs in the blood of some tumor patients [54–56], which has a greater benefit as a diagnostic and prognostic indicator for patients with metastatic disease [34, 37]. However, imposing EpCAM bias on the enriched CTCs population is an obvious drawback of immunocapture methods, including CellSearch [57]. Therefore, numerous new methods have been developed, such as AdnaTest, isolation by size of epithelial tumor cells, density gradient, microfiltration, microflow, and size-determining immunocapture microarrays in recent years [58, 59]. Furthermore, recent technological advances have enabled the isolation and analysis of single intact CTCs [60, 61].

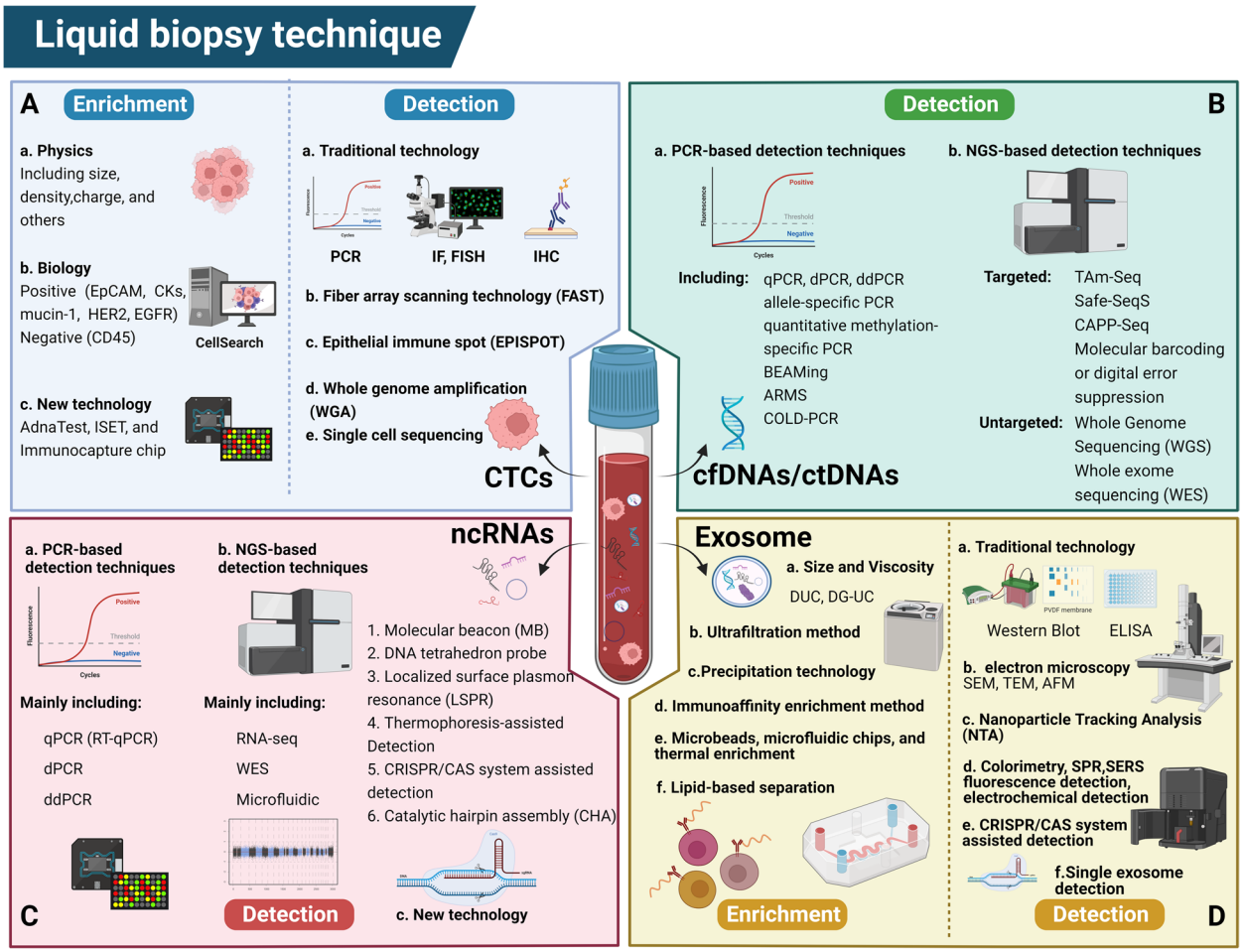

Fig. 2 Techniques for detection of liquid biopsy biomarkers in gastric cancer (GC). Detection of CTCs (A), cfDNA/ctDNA (B), ncRNAs (C), and exosomes (D) in body fluids using different techniques can help in early diagnosis, prognosis prediction, treatment, and recurrence monitoring, and targeted therapy of GC. Created with BioRender.com

CTCs detection and analysis techniques include traditional polymerase chain reaction (PCR) and cellular protein detection methods such as immunofluorescence, immunohistochemistry, and fluorescence-assisted in situ hybridization. The latest methods, such as high-throughput fiber-optic array scanning technology and epithelial immunospotting, can screen CTCs and detect proteins secreted by CTCs [62–65]. The most widely used technique remains PCR, particularly quantitative real-time PCR, which can reduce false-positive results in the data by determining the "cut-off" value. We can count CTCs in the blood by detecting traditional markers of CTCs such as CKs, CEA, TWIST [33], KRAS [66], and PRRX1 [67] and non-coding RNAs, including microRNAs (miRNAs) and Piwi-interacting RNAs [68–70]. With the advancement of technology, sequencing and histological techniques, such as whole genome amplification, single-cell sequencing methods, and proteomics methods, are increasingly used to detect CTCs [71]. Nagrath et al. [72] developed the "CTCs chip," a microfluidic-based device for detecting CTCs with a significantly higher yield and purity. Using this innovative technology, CTCs can be captured, stained, and scanned directly from small volumes of blood. Several studies have demonstrated the efficacy of this method [73–77]. Significant advances in the technology for detecting CTCs in various tumors have opened more opportunities for diagnosing and treating GC.

cfDNA / ctDNA

Mandel et al. [78] discovered DNA fragments in blood in 1948 and coined the term cfDNA. Thierry et al. [79, 80] used cfDNA to discover specific mutations and genetic changes in tumors in 1994. Since then, cfDNAs have received increasing attention. ctDNA is a subset of cfDNA derived from tumor cells [81, 82]. ctDNA

is a small gene fragment derived from primary tumors, metastatic tumors, or even CTCs released into the bloodstream [83]. Most cfDNA fragments are 160–200 base pairs long, whereas ctDNA fragments are much shorter; both are double-stranded fragments [84, 85] with a short half-life (approximately 2 h) [86–88]. According to subsequent research, tumor load, tumor status, DNA elimination, degradation mechanisms, the inflammatory response, or tissue damage can affect the amount and nature of ctDNA [89, 90], allowing it to reflect tumorigenesis and progression in vivo in real time. Furthermore, ctDNA carries tumor-specific genetic/epigenetic variants (including point mutations, structural variants, copy number variants, microsatellite alterations, and methylation) that vary greatly between individuals [91], making ctDNA collection from blood non-invasive compared to traditional tumor biopsy and facilitating the identification and screening of GC. The clinical application of cfDNA/ctDNA as liquid biopsy markers in GC is listed in Table 2.

Clinical application of cfDNA/ctDNA in GC liquid biopsy

Increasing evidence suggests that cfDNA/ctDNA is a new potential biomarker that can guide diagnosis, predict prognosis, monitor recurrence, and treat GC [107, 108]. In evaluating the potential therapeutic utility of plasma ctDNA levels as a diagnostic tool for early GC, Kim et al. [92] found that the area under the curve (AUC) value of cfDNA diagnostic GC was 0.991, and its sensitivity and specificity reached 96.67% and 94.11%. They also found that the level of cfDNA expression increased proportionally among healthy participants, patients with early GC, and patients with late GC, and that was associated with tumor node metastasis (TNM) staging. Furthermore, cfDNA levels are significantly reduced 24 h after surgery compared to pre-GC levels. Qian et al. [93] found that the level of serum cfDNA expression in patients with stage I GC was about 6 times that of the healthy control participants, and the level of cfDNA expression in patients with stage III to IV GC was significantly higher than that in patients with stage I GC. The AUC for diagnosing GC with cfDNA was 0.94, with sensitivity and specificity of 78.96% and 91.81%, respectively. Similarly, Park et al. [94] found that the average concentration of plasma cfDNA in patients with GC was 2.4 times that of the normal control participants, and the sensitivity and specificity of using cfDNA to distinguish between patients with GC and normal people reached 75% and 63%, respectively. Fang et al. [95] and Yang et al. [98] found that ctDNA positivity was related to the disease stage, that ctDNA could hardly be detected in early GC patients before surgery, that patients with high ctDNA levels have a significantly lower OS and a higher risk of peritoneal recurrence, and that ctDNA mutations are associated with a poor prognosis in patients with advanced GC. Similarly, Pu et al. [96] demonstrated that ctDNA could distinguish patients with advanced GC from patients with early GC and healthy controls, with AUC values of 0.565 and 0.744, respectively. Interestingly, they also found that ctDNA concentrations are high up to 21 days postoperatively but reduce after 3 months. In a separate investigation, Normando et al. [97] found significantly prolonged disease-free survival in patients with low ctDNA levels in GC after one cycle of chemotherapy. According to Lan et al. [99] cfDNA levels are more sensitive than CEA levels for predicting recurrence in surgically followed patients. Zhong et al. [100] found that plasma cfDNA concentrations tend to increase with GC progression by analyzing changes in plasma cfDNA concentrations during the chemotherapy treatment of patients with advanced GC. There is no significant difference in the trend of plasma cfDNA concentrations over time in patients with stable disease. The findings of the above studies indicate that cfDNA levels can be used to monitor the progression of GC.

As an essential component of liquid biopsy, cfDNA/ctDNA is also used in immuno-oncology. Numerous studies have demonstrated the efficacy of PD-1 targeted therapy for some patients with metastatic GC (MGC). To investigate the potential role of cfDNA/ctDNA in GC-targeted therapy, Kim et al. [101] conducted a prospective analysis of 61 MGC patients treated with pembrolizumab and observed ctDNA levels drop after 6 weeks of patient treatment. Moreover, microsatellite instability (MSI) predicted the ICB therapeutic benefit of PD-1/PD-L1 inhibitors [109]. Willis et al. [102] detected MSI in GC using a targeted next-generation sequencing (NGS) approach. They reported that using cfDNA, they could accurately detect 87% of tissue MSI positivity and 99.5% of tissue microsatellite stability, with an overall accuracy of 98.4%. In addition, immunotherapy is clinically active in cfDNA-MSI-positive patients with advanced GC, with more than half achieving complete or partial remission and sustaining therapeutic benefits. However, research on immune-targeted therapy for cfDNA/ctDNA and GC remains in its preliminary stages.

Aberrant DNA methylation is an epigenetic alteration occurring in organ disease specificity. Methylation of promoter regions has been widely used to identify cfDNA/ctDNA in GC plasma and serum, and frequent promoter hypermethylation and subsequent loss of protein expression are associated with GC [15]. Thus, detecting the cfDNA/ctDNA methylation signature has become an early diagnostic, prognostic prediction, and screening marker for GC. Ren et al. [103] developed a methylation CpG tandem amplification and sequencing (MCTA-Seq) and identified 153 cfDNA methylation

Table 2 Clinical application of cfDNA/ctDNA in GC

Object	Sample Type	Sample size	Sensitivity (%)	Specificity (%)	AUC	Clinical significance	References
cfDNA	Plasma	30 patients with GC	96.67	94.11	0.991	For early detection of cancer and assessment of tumor load	[92]
cfDNA	Serum	124 patients with GC	78.96	91.81	0.94	For early screening of GC	[93]
cfDNA	Serum/ Plasma	130 patients with GC	75	63	0.784	Plasma cfDNA levels are lower than serum cfDNA levels, and plasma cfDNA levels may help predict GC patients	[94]
cpDNA	Plasma	277 patients with progressive GC	34	[a]	[a]	Associated with poor prognosis in GC	[95]
ccf[a]DNA	Plasma	73 patients with GC	[a]	[a]	0.744	Distinguish between patients with stage III and IV GC and healthy controls, and monitor postoperative	[96]
cfDNA	Plasma	30 patients with locally progressive, unresectable, or metastatic GC	[a]	[a]	[a]	Positive correlation between tDNA levels and DFS in patients with progressive GC receiving systemic chemotherapy 3 months after the start of chemotherapy	[97]
ctDNA	Plasma	46 patients with stage I-III GC	39	100	[a]	MRD with ctDNA testing identifies patients at high risk of recurrence	[98]
cfDNA	Serum	428 patients with GC	68.9	95.8	0.98	Predicts response to chemotherapy and surgery in patients with colorectal cancer; tumor recurrence should be considered in GC with persistently elevated cfDNA levels after surgery	[99]
cfDNA	Plasma	106 patients with progressive GC treated with chemotherapy	93.7	45.2	0.8099	Tumor biomarkers as monitoring the efficacy of chemotherapy for GC	[100]
ctDNA	Plasma	61 cases of partially metastatic GC	[a]	[a]	[a]	Associated with improved prognosis	[101]
cfDNA	Plasma	1145 patients with GC	87	[a]	0.984	Potential to expand access to targeted therapies and immunotherapy to all patients with advanced cancer	[102]
Methylation of cfDNA	Plasma	89 patients with GC	40–60	92	0.89	Effective differentiation between early GC, colorectal cancer, and liver cancer	[103]
Methylation of cfDNA	Plasma	99 patients with GC	[a]	[a]	0.81	High postoperative long fragment LINE-1 concentrations suggest high risk of MRD and recurrence	[104]
Methylation of RASSF1A, SOX17 and WiF-1	Plasma	70 patients with progressive GC	[a]	[a]	[a]	Associated with worse progression-free survival and overall survival	[105]
Methylation of cfDNA	Plasma	1781 Gastrointestinal tumors	[a]	[a]	0.9	Improved diagnostic accuracy of gastrointestinal cancers	[106]

[a] indicates that this data was not presented in the study

biomarkers, including DOCK10, CABIN1, and KCNQ5, and their work showed that MCTA-Seq could distinguish early GC using a highly specific algorithm. MCTA-Seq detected early-stage GC at a sensitivity of 40%–60% with a specificity of 92%. While Kandimalla et al. [106] developed EpiPanGI Dx, a sensitive and targeted methylation-based assay for cfDNA that increases the prediction accuracy of large log GC to 85–95%. In a separate study, Ko et al. [104] found that LINE-1 methylation in cfDNA may serve as a novel biomarker for screening GC, with an AUC of 0.81 for its diagnostic GC. The findings also indicated that patients with lower pre-treatment LINE-1 methylation levels have significantly lower OS, and patients with low preoperative LINE-1 methylation have worse recurrence-free survival and OS. Karamitrousis et al. [105] used methylation-specific PCR to detect the methylation status of oncogenes *RASSF1A*, *SOX17*, and *WiF-1* in the cfDNA of 70 patients with progressive GC and found that promoter methylation of the examined genes is significantly associated with decreased PFS and OS compared with patients without methylation and that simultaneous methylation of the above genes results in worse PFS and OS in GC patients. Given the above findings, changes in cfDNA/ctDNA concentrations may be a reliable biomarker for detecting early GC.

Detection methods of cfDNA/ctDNA

Like CTCs, cfDNA/ctDNA levels are low in vivo and have a short half-life, necessitating sensitive and specific detection methods [110], which can be divided primarily into PCR-based and NGS-based methods (Fig. 2B). PCR remains the most widely used method among these. PCR-based techniques include standard quantitative PCR (qPCR), digital PCR (dPCR) and droplet digital PCR (ddPCR), allele-specific PCR, quantitative methylation-specific PCR, BEAMing (beads, emulsions, amplification, and magnetism) [111–113], ARMS [114], and co-amplification at lower denaturation temperature-PCR [115]. The sensitivity of PCR-based assays has increased dramatically over the past decade. On the other hand, dPCR and ddPCR techniques allow absolute quantification of target molecules, gradually allowing this technique to replace traditional detection methods. Although PCR-based techniques are typically more sensitive and less expensive, numerous experiments have shown that the number of mutations detected is limited, the detection area is somewhat restricted, and the sample size increases as the assay is used more frequently. This has increased the use of NGS technology for cfDNA/ctDNA detection, which can detect multiple mutations and new mutations.

NGS-based assays are categorized as targeted and untargeted, with targeted NGS technologies such as tagged-amplicon deep sequencing (TAm-Seq) [116], Safe-Sequencing System (Safe-SeqS) [117], and personalized profiling by deep sequencing (CAPP-Seq) [118], detecting multiple rare mutations in the genome and ctDNA simultaneously without tumor sequencing. Molecular barcoding or digital error suppression are additional techniques to differentiate between actual low-frequency mutations and artifactual mutations that appear during PCR amplification [119, 120]. Although targeted ctDNA analysis can identify tumor mutations in some patients, it does not rule out the possibility of patients developing novel, unknown mutations. Non-targeted NGS technologies, such as whole genome sequencing (WGS) and whole exome sequencing, can be used to detect all tumor mutations in patients and can also be used for whole genome copy number analysis and large structural variant detection, despite their low sensitivity and prohibitive cost [121].

Due to their respective advantages and disadvantages, there is currently no uniform standard for cfDNA/ctDNA detection assays. Combining the two approaches can increase the number and detection limits of cfDNA/ctDNA using WGS to map tumor-specific chromosomes in tumor tissues, followed by quantitative analysis of cfDNA/ctDNA in plasma using PCR- or NGS-based quantitative methods [122, 123]. However, cfDNA/ctDNA assays have limitations, and there is a need for sensitive, high-target volume, and low-cost assays.

ncRNAs

In addition to the NGS-based genome sequencing described above, various transcriptome sequencing technologies have emerged, resulting in many coding RNAs/messenger RNAs (mRNAs) and ncRNAs. Although ncRNAs cannot be translated, they can act as "regulators" of many genes or proteins. Among the major ncRNAs, miRNAs, tRNA-derived small RNAs (tsRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) have received the most attention [124]. miRNAs are small non-coding RNAs (sncRNAs) of about 22 nucleotides in length that can affect tumor biological progression by regulating tumor proto-oncogenes or oncogenes at the post-transcriptional level [125, 126]. Similarly, shorter-length tsRNAs are derived fragments generated after the cleavage of pre-tRNAs or mature tRNAs in specific environments [127, 128], which can be classified into tRNA-derived fragments and tRNA halves depending on the location of cleavage [129].

Furthermore, tsRNAs have more modifications than other sncRNAs, making them more stable in the blood. The mechanism of action of tsRNAs is similar to that of miRNAs [130, 131], and they play a role in tumor progression by regulating gene expression, translation, and epigenetics [132]. Unlike the sncRNAs mentioned

above, lncRNAs are ncRNAs of more than 200 nucleotides in length, most of which are by-products of RNA polymerase II transcription and can play a key role in tumor progression through various mechanisms such as regulating target gene expression, recruiting chromatin modifications, interfering with mRNA splicing, translation, and degradation, and acting as miRNA sponges [124, 133–135]. circRNAs are covalently closed single-stranded cyclic molecules whose structure resists degradation by most ribonucleases R, making their expression more stable [136]. circRNAs can influence tumor biology by acting as "miRNAs sponges" or translation templates for certain peptides and proteins and binding to specific RNA-binding proteins [137–139]. Because of these ncRNAs' unique structure and properties, their expression in blood is relatively stable, allowing them to serve as important biomarkers and therapeutic targets for many tumor liquid biopsies, including GC [107, 132, 140, 141]. The clinical application of ncRNAs as liquid biopsy markers in GC is listed in Table 3.

Clinical application of ncRNAs in GC liquid biopsy

Tumor cells can deliver ncRNAs into body fluids via special mechanisms known as circulating ncRNAs. They are widely present in body fluids such as plasma, serum, and tumor patients' exocytosis. Moreover, increasing studies have shown that circulating ncRNAs in the blood of tumor patients can be detected in large quantities and used as biomarkers for GC [169, 170]. Our previous studies have shown that circPTPN22, which is up-regulated in GC plasma, can effectively differentiate GC patients from healthy controls; its AUC value can reach 0.857. In addition, the level of expression of circPTPN22 was higher in patients with advanced GC (stages III-IV) than in patients with early GC (stages I-II). The expression level of circPTPN22 in n patients with GC decreased significantly following surgery, and its high expression predicted the existence of GC. The high expression of circPTPN22 indicated poor survival for patients with GC. This suggests that circPTPN22 can be used as an early diagnostic and prognostic marker for GC [146]. Meanwhile, another study also found that hsa_tsr016141, which was up-regulated in GC serum, could also effectively distinguish GC patients from healthy controls, its AUC value can reach 0.814 [148]. Recently, Roy et al. [147] developed a risk diagnosis prediction model based on 8 CircRNAs that are up-regulated in both tissues and plasma of GC patients. This model can accurately and effectively distinguish GC patients from non-disease control groups, and the AUC values of GC diagnosed by this model in the training cohort and testing cohort are 0.87 and 0.83, respectively. It is worth noting that this model can effectively identify early (stages I-II) GC patients in the training cohort and the validation cohort, and their AUC values can reach 0.87 and 0.82 respectively. In a recent meta-analysis, Xu et al. [142] found that miR-21, miR-106, miR-421, and miR-223 have better diagnostic efficacy for GC, with miR-421 particularly useful as an auxiliary diagnostic indicator for GC; the AUC of its diagnostic GC can reach 0.92. More studies found that miR-548d-3p [153], miR-4742-5p [154], and miR-23b [145], which are significantly up-regulated in GC, and miR-137-3p [152], miR-148a [144], and miR-875-5p [143], which are significantly down-regulated in GC, are effective in distinguishing GC patients from healthy control individuals. In terms of metastasis, miR-17-5p and miR-4742-5p are up-regulated in GC. Meanwhile, the knockdown of either miR-17-5p or miR-4742-5p significantly inhibits GC cell proliferation, invasion, and metastasis [154, 155]. Furthermore, down-regulated PTCSC3 expression in GC patients correlates with HULC, which is up-regulated in GC patients. PTCSC3 inhibits GC cell invasion and migration, whereas HULC promotes it. Both act on cell invasion and migration via the Wnt/β-catenin signaling pathway [156]. Another study found that knocking down the expression of up-regulated CircPVT1, or circ 0,006,089, in GC cells reduces GC cell growth, invasion, and migration [157, 158]. Zheng et al. [159] found that the tiRNA-Val-CAC-001, which is down-regulated in expression in GC tissues and cells, may affect GC cell metastasis by targeting LRP6 and regulating the Wnt/β-catenin signaling pathway. All the above studies suggest that ncRNAs play a crucial role in the early diagnosis, prognosis prediction, and metastasis of GC.

In addition to the early diagnosis and prognostic significance of GC, ncRNAs have been linked to susceptibility and resistance to anti-treatment in GC. Azimi et al. [162] and Kim et al. [163] used data mining and high-throughput miRNA microarray analysis to identify numerous miRNAs (miR100, miR-34a, miR-23a, miR-30a, let-7 g, miR-342, miR-16, miR-181, miR-1, and miR-34) associated with chemotherapy sensitivity in GC. Zhang et al. found that CRNDE, which is lowly expressed in human GC, may play an important role in autophagy-mediated chemoresistance by binding to SRSF6. When GC patients develop chemoresistance, CRNDE expression could be restored to improve the effect of chemotherapy, implying that CRNDE may be a new biomarker for GC prognosis and treatment [165]. In addition, ncRNAs have gained interest as GC-targeted drugs for treating GC. Cisplatin (CDDP) chemotherapy significantly reduces the level of miR-30a in GC cells, and interestingly, up-regulation of miR-30a inhibits GC cell sensitivity to CDDP [164]. Furthermore, CDDP-resistant GC cells that up-regulated SNHG6 and circ_AKT3 expression could regulate CDDP

Table 3 Clinical application of ncRNAs and exosomes in GC

Projects	Category	Biomarker	Sample Type	Expression	Clinical significance	References
Diagnosis, prognosis, and treatment	miRNAs	miR-21、miR-106、miR-421 and miR-223	Serum, plasma, gastric juice, and blood	All were significantly different	Helps in early diagnosis and mass screening of GC	[142]
		miR-875-5p	Tissue and cells	downregulated	May serve as a potential diagnostic and therapeutic target for GC	[143]
		miR-148a	Plasma	downregulated	Associated with tumor progression and poor prognosis. Restoration of blood miR-148A levels may be a new nucleic acid anti-cancer therapy	[144]
		miR-23b	Plasma	up regulated	Associated with poor clinical prognosis	[145]
	circRNAs	circPTPN22	Plasma	up regulated	Can effectively identify GC patients and healthy controls	[146]
		hsa_circ_0045602、hsa_circ_0008768、hsa_circ_0007380、hsa_circ_0002019、hsa_circ_0006089、hsa_circ_0034398、hsa_circ_0052001 and hsa_circ_0001013	Plasma	up regulated	Powerful identification of early GC patients	[147]
	tsRNAs	hsa_tsr016141	Tissue and serum	up regulated	Clearly distinguish GC patients from healthy controls or patients with gastritis	[148]
	Exosome	Exosome miR-4741、miR-32、miR-3149 and miR-6727	Tissue and plasma	Expression of exosome miR-4741 was upregulated, and expression of exosome miR-32, miR-3149 and miR-6727 was downregulated	Act as a diagnostic marker for GC and an influential factor in inhibiting GC progression	[149]
		Exosome LncRNAH19GC	Serum	downregulated	A potential biomarker with diagnostic and prognostic value	[150]
		Exosome hsa_circ_0015286	Tissue, plasma, and cells	up regulated	May be a non-invasive biomarker for GC diagnosis and prognostic assessment	[151]

Table 3 (continued)

Projects	Category	Biomarker	Sample Type	Expression	Clinical significance	References
Transfers	miRNAs	miR-137-3p	Cells	downregulated	Tumor-suppressing effect, may promote GC progression by affecting immune infiltration	[152]
		miR-548d-3p	Cells	up regulated	Accelerated proliferation and migration of GC cells	[153]
		miR-4742-5p	Cells	up regulated	Effective treatment to inhibit cancer metastasis	[154]
		miR-17-5p	Cells	up regulated	Influence the transfer and progression of GC	[155]
	lncRNAs	PTCSC3	Tissue and cells	downregulated	Regulation of cell invasion and migration	[156]
	circRNAs	CircPVT1	Cells	up regulated	Regulation of GC cell proliferation, migration, and invasion	[157]
		circ_0006089	Cells	up regulated	Regulation of GC cell proliferation, migration, and invasion	[158]
	tsRNAs	tiRNA-Val-CAC-001	Tissue and cells	downregulated	As a promising biomarker and by targeting LRP6, regulates the Wnt/β-catenin signaling pathway to affect GC cell metastasis	[159]
	Exosome	Exosome TRIM3	Serum	downregulated	Inhibition of GC growth and metastasis in vitro and in vivo	[160]
		Exosome MET	Cells	up regulated	Promotes tumor growth and progression in vitro and in vivo	[161]

Table 3 (continued)

Projects	Category	Biomarker	Sample Type	Expression	Clinical significance	References
Drug resistance	miRNAs	miR100、miR-34a、miR-23a and miR-30a	_	All were significantly different	Modulated sensitivity of GC to chemotherapeutic agents	[162]
		let-7 g、miR-342、miR-16、miR-181、miR-1 and miR-34	_	All were significantly different	Correlation with chemotherapy sensitivity	[163]
		miR-30a	Cells	up regulated	Led to Beclin 1-mediated autophagy and promoted cisplatin-induced apoptosis and G2/M phase cell cycle arrest in GC cells	[164]
	lncRNAs	CRNDE	Cells	downregulated	Playing a key role in autophagy-mediated chemoresistance through binding to SRSF6	[165]
		SNHG6	Cells	up regulated	Progression of cisplatin resistance and GC is regulated by sponge miR-1297	[166]
	circRNAs	Hsacirc_004413	Cells	up regulated	GC cells can be made resistant to 5-fluorouracil by adsorption of miR-145-5p	[167]
		circ_AKT3	Tissue and cells	up regulated	Interaction with miR-206 regulates cisplatin resistance	[168]

resistance and GC progression via sponge miR-1297 and miR-206, respectively, indicating that targeting SNHG6 and circ_AKT3 may be a promising option to address GC chemoresistance [166, 168]. In 5-fluorouracil (5-FU)-resistant GC cells, hsacirc_004413 could make GC cells resistant to 5-FU by adsorbing miR-145-5p [167]. circCPM up-regulates another 5-FU-resistant GC cell line and tissues and increases PRKAA2 expression by directly binding to miR-21-3p, activating GC cell autophagy and chemoresistance [171]. ncRNAs have been considered biomarkers for multiple-stage cancers. If ncRNA assays can be used effectively in clinical practice, these methods can detect tumors in patients early and mitigate their suffering [172].

Detection methods of ncRNAs

Because ncRNAs and cfDNA are nucleic acid products, they are detected similarly. RT-qPCR, dPCR, and ddPCR based on PCR reactions, gene chips, and NGS-based RNA-sequencing are the most common methods for detecting ncRNAs (Fig. 2C). RT-qPCR is the gold standard method for quantitatively detecting trace ncRNAs, with high sensitivity, reproducibility, and accuracy [173]. dPCR and ddPCR aid in quantifying ncRNAs and allow for the precise quantification of target nucleic acids in samples. For initial screening and obtaining profiles of ncRNAs, gene microarray and NGS methods are commonly used [174], whereas RT-qPCR and dPCR are used to validate previous results [175].

Chen et al. [176] recently proposed RNAdetect, a computational method that incorporates novel predictive features based on generalized ensemble defects. Furthermore, n-gram models extract features that effectively capture sequence homology with known ncRNA families. Novel methods for detecting ncRNAs have emerged as technological advancements. Catalytic hairpin assembly (CHA), molecular beacon, DNA tetrahedron probe, localized surface plasmon resonance, thermophoresis-assisted detection, and CRISPR/CAS system-assisted detection [177–180] are just a few examples. Bellassai et al. [181] developed a hairpin-probe-based isothermal strand replacement polymerization method for detecting miRNAs and used it to quantify osteoarthritis-associated miR-127 in joint fluid. Furthermore, Chen et al. [182] coupled an RNA-based CHA circuit with CRISPR-Cas12a for detecting miRNAs under isothermal conditions. As a result, the circuit they developed achieved the nanomolar detection limit and allowed accurate detection of miRNA levels in different cell lines. Similarly, Yang et al. [183] proposed an isothermal amplification system based on double CHA and chameleon DNA template silver nanoclusters for label-free ratio detection of circRNAs. This system can detect and visually distinguish circRNAs. Its lower limit of detection for target circRNAs can reach 1 pm. Introducing these new technologies revitalizes the detection line of ncRNAs in blood and provides a new reference for further improving liquid biopsy rules.

Exosomes

Exosomes are lipid bilayer extracellular vesicles with a diameter of 30–150 nm found in nearly all body fluids (blood, urine, cerebrospinal fluid, and saliva) [160, 184]. Exosomes secrete various mRNAs, ncRNAs, transmembrane or encapsulated cytoplasmic proteins, and lipids from tumors or normal cells into body fluids, allowing intercellular communication or releasing contents to facilitate fluid biopsy [185–189]. Because of the lipid bilayer structure, exosomes are relatively abundant and stable in circulation, making them potential biomarkers for early diagnosis, prognosis, treatment efficacy, and drug resistance in GC [190, 191]. The clinical application of exosomes as liquid biopsy markers in GC is listed in Table 3.

Clinical application of exosomes in GC liquid biopsies

Exosomes' properties make them a promising candidate for liquid biopsy [185, 192]. First, blood exosomes contain many ncRNAs as biomarkers for early diagnosis, prognosis prediction, metastasis, and drug resistance in GC. Tang et al. [149] used precipitation technology to separate and extract exosomes from the plasma of 60 patients with early GC, 60 patients with intermediate and late GC, and 57 healthy people who served as controls. After analysis, the expression of exosome miR-4741 was up-regulated in GC tissues and plasma, while exosomes miR-32, miR-3149, and miR-6727 were down-regulated in GC tissues or plasma. The expression levels of miR-4741 and miR-3149 in the plasma of patients with early and middle-late GC were significantly different. The AUC values of the secretions miR-4741, miR-32, miR-3149, and miR-6727 for GC diagnosis were 0.8554, 0.9456, 0.7683, and 0.8923, respectively, suggesting that the above four secretions miRNAs can be used as GC diagnostic markers. Another study found that the expression level of exosomal LncRNAH19 in the serum of patients before and after GC surgery is significantly elevated, but the serum level of exosomal LncRNAH19 is significantly lower in patients after GC surgery compared to before surgery, the AUC of its diagnostic GC can reach 0.849, the sensitivity and specificity are 74.36% and 83.95%, respectively, and its expression level is significantly correlated with TNM stage [150]. Zheng et al. [151] detected that the up-regulation of hsa_circ_0015286 in GC tissue, plasma, and cancer cell exosomes is closely correlated

with tumor size, clinical stage, and lymph node metastasis and that the AUC of GC was 0.778, with sensitivity and specificity of 82.1% and 65.7%, respectively. After surgery, the expression level of exosomal hsa_circ_0015286 is significantly lower in GC patients, and the OS time is significantly longer in patients with low exosomal hsa_circ_0015286 expression.

In addition to ncRNAs, exosomes contain cell-specific proteins that play an important role in GC diagnosis, prognosis, metastasis, and drug resistance. TRIM3 protein levels in serum exosomes of GC patients are significantly lower than those of healthy control individuals. Exosomes-mediated delivery of TRIM3 protein inhibits GC growth and metastasis in vitro and in vivo, suggesting that TRIM3 could be used as a diagnostic marker and therapeutic target for GC [193]. Furthermore, exosomes released by *Helicobacter pylori*-infected GC cells increase MET expression. These exosomes containing MET protein are delivered and internalized by macrophages, promoting tumor growth and progression in vitro and in vivo [161].

Taken together, circulating exosomes and their derived "products" may open new avenues for GC liquid biopsy.

Detection techniques of exosomes

Exosomes are difficult to isolate with high efficiency and purity in liquid biopsies due to their unique formation and delivery processes [194, 195]. Furthermore, because tumor exosomes constitute only a small proportion of all exosomes in body fluids, high-sensitivity and specificity exosome detection methods are needed for subsequent detection and analysis. Several methods have been developed for isolating exosomes and detecting exosomal proteins and nucleic acids (Fig. 2D) [196–198]. Exosome isolation and enrichment methods are based on exosome characteristics such as density, size, surface composition, and exosome precipitation [194, 199]. 1. Ultracentrifugation (UC) is based on particle size and solution viscosity and includes differential UC and density gradient UC [200]. UC is the gold standard for exosome isolation and the most widely used method [201]. 2. Ultrafiltration is a simple method with high purification but low yield based on exosome size [202]. Combination UC and ultrafiltration methods are now widely used, combining the advantages of both methods and making exosome extraction easier and yielding more exosomes. 3. The precipitation-based exosome separation and enrichment method allows exosome separation using highly hydrophilic polymers competitively bound to water molecules around the exosome membrane, reducing solubility [203]. 4. The immunoaffinity enrichment method for exosome isolation allows good differentiation between cancer cell-originated exosomes and normal cell-originated exosomes using antibodies and inducers targeting tumor-associated proteins such as CD81, GPC-1, and EpCAM [204, 205]. 5. The separation and enrichment of exosomes were mediated by lipid bimolecular affinity. This method can substantially shorten the separation procedure for exosomes [206]. 6. The use of microbeads, microfluidic chips, and thermal enrichment makes the enrichment of exosomes fast and simple [207–209].

The traditional method for detecting exosomes is using western blot analysis or an enzyme-linked immunosorbent assay for exosomal membrane proteins or other marker proteins, but the method is complicated, insensitive, and unsuitable for mass detection [210]. Therefore, new techniques for detecting exosomes have been developed, such as scanning electron microscopy, transmission electron microscopy, atomic force microscopy, dynamic light scattering, nanoparticle tracking analysis, colorimetric assay, fluorescence detection, surface plasmon resonance, surface-enhanced Raman scattering, magnetic detection, electrochemical detection, CRISPR/CAS system-assisted detection, single exosome detection, and others [211–218]. In the CRISPR/Cas9 system, Cas9 nuclease can recognize specific complementary double-stranded DNA (dsDNA) sequences containing proto-spacer adjacent motifs using guide RNA to efficiently shear dsDNA sequences. Protein detection can be transformed into nucleic acid quantification by targeting extracellular protein aptamers [219]. Recently, Zhao et al. [220] proposed an aptamer-based extracellular membrane protein recognition combined with CRISPR/Cas12-assisted fluorescent signal amplification, making the CRISPR/Cas system promising as a sensitive tool for exosomal protein identification and quantification, which may aid in cancer diagnosis and therapeutic monitoring. Endogenous heterogeneity currently impedes the analysis of humoral exosomes, and in vivo mutations may lead to erroneous analysis results. Single exosome detection may offer a solution to this problem [221]. Guo et al. [218] used DNA nanostructure labeling and exosome membrane staining to establish a single-strand DNA-assisted single exon detection platform. The authors used long, single-stranded DNA amplified by the roll ring grown in situ on the surface of the exosome to identify the surface protein of a single exosome. This method can also analyze the protein mass spectra of different exosomes without calibration, allowing exosomes to be clearly distinguished based on their source cells.

Table 4 Liquid biopsy technique in GC

Testing Target	Testing purpose	Testing Technology	Testing Advantages	Testing Disadvantages	References
CTCs	Isolation and enrichment	Micro-electro-mechanical system (MEMS)	Simple and easy to operate	The large difference in size of different tumor cells, etc., makes it impossible to uniformly	[47]
		Isolation by size of epithelial tumor cells(ISET)			[48]
		Microfluidics			[49]
		Dual-Aptamer-Targeted Immunomagnetic Nanoparticles	Dependence on immunological antibodies for specific capture of CTCs	Recovery of EpCAM + CTCs only	[50]
		CellSearch	Semi-automatic and can process multiple samples at once	Enriched CTCs population exerts EpCAM bias	[37]
	Testing	qPCR and protein detection techniques (immunofluorescence, immunohistochemistry, fluorescence-assisted in situ hybridization.)	Enables quantitative and accurate testing	Longer detection time and less specificity	[33, 66, 68]
		Fiber-optic array scanning technology (FAST)	Detect low levels of CTCs without enrichment	Higher cost	[63]
		Epithelial immune spots	Detection of proteins secreted/released/shed by individual epithelial cancer cells	Smaller sample size for one-time processing	[64]
		Whole genome amplification (WGA)	Rapid and efficient detection of specific proteins or markers of CTCs	Lower specificity and higher cost	[71]
		Single-cell sequencing	Ability to detect its properties at the individual cell level	Lower level reflected	[71]
cfDNA/ctDNA	PCR-based detection	qPCR、dPCR、ddPCR、allele-specific PCR, quantitative methylation-specific PCR, BEAMing, ARMS, and COLD-PCR	Quantitative detection of target molecules with improved detection sensitivity and lower cost	The number of mutations detected is limited, the detection area is restricted, and the sample size increases with the number of times the assay is used	[111–115]
	NGS-based detection	Targeted NGS Technology(TAm-Seq, Safe-SeqS, CAPP-Seq and molecular barcoding or digital error suppression)	Can identify tumor mutations in some patients with high sensitivity	The number of detected tumor mutations is low and contains a large number of positional tumor mutations that are not detected	[116–120]
		Non-targeted NGS technology(WGS and WES)	Detects all tumor mutations in patients and can be used for genome-wide copy number analysis and large structural variant detection	Lower sensitivity and higher cost	[121]
ncRNAs	PCR-based detection	RT-qPCR, dPCR, and ddPCR	Better sensitivity, repeatability, and accuracy	The number of molecules detected is limited and the sample size increases with the number of times the assay is used	[174]
	NGS-based detection	Gene chips	Preliminary screening and mapping of ncRNAs can be obtained	Lower specificity and higher cost	[175]
		RNA-Seq	Preliminary screening and mapping of ncRNAs can be obtained	Lower specificity and higher cost	[175]
	Detecting the presence of functional ncRNAs	RNAdetect	Accurate detection of the presence of functional ncRNAs	High dependency	[176]
	Isothermal amplification technique detection	Catalytic hairpin assembly (CHA)	The detection conditions are relatively simple and allow for amplification of the target molecule, making it easy to detect	Poor specificity	[183]
	Detection of new technologies	Molecular beacons (MB), DNA tetrahedron probe, Localized surface plasmon resonance (LSPR), Thermophoresis-assisted detection and CRISPR/CAS system-assisted detection	Easy and convenient operation, lower detection limit	Poor specificity or low sensitivity	[177–180]

Table 4 (continued)

Testing Target	Testing purpose	Testing Technology	Testing Advantages	Testing Disadvantages	References
Exosome	Isolation and enrichment	Differential Ultracentrifugation (DUC) and Density Gradient Ultracentrifugation (DG-UC)	Exosome isolation and enrichment based on particle size and solution viscosity, simple operation and high extraction purity	Lower recovery efficiency and purity	[201]
		Ultrafiltration	Simple operation, high purification	Lower yield	[202]
		Sedimentation Technology	Highly hydrophilic polymers can be used to competitively bind to water molecules around the exosome membrane, thereby reducing solubility and enabling exosome separation	Lack of specificity, serious contamination, difficult to expand	[203]
		Immunoaffinity enrichment method	High specificity, better differentiation between exosomes of cancer cell origin and exosomes of normal cell origin	Higher cost and high dependency	[205]
		Microbeads, microfluidic chips, and thermal enrichment	Improved efficiency and detection limits of exosome enrichment	Higher cost and high dependency	[207–209]
		Lipid-based separation	Reduce the damage of exosome and extract effectively	With other pollution and lack of specificity	[206]
	Testing	Traditional Western Blot (WB) or enzyme-linked immunosorbent assay (ELISA)	Specifically detects exosomal membrane proteins or other marker proteins	Complex steps and low sensitivity	[210]
		Scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and dynamic light scattering (DLS)	Easy, fast, and visual operation	Samples are susceptible to damage from external conditions and are more expensive	[211]
		Nanoparticle Tracking Analysis (NTA)	Visualize and provide numerous information on exosomes	Complex operation and poor repeatability	[160]
		Colorimetric method	Easy to operate and visualize	High dependent and subject to external interference	[215]
		Fluorescence detection	Low cost, non-destructive, multiplex detection capability, easy automation	High dependent and subject to external interference	[213]
		Surface Plasmon Resonance (SPR)	No sample preparation and a label-free, real-time molecular sensing technique	High dependent	[214]
		Surface enhanced Raman scattering (SERC)	Enabled cheap, portable, and easily available establishment of detection	Higher cost and high dependency	[217]
		Electrochemical testing	High sensitivity, high selectivity, low cost, easy to use	High dependent	[212]
		CRISPR/CAS system-assisted detection	High specificity	Complex operation and low sensitivity	[187, 213, 215]
		Single exosome detection	Provide more accurate tumor progression information and reflect individual differences, and have strong specificity	Low detection rate and high personalization cost	[218]

Challenges and future developments in liquid biopsy

Although liquid biopsy technology has promising applications, numerous issues must be addressed before clinical promotion. Table 4 summarizes the advantages and disadvantages of liquid biopsy techniques in GC.

First, a highly sensitive assay must be developed. For example, CTCs and cfDNA/ctDNA concentrations in GC patients' blood are low, but the sensitivity of current detection and extraction techniques for both is low, making detection more challenging [222, 223]. Furthermore, although NGS technology can improve detection sensitivity through enrichment and amplification, it also introduces issues such as gene information mismatches, incomplete detection information, and false positives [224]. Furthermore, most current technologies have long detection times and high costs, limiting their use to large-scale promotion. Therefore, technologies with high sensitivity and high detection efficiency are required to support liquid biopsy.

Second, standardized operational procedures and data processing methods must be developed. Since existing clinical guidelines for liquid biopsy do not provide uniform and prudent evidence, the liquid biopsy results are not very comparable, and the assay quality is not uniform. In addition, the sensitivity and specificity may differ when using different techniques or assays to detect markers [15]. Therefore, before liquid biopsy can be used in clinical practice as a precision medicine tool to drive GC management, pre-analytical procedures, and post-analytical data processing, such as counting CTCs and ctDNA, characterization of CTCs and genetic or epigenetic changes in ctDNA analysis, and quantification of circulating ncRNAs, must be standardized to ensure reproducibility and data comparability.

Third, large-scale clinical study validation is an important aspect of the clinical application of liquid biopsy technology. Current liquid biopsy marker studies include fewer samples and have a short validation period. In addition, most of them focus only on the specificity and sensitivity of the liquid biopsy detection system without a comprehensive test of the assay's reproducibility, accuracy, consistency, reference range, and minimum detection limit. For example, CTCs and cfDNA studies have primarily focused on the late-stage or postoperative blood of tumor patients, with few studies on their different stages. Studies on cfDNAs, ctDNAs, and ncRNAs, on the other hand, have focused on detecting the sensitivity and specificity of markers in different stages or states of tumors, with few studies exploring the scope of their detection. In contrast, histology studies are more pre-screened, which results in more markers being obtained at the end, lowering the final specificity. These issues require large-scale clinical samples for subsequent demonstration and provide sufficient evidence and support for liquid biopsy technology's early entry into clinical applications.

Fourth, novel biomarker types are being investigated as liquid biopsy reserves. There are currently fewer types of liquid biopsies, and the relatively small amounts of CTCs, ctDNAs, ncRNAs, and exosomes included compared to other components of blood make detection more difficult [225]. Therefore, researchers have also developed more content-rich biomarkers to complement them. Currently, tumor-educated platelets, circulating endothelial cells, and tumor microenvironment components have been identified as liquid biomarkers for biopsy [226–228], but no application of these three markers in GC diagnosis has been found. Therefore, more research is needed to complement the effective GC markers and find new markers that can compensate for the shortcomings of current biomarkers and thus provide clinical guidance.

Finally, the future of liquid biopsy will be a combined diagnosis. Although many liquid biopsy techniques have been developed and many biomarkers have been screened using various techniques, studies have shown that combining different biomarkers for a tumor or disease diagnosis and drug use improves overall accuracy. For example, Li et al. [229] found that using tRNA-GlyGCC-5 and sRESE alone has a much lower effect on identifying esophageal squamous cell carcinoma than using both. Hong et al. [230] and Tang et al. [231] found that the combination of exosome miRNAs or the combination of exosome miRNAs and CEA surpassed a single exosome miRNA marker in the diagnosis of GC. Our previous research found that the AUC value of GC diagnosis using circPTPN22 alone was 0.857, but the AUC value increased to 0.892 after combining traditional tumor markers CEA and CA199, demonstrating that the combined use of markers could compensate for the limitations of a single marker [146]. Therefore, if the benefits of various liquid biopsy techniques can be fully utilized and the combination of these techniques can be used for tumor diagnosis, it will significantly impact the future diagnosis and treatment of tumors and diseases.

Conclusion

In conclusion, as a non-invasive detection method, liquid biopsy has emerged as a promising new modality for GC in early screening or diagnosis, postoperative monitoring, treatment response, and tumor drug resistance [232]. The method provides tumor molecular information and overcomes tumor heterogeneity, allowing real-time monitoring of tumor progression and personalized patient treatment. Although liquid biopsy has demonstrated significant benefits, the lack of novel

liquid biopsy markers, low specificity and sensitivity, a lack of standard operating procedures and data analysis methods, and prohibitive costs currently prevent liquid biopsy from being widely used in the clinic. We believe that as the mechanisms of GC development and detection technology advance, the role of liquid biopsy technology in GC will gradually be revealed. It will be of great value in the clinical application of GC diagnosis and prognosis.

Abbreviations

GC	Gastric cancer
CEA	Carcinoembryonic antigen
CA	Carbohydrate antigen
CTCs	Circulating tumor cells
cfDNA	Circulating free DNA
ctDNA	Circulating tumor DNA
ncRNAs	Non-coding RNAs
EpCAM	Epithelial cell adhesion molecules
CKs	Cytokeratins
OS	Overall survival
PFS	Progression-free survival
PD-L1	Programmed cell death protein 1
CSV	Cell-surface vimentin
FDA	Food and Drug Administration
PCR	Polymerase chain reaction
miRNAs	MicroRNAs
TNM	Tumor node metastasis
MGC	Metastatic GC
MSI	Microsatellite instability
NGS	Next-generation sequencing
qPCR	Quantitative PCR
dPCR	Digital PCR
ddPCR	Droplet digital PCR
TAm-Seq	Tagged-amplicon deep sequencing
Safe-SeqS	Safe-Sequencing System
CAPP-Seq	Personalized profiling by deep sequencing
WGS	Whole genome sequencing
mRNAs	Messenger RNAs
tsRNAs	TRNA-derived small RNAs
lncRNAs	Long non-coding RNAs
circRNAs	Circular RNAs
sncRNAs	Small non-coding RNAs
AUC	Area under the curve
CDDP	Cisplatin
5-FU	5-Fluorouracil
CHA	Catalytic hairpin assembly
UC	Ultracentrifugation
dsDNA	Double-stranded DNA

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Authors' contributions
SM drafted the manuscript and created the diagrams. MLZ, YHX, and XLG revised the manuscript. MYZ, GA, and YMY revised the article format and added references. XBF, and GQW directed the writing of the manuscript and provided financial support. The author(s) read and approved the final manuscript.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2. Ugai T, Sasamoto N, Lee HY, Ando M, Song M, Tamimi RM, et al. Is early-onset cancer an emerging global epidemic? Current evidence and future implications. Nat Rev Clin Oncol. 2022;19(10):656–73.
3. Pasechnikov V, Chukov S, Fedorov E, Kikuste I, Leja M. Gastric cancer: prevention, screening and early diagnosis. World J Gastroenterol. 2014;20(38):13842–62.
4. Huang ZB, Zhang HT, Yu B, Yu DH. Cell-free DNA as a liquid biopsy for early detection of gastric cancer. Oncol Lett. 2021;21(1):3.
5. Tan P, Yeoh KG. Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma. Gastroenterology. 2015;149(5):1153-1162.e1153.
6. Levy I, Gralnek IM. Complications of diagnostic colonoscopy, upper endoscopy, and enteroscopy. Best Pract Res Clin Gastroenterol. 2016;30(5):705–18.
7. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92.
8. Gupta N, Bansal A, Wani SB, Gaddam S, Rastogi A, Sharma P. Endoscopy for upper GI cancer screening in the general population: a cost-utility analysis. Gastrointest Endosc. 2011;74(3):610-624.e612.
9. Allgayer H, Heiss MM, Schildberg FW. Prognostic factors in gastric cancer. Br J Surg. 1997;84(12):1651–64.
10. Pantel K, Alix-Panabières C. Liquid biopsy in 2016: Circulating tumour cells and cell-free DNA in gastrointestinal cancer. Nat Rev Gastroenterol Hepatol. 2017;14(2):73–4.
11. Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med. 2010;16(9):398–406.
12. Pérez-Callejo D, Romero A, Provencio M, Torrente M. Liquid biopsy based biomarkers in non-small cell lung cancer for diagnosis and treatment monitoring. Translational lung cancer research. 2016;5(5):455–65.
13. Zhou H, Zhu L, Song J, Wang G, Li P, Li W, et al. Liquid biopsy at the frontier of detection, prognosis and progression monitoring in colorectal cancer. Mol Cancer. 2022;21(1):86.
14. Li TT, Liu H, Yu J, Shi GY, Zhao LY, Li GX. Prognostic and predictive blood biomarkers in gastric cancer and the potential application of circulating tumor cells. World J Gastroenterol. 2018;24(21):2236–46.
15. Jelski W, Mroczko B: Molecular and Circulating Biomarkers of Gastric Cancer. Int J Mol Sci 2022, 23(14).
16. Praharaj PP, Bhutia SK, Nagrath S, Bitting RL, Deep G. Circulating tumor cell-derived organoids: Current challenges and promises in medical research and precision medicine. Biochim Biophys Acta. 2018;1869(2):117–27.
17. van de Stolpe A, Pantel K, Sleijfer S, Terstappen LW, den Toonder JM. Circulating tumor cell isolation and diagnostics: toward routine clinical use. Cancer Res. 2011;71(18):5955–60.

18. Mishima Y, Paiva B, Shi J, Park J, Manier S, Takagi S, et al. The Mutational Landscape of Circulating Tumor Cells in Multiple Myeloma. Cell Rep. 2017;19(1):218–24.
19. Keller L, Pantel K. Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. Nat Rev Cancer. 2019;19(10):553–67.
20. Krebs MG, Metcalf RL, Carter L, Brady G, Blackhall FH, Dive C. Molecular analysis of circulating tumour cells-biology and biomarkers. Nat Rev Clin Oncol. 2014;11(3):129–44.
21. Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, et al. Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res. 2004;10(24):8152–62.
22. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004;10(20):6897–904.
23. Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. Mol Oncol. 2016;10(3):374–94.
24. Gkountela S, Castro-Giner F, Szczerba BM, Vetter M, Landin J, Scherrer R, et al. Circulating Tumor Cell Clustering Shapes DNA Methylation to Enable Metastasis Seeding. Cell. 2019;176(1–2):98-112.e114.
25. Koike H, Ichikawa D, Ikoma H, Otsuji E, Kitamura K, Yamagishi H. Comparison of methylation-specific polymerase chain reaction (MSP) with reverse transcriptase-polymerase chain reaction (RT-PCR) in peripheral blood of gastric cancer patients. J Surg Oncol. 2004;87(4):182–6.
26. Seo JH, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS, et al. Follow-up study of peripheral blood carcinoembryonic antigen mRNA using reverse transcription-polymerase chain reaction as an early marker of clinical recurrence in patients with curatively resected gastric cancer. Am J Clin Oncol. 2005;28(1):24–9.
27. Kang HM, Kim GH, Jeon HK, Kim DH, Jeon TY, Park DY, et al. Circulating tumor cells detected by lab-on-a-disc: Role in early diagnosis of gastric cancer. PLoS ONE. 2017;12(6): e0180251.
28. Tang L, Zhao S, Liu W, Parchim NF, Huang J, Tang Y, et al. Diagnostic accuracy of circulating tumor cells detection in gastric cancer: systematic review and meta-analysis. BMC Cancer. 2013;13:314.
29. Negishi R, Yamakawa H, Kobayashi T, Horikawa M, Shimoyama T, Koizumi F, et al. Transcriptomic profiling of single circulating tumor cells provides insight into human metastatic gastric cancer. Communications biology. 2022;5(1):20.
30. Huang X, Gao P, Sun J, Chen X, Song Y, Zhao J, et al. Clinicopathological and prognostic significance of circulating tumor cells in patients with gastric cancer: a meta-analysis. Int J Cancer. 2015;136(1):21–33.
31. Liu M, Wang R, Sun X, Liu Y, Wang Z, Yan J, et al. Prognostic significance of PD-L1 expression on cell-surface vimentin-positive circulating tumor cells in gastric cancer patients. Mol Oncol. 2020;14(4):865–81.
32. Cheng B, Tong G, Wu X, Cai W, Li Z, Tong Z, et al. Enumeration And Characterization Of Circulating Tumor Cells And Its Application In Advanced Gastric Cancer. Onco Targets Ther. 2019;12:7887–96.
33. Jhi JH, Kim GH, Park SJ, Kim DU, Lee MW, Lee BE et al: Circulating Tumor Cells and TWIST Expression in Patients with Metastatic Gastric Cancer: A Preliminary Study. Journal of clinical medicine 2021, 10(19).
34. Matsusaka S, Chìn K, Ogura M, Suenaga M, Shinozaki E, Mishima Y, et al. Circulating tumor cells as a surrogate marker for determining response to chemotherapy in patients with advanced gastric cancer. Cancer Sci. 2010;101(4):1067–71.
35. Ito H, Sato J, Tsujino Y, Yamaguchi N, Kimura S, Gohda K, et al. Long-term prognostic impact of circulating tumour cells in gastric cancer patients. World J Gastroenterol. 2016;22(46):10232–41.
36. Hiraiwa K, Takeuchi H, Hasegawa H, Saikawa Y, Suda K, Ando T, et al. Clinical significance of circulating tumor cells in blood from patients with gastrointestinal cancers. Ann Surg Oncol. 2008;15(11):3092–100.
37. Zeng CDD, Jin CC, Gao C, Xiao AT, Tong YX, Zhang S. Preoperative Folate Receptor-Positive Circulating Tumor Cells Are Associated With Occult Peritoneal Metastasis and Early Recurrence in Gastric Cancer Patients: A Prospective Cohort Study. Front Oncol. 2022;12: 769203.
38. Yu P, Zhu S, Luo Y, Li G, Pu Y, Cai B, et al. Application of Circulating Tumor Cells and Circulating Free DNA from Peripheral Blood in the Prognosis of Advanced Gastric Cancer. J Oncol. 2022;2022:9635218.
39. Venerito M, Vasapolli R, Rokkas T, Malfertheiner P. Gastric cancer: epidemiology, prevention, and therapy. Helicobacter. 2018;23(Suppl 1): e12518.
40. Lee MW, Kim GH, Jeon HK, Park SJ. Clinical Application of Circulating Tumor Cells in Gastric Cancer. Gut Liver. 2019;13(4):394–401.
41. Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol. 2016;17(6):717–26.
42. Lin C, He H, Liu H, Li R, Chen Y, Qi Y, et al. Tumour-associated macrophages-derived CXCL8 determines immune evasion through autonomous PD-L1 expression in gastric cancer. Gut. 2019;68(10):1764–73.
43. Satelli A, Batth IS, Brownlee Z, Rojas C, Meng QH, Kopetz S, et al. Potential role of nuclear PD-L1 expression in cell-surface vimentin positive circulating tumor cells as a prognostic marker in cancer patients. Sci Rep. 2016;6:28910.
44. Strati A, Koutsodontis G, Papaxoinis G, Angelidis I, Zavridou M, Economopoulou P, et al. Prognostic significance of PD-L1 expression on circulating tumor cells in patients with head and neck squamous cell carcinoma. Ann Oncol. 2017;28(8):1923–33.
45. Kowalik A, Kowalewska M, Góźdź S. Current approaches for avoiding the limitations of circulating tumor cells detection methods-implications for diagnosis and treatment of patients with solid tumors. Transl Res. 2017;185:58-84.e15.
46. Tan Y, Wu H. The significant prognostic value of circulating tumor cells in colorectal cancer: A systematic review and meta-analysis. Curr Probl Cancer. 2018;42(1):95–106.
47. Zheng S, Lin H, Liu JQ, Balic M, Datar R, Cote RJ, et al. Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells. J Chromatogr A. 2007;1162(2):154–61.
48. Vona G, Sabile A, Louha M, Sitruk V, Romana S, Schütze K, et al. Isolation by size of epithelial tumor cells : a new method for the immunomorphological and molecular characterization of circulatingtumor cells. Am J Pathol. 2000;156(1):57–63.
49. Tan SJ, Yobas L, Lee GY, Ong CN, Lim CT. Microdevice for the isolation and enumeration of cancer cells from blood. Biomed Microdevices. 2009;11(4):883–92.
50. Li C, Yang S, Li R, Gong S, Huang M, Sun Y, et al. Dual-Aptamer-Targeted Immunomagnetic Nanoparticles to Accurately Explore the Correlations between Circulating Tumor Cells and Gastric Cancer. ACS Appl Mater Interfaces. 2022;14(6):7646–58.
51. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. Nat Rev Clin Oncol. 2019;16(7):409–24.
52. Lianidou ES, Mavroudis D, Georgoulias V. Clinical challenges in the molecular characterization of circulating tumour cells in breast cancer. Br J Cancer. 2013;108(12):2426–32.
53. Tsujiura M, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Otsuji E. Liquid biopsy of gastric cancer patients: circulating tumor cells and cell-free nucleic acids. World J Gastroenterol. 2014;20(12):3265–86.
54. Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2004;351(8):781–91.
55. Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res. 2007;13(23):7053–8.
56. Sastre J, Maestro ML, Puente J, Veganzones S, Alfonso R, Rafael S, et al. Circulating tumor cells in colorectal cancer: correlation with clinical and pathological variables. Ann Oncol. 2008;19(5):935–8.
57. Rushton AJ, Nteliopoulos G, Shaw JA, Coombes RC: A Review of Circulating Tumour Cell Enrichment Technologies. Cancers (Basel) 2021, 13(5).
58. Bahnassy AA, Salem SE, Mohanad M, Abulezz NZ, Abdellateif MS, Hussein M, et al. Prognostic significance of circulating tumor cells (CTCs) in Egyptian non-metastatic colorectal cancer patients: A comparative study for four different techniques of detection (Flowcytometry, Cell Search, Quantitative Real-time PCR and Cytomorphology). Exp Mol Pathol. 2019;106:90–101.
59. Cho H, Chung JS, Han KH: A Direct Comparison between the Lateral Magnetophoretic Microseparator and AdnaTest for Isolating Prostate Circulating Tumor Cells. Micromachines 2020, 11(9).
60. Sakaizawa K, Goto Y, Kiniwa Y, Uchiyama A, Harada K, Shimada S, et al. Mutation analysis of BRAF and KIT in circulating melanoma cells at the single cell level. Br J Cancer. 2012;106(5):939–46.

61. Heitzer E, Auer M, Gasch C, Pichler M, Ulz P, Hoffmann EM, et al. Complex tumor genomes inferred from single circulating tumor cells by array-CGH and next-generation sequencing. Cancer Res. 2013;73(10):2965–75.
62. Krivacic RT, Ladanyi A, Curry DN, Hsieh HB, Kuhn P, Bergsrud DE, et al. A rare-cell detector for cancer. Proc Natl Acad Sci U S A. 2004;101(29):10501–4.
63. Hsieh HB, Marrinucci D, Bethel K, Curry DN, Humphrey M, Krivacic RT, et al. High speed detection of circulating tumor cells. Biosens Bioelectron. 2006;21(10):1893–9.
64. Alix-Panabières C. EPISPOT assay: detection of viable DTCs/CTCs in solid tumor patients. Recent Results Cancer Res. 2012;195:69–76.
65. Alix-Panabières C, Vendrell JP, Pellé O, Rebillard X, Riethdorf S, Müller V, et al. Detection and characterization of putative metastatic precursor cells in cancer patients. Clin Chem. 2007;53(3):537–9.
66. Chen Y, Li Y, Qi C, Zhang C, Liu D, Deng Y, et al. Dysregulated KRAS gene-signaling axis and abnormal chromatin remodeling drive therapeutic resistance in heterogeneous-sized circulating tumor cells in gastric cancer patients. Cancer Lett. 2021;517:78–87.
67. Zhang Y, Yao J, Feng J, Wang S, Yang Z, Huang W, et al. Relationship between PRRX1, circulating tumor cells, and clinicopathological parameter in patients with gastric cancer. J BUON. 2020;25(3):1455–62.
68. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834–8.
69. Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet. 2009;10(10):704–14.
70. Mei Y, Clark D, Mao L. Novel dimensions of piRNAs in cancer. Cancer Lett. 2013;336(1):46–52.
71. Heidrich I, Abdalla TSA, Reeh M, Pantel K: Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as a Liquid Biopsy Marker in Colorectal Cancer. Cancers (Basel) 2021, 13(18).
72. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007;450(7173):1235–9.
73. Stott SL, Lee RJ, Nagrath S, Yu M, Miyamoto DT, Ulkus L et al: Isolation and characterization of circulating tumor cells from patients with localized and metastatic prostate cancer. Sci Transl Med 2010, 2(25):25ra23.
74. Green BJ, Marazzini M, Hershey B, Fardin A, Li Q, Wang Z, et al. PillarX: A Microfluidic Device to Profile Circulating Tumor Cell Clusters Based on Geometry, Deformability, and Epithelial State. Small. 2022;18(17): e2106097.
75. Quan Y, Chen K, Xiang N, Ni Z. A single-view field filter device for rare tumor cell filtration and enumeration. Electrophoresis. 2020;41(23):2000–6.
76. Konno N, Suzuki R, Takagi T, Sugimoto M, Asama H, Sato Y, et al. Clinical utility of a newly developed microfluidic device for detecting circulating tumor cells in the blood of patients with pancreatico-biliary malignancies. J Hepatobiliary Pancreat Sci. 2021;28(1):115–24.
77. Gwak H, Kim J, Kashefi-Kheyrabadi L, Kwak B, Hyun KA, Jung HI: Progress in Circulating Tumor Cell Research Using Microfluidic Devices. Micromachines 2018, 9(7).
78. Mandel P, Metais P. Nuclear Acids In Human Blood Plasma. C R Seances Soc Biol Fil. 1948;142(3–4):241–3.
79. Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, et al. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acids Res. 2010;38(18):6159–75.
80. Thierry AR, Mouliere F, El Messaoudi S, Mollevi C, Lopez-Crapez E, Rolet F, et al. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nat Med. 2014;20(4):430–5.
81. Alimirzaie S, Bagherzadeh M, Akbari MR. Liquid biopsy in breast cancer: A comprehensive review. Clin Genet. 2019;95(6):643–60.
82. Thierry AR, El Messaoudi S, Gahan PB, Anker P, Stroun M. Origins, structures, and functions of circulating DNA in oncology. Cancer Metastasis Rev. 2016;35(3):347–76.
83. Chen W, Yan H, Li X, Ge K, Wu J. Circulating tumor DNA detection and its application status in gastric cancer: a narrative review. Translational cancer research. 2021;10(1):529–36.
84. Fujisawa R, Iwaya T, Endo F, Idogawa M, Sasaki N, Hiraki H, et al. Early dynamics of circulating tumor DNA predict chemotherapy responses for patients with esophageal cancer. Carcinogenesis. 2021;42(10):1239–49.
85. Qi Q, Pan YF, Shen JJ, Gu XQ, Han SW, Liao HH, et al. Circulating DNA for detection of gastric cancer. Eur Rev Med Pharmacol Sci. 2016;20(12):2558–64.
86. Wang Z, Cheng Y, An T, Gao H, Wang K, Zhou Q, et al. Detection of EGFR mutations in plasma circulating tumour DNA as a selection criterion for first-line gefitinib treatment in patients with advanced lung adenocarcinoma (BENEFIT): a phase 2, single-arm, multicentre clinical trial. Lancet Respir Med. 2018;6(9):681–90.
87. Nordgård O, Tjensvoll K, Gilje B, Søreide K. Circulating tumour cells and DNA as liquid biopsies in gastrointestinal cancer. Br J Surg. 2018;105(2):e110–20.
88. Alix-Panabières C, Bartkowiak K, Pantel K. Functional studies on circulating and disseminated tumor cells in carcinoma patients. Mol Oncol. 2016;10(3):443–9.
89. Liao W, Yang H, Xu H, Wang Y, Ge P, Ren J, et al. Noninvasive detection of tumor-associated mutations from circulating cell-free DNA in hepatocellular carcinoma patients by targeted deep sequencing. Oncotarget. 2016;7(26):40481–90.
90. Labgaa I, Villacorta-Martin C, D'Avola D, Craig AJ, von Felden J, Martins-Filho SN, et al. A pilot study of ultra-deep targeted sequencing of plasma DNA identifies driver mutations in hepatocellular carcinoma. Oncogene. 2018;37(27):3740–52.
91. Vymetalkova V, Cervena K, Bartu L, Vodicka P: Circulating Cell-Free DNA and Colorectal Cancer: A Systematic Review. Int J Mol Sci 2018, 19(11).
92. Kim K, Shin DG, Park MK, Baik SH, Kim TH, Kim S, et al. Circulating cell-free DNA as a promising biomarker in patients with gastric cancer: diagnostic validity and significant reduction of cfDNA after surgical resection. Annals of surgical treatment and research. 2014;86(3):136–42.
93. Qian C, Ju S, Qi J, Zhao J, Shen X, Jing R, et al. Alu-based cell-free DNA: a novel biomarker for screening of gastric cancer. Oncotarget. 2017;8(33):54037–45.
94. Park JL, Kim HJ, Choi BY, Lee HC, Jang HR, Song KS, et al. Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients. Oncol Lett. 2012;3(4):921–6.
95. Fang WL, Lan YT, Huang KH, Liu CA, Hung YP, Lin CH, et al. Clinical significance of circulating plasma DNA in gastric cancer. Int J Cancer. 2016;138(12):2974–83.
96. Pu WY, Zhang R, Xiao L, Wu YY, Gong W, Lv XD, et al. Prediction of cancer progression in a group of 73 gastric cancer patients by circulating cell-free DNA. BMC Cancer. 2016;16(1):943.
97. Normando SRC, Delgado PO, Rodrigues A, David Filho WJ, Fonseca FLA, Cruz F, et al. Circulating free plasma tumor DNA in patients with advanced gastric cancer receiving systemic chemotherapy. BMC Clin Pathol. 2018;18:12.
98. Yang J, Gong Y, Lam VK, Shi Y, Guan Y, Zhang Y, et al. Deep sequencing of circulating tumor DNA detects molecular residual disease and predicts recurrence in gastric cancer. Cell Death Dis. 2020;11(5):346.
99. Lan YT, Chen MH, Fang WL, Hsieh CC, Lin CH, Jhang FY, et al. Clinical relevance of cell-free DNA in gastrointestinal tract malignancy. Oncotarget. 2017;8(2):3009–17.
100. Zhong Y, Fan Q, Zhou Z, Wang Y, He K, Lu J. Plasma cfDNA as a Potential Biomarker to Evaluate the Efficacy of Chemotherapy in Gastric Cancer. Cancer Manag Res. 2020;12:3099–106.
101. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med. 2018;24(9):1449–58.
102. Willis J, Lefterova MI, Artyomenko A, Kasi PM, Nakamura Y, Mody K, et al. Validation of Microsatellite Instability Detection Using a Comprehensive Plasma-Based Genotyping Panel. Clin Cancer Res. 2019;25(23):7035–45.
103. Ren J, Lu P, Zhou X, Liao Y, Liu X, Li J, et al. Genome-Scale Methylation Analysis of Circulating Cell-Free DNA in Gastric Cancer Patients. Clin Chem. 2022;68(2):354–64.
104. Ko K, Kananazawa Y, Yamada T, Kakinuma D, Matsuno K, Ando F, et al. Methylation status and long-fragment cell-free DNA are prognostic biomarkers for gastric cancer. Cancer Med. 2021;10(6):2003–12.
105. Karamitrousis EI, Balgkouranidou I, Xenidis N, Amarantidis K, Biziota E, Koukaki T, et al. Prognostic Role of RASSF1A, SOX17 and Wif-1 Promoter Methylation Status in Cell-Free DNA of

Advanced Gastric Cancer Patients. Technol Cancer Res Treat. 2021;20:1533033820973279.
106. Kandimalla R, Xu J, Link A, Matsuyama T, Yamamura K, Parker MI, et al. EpiPanGI Dx: A Cell-free DNA Methylation Fingerprint for the Early Detection of Gastrointestinal Cancers. Clin Cancer Res. 2021;27(22):6135–44.
107. Chivu-Economescu M, Necula L, Matei L, Dragu D, Bleotu C, Diaconu CC. Clinical Applications of Liquid Biopsy in Gastric Cancer. Front Med. 2021;8: 749250.
108. Lengyel CG, Hussain S, Trapani D, El Bairi K, Altuna SC, Seeber A et al: The Emerging Role of Liquid Biopsy in Gastric Cancer. Journal of clinical medicine 2021, 10(10).
109. Benson AB 3rd, Arnoletti JP, Bekaii-Saab T, Chan E, Chen YJ, Choti MA, et al. Colon cancer. J Natl Compr Canc Netw. 2011;9(11):1238–90.
110. Heeke S, Hofman V, Benzaquen J, Otto J, Tanga V, Zahaf K, et al. Detection of EGFR Mutations From Plasma of NSCLC Patients Using an Automatic Cartridge-Based PCR System. Front Pharmacol. 2021;12: 657743.
111. Chang L, Li J, Zhang R. Liquid biopsy for early diagnosis of non-small cell lung carcinoma: recent research and detection technologies. Biochim Biophys Acta. 2022;1877(3): 188729.
112. Olmedillas-López S, García-Arranz M, García-Olmo D. Current and Emerging Applications of Droplet Digital PCR in Oncology. Mol Diagn Ther. 2017;21(5):493–510.
113. Hudecova I. Digital PCR analysis of circulating nucleic acids. Clin Biochem. 2015;48(15):948–56.
114. Feng Q, Yang ZY, Zhang JT, Tang JL. Comparison of direct sequencing and amplification refractory mutation system for detecting epidermal growth factor receptor mutation in non-small-cell lung cancer patients: a systematic review and meta-analysis. Oncotarget. 2017;8(35):59552–62.
115. Mauger F, How-Kit A, Tost J. COLD-PCR Technologies in the Area of Personalized Medicine: Methodology and Applications. Mol Diagn Ther. 2017;21(3):269–83.
116. Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DW, Kaper F et al: Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med 2012, 4(136):136ra168.
117. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. Proc Natl Acad Sci U S A. 2011;108(23):9530–5.
118. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54.
119. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, Scherer F, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. Nat Biotechnol. 2016;34(5):547–55.
120. Kukita Y, Matoba R, Uchida J, Hamakawa T, Doki Y, Imamura F, et al. High-fidelity target sequencing of individual molecules identified using barcode sequences: de novo detection and absolute quantitation of mutations in plasma cell-free DNA from cancer patients. DNA Res. 2015;22(4):269–77.
121. Koldby KM, Mortensen MB, Detlefsen S, Pfeiffer P, Thomassen M, Kruse TA. Tumor-specific genetic aberrations in cell-free DNA of gastroesophageal cancer patients. J Gastroenterol. 2019;54(2):108–21.
122. Olsson E, Winter C, George A, Chen Y, Howlin J, Tang MH, et al. Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med. 2015;7(8):1034–47.
123. Reinert T, Schøler LV, Thomsen R, Tobiasen H, Vang S, Nordentoft I, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. Gut. 2016;65(4):625–34.
124. Cech TR, Steitz JA. The noncoding RNA revolution-trashing old rules to forge new ones. Cell. 2014;157(1):77–94.
125. Ding L, Xu Y, Zhang W, Deng Y, Si M, Du Y, et al. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. Cell Res. 2010;20(7):784–93.
126. Petrocca F, Visone R, Onelli MR, Shah MH, Nicoloso MS, de Martino I, et al. E2F1-regulated microRNAs impair TGFbeta-dependent cell-cycle arrest and apoptosis in gastric cancer. Cancer Cell. 2008;13(3):272–86.
127. Kim HK, Yeom JH, Kay MA. Transfer RNA-Derived Small RNAs: Another Layer of Gene Regulation and Novel Targets for Disease Therapeutics. Mol Ther. 2020;28(11):2340–57.
128. Zhu L, Ge J, Li T, Shen Y, Guo J. tRNA-derived fragments and tRNA halves: The new players in cancers. Cancer Lett. 2019;452:31–7.
129. Xie Y, Yao L, Yu X, Ruan Y, Li Z, Guo J. Action mechanisms and research methods of tRNA-derived small RNAs. Signal Transduct Target Ther. 2020;5(1):109.
130. Ma B, Wang Y, Zhou X, Huang P, Zhang R, Liu T, et al. Synergistic suppression effect on tumor growth of hepatocellular carcinoma by combining oncolytic adenovirus carrying XAF1 with cisplatin. J Cancer Res Clin Oncol. 2015;141(3):419–29.
131. Guo R, Yang J, Liu X, Wu J, Chen Y: Increased von Willebrand factor over decreased ADAMTS-13 activity is associated with poor prognosis in patients with advanced non-small-cell lung cancer. J Clin Lab Anal 2018, 32(1).
132. Gu X, Zhang Y, Qin X, Ma S, Huang Y, Ju S. Transfer RNA-derived small RNA: an emerging small non-coding RNA with key roles in cancer. Exp Hematol Oncol. 2022;11(1):35.
133. Iyer MK, Niknafs YS, Malik R, Singhal U, Sahu A, Hosono Y, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199–208.
134. Gong C, Maquat LE. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3' UTRs via Alu elements. Nature. 2011;470(7333):284–8.
135. Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. J Clin Invest. 2017;127(3):761–71.
136. Chen LL, Yang L. Regulation of circRNA biogenesis. RNA Biol. 2015;12(4):381–8.
137. Holcik M, Sonenberg N. Translational control in stress and apoptosis. Nat Rev Mol Cell Biol. 2005;6(4):318–27.
138. Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, et al. 5' UTR m(6)A Promotes Cap-Independent Translation. Cell. 2015;163(4):999–1010.
139. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019;20(11):675–91.
140. Zhou M, Dong J, Huang J, Ye W, Zheng Z, Huang K et al: Chitosan-Gelatin-EGCG Nanoparticle-Meditated LncRNA TMEM44-AS1 Silencing to Activate the P53 Signaling Pathway for the Synergistic Reversal of 5-FU Resistance in Gastric Cancer. Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2022, 9(22):e2105077.
141. Zang X, Jiang J, Gu J, Chen Y, Wang M, Zhang Y, et al. Circular RNA EIF4G3 suppresses gastric cancer progression through inhibition of β-catenin by promoting δ-catenin ubiquitin degradation and upregulating SIK1. Mol Cancer. 2022;21(1):141.
142. Xu Y, Wang G, Hu W, He S, Li D, Chen P, et al. Clinical role of miR-421 as a novel biomarker in diagnosis of gastric cancer patients: A meta-analysis. Medicine (Baltimore). 2022;101(19): e29242.
143. Gao S, Zhang Z, Wang X, Ma Y, Li C, Liu H, et al. hsa-miR-875-5p inhibits tumorigenesis and suppresses TGF-β signalling by targeting USF2 in gastric cancer. J Transl Med. 2022;20(1):115.
144. Komatsu S, Imamura T, Kiuchi J, Takashima Y, Kamiya H, Ohashi T, et al. Depletion of tumor suppressor miRNA-148a in plasma relates to tumor progression and poor outcomes in gastric cancer. Am J Cancer Res. 2021;11(12):6133–46.
145. Zhuang K, Han K, Tang H, Yin X, Zhang J, Zhang X, et al. Up-Regulation of Plasma miR-23b is Associated with Poor Prognosis of Gastric Cancer. Med Sci Monit. 2016;22:356–61.
146. Ma S, Kong S, Gu X, Xu Y, Tao M, Shen L, et al. As a biomarker for gastric cancer, circPTPN22 regulates the progression of gastric cancer through the EMT pathway. Cancer Cell Int. 2021;21(1):44.
147. Roy S, Kanda M, Nomura S, Zhu Z, Toiyama Y, Taketomi A, et al. Diagnostic efficacy of circular RNAs as noninvasive, liquid biopsy biomarkers for early detection of gastric cancer. Mol Cancer. 2022;21(1):42.
148. Gu X, Ma S, Liang B, Ju S. Serum hsa_tsr016141 as a Kind of tRNA-Derived Fragments Is a Novel Biomarker in Gastric Cancer. Front Oncol. 2021;11: 679366.
149. Tang G, Wang J, Dong W, Dai K, Du J. Exosomal miRNA Expression Profiling and the Roles of Exosomal miR-4741, miR-32, miR-3149,

and miR-6727 on Gastric Cancer Progression. Biomed Res Int. 2022;2022:1263812.
150. Zhou H, Shen W, Zou H, Lv Q, Shao P. Circulating exosomal long non-coding RNA H19 as a potential novel diagnostic and prognostic biomarker for gastric cancer. J Int Med Res. 2020;48(7):300060520934297.
151. Zheng P, Gao H, Xie X, Lu P. Plasma Exosomal hsa_circ_0015286 as a Potential Diagnostic and Prognostic Biomarker for Gastric Cancer. Pathol Oncol Res. 2022;28:1610446.
152. Yang M, Lu Z, Yu B, Zhao J, Li L, Zhu K et al: COL5A1 Promotes the Progression of Gastric Cancer by Acting as a ceRNA of miR-137–3p to Upregulate FSTL1 Expression. Cancers (Basel) 2022, 14(13).
153. Yu S, Meng H, Shi S, Cao S, Bian T, Zhao H. miR-548d-3p inhibits the invasion and migration of gastric cancer cells by targeting GKN1. J Clin Lab Anal. 2022;36(7): e24520.
154. Bae WJ, Woo KJ, Ahn JM, Yang CM, Kim YS, Kim S, et al. miR-4742-5p promotes invasiveness of gastric cancer via targeting Rab43: An in vitro study. Biochem Biophys Res Commun. 2022;613:180–6.
155. Yifei S, Chunxiao H, Dinuo L: MiR-17–5p Inhibits the Proliferation and Metastasis of Gastric Cancer Cells by Targeting PTEN Protein. Altern Ther Health Med 2022.
156. LncRNA PTCSC3 and lncRNA HULC Negatively Affect Each Other to Regulate Cancer Cell Invasion and Migration in Gastric Cancer [Retraction]. Cancer Manag Res 2021, 13:8003–8004.
157. Li H, Xue S, Zhang X, Li F, Bei S, Feng L. CircRNA PVT1 modulated cell migration and invasion through Epithelial-Mesenchymal Transition (EMT) mediation in gastric cancer through miR-423-5p/Smad3 pathway. Regenerative therapy. 2022;21:25–33.
158. Wang X, Song Z, Meng Q, Xia S, Wang C, Huang X: Circular RNA circ_0006089 regulates the IGF1R expression by targeting miR-143–3p to promote gastric cancer proliferation, migration and invasion. Cell cycle (Georgetown, Tex) 2022:1–14.
159. Zheng J, Li C, Zhu Z, Yang F, Wang X, Jiang P, et al. A 5`-tRNA Derived Fragment NamedtiRNA-Val-CAC-001 Works as a Suppressor in Gastric Cancer. Cancer Manag Res. 2022;14:2323–37.
160. !!! INVALID CITATION !!! {}.
161. Che Y, Geng B, Xu Y, Miao X, Chen L, Mu X, et al. Helicobacter pylori-induced exosomal MET educates tumour-associated macrophages to promote gastric cancer progression. J Cell Mol Med. 2018;22(11):5708–19.
162. Azimi M, Totonchi M, Ebrahimi M. Determining The Role of MicroRNAs in Self-Renewal, Metastasis and Resistance to Drugs in Human Gastric Cancer Based on Data Mining Approaches: A Systematic Review. Cell J. 2022;24(1):1–6.
163. Kim CH, Kim HK, Rettig RL, Kim J, Lee ET, Aprelikova O, et al. miRNA signature associated with outcome of gastric cancer patients following chemotherapy. BMC Med Genomics. 2011;4:79.
164. Abbasi A, Hosseinpourfeizi M, Safaralizadeh R. All-trans retinoic acid-mediated miR-30a up-regulation suppresses autophagy and sensitizes gastric cancer cells to cisplatin. Life Sci. 2022;307: 120884.
165. Zhang F, Wang H, Yu J, Yao X, Yang S, Li W, et al. LncRNA CRNDE attenuates chemoresistance in gastric cancer via SRSF6-regulated alternative splicing of PICALM. Mol Cancer. 2021;20(1):6.
166. Mei J, Liu G, Li R, Xiao P, Yang D, Bai H et al: LncRNA SNHG6 knockdown inhibits cisplatin resistance and progression of gastric cancer through miR-1297/BCL-2 axis. Biosci Rep 2021, 41(12).
167. Zhou F, Ding W, Mao Q, Jiang X, Chen J, Zhao X, et al. The regulation of hsacirc_004413 promotes proliferation and drug resistance of gastric cancer cells by acting as a competing endogenous RNA for miR-145-5p. PeerJ. 2022;10: e12629.
168. Shi W, Wang F. circ_AKT3 knockdown suppresses cisplatin resistance in gastric cancer. Open medicine (Warsaw, Poland). 2022;17(1):280–91.
169. Song J, Lin Z, Liu Q, Huang S, Han L, Fang Y, et al. MiR-192-5p/RB1/NF-κBp65 signaling axis promotes IL-10 secretion during gastric cancer EMT to induce Treg cell differentiation in the tumour microenvironment. Clin Transl Med. 2022;12(8): e992.
170. Yang J, Lu J, Yin N, Sun J, Pu J, Zang J. miR-622 Counteracts the NUAK1-Induced Gastric Cancer Cell Proliferation and the Antioxidative Stress. Dis Markers. 2022;2022:9616764.
171. Fang L, Lv J, Xuan Z, Li B, Li Z, He Z, et al. Circular CPM promotes chemoresistance of gastric cancer via activating PRKAA2-mediated autophagy. Clin Transl Med. 2022;12(1): e708.
172. Ma S, Kong S, Wang F, Ju S. CircRNAs: biogenesis, functions, and role in drug-resistant Tumours. Mol Cancer. 2020;19(1):119.
173. Wu HJ, Chu PY: Current and Developing Liquid Biopsy Techniques for Breast Cancer. Cancers (Basel) 2022, 14(9).
174. Ozawa PMM, Jucoski TS, Vieira E, Carvalho TM, Malheiros D, Ribeiro E. Liquid biopsy for breast cancer using extracellular vesicles and cell-free microRNAs as biomarkers. Transl Res. 2020;223:40–60.
175. Lee I, Baxter D, Lee MY, Scherler K, Wang K. The Importance of Standardization on Analyzing Circulating RNA. Mol Diagn Ther. 2017;21(3):259–68.
176. Chen CC, Qian X, Yoon BJ. RNAdetect: efficient computational detection of novel non-coding RNAs. Bioinformatics. 2019;35(7):1133–41.
177. Lee JH, Kim JA, Kwon MH, Kang JY, Rhee WJ. In situ single step detection of exosome microRNA using molecular beacon. Biomaterials. 2015;54:116–25.
178. Xue T, Liang W, Li Y, Sun Y, Xiang Y, Zhang Y, et al. Ultrasensitive detection of miRNA with an antimonene-based surface plasmon resonance sensor. Nat Commun. 2019;10(1):28.
179. Gao J, Zhang H, Wang Z. A DNA tetrahedron nanoprobe-based fluorescence resonance energy transfer sensing platform for intracellular tumor-related miRNA detection. Analyst. 2020;145(10):3535–42.
180. Duan J, Lu G, Xie Z, Lou M, Luo J, Guo L, et al. Genome-wide identification of CRISPR/Cas9 off-targets in human genome. Cell Res. 2014;24(8):1009–12.
181. Bellassai N, D'Agata R, Spoto G. Isothermal circular strand displacement-based assay for microRNA detection in liquid biopsy. Anal Bioanal Chem. 2022;414(22):6431–40.
182. Chen P, Wang L, Qin P, Yin BC, Ye BC. An RNA-based catalytic hairpin assembly circuit coupled with CRISPR-Cas12a for one-step detection of microRNAs. Biosens Bioelectron. 2022;207: 114152.
183. Yang M, Li H, Li X, Huang K, Xu W, Zhu L. Catalytic hairpin self-assembly regulated chameleon silver nanoclusters for the ratiometric detection of CircRNA. Biosens Bioelectron. 2022;209: 114258.
184. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. Ann Oncol. 2021;32(4):466–77.
185. Kalluri R, LeBleu VS: The biology, function, and biomedical applications of exosomes. Sci 2020, 367(6478).
186. Tkach M, Théry C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell. 2016;164(6):1226–32.
187. Lin S, Yu Z, Chen D, Wang Z, Miao J, Li Q, et al. Progress in Microfluidics-Based Exosome Separation and Detection Technologies for Diagnostic Applications. Small. 2020;16(9): e1903916.
188. Zhang YC, Zhou Q, Wu YL. The emerging roles of NGS-based liquid biopsy in non-small cell lung cancer. J Hematol Oncol. 2017;10(1):167.
189. Becker A, Thakur BK, Weiss JM, Kim HS, Peinado H, Lyden D. Extracellular Vesicles in Cancer: Cell-to-Cell Mediators of Metastasis. Cancer Cell. 2016;30(6):836–48.
190. Nair S, Tang KD, Kenny L, Punyadeera C. Salivary exosomes as potential biomarkers in cancer. Oral Oncol. 2018;84:31–40.
191. Chen H, Huang C, Wu Y, Sun N, Deng C: Exosome Metabolic Patterns on Aptamer-Coupled Polymorphic Carbon for Precise Detection of Early Gastric Cancer. ACS nano 2022.
192. Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New Technologies for Analysis of Extracellular Vesicles. Chem Rev. 2018;118(4):1917–50.
193. Fu H, Yang H, Zhang X, Wang B, Mao J, Li X, et al. Exosomal TRIM3 is a novel marker and therapy target for gastric cancer. J Exp Clin Cancer Res. 2018;37(1):162.
194. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. Theranostics. 2017;7(3):789–804.
195. He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018;8(1):237–55.
196. Dobhal G, Datta A, Ayupova D, Teesdale-Spittle P, Goreham RV. Isolation, characterisation and detection of breath-derived extracellular vesicles. Sci Rep. 2020;10(1):17381.
197. An M, Wu J, Zhu J, Lubman DM. Comparison of an Optimized Ultracentrifugation Method versus Size-Exclusion Chromatography for Isolation of Exosomes from Human Serum. J Proteome Res. 2018;17(10):3599–605.

198. Hu T, Wolfram J, Srivastava S. Extracellular Vesicles in Cancer Detection: Hopes and Hypes. Trends in cancer. 2021;7(2):122–33.
199. Wang W, Luo J, Wang S. Recent Progress in Isolation and Detection of Extracellular Vesicles for Cancer Diagnostics. Adv Healthcare Mater. 2018;7(20): e1800484.
200. Royo F, Théry C, Falcón-Pérez JM, Nieuwland R, Witwer KW: Methods for Separation and Characterization of Extracellular Vesicles: Results of a Worldwide Survey Performed by the ISEV Rigor and Standardization Subcommittee. Cells 2020, 9(9).
201. Gardiner C, Di Vizio D, Sahoo S, Théry C, Witwer KW, Wauben M, et al. Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. Journal of extracellular vesicles. 2016;5:32945.
202. Taylor DD, Shah S. Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. Methods. 2015;87:3–10.
203. Weng Y, Sui Z, Shan Y, Hu Y, Chen Y, Zhang L, et al. Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling. Analyst. 2016;141(15):4640–6.
204. Zhang P, He M, Zeng Y. Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/polydopamine coating. Lab Chip. 2016;16(16):3033–42.
205. Lin B, Tian T, Lu Y, Liu D, Huang M, Zhu L, et al. Tracing Tumor-Derived Exosomal PD-L1 by Dual-Aptamer Activated Proximity-Induced Droplet Digital PCR. Angew Chem Int Ed Engl. 2021;60(14):7582–6.
206. Wan Y, Cheng G, Liu X, Hao SJ, Nisic M, Zhu CD et al: Rapid magnetic isolation of extracellular vesicles via lipid-based nanoprobes. Nature biomedical engineering 2017, 1.
207. Liu C, Zhao J, Tian F, Cai L, Zhang W, Feng Q, et al. Low-cost thermophoretic profiling of extracellular-vesicle surface proteins for the early detection and classification of cancers. Nature biomedical engineering. 2019;3(3):183–93.
208. Li B, Pan W, Liu C, Guo J, Shen J, Feng J, et al. Homogenous Magneto-Fluorescent Nanosensor for Tumor-Derived Exosome Isolation and Analysis. ACS sensors. 2020;5(7):2052–60.
209. Wu M, Ouyang Y, Wang Z, Zhang R, Huang PH, Chen C, et al. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc Natl Acad Sci U S A. 2017;114(40):10584–9.
210. Doldán X, Fagúndez P, Cayota A, Laíz J, Tosar JP. Electrochemical Sandwich Immunosensor for Determination of Exosomes Based on Surface Marker-Mediated Signal Amplification. Anal Chem. 2016;88(21):10466–73.
211. Ashcroft BA, de Sonneville J, Yuana Y, Osanto S, Bertina R, Kuil ME, et al. Determination of the size distribution of blood microparticles directly in plasma using atomic force microscopy and microfluidics. Biomed Microdevices. 2012;14(4):641–9.
212. Cao Y, Li L, Han B, Wang Y, Dai Y, Zhao J. A catalytic molecule machine-driven biosensing method for amplified electrochemical detection of exosomes. Biosens Bioelectron. 2019;141: 111397.
213. Liu C, Yang Y, Wu Y. Recent Advances in Exosomal Protein Detection Via Liquid Biopsy Biosensors for Cancer Screening, Diagnosis, and Prognosis. AAPS J. 2018;20(2):41.
214. Thakur A, Qiu G, Ng SP, Guan J, Yue J, Lee Y, et al. Direct detection of two different tumor-derived extracellular vesicles by SAM-AuNIs LSPR biosensor. Biosens Bioelectron. 2017;94:400–7.
215. Saad MG, Beyenal H, Dong WJ: Exosomes as Powerful Engines in Cancer: Isolation, Characterization and Detection Techniques. Biosensors (Basel) 2021, 11(12).
216. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, et al. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. Nanomedicine. 2011;7(6):780–8.
217. Kwizera EA, O'Connor R, Vinduska V, Williams M, Butch ER, Snyder SE, et al. Molecular Detection and Analysis of Exosomes Using Surface-Enhanced Raman Scattering Gold Nanorods and a Miniaturized Device. Theranostics. 2018;8(10):2722–38.
218. Guo K, Li Z, Win A, Coreas R, Adkins GB, Cui X, et al. Calibration-free analysis of surface proteins on single extracellular vesicles enabled by DNA nanostructure. Biosens Bioelectron. 2021;192: 113502.
219. Hiranniramol K, Chen Y, Wang X. CRISPR/Cas9 Guide RNA Design Rules for Predicting Activity. Methods Mol Biol. 2020;2115:351–64.
220. Zhao X, Zhang W, Qiu X, Mei Q, Luo Y, Fu W. Rapid and sensitive exosome detection with CRISPR/Cas12a. Anal Bioanal Chem. 2020;412(3):601–9.
221. Yu D, Li Y, Wang M, Gu J, Xu W, Cai H, et al. Exosomes as a new frontier of cancer liquid biopsy. Mol Cancer. 2022;21(1):56.
222. Kilgour E, Rothwell DG, Brady G, Dive C. Liquid Biopsy-Based Biomarkers of Treatment Response and Resistance. Cancer Cell. 2020;37(4):485–95.
223. Alix-Panabières C. The future of liquid biopsy. Nature. 2020;579(7800):S9.
224. Morganti S, Tarantino P, Ferraro E, D'Amico P, Duso BA, Curigliano G. Next Generation Sequencing (NGS): A Revolutionary Technology in Pharmacogenomics and Personalized Medicine in Cancer. Adv Exp Med Biol. 2019;1168:9–30.
225. Bai Y, Zhao H. Liquid biopsy in tumors: opportunities and challenges. Annals of translational medicine. 2018;6(Suppl 1):S89.
226. Tesfamariam B. Involvement of platelets in tumor cell metastasis. Pharmacol Ther. 2016;157:112–9.
227. Manzoni M, Comolli G, Torchio M, Mazzini G, Danova M. Circulating endothelial cells and their subpopulations: role as predictive biomarkers in antiangiogenic therapy for colorectal cancer. Clin Colorectal Cancer. 2015;14(1):11–7.
228. Galindo-Pumariño C, Collado M, Herrera M, Peña C: Tumor Microenvironment in Metastatic Colorectal Cancer: The Arbitrator in Patients' Outcome. Cancers (Basel) 2021, 13(5).
229. Li K, Lin Y, Luo Y, Xiong X, Wang L, Durante K, et al. A signature of saliva-derived exosomal small RNAs as predicting biomarker for esophageal carcinoma: a multicenter prospective study. Mol Cancer. 2022;21(1):21.
230. Hong L, Xu L, Jin L, Xu K, Tang W, Zhu Y, et al. Exosomal circular RNA hsa_circ_0006220, and hsa_circ_0001666 as biomarkers in the diagnosis of pancreatic cancer. J Clin Lab Anal. 2022;36(6): e24447.
231. Tang S, Cheng J, Yao Y, Lou C, Wang L, Huang X, et al. Combination of Four Serum Exosomal MiRNAs as Novel Diagnostic Biomarkers for Early-Stage Gastric Cancer. Front Genet. 2020;11:237.
232. Heidrich I, Ačkar L, Mossahebi Mohammadi P, Pantel K. Liquid biopsies: Potential and challenges. Int J Cancer. 2021;148(3):528–45.

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