Am J Cancer Res 2019;9(12):2567-2579
www.ajcr.us /ISSN:2156-6976/ajcr0103511

Review Article

Clinical applications of liquid biopsies for early lung cancer detection

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Received October 14, 2019; Accepted November 15, 2019; Epub December 1, 2019; Published December 15, 2019

Abstract: Over the past decade, the clinical utility of liquid biopsies in lung cancer has drawn increasing attention. Having been successfully applied in targeted therapy for late stage lung cancer, liquid biopsies are being further investigated regarding their potential role for early detection of lung cancer. Novel biomarkers with high sensitivity and specificity are crucial for identifying patients at early stages as well as for monitoring high-risk populations. A variety of bodily fluids (such as plasma, serum, and sputum) and biomarkers (such as cfDNA, CTCs, gene methylation, and miRNA) have been investigated for their potential role in the diagnosis of lung cancer. In this review, we summarize recent advances in circulating biomarkers regarding the early detection of lung cancer and discuss their potential applications and challenges in clinical settings.

Keywords: Liquid biospy, early lung cancer detection, circulating biomarkers

Introduction

Circulating free DNA (cfDNA) and RNA in the blood were first identified in 1948. This was the first step in so-called 'liquid biopsy' [1]. For the past decade, liquid biopsies have been shown to be an alternative method for cancer diagnosis. These are tests which utilize bodily fluids such as blood and sputum. Liquid biopsies are used to identify tumor cells and tumor-derived products in body fluids. The most investigated analytes of liquid biopsies are circulating tumor cells (CTCs) and circulating cell-free tumor DNA (ctDNA) [2]. Recently, other liquid biopsy biomarkers and analytes including circulating microRNAs (miRNAs), tumor-derived exosomes, and tumor-educated platelets (TEP) have begun to draw more attention from researchers [2].

Compared to regular biopsies, which require surgical extraction of sample cells or tissues, liquid biopsies are non-invasive. Additionally, due to the heterogeneity of tumor tissue, small biopsy samples may not reflect the full genetic image of tumors [3]. For specific cancer types, including lung cancer, it is not only difficult to extract tissue samples, but regular biopsies may increase patients' risk of complications [4, 5]. Hence, liquid biopsies have been largely used to provide diagnostic results for late-stage lung cancer patients [6]. Liquid biopsies provide an excellent alternative method when multiple analyses are needed to monitor tumor progression and treatment response.

In lung cancer, early diagnosis can improve overall treatment outcomes, while the current diagnosis method using imaging techniques often leads to high false positive rates [7]. Hence, liquid biopsies may provide a good alternative and/or complementary method for the early detection of lung cancer. In this review, we will discuss studies utilizing liquid biopsies as tools for lung cancer diagnosis, especially the selection of biomarkers for early detection.

Liquid biopsy in lung cancer

Approximately 80% of lung cancer patients have non-small cell lung cancer (NSCLC) [8], which is one of the leading causes of cancer-related death in the developed world. Diagnosis, disease monitoring, and targeted therapy guided by ctDNA and CTC have been extensively studied. ctDNA has been approved by the United States Food and Drug Administration (FDA) as well as the European Medical Association (EMA).

ctDNA released by tumor cells mainly originate from apoptotic and necrotic cells [9, 10]. A number of somatic mutations in ctDNA, which provide guidance in targeted therapy have been reported [2]. For example, clinical guidelines are available which provide patients harboring epidermal growth factor receptor (EGFR) mutations with personalized treatments utilizing tyrosine kinase inhibitors (TKIs) [11, 12]. Rearrangements of echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the *ALK* gene are another importable, actionable mutation for NSCLC patients [13, 14]. Currently, three US FDA-approved ALK inhibitors are available-crizotinib, ceritinib, and alectinib-while new drugs are being developed. There are several other actionable mutations in genes such as *ROS1* [15], and *PDL1* [16]. However, most mutations identified are in late stage cancer patients as ctDNA concentration is significantly correlated with tumor volume [17], and to identify tumor specific mutations at a low allele frequency, sufficient ctDNA molecules are needed in the circulation system. In recent studies, researchers have indicated an additional complexity in interpreting mutations in peripheral blood cells due to the clonal hematopoiesis (CH) process [18]. For example, a *KRAS* mutation identified in cfDNA does not necessarily originate from tumors, but rather may be the consequence of CH [19].

However, the clinical application of CTCs in NSCLC is still being tested, and is not used in routine clinical practices. This is primarily due to the difficulty in identifying reliable lung CTC markers [21].

Liquid biopsy in early-stage lung cancer

It is critical to diagnose primary lung cancer at an early stage, as this leads to better survival rates. The five-year survival rate is approximately 33% for patients discovered at stage I or II, whereas fewer than 15% of NSCLC patients live more than five years following diagnosis at a later stage [22]. However, early diagnosis of lung cancer is difficult. Currently, the classic detection of early stage lung cancer is carried out using imaging techniques such as low-dose computed tomography (LDCT). However, LDCT screening has a low specificity, which can result in a high false positive rate (96.4%), overdiagnosis, and unnecessary treatment [7].

Clinical applications of ctDNA in the diagnosis and surveillance of resistant mutations in late-stage NSCLC patients have been demonstrated. The next area to explore is its value for early detection. ctDNA concentration in peripheral blood and other bodily fluids increases with tumor size and cancer stage [23]. In early-stage cancer patients, ctDNA in circulation is less representative than in patients with advanced and metastatic diseases [24]. Additionally, free DNA also exists in the circulatory systems of healthy individuals [25], and is present at a high level in patients with benign diseases such as hepatic disorders, diabetes, cardiovascular diseases, non-neoplastic lung diseases or infections [26-28]. It is therefore extremely difficult to extract adequate amounts of tumor DNA molecules in cfDNA, or to identify somatic mutations originating from tumor cells. It has recently been estimated that a tumor volume of 10 cm^3 is needed to sensitively measure variant allele frequencies of 0.1% [29]. However, this required tumor size is far larger than an early stage or asymptomatic tumor would be [29].

Despite the difficulties in utilizing tumor-specific mutation for distinguishing tumor DNA from typical cfDNA, a number of investigators have applied ctDNA for NSCLC early-detection [17, 30-33]. However, the diagnosis sensitivity of mutation-based methods for early-stage cancer patients is either low [17, 30, 33] or has only been explored with a small cohort of samples [31, 32]. To separate tumor DNA from the large amount of background wild-type DNA, novel techniques have been developed, such as targeted deep sequencing, denaturing capillary electrophoresis, mutant enrichment, digital polymerase chain reaction (PCR), and single-molecule sequencing [34].

Biomarker selection in early detection

Alternatively, new liquid biopsy biomarkers in different bodily fluids are been discovered which may be more suitable for early detection [35]. **Table 1** summarizes some of the most recent studies of biomarkers, other than mutation-based methods, which have potential for early detection of lung cancer, with the type of analyte (blood, serum, plasma), detection techniques (PCR based, sequencing based), diagnostic sensitivity and specificity, and cohort size all reported.

cfDNA concentration

In one early study, Sozzi *et al*. found that the amount of cfDNA measured by quantitative reverse transcriptase PCR (qRT-PCR) was much higher in cancer-free heavy smokers [36]. This highlights the option of utilizing the amount of free DNA in plasma to monitor high-risk populations such as heavy smokers [36]. The same authors later measured cfDNA levels in a large cohort of 1,035 heavy-smokers [37]. While no additional diagnosis value was added by cfDNA to spiral computed tomography, it was found that cfDNA levels were associated with patients' survival and may also represent disease aggressiveness. Two other studies using the same method also demonstrated differences in cfDNA concentrations between cancer patients and healthy donors [38, 39]. In one study, diagnosis sensitivity and specificity were 79% and 83%, respectively. However, only four of 104 cases were at an early stage, meaning diagnosis values for early lung cancer may not been accurately estimated [39]. In a study by Paci *et al*., more than half of the 151 patients were at an early stage at the time of measurement; however, relative high sensitivity (86.8%) was accomplished by sacrificing specificity (46.8%) [38]. Unless more studies are carried out with larger cohorts at early stages to demonstrate improved diagnostic accuracy, cfDNA concentration alone does not appear to be a promising biomarker.

Gene methylation

Compared to normal cells, methylation patterns of tumor cells are significantly altered [40]. This epigenetic alteration in DNA during cancer development may occur in a pattern of genome-wide hypomethylation, locus-specific gain or loss of cytosine methylation in promoters associated with CpG islands [41, 42]. In cancer patients, many tumor suppressor genes are methylated and silenced. Many studies have been carried out so that authors can understand the methylation pattern and mechanisms of molecular pathways that lead to cancer [43]. Additionally, gene methylation patterns have emerged as a sensitive and specific biomarker for early cancer detection [44-46].

Methylation of single or multiple tumor suppressor genes in the plasma or serum of lung cancer patients as potential biomarkers have both been reported (**Table 1**). Several studies were conducted to detect the methylation status of short stature homeobox 2 gene (*SHOX2*), which is located at the third chromosome and is 10k bp in size [47]. Two CpG islands are located within *SHOX2*, with one island covering 1k bp in the 5'-region and the other a 500 bp island in the 3'-region of the gene. In lung cancer, hypermethylation of *SHOX2* is associated with SCLC and squamous cell carcinoma [48]. Two separate studies using real-time PCR and methylation-specific HeavyMethyl assay have shown that lung cancer patients and non-cancer individuals can be distinguished by plasma *SHOX2* methylation status at a sensitivity of 60% and 81%, and specificity of 90% and 79%, respectively [48, 49]. Methylation of *SHOX2* genes can also be analyzed in bronchial aspirates [50, 51], resulting in a comparable performance to plasma (**Table 1**). An epigenetic signature of four genes, namely *BCAT1*, *CDO1*, *TRIM58*, and *ZNF177*, was discovered, all four of which were hypermethylated in stage I lung cancer patients [52]. The discovery of this epigenetic signature also validated using bronchial aspirates and bronchoalveolar lavages [52]. All of this highlights the possibility of using methylation status of one or more genes as biomarkers in early lung cancer diagnosis.

Circulating tumor cells

It has been demonstrated that circulating tumor cell counts are a promising biomarker for lung cancer diagnosis [53] and prognosis [54] as well as monitoring of therapy response [55]. Sequential CTC monitoring can provide relevant clinical information for patients' responses to treatment, meaning they have been used as biomarkers in ongoing clinical trials [56, 57].

Table 1. Biomarker and analyte of liquid biopsy in early lung cancer detection

Biomarker	Analyte	Gene	Technology	Sensitivity (%)	Specificity (%)	Cancer stage and the number of patients	Reference
CfDNA amount	Plasma	*TERT*	qRT-PCR	90	86	I: 34 II: 25 III: 38 IV: 3	[36]
CfDNA amount	Plasma	*TERT*	qRT-PCR	79	83	I-II: 4 III-IV: 100	[39]
CfDNA amount	Plasma	*TERT*	qRT-PCR	85.8	46.8	I: 77 II: 18 III: 42 IV: 14	[38]
Gene methylation	Plasma	*SHOX2*	Real-time PCR and methylation-specific HeavyMethyl assay	60	90	I: 37 II: 29 III: 53 IV: 42	[48]
Gene methylation	Plasma	*SHOX2*	Plasma Methylation-specific real-time PCR	80.65	78.57	I: 1 II: 4 III: 8 IV: 22	[49]
Gene methylation	Bronchial aspirates	*SHOX2*	Real-time PCR and methylation-specific HeavyMethyl assay	68	95	I: 59 II: 43 III: 108 IV: 62	[50]
Gene methylation	Bronchial aspirates	*SHOX2*	Real-time PCR and methylation-specific HeavyMethyl assay	78	96	I-IV: 63	[51]
Gene methylation	Bronchial aspirates and bronchoalveolar lavages	*CDO1, BCAT1, TRIM58,* and *ZNF177*	Pyrosequencing	84.6	81	I: 5 II: 6 III: 21 IV: 18	[52]
miRNA	Plasma	12 miRNAs	qRT-PCR	73.3	96.5	I: 28	[74]
miRNA	Plasma	miR-21, miR210, miR-486-5p	qRT-PCR	75	85	I: 24 II: 30 III and IV: 22	[73]
miRNA	Plasma	miR-155, miR-197, miR-182	Real-time RT-PCR	81.3	86.8	I: 21 II: 12 III: 11 IV: 30	[75]
miRNA	Plasma	15 miRNAs	Microfluidic cards	80	90	I: 15 II-III-IV: 7	[76]
miRNA	Plasma	24 miRNAs	qRT-PCR	87	81	I: 37 II-III: 12 IV: 19	[77]
miRNA	Serum	miR-1254, miR-574-5p	Microarray profiling	82	77	I and II: 10	[80]
miRNA	Serum	30 miRNAs	qRT-PCR	Overall: 71 Stage I: 59	Overall: 90 I: 90	I: 22 II-IV: 12	[81]

miRNA	Serum	miR-146b, miR-221, let-7a, miR-155, miR-17-5p, miR-27a, miR-106a, miR-29c	qRT-PCR	NA	NA	I: 180 II: 40	[22]
miRNA	Serum	13 miRNAs	qRT-PCR	77.8	74.8	I: 31 II-III: 5	[82]
miRNA	Serum	miR-15b-5p, miR-16-5p, miR-20a-5p	Fluorescence quantum dots liquid bead array	94.3	94.2	I: 49 II-III: 21	[83]
miRNA	Sputum	miR-21	Real-time RT-PCR	69.7	100	I: 3 II: 5 III: 7 IV: 8	[96]
miRNA	Sputum	miR-21, miR-486, miR-375, miR-200b	Real-time RT-PCR	I: 69.2 II: 69.9 III: 71.5 IV: 70.9	I: 81.7 II: 82.5 III: 79.1 IV: 82.7	I: 16 II: 15 III: 17 IV: 16	[85]
miRNA	Sputum	miR-31, miR-210	qRT-PCR	65.2	89.7	I: 17 II: 18 III-IV: 18	[86]
miRNA	Sputum	miR-21, miR-31, miR-210	qRT-PCR	Set 1: 82.1	Set 1: 88.4	Set 1 I: 45 II: 22	[87]
				Set 2: 80.5	Set 2: 86.1	Set 2 I: 51 II: 25	
miRNA	Sputum	miR-223	Real-time RT-PCR	82	95	I: 2 II: 3 III: 5 IV: 7	[88]
Exosome miRNA	Plasma	Set 1: miR-378a, miR-379, miR-139-5p, miR-200b-5p	qRT-PCR	Set 1: 97.5	Set 1: 72	Set 1 I: 30	[90]
		Set 2: miR-151a-5p, miR-30a-3p, miR200b-5p, miR-629, miR-100, miR-154-3p		Set 2: 90.8	Set 2: 96.0	Set 2 I: 50	
Exosome miRNA	Plasma	let-7b-5p, let-7e-5p, miR-23a3p, miR-486-5p	qRT-PCR	80.3	92.3	I: 60	[71]
TEP mRNA profile	Blood	1072 RNAs	RNA-seq	All cancer types: 97	All cancer types: 94	NSCLC (stage: NA): 24	[92]
TEP tumor driver gene	Platelet and plasma	*EML4-ALK* rearrangements	RT-PCR	Platelet: 65 Plasma: 21	Platelet: 100 Plasma: 100	Platelet (stage: NA): 67 Plasma (stage: NA): 32	[94]

CTC surveillance of chronic obstructive pulmonary disease (COPD) patients may allow diagnosis of early-stage lung cancer [58]. Several studies of CTCs as biomarkers in lung cancer have been summarized in a review by Hanssen *et al*. [59]. Techniques for detecting CTCs in blood can be divided into two groups: label-dependent and label-independent methods. In a typical label-dependent method, an enrichment step is required, in which CTCs are enriched using protein markers that are expressed by CTCs rather than the surrounding blood cells. The cell surface protein epithelial cell adhesion molecule (EpCAM) will later be used as capture antigen [59]. Typically, in the label-independent or EpCAM-independent method, epithelial tumor cells will be isolated by size [59].

In **Table 1**, we have also summarized examples of CTCs in lung cancer diagnostics which include patients with early-stage cancers. Overall, performance of CTCs has low sensitivity (30-89%) but high specificity (88-100%) for distinguishing lung cancer patients from healthy individuals (**Table 1**). The EpCAM-based method has a poorer performance than the cell size based method, which is consistent with the positivity rate summarized in a previous review [59]. CTCs were detected in 30.6% of lung cancer patients in a study of 150 patients, which illustrated the utility of CTCs for predicting metastatic disease [60]. In another study, CTC type was associated with the outcome of systematic therapies, although no sensitivity or specificity was given for the diagnostic purpose of CTCs [61]. One group of researchers conducted two studies with a cohort in which 65%-70% of the patients were at stage I or II. They consistently found no detection of CTC in healthy donors, while 49% of patients had detectable CTCs [62, 63]. They also indicated a correlation between levels of CTCs and the risk of recurrence and death in early-stage, resectable NSCLC [62]. In a later study, 77 patients were recruited, 44 of which were at early stages. A better diagnosis performance was reported (sensitivity: 89% and specificity: 100%), using CTCs isolated by size [64]. Additionally, CTCs were found to be predictive of survival in a cohort of 97 I-IV stage SCLC patients [65]. In a consolidation of 20 studies with a total of 1576 NSCLC patients, an association between CTC and patients' overall survival and cancer-free survival was indicated [66].

It would appear that CTCs have values as biomarkers in clinical lung cancer diagnosis and prognosis. However, techniques for isolating and estimating CTCs still need to be improved to achieve reliable and consistent results with better sensitivity. This also requires a standardization of experimental processes once the optimal method has been developed and validated.

microRNAs

While RNA is not stable in circulating bodily fluids, miRNAs, which are fragments of single-stranded non-coding RNA and have a length of 19 to 25 nucleotides, are extractable and detectable in blood [67]. In the past decade, the application of miRNAs in clinical settings as biomarkers for lung cancer early detection has drawn much attention, as reviewed by Han *et al*. [68]. miRNAs can regulate the expression of genes in cancer processes by targeting messenger RNAs (mRNAs). miRNA profiles in plasma, serum, sputum, and exosome are distinguishable between lung cancer patients and healthy donors [69-72].

Researchers of several studies have adopted a quantitative or real-time PCR-based method to quantify miRNA profiles in plasma, demonstrating the potential utility of plasma miRNA expressions for early detection of lung cancer (**Table 1**). Sensitivity and specificity utilizing plasma miRNA profiles to differentiate tumor patients, healthy donors or individuals with solitary pulmonary nodules (SPNs) range from 73 to 87%, and 81 to 97%, respectively (**Table 1**). Shen *et al*. found that expression levels of plasma miR-21 and miR-210 were higher, whereas miR-486-5p had a lower expression level in patients with malignant SPNs compared to healthy donors or individuals with benign SPNs [73]. In a separate study with a cohort of stage I patients, the same group of researchers found that the miRNA predictor of four miRNAs: miR-21, miR-210, and miR-486-5p, and miRNA-126, was able to distinguish NSCLC patients from healthy controls at a sensitivity of 86.22% and specificity 96.55% [74]. Three miRNAs (miR-155, miR-197, and miR-182) in the plasma were reported to be elevated in lung cancer patients, including those at stage I, compared to controls [75]. In addition to the four miRNAs discovered by Shen et al. [73, 74], 52 more miRNAs were differentially expressed in the plasma

of cancer patients and healthy donors. An miRNA signature of 16 ratios consisting of 15 miRNAs was identified which had a diagnosis value and could predict the disease up to 28 months before detection by computed tomography (CT) [76]. The prognosis value of miRNA profiles has been further demonstrated by the association between a separately identified miRNA signature and disease aggressiveness [76]. Sozzi *et al*. demonstrated the predictive, diagnosis, and prognosis values of miRNAs and found that miRNAs can reduce false positive rates by LDCT [77]. Sozzi *et al*. identified four different miRNA signatures using 24 miRNAs for the purpose of assessing risk of disease, risk of aggressive disease, presence of disease, and presence of aggressive disease [77]. Among the 24 miRNAs identified, miR-21, miR-486-5p, miR-126, and miR-197 overlapped with previous studies [74, 75]. Given that most of these studies have small sample sizes, more studies consisting of larger cohorts with high-risk population are needed, such as the BIOMILD study [78].

miRNAs can also be detected in serum. miRNA profiles in serum and plasma can differ due to the impact of the coagulation process on the spectrum of extracellular miRNA in blood [79]. A number of studies using serum miRNAs in lung cancer early detection are included in **Table 1**. In most of these, more than half or all patients recruited were at early stages (**Table 1**). Overall, serum miRNA profiles have comparable diagnosis performances with plasma miRNA profiles, of which sensitivity and specificity range from 59 to 94%, and 75 to 94%, respectively (**Table 1**). The miRNAs found in these studies varied dramatically. Foss *et al*. reported two miRNAs in serum, miR-1254 and miR-574-5p, which had increased expression levels in early stage lung cancers [80]. Bianchi *et al*. developed a model based on detection of 34 miRNAs from serum to identify patients with early stage NSCLC when screening high-risk individuals [81]. A set of miRNAs (miR-146b, miR-221, let-7a, miR-155, miR-17-5p, miR-27a, and miR-106a) was expressed in serum at a lower level in early-stage NSCLC compared to non-cancer controls, while miR-29c was expressed at a higher level [22]. These researchers also found no correlation between serum and plasma miRNA profiles, which is consistent with the speculation of the impact in the coagulation process. They also implied the prognosis value of miR-223 for predicting cancer-specific mortality in stage I patients [22]. Subsequently, a miRNA signature of 13 miRNAs was validated in a lung cancer screening among 1,115 high-risk individuals [82]. Those 13 miRNAs are miR-92a-3p, miR-30b-5p, miR-191-5p, miR-484, miR-328-3p, miR-30c-5p, miR-374a-5p, let-7d-5p, miR-331-3p, miR-29a-3p, miR-148a-3p, miR-223-3p, and miR-140-5p. In another study, a model of three miRNAs (miR-15b-5p, miR-16-5p, and miR-20a-5p) was developed for lung cancer diagnosis with a cohort of 70 NSCLC patients and 54 healthy controls [83]. Geographical or racial factors need to be taken into consideration as it has been found that cohorts from different countries display different miRNA profiles. For example, concentration of miR-214 was higher in an American cohort than a Chinese cohort [84].

In addition to miRNAs expressed in serum or plasma, miRNAs extracted from sputum also provide a good candidate biomarker in lung cancer early detection. Researchers of several studies using sputum miRNA profiles have demonstrated a comparable diagnosis performance (**Table 1**), while sputum is even less invasive than a blood draw. Preliminary results have less variability in the selection of miRNAs in sputum samples. miR-21, miR-31, and miR-210 have been found in several studies [85-87], while miR-223 was only reported by Bagheri *et al*. [88].

Exosomes are micro-vehicles, which transport different types of molecules and are critical in cell-cell communication [69]. Tumor-derived exosomes play significant roles in cancer pathogenesis, therapy, and diagnosis [89]. These extracellular vesicles also contain miRNAs, which can be transferred to target cells. Cazzoli *et al*. found two sets of exosome miRNAs, which not only allowed them to distinguish lung nodule patients (lung adenocarcinomas and carcinomas) from healthy former smokers, but also to distinguish lung adenocarcinoma and granuloma [90]. A more recent study also indicated that exosome miRNAs could discriminate between subtypes of NSCLC, such as adenocarcinoma and squamous cell carcinoma [71].

miRNAs have so far drawn the most attention in early lung cancer detection, where they have indicated acceptable diagnosis performances. However, some hurdles are yet to be overcome

when comparing and consolidating data. These hurdles include: a) the variation in analytes such as plasma, serum, sputum and the selection of specific miRNAs; b) the methods to use for determining miRNA profiles: directly comparing miRNA expression levels or models based on multiple miRNA ratios and/or expression levels. The use of miRNA for the diagnosis of early lung cancer also requires a standardization of the process for its application in clinical settings, and studies with large cohorts are required to validate its stability and reproducibility.

Tumor-educated platelets

Blood platelets are the second most-abundant cell type in peripheral blood and tend to be contained in the so-called 'buffy coat' of blood. Blood platelets are known for their role in hemostasis and initiation of wound healing [91]. Tumors can 'educate' platelets by changing mRNA profile, thus providing a valuable platform for diagnostics across different cancer types [92]. More recently, the focus of studies of liquid biopsies for early detection has expanded to tumor-educated platelets. Blood platelets can interact with tumor cells and affect tumor microenvironment, in turn impacting tumor metastasis and growth [93]. The altered RNA profile of blood platelets not only allows differentiation between patients with localized and metastasized tumors from 55 healthy individuals, but also allows identification of specific tumor mutations in genes such as *MET*, *HER2*, *KRAS*, *EGFR*, and *PIK3CA* [92]. This is a promising start in the utilization of TEP as biomarker, although larger cohorts of high-risk individuals need to be screened, tested and followed-up. Researchers of another study using both TEP and plasma to identify *EML4-ALK* rearrangements pointed out the superior performance of TEP as an analyte with a sensitivity of 65% compared to 21% sensitivity in plasma, while specificity for both were 100% [94]. Additionally, TEPs can pinpoint the location of the primary tumor, which may facilitate the use of liquid biopsies for cancer diagnostics [92]. However, the cancer stage of patients in these studies was not specified. Hence, the value of TEP in early cancer detection is yet to be determined.

Conclusions

Liquid biopsies are a promising tool in lung cancer diagnosis and prognosis, and may be able to guide treatment as well as monitor response to treatments. Crucially, liquid biopsy approaches based on ctDNA, CTC, gene methylation, miRNAs, and mRNA provide new avenues for early detection of primary cancers. While the feasibility of these liquid biopsy analyses using various analytes and biomarkers have been demonstrated, the combination of classic methods such as imaging techniques with liquid biopsy may lead to a better sensitivity. However, challenges remain due to limitations in the current understanding of carcinogenesis biology.

While multiple analytes and biomarkers are available for early detection, there are as yet no biomarkers, which could provide a highly sensitive, screening test or that have been validated in a large cohort of samples. The variation in biomarker selection remains one of the most challenging problems as, for example, different miRNAs have been identified in a variety of studies. Multiple platforms such as RT-PCR-based method and next generation sequencing (NGS) have great potential, whilst bringing in other challenges such as process standardization for different methods, and cross-platform assay validation. Most of the studies in this review were conducted in one research or medical institute. Hence, numbers of patients or high-risk individuals recruited were sometimes too limited to validate early detection biomarkers. Larger-scale, multi-center collaboration is therefore highly encouraged.

cfDNA-guided personalized therapy has already emerged in clinical practice. For example, NSCLC patients who are harboring *EGFR* T790M mutation based on cfDNA can receive treatment with TKIs [95]. However, many aspects are yet to be explored for utilizing liquid biopsy-based methods for early detection. For clinical implementation, highly specific, sensitive and reproducible tools are needed for lung cancer screening and early detection. Multiple clinical trials for early lung cancer using liquid biopsies are ongoing (www.clinicaltrials.gov). The future of liquid biopsy methods for improving cancer management is dependent on outcomes compared to the current standard of care.

Acknowledgements

This study was supported by the Science and Technology Project of Guangdong Province (no. 2017B090901067).

Disclosure of conflict of interest

None.

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