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Liquid biopsy leads to a paradigm shift in the treatment of pancreatic cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most cancers. Its 5-year survival rate is very low. The recent induction of neoadjuvant chemotherapy and improvements in chemotherapy for patients with pancreatic cancer have resulted in improved survival outcomes. However, the prognosis of pancreatic cancer is still poor. To dramatically improve the prognosis, we need to develop more tools for early diagnosis, treatment selection, disease monitoring, and response rate evaluation. Recently, liquid biopsy (circulating free DNA, circulating tumor DNA, circulating tumor cells, exosomes, and microRNAs) has caught the attention of many researchers as a new biomarker that is minimally invasive, confers low-risk, and displays an overall state of the tumor. Thus, liquid biopsy does not employ the traditional difficulties of obtaining tumor samples from patients with advanced PDAC to investigate their molecular biological status. In addition, it allows for long-term monitoring of the molecular profile of tumor progression. These could help in identifying tumor-specific alterations that use the target structure for tailor-made therapy. Through this review, we highlighted the latest discoveries and advances in liquid biopsy technology in pancreatic cancer research and showed how it can be applied in clinical practice.

Key Words: Pancreatic cancer; Cell-free DNA; Circulating tumor DNA; Circulating tumor cells; Exosomes; MicroRNAs

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Core Tip: We focused on liquid biopsy technology for pancreatic ductal adenocarcinoma (PDAC), including circulating free DNA, circulating tumor DNA, circulating tumor cells, exosomes, and microRNAs. We equally described the characteristics of these technologies and reviewed the clinical significance according to the purpose of these biomarkers: early diagnosis, prognosis, prediction of recurrence, and therapeutic response. Although liquid biopsy still has many limitations to its widespread utilization in clinical practice, liquid biopsy has the potential to be applied from diagnosis to treatment. It is expected to improve the prognosis of PDAC radically.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the seventh most common cause of cancer-related death worldwide, and its incidence is increasing[1-3]. In the United States and Japan, it ranks as the fourth leading cause of cancer-related mortality[4,5]. Its 5-year survival rate remains as low as 6% in the United States[6]. Without novel diagnostic methods and/or treatments, it is expected to become the second leading cause of cancer-related deaths by 2030[7]. Due to its early metastatic nature, up to 20% of patients with PDAC are eligible for initial resection[6]. The poor prognosis results from the low resectability rate at diagnosis, with surgery being the only potentially curative treatment. However, even with radical resection, most patients relapse within a year. Moreover, due to the high resistance rate to chemotherapy, radiotherapy, and immunotherapy[8], non-operative treatment has a poorer prognosis with a median survival of 5-9 mo[9].

A variety of genetic and molecular alterations have been identified in PDAC, including mutations in *KRAS*, *p16*, *p53*, *BRCA2*, *Smad4*, etc[10]. However, translating this scientific knowledge into clinical treatment regimens has not yet been achieved. Tumor marker-adjusted treatments for PDAC have been discussed by several authors[11-16]. Carbohydrate antigen 19-9 (CA19-9) is considered the best tumor marker for patients with PDAC. CA19-9 values correlate with tumor size, stage, and burden[17,18]. Therefore, CA19-9 is commonly used to diagnose PDAC, assess resectability, monitor progression, and determine prognosis[19]. Preoperative and postoperative CA19-9 levels predict prognosis[20-24]. Although CA19-9 is a helpful prognostic factor of PDAC, its usefulness remains controversial[19,25-27]. Better tumor markers that correlate with tumor size, predict recurrence after surgery, reflect tumor progression and metastasis, and indicate response to chemotherapy are urgently needed to improve the prognosis of PDAC.

This ideal tumor biomarker should be disease-specific and sensitive. It should also have a high positive predictive value and allows for the detection of disease at very early stages. Furthermore, it should be easy to collect, and the test should be simple and economically feasible. To replace CA19-9 as an alternative biomarker, many researchers have reported the usefulness of liquid biopsy in various cancers. Liquid biopsy is used to diagnose cancer through the detection of circulating tumor cells (CTCs), cell-free and circulating tumor DNA (cfDNA and ctDNA), and microvesicles such as exosomes containing nucleic acids and proteins released from primary tumors and metastases into body fluids. In contrast to surgical or needle biopsy, liquid biopsy is a non-invasive diagnostic method. As a result, it can be detected in real-time and can provide valid information[28-33].

Through this review, we aimed at exploring cfDNA, ctDNA, CTCs, exosomes, and microRNAs (miRNAs) in liquid biopsy technology for early detection, prognostic evaluation, prediction of response to chemotherapy, development of acquired resistance, and early detection of disease relapse, and to evaluate their clinical utility.

CFDNA

cfDNA in plasma possibly originates from necrosis, apoptosis, and/or macrophage digestion of tumoral and healthy cells. Previous studies demonstrated that most of the plasma cfDNA molecules originate from the hematopoietic system in healthy individuals[34,35]. However, in certain physiological or pathological conditions, such as pregnancy, organ transplantation, and cancers, the related/affected tissues could release additional DNA into peripheral circulation[36-38]. In 1948, Mandel *et al*[39] first discovered circulating cfDNA. However, it was not until 1977 that the utility of cfDNA was appreciated when Leon *et al*[36] discovered that significantly elevated levels were detected in patients with cancer compared to healthy controls. In 1989, Stroun *et al*[40] found that some of the cfDNA in the plasma of

cancer patients was derived from cancer cells (called ctDNA). Subsequent studies revealed that cancer cells release ctDNA fragments into blood and other biological fluids such as urine, saliva, and cerebrospinal fluid. ctDNA is highly fragmented, with a shorter fragment length found in cancer patients (134-145 bp) than that of healthy individuals (165-167 bp)[41]. ctDNA is derived from various tumor sites and can provide much more comprehensive genomic and epigenomic information than single-site biopsies. Thus, ctDNA overcomes the issue of tumor heterogeneity, a significant limitation of conventional tissue biopsy. Furthermore, its non-invasive nature allows for continuous real-time monitoring of the molecular status of cancer.

CTCS

CTCs are defined as cells derived from primary, recurrent, or metastatic tumors. CTCs were reported in 1869, and their significance in peripheral blood has been extensively studied in various malignancies[42-46]. It potentially correlated with tumor metastasis and recurrence in breast cancer[42,43], prostate cancer[44], lung cancer[45], and colorectal cancer[46]. Multiple techniques have been developed to explore CTCs, tumor-specific epitopes that are not present in normal blood cells, changes in physical properties such as size, density, and electromechanical properties, or high-throughput imaging to uncollected blood cell preparations[47]. CTCs can travel in the interstitium and bloodstream as single cells as well as clusters[48,49]. Several studies have depicted that CTC clusters correlate with high metastatic potential and poor outcomes. CTC clusters were more significantly associated with distant metastasis than single CTCs[50,51]. Its predictive value has been explored in recent studies in patients with lung, breast, and colon cancer[48,52,53].

EXOSOMES

In 1967, Peter Wolf first discovered that platelets release numerous vesicles[54]. At the time, these vesicles were regarded as cell fragments with no associated biological function. In 1983, exosomes were first observed in sheep reticulocytes, and Johnstone named them "exosomes" in 1987[55]. They are one of the extracellular vesicles (EVs) and have attracted attention in tumor biology recently. Exosomes are 40-150 nm in diameter containing transmembrane proteins, heat shock proteins, nucleic acids (DNA, mRNA, miRNA, long and short non-coding RNA), and enzymes (GAPDH, ATPase, pgk1, RAB)[56-58]. The molecular content of exosomes reflects the nature and state of the cell from which they originate, and their content can alter the function of the recipient cell[59]. In 1996, B lymphocyte-derived exosomes were found to exhibit antigen-presenting properties and induce T-cell responses[60]. Similarly, antigen-presenting exosomes derived from dendritic cells retarded the progression of cancer[61]. These lipid-bilayer vesicles apparently regulate tumor drug resistance, metastasis, and suppression of immune responses. From tumor growth to cellular metastasis, a complex exosome communication network between tumor and non-tumor cells directs all stages[62]. Tumor cells develop exosome-based mechanisms that promote a favorable microenvironment to support tumor growth by facilitating cell metastasis, evading apoptosis, establishing a premetastatic niche, and transporting information from cell to cell[62]. Tumor-derived exosomes can efficiently be captured by various separation methods and gives substantial information about the tumor. In addition, since exosomes are ubiquitously present in body fluids, diffuse through them, and fuse with cell membranes to exert their effects, they are potential tumor therapeutic drug carriers.

MIRNAS

miRNAs are non-coding RNAs that are involved in regulating gene expression. Most miRNAs are transcribed from DNA sequences to become primary miRNAs, then processed into precursor miRNAs and finally mature miRNAs. In most cases, miRNAs interact with 3' untranslated region (3' UTR) of the target mRNA to induce mRNA degradation and translational repression. However, interactions with other regions, such as the 5' UTR, coding sequences, and gene promoters, have also been reported. Under certain conditions, they activate translation or regulate transcription. The interaction of miRNAs with their target genes is dynamic. It depends on many factors, including the intracellular location of the miRNA, the amount of miRNA and target mRNA present, and the affinity of the miRNA-mRNA interaction. MiRNAs are secreted into the extracellular fluid and transported to target cells *via* vesicles such as exosomes or binding to proteins such as Argonaute. Extracellular miRNAs mediate cell-to-cell communication as chemical messengers. The first miRNAs were reported in the nematode, *C. elegans* by the Ambros group at Harvard University in 1993[63]. Ambros *et al*[63] screened for mutants that affect the timing of cell fate switching during nematode development. They identified two genes: *lin-4* and *let-7*. Surprisingly, these genes did not code for proteins but for small RNAs, later called miRNAs. So far,

over 38000 miRNAs have been identified and catalogued in the public database, miRBase (www.mirbase.org). miRNAs are involved in a wide range of processes, including metabolism, cell proliferation, apoptosis, and developmental timing[64]. Overexpressed miRNAs act as regulators of oncogenes (through downregulation of tumor suppressor genes) and/or cellular processes such as cell differentiation and apoptosis. Thus, miRNAs are associated with the development of various types of cancer, including colorectal, breast, ovarian, and endometrial cancer[65-68], but their exact pathways are not fully understood. MiRNAs are involved in cell growth and differentiation regulators and have been proposed to be good candidates for diagnosis and treatment of cancers[69].

CLINICAL SIGNIFICANCE OF LIQUID BIOPSY IN PANCREATIC CANCER

Early diagnosis

KRAS mutations in tumoral tissues have been detected in 90% of PDAC[9,70,71] and the heterogeneity of *KRAS* mutations between primary tumor and metastasis in patients with PDAC[72,73]. Due to high penetration of *KRAS* mutations in PDAC patients, identification of *KRAS* mutations in cfDNA could be a suitable cancer biomarker. CfDNA, however, is immediately removed from the circulatory system by nuclease action and urinary excretion. In addition, uptake by the liver and spleen and degradation by macrophages may also affect its removal from the circulation[74,75]. This short half-life, a few hours at most[74], makes it complicated to detect cfDNA and ctDNA in early-stage cancer.

CTCs was not highly observed in early-stage PDAC, with a sensitivity of < 50%[76,77]. The detection rate of CTCs is 0.0%, 60.7%, 78.6%, and 96.3% of American Joint Commission on Cancer stages I, II, III, and IV patients, respectively, and increases dependence on cancer progression[76]. On the other hand, CTCs have also been detected in patients with early-stage PDAC[78-80], with relatively high sensitivity of over 70%. Kulemann *et al*[81] reported that no difference in the detection rate of CTCs between early-stage and advanced PDAC, which may suggest that CTCs are disseminated from the primary tumor in the early stages of the disease and may be used to diagnose PDAC in the initial stages.

Exosomes have shown promise for early detection of PDAC and proved to be a useful tool clinically [33,82]. Melo *et al*[33] found glypican-1 (the cell surface proteoglycan glypican-1) on tumor-derived exosomes as a diagnostic biomarker in PDAC, which enabled to distinguish healthy persons from patients with benign diseases and patients with early- and late-stage pancreatic cancer with 100% of the sensitivity and specificity. Another study, however, failed to show significant differences in GPC1 between healthy, PDAC and chronic pancreatitis samples while a combination of detection with high levels of exosomal miR-10b, miR-21, miR-30c, and miR-181a and low levels of miR-let7a succeeded to distinguish PDAC from healthy and chronic pancreatitis samples[83]. The EV is also a potent biomarker for early detection. Liang *et al*[84] identified ephrin type-A receptor 2 (EphA2) to distinguish PDAC patients from pancreatitis patients and healthy control. In addition, it discriminated against PDAC patients with early disease (stage I/II), who were potentially benefit from curative surgical resection, from normal healthy control (NC) and pancreatitis cases. The blood levels of EphA2-EV before treatments distinguished stage I/II PDAC patients from NC and pancreatitis accurately.

Differences in expression of miRNA are considered precursors to prominent diagnostic biomarkers of PDAC[85-89]. High expressions of serum exosomal miR-17-5p and miR-21 are likely observed in PDAC patients in connection with metastasis and the advanced stage of PDAC[85]. Studies of miR-10b[86,87] also showed increased levels in exosomes isolated from the plasma of PDAC patients compared to those with chronic pancreatitis or normal controls. Peng *et al*[90] conducted the meta-analysis including 46 studies and found that the sensitivity, specificity, and AUC of circulating miRNAs for discriminating early-stage PDAC patients (0-IIa) from non-PDAC controls were 0.78 (0.76-0.81), 0.78 (0.75-0.80) and 0.85 (0.82-0.88), respectively.

cfDNA, ctDNA, and CTCs are highly detectable in patients with advanced PDAC, but less noticeable in early-stage PDAC while exosomes are secreted into cells from early stages and miRNAs are abundant and relatively easy to detect. These biomarkers, however, are not yet appropriate for early diagnosis in clinical practice and liquid biopsy (especially cfDNA and ctDNA) needs to be improved for the detection capability as a screening tool for PDAC (Table 1).

Prognosis assessment

Numerous articles have been published regarding the prognosis of PDAC using liquid biopsy. It is challenging to detect cfDNA in early-stage PDAC due to small amount of cfDNA at the instance. On the other hand, cfDNA is detected with high probability in plasma from patients with advanced PDAC and high levels of cfDNA are significantly associated with poor prognosis[91-93]. Higher levels of plasma DNA (> 62 ng/mL) is significantly associated with poor outcome in overall survival (OS), presence of vascular encasement and metastasis[91]. Fragment size of cfDNA and cfDNA are investigated in patients with advanced PDAC, where a pretreatment cfDNA fragment size of 167 bp or less and a high pretreatment cfDNA level are associated with shorter progression-free survival (PFS) and OS[92]. Regarding ctDNA, *KRAS* mutations in plasma from PDAC patients were detected in 1999 in connection with poor survival[94]. Then, several studies reported the feasibility of detecting circulating mutant

Table 1 Liquid biopsy in the early diagnosis of pancreatic cancer

Ref.	Journal	No. of patients	Biomarker	Method	Main findings
Ankeny <i>et al</i> [76], 2016	<i>Br J Cancer</i>	72	CTC	Microfluidic NanoVelcro CTC chip	Detection rate of PDAC: 54/72 (sensitivity = 75.0%, specificity = 96.4%)
Rhim <i>et al</i> [77], 2014	<i>Gastroenterology</i>	51	CTC	Microfluidic geometrically enhanced differential immunocapture	CTC (≥ 3) in 33% of patients with cystic lesions and no clinical diagnosis of cancer, 73% with PDAC, and 0% of controls
Xu <i>et al</i> [78], 2017	<i>Int J Mol Sci</i>	40	CTC	NE-iFISH	The positive rate of diagnosis of PDAC is nearly 97% in combination with CA19-9
Poruk <i>et al</i> [79], 2017	<i>Clin Cancer Res</i>	60	CTC	ISSET method & immunofluorescence	The positive rate is 12% in stage I PDAC
Gao <i>et al</i> [80], 2016	<i>J Exp Clin Cancer Res</i>	25	CTC	SE-iFISH platform	Sensitivity of 88 % and specificity of 90 % in PDAC
Kulemann <i>et al</i> [81], 2015	<i>Pancreas</i>	11	CTC	ScreenCell; Cyto kit	No difference in the rate of CTC detection between early-stage and advanced-stage diseases ($P = 0.71$)
Melo <i>et al</i> [33], 2015	<i>Nature</i>	56	Exosome	FACS analysis	The sensitivity and specificity of GPC1+ circulating exosomes in diagnosing PDAC were both 100%
Buscail <i>et al</i> [82], 2019	<i>Cancers</i>	30	Exosome and CTC	FACS, Cellsearch and RosetteSepTM	Combining quantification of GPC1-positive exosomes and CTC detection identified all the PDAC patients, showed a negative predictive value of 100%, and an overall diagnostic accuracy of 91%
Lai <i>et al</i> [83], 2017	<i>Cancer Lett</i>	40	Exosome and miRNA	LC-MS & RT-qPCR	High levels of exosomal miR-10b, miR-21, miR-30c, and miR-181a and low levels of miR-let7a differentiated PDAC from healthy and chronic pancreatitis samples
Liang <i>et al</i> [84], 2017	<i>Nat Biomed Eng</i>	23	EV	nPES assay	Pre-therapy EphA2-EV blood levels accurately distinguished stage I/II pancreatic cancer patients from NC (AUC = 0.96) and pancreatitis patients (AUC = 0.93)
Que <i>et al</i> [85], 2013	<i>World J Surg Oncol</i>	49	miRNA	RT-PCR	Serum exosomal miR-17-5p was higher in PDAC patients than in non-PDAC patients and healthy participants
Cote <i>et al</i> [86], 2014	<i>Am J Gastroenterol</i>	215	miRNA	RT-PCR	Increased expression of miRNA-10b, -155, and -106b in plasma appears highly accurate in diagnosing PDAC
Ouyang <i>et al</i> [87], 2015	<i>Oncogene</i>	42	miRNA	RT-PCR	Plasma miR-10b levels significantly increased in comparison with normal controls
Slater <i>et al</i> [88], 2014	<i>Transl Oncol</i>	59	miRNA	Real-time PCR	A combination test of miRNA-196a and miRNA-196b, whose expression is upregulated from the PanIN state, can identify patients with PanIN 2/3
Madhavan <i>et al</i> [89], 2015	<i>Int J Cancer</i>	220	Exosome and miRNA	miRNeasyMinikit, RT-PCR, qRT-PCR and flow cytometry	The selected miR-1246, miR-4644, miR-3976 and miR-4306 were significantly upregulated in 83% of PDAC serum-exosomes, but rarely in control groups

AUC: Area under the curve; CA19-9: Carbohydrate antigen 19-9; CTC: Circulating tumor cell; EphA2: Ephrin type-A receptor 2; EV: Extracellular vesicles; FACS: Fluorescence-activated cell sorting; ISSET: Isolation by size of epithelial tumor cells; LC-MS: Liquid chromatography-tandem mass spectrometry; miRNA: microRNA; nPES: Nanoplasmon-enhanced scattering; NC: Normal healthy control; NE-iFISH: Negative enrichment immunofluorescence and in situ hybridization of chromosome 9; PDAC: Pancreatic ductal adenocarcinoma; RT-qPCR: Quantitative reverse transcription polymerase chain reaction; SE-iFISH: Subtraction enrichment and immunostaining-fluorescence in situ hybridization.

KRAS genes in the blood of PDAC patients, as well as the prognostic relevance of *KRAS* genes[95-103]. Hadano *et al*[96] assessed the prognosis of patients who underwent curative pancreatoduodenectomy for PDAC according to the presence of *KRAS*-mutated ctDNA before surgery. The median OS of patients with PDAC with ctDNA was significantly worse than that of those without ctDNA (13.6 and 27.6 mo, respectively). ctDNA before surgery is not associated with prognosis or recurrence[97,98]. Bernard *et al*[99] validated the significance of change in ctDNA and exosome DNA (exoDNA) during neoadjuvant chemotherapy in resectable PDAC patients and found that increased exoDNA levels after neoadjuvant chemotherapy were significantly associated with disease progression, whereas ctDNA showed no correlation with outcome. They also elucidated the significance of the presence of ctDNA and exoDNA before chemotherapy in unresectable PDAC patients demonstrating that detection of ctDNA and exoDNA mutant allele frequency (MAFs) $\geq 5\%$ at baseline status were a significantly poor prognosis. Kinugasa *et al*[100] reported that *KRAS* mutations were observed in 62.5% of serum samples of 75 patients with PDAC at all stages using droplet digital polymerase chain reaction (PCR) and were correlated with worse OS. *KRAS* mutations in ctDNA were an independent negative predictor of survival in unresectable pretreatment PDAC patients[101-103].

CTCs serve as prognostic markers in several studies. A recent 9-cohort meta-analysis of separate studies using CellSearch and reverse transcription PCR detection methods involving 623 PDAC patients found an association between detection of CTCs and poor prognosis. Among the 623 patients, 268 patients (43%) with CTCs showed poor PFS and OS compared to the those without CTCs[104]. The different methods of enrichment and detection of CTCs demonstrate that the abundance of CTCs[76, 105-108], circulating tumor microemboli (CTMs)[109], could be predictive of worse survival. Results have varied depending on the isolation techniques, detection methods, and the population of patients. Bidard *et al*[110] investigated the CTC detection rate using CellSearch® in a subgroup of 79 patients with locally advanced PDAC enrolled in the LAP 07 trial; 11% with CTCs had worse OS. Using the CellSearch enrichment method, Kurihara *et al*[111] investigated the significance of CTCs as a biomarker of clinical outcomes in 26 patients. Eleven of 26 patients (42%) showed CTCs. PDAC patients without CTCs exhibited significant longer median survival times of 375.8 d than those with CTCs (110.5 d, $P < 0.001$). de Albuquerque *et al*[112] also reported a worse median PFS in patients with CTC (47% of patients). Detection of CTCs using immunomagnetic epithelial cell adhesion molecule and mucin1 demonstrated longer PFS of 138.0 d in patients without CTCs than in those with CTC (66.0 d). Intriguingly, CTC enumeration is not correlated with clinicopathological features of the disease, including metastasis status and tumor stages. The detection of CTCs alone makes no difference in the prognosis of PDAC patients[113].

The exosomal miRNA-mediated cell-to-cell signaling in the tumor microenvironment plays a significant role in the progression of cancer[114,115]. The proliferation and invasive properties of surrounding cancer cells were shown to be modulated by PDAC exosomal miR-222[116], which was related to poor outcome in PDAC patients[116]. PDAC cells releases exosomes enriched with miR-301a under hypoxic conditions, where circulating exosomal miR-301a-3p levels were positively associated with depth of invasion, lymph node metastasis, late TNM stage, and poor prognosis of PDAC patients[117]. Exosomal miRNAs have been studied to assess PDAC growth, migration, and invasion; further longitudinal studies are essential to identify exosomal miRNAs as prognostic biomarkers for PDAC. Exosomal proteins also play a significant role in PDAC diagnosis. The levels of GPC1 are associated with tumor size and disease burden of PDAC[118]. Macrophage migration inhibitory factor (MIF) is highly expressed in PDAC-derived exosomes and its blockade prevented liver pre-metastatic niche formation and metastasis[119]. MIF was markedly higher in exosomes from patients of stage I PDAC, who later developed liver metastasis, in comparison with advanced PDAC patients, suggesting that increased levels of exosomal MIF could be a biomarker for developing liver metastasis in PDAC patients (Table 2).

Recurrence monitoring

A liquid biopsy is a promising tool to detect minimal residual disease in various gastrointestinal malignancies. Predicting cancer recurrence earlier improves prognosis and provides more options for early consideration of anticancer drugs and operations. Longitudinal monitoring of ctDNA demonstrated to show tumor dynamics in colorectal cancer due to its short half-life[74], which could be available for PDAC.

The monitoring of ctDNA in PDAC patients after surgery reflects early recurrence[97,98,103,120-123]. Multivariate analysis revealed that detection of *KRAS* mutations in postoperative serum was an independent prognostic factor for disease-free survival (DFS), which is associated with recurrence ($P = 0.027$)[97]. Increase of *KRAS* mutated ctDNA after surgery, during periods of adjuvant chemotherapy and observation, was a highly predictive dynamic marker of early relapse[120]. We reported that detection of *KRAS* mutated ctDNA after surgery was associated with OS regardless of recurrence ($P = 0.005$) while increase of CA19-9 was associated with recurrence ($P < 0.001$) but not OS ($P = 0.692$)[98]. Yamaguchi *et al*[124] reported that detection of ctDNA before surgery was significantly associated with poor DFS. Detection of ctDNA before surgery is likely to be associated with recurrence comparing to the monitoring of ctDNA after surgery[125-128]. *KRAS* mutated ctDNA before surgery, especially *KRAS* G12D mutation, serves as a biomarker for early relapse in resectable PDAC patients[125]. Lee *et al*[126]

Table 2 Liquid biopsy in the predicting prognosis of pancreatic cancer

Ref.	Journal	year	No. of patients	Biomarker	Method	Main findings
Singh <i>et al</i> [91], 2015	<i>Cancer Invest</i>	2015		cfDNA		Higher level of plasma DNA (> 62 ng/mL) was found to associate significantly with lower overall survival time ($P = 0.002$), presence of vascular encasement ($P = 0.030$) and metastasis ($P = 0.001$)
Lapin <i>et al</i> [92], 2018	<i>J Transl Med</i>	2018	61	cfDNA	2100 Bioanalyzer	Pre-treatment cfDNA levels could independently predict prognosis for both PFS (HR = 3.049, $P = 0.005$) and OS (HR = 2.236, $P = 0.028$)
Wang <i>et al</i> [93], 2021	<i>Pancreas</i>	2021	97	cfDNA	PCR	The 1- and 5-year survivals for those with high cfDNA were poorer; 70.2% and 21.2%, respectively, as compared with 93.4% and 23.7% for those with low cfDNA level
Castells <i>et al</i> [94], 1999	<i>J Clin Oncol</i>	1999	47	ctDNA	PCR-RFLP and SSCP	Plasma <i>KRAS</i> mutations were identified as the only independent prognostic factor (odds ratio, 1.51; 95%CI: 1.02 to 2.23)
Ako <i>et al</i> [95], 2017	<i>Pancreatolgy</i>	2017	40	ctDNA	ddPCR	<i>KRAS</i> mutation at G12V in the plasma or serum conferred a significantly poorer prognosis than without the mutation ($P < 0.01$)
Hadano <i>et al</i> [96], 2016	<i>Br J Cancer</i>	2016	105	ctDNA	ddPCR	Patients who were preoperative ctDNA+ had a significantly poorer prognosis with respect to OS ($P < 0.0001$)
Nakano <i>et al</i> [97], 2018	<i>Br J Cancer</i>	2018	45	ctDNA	PNA directed, PCR clamping	There were no significant differences in DFS and OS between patients with and without <i>KRAS</i> mutations from preoperative serum
Watanabe <i>et al</i> [98], 2019	<i>PLoS One</i>	2019	78	ctDNA	ddPCR	No effect of the presence of <i>KRAS</i> -mutated ctDNA before surgery on RFS (median: 16.9 mo <i>vs</i> 32.4 mo) was observed
Bernard <i>et al</i> [99], 2019	<i>Gastroenterology</i>	2019	34	ctDNA and exosome DNA	ddPCR	Increased exosome DNA levels after neoadjuvant therapy were significantly associated with disease progression ($P = 0.003$)
Kinugasa <i>et al</i> [100], 2015	<i>Cancer</i>	2015	75	ctDNA	ddPCR	<i>KRAS</i> mutations in plasma correlated with poor OS ($P = 0.002$)
Tjensvoll <i>et al</i> [101], 2016	<i>Mol Oncol</i>	2016	14	ctDNA	PNA clamp PCR	Kaplan-Meier survival analyses indicated that patients with a positive ctDNA before or after initiation of chemotherapy had shorter PFS and OS
Chen <i>et al</i> [102], 2010	<i>Eur J Surg Oncol</i>	2010	91	ctDNA	Direct sequencing	<i>KRAS</i> codon 12 mutation from plasma DNA was an independent negative prognostic factor (HR, 7.39; 95%CI: 3.69-14.89)
Sausen <i>et al</i> [103], 2015	<i>Nat Commun</i>	2015	101	ctDNA	Next-generation sequencing and digital PCR	ctDNA was an independent prognostic marker of OS in advanced disease, with OS of 6.5 mo <i>vs</i> 19.0 mo for ctDNA-positive and negative patients, respectively
Khoja <i>et al</i> [105], 2012	<i>Br J Cancer</i>	2012	54	CTC	Cellsearch and ISET	The PFS and OS for patients without <i>vs</i> those with CTCs was 140 d <i>vs</i> 94 d ($P = 0.13$) and 164 d <i>vs</i> 127 d ($P = 0.26$), respectively
Earl <i>et al</i> [106], 2015	<i>BMC Cancer</i>	2015	45	CTC	Cellsearch	A Cox regression analysis showed a significant difference in OS for CTC positive <i>vs</i> negative patients with a HR of 3.0 ($P = 0.023$)

Zhang <i>et al</i> [107], 2015	<i>Int J Cancer</i>	2015	61	CTC	The EpCAM-independent method	CTCs positive pancreatic cancer patients exhibit a worse ($P = 0.0458$) survival rate
Okubo <i>et al</i> [108], 2017	<i>Eur J Surg Oncol</i>	2017	65	CTC	Cellsearch	A multivariate analysis identified the presence or absence of CTCs as an independent prognostic factor ($P = 0.049$)
Ankeny <i>et al</i> [76], 2016	<i>Br J Cancer</i>	2016	100	CTC	Microfluidic NanoVelcro CTC chip	A cut-off of 3 CTCs in 4 mL venous blood was able to discriminate between local/regional and metastatic disease (AUROC = 0.885; 95%CI: 0.800-0.969; and $P < 0.001$)
Chang <i>et al</i> [109], 2016	<i>Clin Chem</i>	2016	63	CTM	anti-EpCAM conjugated supported lipid bilayer-coated microfluidic chips	CTM was an independent prognostic factor of OS and PFS ($P = 0.0001$ and $P = 0.003$, respectively)
Bidard <i>et al</i> [110], 2013	<i>Ann Oncol</i>	2013	79	CTC	Cellsearch	CTC positivity was associated with poor tumor differentiation ($P = 0.04$), and with shorter OS in multivariable analysis ($P = 0.01$)
Kurihara <i>et al</i> [111], 2008	<i>J Hepatobiliary Pancreat Surg</i>	2008	47	CTC	Cellsearch	MST of the CTC-positive and -negative patients were 110.5 and 375.8 d ($P < 0.001$)
de Albuquerque <i>et al</i> [112], 2008	<i>Oncology</i>	2012	74	CTC		Median PFS time was 66.0 d for patients with baseline CTC positivity and 138.0 days for CTC-negative patients ($P = 0.01$)
Kulemann <i>et al</i> [81], 2015	<i>Pancreas</i>	2015	21	CTC	ScreenCell	The presence of CTC did not adversely affect MST: 16 mo in CTC-positive ($n = 18$) vs 10 mo in CTC-negative ($n = 3$) patients
Li <i>et al</i> [116], 2018	<i>Cell Physiol Biochem</i>	2018	73	miRNA	Arraystar Human miRCURYTM LNA Array	Multivariate analyses showed that exosomal miR-222 was independent risk factors for PDAC survival ($P = 0.046$)
Wang <i>et al</i> [117], 2018	<i>Cancer Res</i>	2018	50	miRNA	qRT-PCR	Exosomal miR-301a-3p overexpression predicted late TNM stage and poor survival in human PDAC ($P = 0.0182$)
Frampton <i>et al</i> [118], 2018	<i>Oncotarget</i>	2018	43	GPC1+ circulating exosomes	ELISA	Patients with high crExos GPC1 levels have significantly larger PDACs (> 4 cm; $P = 0.012$)
Costa-Silva <i>et al</i> [119], 2015	<i>Nat Cell Biol</i>	2015	55	Exosome	ELISA	Increased levels of MIF in exosomes isolated from patients with PDAC with progression of disease post-diagnosis compared with PDAC patients with no evidence of disease five years post-diagnosis ($P < 0.01$) and with healthy controls ($P < 0.01$), but not patients with liver metastasis

AUROC: Area under the curve; cfDNA: Cell-free DNA; ctDNA: Circulating tumor DNA; CTC: Circulating tumor cell; CTM: Circulating tumor microemboli; DFS: Disease-free survival; ddPCR: Droplet digital polymerase chain reaction; ELISA: Enzyme-linked immuno-sorbent assay; EPCAM: Epithelial cell adhesion molecule; ISET: Isolation by size of epithelial tumor cells; HR: Hazard ratio; MST: Median survival time; MIF: Macrophage migration inhibitory factor; miRNA: microRNA; OS: Overall survival; PFS: Progression-free survival; PCR: Polymerase chain reaction; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; PNA: Peptide nucleic acid; PDAC: Pancreatic ductal adenocarcinoma; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; RFS: Recurrence-free survival; SSCP: Single-strand conformation polymorphism.

reported that *KRAS* mutated ctDNA before and after surgery was associated with shorter recurrence-free survival in resectable PDAC patients and all patients with ctDNA had a recurrence. Recurrence after surgery is likely identified earlier by ctDNA than imaging study of computed tomography[103, 121], suggesting that ctDNA may serve as postoperative surveillance.

While preoperative CTCs have been reported to predict tumor recurrence, postoperative CTC monitoring has been rarely evaluated. Park *et al*[129] reported that 12 of 40 patients with CTC (33.3%) in preoperative blood showed a significantly frequent rate of systemic recurrence (distant metastases and peritoneal dissemination). Multivariable logistic regression analysis showed the detection of CTC was an independent risk factor for early recurrence and systemic recurrence.

The detection of exosome-derived KRAS with MAFs of > 1% is associated with worse DFS after resection in patients with localized PDAC[130]. Exosomal miR-451a in plasma of PDAC patients is linked to recurrence after surgery[131]. miRNA derived from portal vein blood exosomes (miR-4525, miR-451a, and miR-21), as well as CTCs, can be utilized for the evaluation of PDAC recurrence[132] (Table 3).

Therapeutic effect monitoring

An accurate real-time understanding of tumor dynamics during chemotherapy helps to select adequate drugs and avoid unnecessary side effects. For this purpose, more sensitive and novel biomarkers are required to overcome disadvantages of conventional biomarkers including CA19-9; however, only few studies have described monitoring using liquid biopsy during chemotherapy in unresectable PDAC, and most of them reported that the prognosis is poor when each marker is detected [78,84,98,99,108,133-137].

The importance of long-term monitoring of ctDNA its predictive ability for the prognosis of PDAC [98,133,134]. In 2018, Kruger *et al*[134] highlighted the cut-off value of ctDNA for detecting early drug response in metastatic PDAC patients who underwent chemotherapy: an increase in ctDNA at day 14 correlated with disease progression determined by subsequent imaging study with a sensitivity of 83% and specificity of 100%. Yin *et al*[138] evaluated ctDNA in patients with PDAC with pathologic complete response (pCR) to neoadjuvant chemotherapy and found its associations with the outcome. They reported that ctDNA existed even in patients with PDAC with pCR to neoadjuvant chemotherapy, thereby predicting early recurrence and reduced survival. These data were obtained using somatic mutations in tumor tissues and CTCs in addition to ctDNA.

Change in number of CTCs was reported in response to neoadjuvant chemotherapy in patients with advanced stage PDAC[138-140]. In a study of 57 PDAC patients who underwent surgery, patients who received neoadjuvant chemotherapy had significantly lower number of CTC than those who were chemotherapy-naïve at the time of surgery[139]. Another study, however, demonstrated no differences in 16 patients who received neoadjuvant chemotherapy[140]. Wei *et al*[135] evaluated CTCs in patients with PDAC who underwent surgery and chemotherapy (modified FOLFIRINOX; oxaliplatin, leucovorin, irinotecan, and fluorouracil, or gemcitabine plus nab-paclitaxel chemotherapy). They reported that the number of CTC decreased or remained same in 12 (92.3%) patients with drug response (either tumor shrinkage or stable tumor burden). Negative enrichment, immunofluorescence, and *in situ* hybridization of chromosome 8 was applied to capturing CTC and determine the role of chromosomal instability in patients with PDAC. Improvement in detection rate of CTCs by using this system revealed the significance of triploid CTCs for the prediction of drug response to chemotherapy in these patients. In addition, CTMs correlated with poor response to chemotherapy in patients having stage IV PDAC [78].

The abundance of exosomal miRNAs was applied to assess therapeutic response. The expression levels of serum miRNAs (miR-221) was significantly upregulated at an earlier time, 3-6 wk of chemotherapy in patients with PDAC without drug response to lapatinib and capecitabine, compared to those without drug response. The expression levels of miRNA (specifically miR-221) increased in PDAC patients who did not respond to lapatinib and 5-fluorouracil (the active form of capecitabine), indicating that the increased level of specific serum miRNAs was associated with resistance to lapatinib and capecitabine treatment[136]. The levels of exosomes in serum at serial time points throughout chemoradiotherapy correlated with treatment resistance in 10 patients with locally advanced PDAC[137]. The levels of EphA2-EV in plasma were strongly associated with treatment response described in the early diagnosis section, reflecting the drug response to neoadjuvant therapy in 23 PDAC patients[84] (Table 4).

CONCLUSION

Liquid biopsy is gaining attention as a non-invasive methodology and is involved in obtaining important tumor information *via* blood-based biomarkers for early diagnosis and treatment of cancer. Tumor components such as CTCs, cfDNA, ctDNA, miRNAs, and exosomes in liquid biopsy have promising value for diagnosis, prognosis, and treatment prediction of surgery and chemotherapy. In particular, the most significant advantage of liquid biopsy over tissue biopsy is its ability to monitor disease progression and treatment efficacy longitudinally in "real time." However, liquid biopsy is not yet considered a standard means of confirming or diagnosing various diseases, including cancer.

The main limitation of liquid biopsy is its lack of sensitivity and accuracy in identifying various types of tumor compared to tissue biopsy. In addition, the ability to detect liquid biopsy is difficult because CTCs, ctDNA, and RNA are relatively scarce compared to other blood components. Furthermore, there are no standard separation, enrichment, and detection methodologies. Applying different techniques and assays to detect CTCs and ctDNA will result in varying sensitivity and specificity[141,142]. Usually, enriched CTCs are identified using tumor-associated biomarkers, either at the protein or mRNA level. However, in patients with epithelial tumors, epithelial markers are downregulated in the process of epithelial mesenchymal transition, making it difficult to identify them, leading to false-negative results

Table 3 Liquid biopsy in recurrence monitoring of pancreatic cancer

Ref.	Journal	No. of patients	Biomarker	Method	Main findings
Nakano <i>et al</i> [97], 2018	<i>Br J Cancer</i>	45	ctDNA	PNA-directed PCR clamping	Multivariate analysis revealed that <i>KRAS</i> mutations in postoperative serum are an independent prognostic factor for DFS ($P = 0.027$). Furthermore, the change from not detecting mutant <i>KRAS</i> in preoperative to mutant <i>KRAS</i> in postoperative cfDNA was an independent prognostic factor for OS ($P = 0.004$)
Hussung <i>et al</i> [120], 2021	<i>BMC Cancer</i>	25	ctDNA	ddPCR, PCR	An increased <i>KRAS</i> mutated ctDNA during adjuvant chemotherapy and follow-up was a highly predictive dynamic marker of early relapse and poor OS
Watanabe <i>et al</i> [98], 2019	<i>PLoS One</i>	78	ctDNA	ddPCR	Detection of mutant <i>KRAS</i> on postoperative ctDNA was associated with OS regardless of recurrence ($P = 0.005$)
Groot <i>et al</i> [121], 2019	<i>Clin Cancer Res</i>	59	ctDNA	ddPCR	ctDNA detected during follow-up predicted clinical recurrence (sensitivity 90%, specificity 88%) with a median lead time of 84 d
Sausen <i>et al</i> [103], 2015	<i>Nat Commun</i>	20 (surgery group)	ctDNA	Next-generation sequencing and digital PCR	Patients with detectable ctDNA after surgical resection ($n = 10$) were more likely to relapse and die from disease compared with those with undetectable ctDNA ($P = 0.0199$)
Jiang <i>et al</i> [122], 2020	<i>Front Oncol</i>	27	ctDNA	Next-generation sequencing	Patients with ctDNA-positive status postoperatively had a markedly reduced DFS compared to those with ctDNA-negative status ($P = 0.019$)
Kim <i>et al</i> [123], 2018	<i>Clin Chem</i>	106	ctDNA	ddPCR	Patients who had increased <i>KRAS</i> MAF values at 6 mo had a shorter OS ($P = 0.036$) than those who had decreased values
Yamaguchi <i>et al</i> [124], 2021	<i>Ann Surg Oncol</i>	97	ctDNA	ddPCR	The multivariate analysis showed that the presence of preoperative ctDNA was associated with poorer OS ($P = 0.008$) and that postoperative ctDNA was not associated with either RFS or OS
Guo <i>et al</i> [125], 2020	<i>Br J Cancer</i>	113 and 44 (discovery and validation cohorts)	ctDNA	ddPCR	Survival analysis showed that plasma <i>KRAS</i> mutations, especially <i>KRAS</i> G12D mutation, had significant association with OS and RFS of resectable PDAC. Plasma <i>KRAS</i> G12D mutation showed a strong correlation with early distant metastasis
Lee <i>et al</i> [126], 2019	<i>Ann Oncol</i>	42	ctDNA	PCR-based-SafeSeqS assays	Preoperative ctDNA detection was associated with inferior RFS ($P = 0.002$) and OS ($P = 0.015$). Detectable ctDNA following curative intent resection was associated with inferior RFS ($P < 0.0001$) and OS ($P = 0.003$)
Pietrasz <i>et al</i> [127], 2017	<i>Clin Cancer Res</i>	31	ctDNA	Next-generation sequencing	The presence of ctDNA was associated with a shorter DFS (4.6 mo <i>vs</i> 17.6 mo; $P = 0.03$) and shorter OS (19.3 mo <i>vs</i> 32.2 mo; $P = 0.027$)
Okada <i>et al</i> [128], 2020	<i>J Gastroenterol</i>	66 (surgery group)	ctDNA	Digital PCR	Patients with preoperative ctDNA MAF $> 0.45\%$ exhibited significantly shorter disease-free survival than those with lower MAF (HR 3.179, 95%CI: 1.025-9.859; $P = 0.0452$)
Park <i>et al</i> [129], 2021	<i>Sci Rep</i>	40	CTC	CD-PRIM kit	On multivariable logistic regression analysis, CTC positivity was an independent risk factor for early recurrence ($P = 0.027$) and systemic recurrence ($P = 0.033$)
Allenson <i>et al</i> [130], 2017	<i>Ann Oncol</i>	142 and 121 (discovery and validation cohort)	Exosome and ctDNA	Electron microscopy, flow cytometry and particle analysis and ddPCR	Higher exosome <i>KRAS</i> MAFs were associated with decreased disease-free survival in patients with localized disease ($P = 0.031$)
Takahasi <i>et al</i> [131], 2018	<i>J Hepatobiliary Pancreat Sci</i>	50	miRNA	qRT-PCR	In cox proportional hazards model analysis, exosomal miR-451a showed significance to OS and DFS ($P = 0.001$, $P = 0.004$)
Kawamura <i>et al</i> [132], 2019	<i>J Hepatobiliary Pancreat Sci</i>	55	miRNA	qRT-PCR	miR-4525, miR-451a, and miR-21 from portal vein can be utilized for the evaluation of pancreatic cancer recurrence ($P = 0.002$, 0.001 and 0.002 , respectively)

ctDNA: Circulating tumor DNA; cfDNA: Cell-free survival; CTC: Circulating tumor cell; DFS: Disease-free survival; ddPCR: Droplet digital polymerase chain reaction; HR: Hazard ratio; MAF: Mutant allele frequency; miRNA: microRNA; OS: Overall survival; PNA: Peptide nucleic acid; PCR: Polymerase chain reaction; PDAC: Pancreatic ductal adenocarcinoma; qRT-PCR: Quantitative reverse transcription polymerase chain reaction; RFS: Recurrence-free survival; SafeSeqS: Safe-sequencing system.

Table 4 Liquid biopsy in the therapeutic effect monitoring of pancreatic cancer

Ref.	Journal	No. of patients	Biomarker	Method	Main findings
Del Re <i>et al</i> [133], 2017	<i>Sci Rep</i>	27	ctDNA	ddPCR	There was a statistically significant difference in PFS and OS in patients with increase <i>vs</i> stability/reduction of ctDNA in the sample collected at day 15 ($P = 0.03$ and $P = 0.009$, respectively)
Kruger <i>et al</i> [134], 2018	<i>Ann Oncol</i>	54	ctDNA	BEAMing	An increase in ctDNA at day 14 correlated with disease progression on subsequent imaging with a sensitivity of 83% and specificity of 100%
Watanabe <i>et al</i> [98], 2019	<i>PLoS One</i>	39	ctDNA	ddPCR	The emergence of <i>KRAS</i> ctDNA in longitudinal tests was associated with prognosis ($P < 0.005$)
Wei <i>et al</i> [135], 2019	<i>Cancer Lett</i>	13 (chemotherapy group)	CTC	Vimentin or EpCAM immobilized microfluidic chip	In patients exhibiting a response, their CTC counts decreased or remained the same, except for one case
Okubo <i>et al</i> [108], 2017	<i>Eur J Surg Oncol</i>	65	CTC	Cellsearch	The overall survival rate was significantly lower in patients with than in those without CTCs even after chemotherapy and chemoradiotherapy ($P = 0.045$)
Xu <i>et al</i> [78], 2017	<i>Int J Mol Sci</i>	83	CTC	NE-iFISH	The proportion of triploid CTC detected by the NE-iFISH was significantly decreased after chemotherapy ($P < 0.001$)
Tian <i>et al</i> [136], 2016	<i>Oncol Lett</i>	17	microRNA	RT-qPCR	Significant upregulation of serum miRNAs (miR-21, miR-210, miR-221 and miR-7), at earlier time points (3-6 wk) was observed in non-responders of chemotherapy compared to responders
Bernard <i>et al</i> [99], 2019	<i>Gastroenterology</i>	104 (chemotherapy group)	exosome and ctDNA	ddPCR	In the longitudinal analysis in chemotherapy group, a MAF peak above 1% in exosome DNA was significantly associated with radiologic progression ($P = 0.0003$)
An <i>et al</i> [137], 2017	<i>J Proteome Res</i>	10	exosome	iTRAQ	They analyzed exosomes before treatment, after one cycle of induction gemcitabine-based chemotherapy, and at 3 wk after starting chemoradiation therapy and compared these samples to serum derived from healthy volunteers. They identified eight proteins that changed during a course of therapy in all patients
Liang <i>et al</i> [84], 2017	<i>Nat Biomed Eng</i>	23 (neoadjuvant chemotherapy group)	EV	nPES	EphA2-EVs were also informative in detecting early responses to neoadjuvant therapy ($P < 0.05$)
Yin <i>et al</i> [138], 2021	<i>Clin Cancer Res</i>	36	somatic mutations, CTCs, and ctDNA	Next-generation sequencing & ISET	Somatic mutations, CTCs, and ctDNA existed even in patients with PDAC with pathologic complete response to NAT, which could possibly predict early recurrence and reduced survival
Poruk <i>et al</i> [140], 2016	<i>Ann Surg</i>	50	CTC	ISET	The detection of CTCs expressing both vimentin and cytokeratin was predictive of recurrence ($P = 0.01$)
Gemenetzi <i>et al</i> [139], 2018	<i>Ann Surg</i>	57	CTC	ISET	Patients who received neoadjuvant chemotherapy had significantly lower total CTCs (tCTCs, $P = 0.007$), eCTCs ($P = 0.007$), and mCTCs ($P = 0.034$), compared with untreated patients eligible for upfront resection

ctDNA: Circulating tumor DNA; CTC: Circulating tumor cell; CR: Complete response; ddPCR: Droplet digital polymerase chain reaction; eCTCs: Epithelial circulating tumor cell; EpCAM: Epithelial cell adhesion molecule; EphA2: Ephrin type-A receptor 2; EV: Extracellular vesicles; iTRAQ: Isobaric tag for relative and absolute quantitation; ISET: Isolation by size of epithelial tumor cells; MAF: Mutant allele frequency; mCTCs: Mesenchymal circulating tumor cell; nPES: Nanoplasm-enhanced scattering; NAT: Neoadjuvant chemotherapy; NE-iFISH: Negative enrichment immunofluorescence and in situ hybridization of chromosome 8; OS: Overall survival; PFS: Progression-free survival; RT-qPCR: Quantitative reverse transcription polymerase chain reaction; tCTC: Total circulating tumor cell.

[143]. Another limitation is the low specificity due to the presence of cfDNA from normal tissue. Furthermore, cfDNA is released from normal cells as a diluent for trace amounts of ctDNA, so an additional pre-step is needed in the analysis to avoid an increase in non-neoplastic cfDNA [144]. Establishing blood noncoding RNA as a biomarker is more complicated than in other common biomarkers due to its lack of suitable housekeeping noncoding RNA reference analytes, and high intra-patient variability. Thus, these limitations can lead to a lack of consistency between biomarkers identified in different studies [145]. Exosomes are more manageable to isolate than CTCs or cfDNA in tumors. Therefore, more studies focus on exosomes in the early diagnosis of cancer. However, they still have limitations in clinical application, such as low targeting efficiency and easy phagocytosis by the immune system. In addition, methods for isolating and purifying exosomes are time and labor-consuming. More multicentered, large-scale, and long-term studies, including clinical trials, are urgently needed to make liquid biopsy clinically available.

Similar to other tumors, cfDNA, ctDNA, CTCs, exosomes, and miRNAs are promising new biomarkers in the treatment of PDAC. However, the use of liquid biopsies in the same manner as conventional tumor markers of PDAC is not clear yet. These liquid biopsies are at least as effective as currently used tumor markers. Furthermore, cost-effectiveness is significant when the marker is used for clinical practice. With the advent of KRAS12c inhibitors, liquid biopsy will be performed many times during treatment. It is also possible that oncogenic carcinomas may acquire somatic mutations, such as EGFRT 790M in lung cancer, which must be monitored. Although liquid biopsy is minimally invasive, new and innovative technologies are needed to reduce the time and effort required for multiple analyses. With advances in cancer genomic medicine, new base mutation-specific inhibitors have been developed or new genetic mutations directly linked to drug resistance have been identified. So, strategies for various cancers may change at an unprecedented pace. Since it is practically difficult to make next-generation sequence analysis mandatory many times during treatment, it will be important in the future to assemble an appropriate analytical system that is as minimal as necessary.

FOOTNOTES

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