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Review Article

Liquid biopsy for early detection of lung cancer

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ABSTRACT

Lung cancer is the leading cause of cancer-related mortality worldwide. Early cancer detection plays an important role in improving treatment success and patient prognosis. In the past decade, liquid biopsy became an important tool for cancer diagnosis, as well as for treatment selection and response monitoring. Liquid biopsy is a broad term that defines a non-invasive test done on a sample of blood or other body fluid to look for cancer cells or other analytes that can include DNA, RNA, or other molecules released by tumor cells. Liquid biopsies mainly include circulating tumor DNA, circulating RNA, microRNA, proteins, circulating tumor cells, exosomes, and tumor-educated platelets. This review summarizes the progress and clinical application potential of liquid biopsy for early detection of lung cancer.

Introduction

Lung cancer is one of the deadliest malignant tumors and the leading cause of cancer death, with almost 1.8 million global deaths a year[1] and over 130,000 deaths in 2022 in the United States alone.[2] There are two main types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for about 85% of all lung cancer types.[3] In the past decade, lung cancer mortality was slowly decreasing, coinciding with changes in medical practice related to cancer screening and introduction of the new treatment options including targeted therapies and immunotherapy.[2] However, over 70% of lung cancer patients are diagnosed at later stages when the disease has already spread, and 5-year relative survival rate for patients with lung cancer is only 22%,[2] therefore it is important to find new efficient methods for early diagnosis of the disease, efficient treatment selection and disease monitoring.

Low-dose spiral computed tomography (LDCT) scanning is a noninvasive medical imaging test that has been used for the early detection of lung cancer for over 20 years.[4] Currently, lung cancer screening is recommended for certain people at high risk of the disease, but who do not have any signs or symptoms.[3] Multiple clinical trials conducted over several years reported reduction in lung cancer specific mortality rate with chest LDCT screening among high-risk individuals, but data varied between different trials,[5] and almost half of people detected as having lung cancer via their participation in LDCT screening are overdiagnosed, which results in unnecessary invasive follow-up procedures and psychological burden.[6] LDCT screening is also associated with repeat radiation exposure, and the rate of compliance is low: in 2018, only 4% of eligible patients in the United States were screened.[7] Tumor tissue biopsy is still required for cancer diagnosis and staging and represents the standard biological sample for molecular analysis. However, this approach has significant limitations due to the invasiveness of the procedure, limited amount of material for molecular testing and difficulty of repeat testing.[8] Tissue biopsies also have limitations due to tumor heterogeneity, and a single biopsy may not be representative of the entire genetic complexity of the tumor.[9]

Finding safe and efficient methods for screening and early detection of lung cancer that can be used as standalone tools or complement existing routine diagnostic procedures could significantly improve patients' survival. Liquid biopsy, a test that can detect products derived from a tumor in body fluids, for example, in a simple blood draw, holds great promise as a non-invasive, easy and accessible tool that can supplement or overcome the limitations of currently used methods for the early detection of lung cancer.[10] Major types of liquid biopsy analytes are summarized in Table 1.

Advantages of liquid biopsy

In recent years, circulating biomarkers became a target of extensive interest, and multiple studies were dedicated to finding novel markers that can aid in diagnostics, detection of minimal residual disease (MRD), prognosis, treatment selection and monitoring of cancer,[11,12] including early detection of lung cancer.[10,13-17] A biomarker is a biological observation that can be objectively measured and evaluated as an indicator of biological and pathogenic processes and ideally predicts a clinically relevant outcome that is more difficult to observe.[18] Circulating biomark-

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Table 1
Comparison of liquid biopsy analytes for early detection of lung cancer.

Analyte	Advantages	Challenges	References
ctDNA, cfDNA mutation analysis	Elevated in cancer patients; genetic alterations represent tumor tissue	ctDNA has low concentration compared to germline cfDNA; low minor allele frequency	Bettegowda et al[25], Duffy[117]
DNA methylation	Representative of tumor tissue; distinct tumor-specific methylation patterns	Low ctDNA concentration; lack of standard detection methods	Farooq and Herman[118], Li et al[46]
DNA fragmentomes	Scalable, cost-effective	Variability; low sensitivity in early-stage disease	Mathios et al[69]
Circulating tumor cells (CTCs)	Reflects molecular characteristics of tumors	Very rare in bloodstream; difficult to isolate	Kapeleris et al[119,120]
MicroRNA	Stable in blood; distinct RNA profiles in early-stage cancers	High variability in different studies; low specificity for a cancer type	Frydrychowicz et al[121]
Exosomes	Increased in cancer patients; contain nucleic acid and protein biomarkers	Lack of standard detection methods; high costs	Cui et al[122]
Tumor-educated platelets	Easy to isolate; distinct RNA profiles; RNA represents tumor transcriptome	High variability; lack of standard detection methods	Best et al[97]
Protein biomarkers	Established analysis methods	Poor sensitivity; low specificity for a cancer type	Casillas et al[103], Baran and Brzeziańska-Lasota[106]

cfDNA: Cell-free DNA; ctDNA: Circulating tumor DNA.

ers have the potential to improve early diagnosis of lung cancer either as a screening or a diagnostic tool used alone or in combination with imaging.

In lung cancer, the application of liquid biopsies may be particularly important due to the invasiveness of lung tumor biopsies and a higher risk of potential complications from the procedure, including occasional reports of death.[19] Liquid biopsies have many advantages including non-invasiveness, lower cost, potential for genomic testing, ability to monitor tumor evolution through treatment, and the ability to overcome tumor heterogeneity.[20] The potential clinical applications of liquid biopsy include screening, diagnostics, detection of MRD after surgery, identifying targetable mutations, monitoring response to treatment including immunotherapy, and identifying relapse and resistance to treatment. While several clinical applications of liquid biopsy have been approved by Food and Drug Administration (FDA) for therapy selection in lung cancer, including Guardant360 CDx and FoundationOne Liquid CDx, the role of liquid biopsy in the early detection of lung cancer, though very promising, is not yet defined. There are hopes that liquid biopsies will become common diagnostic applications in routine lung cancer screening and early diagnosis.

Cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA)

Cell free DNA (cfDNA) is a result of the normal physiological process in the human body, including tissue remodeling and apoptosis.[21] Presence of cell free DNA in the serum of cancer patients was discovered over 40 years ago, when researchers found that in cancer patients and in patients with metastatic disease, cfDNA is present at higher levels compared to the healthy individuals.[22] CfDNA in cancer patients can carry fragments of 180–200 base pairs in length originating from tumor cells called circulating tumor DNA (ctDNA) and provide non-invasive access to the tumor characteristics without requiring a tissue biopsy,[23,24] including mutations in oncogenes or tumor suppressor genes,[25] chromosomal alterations[26] and epigenetic changes[27]. The concentration of ctDNA in plasma can vary significantly and correlates with many factors including type and size of the tumor and disease stage.[25] A study by Newman et al[28] using an ultra-sensitive sequencing technology was able to detect ctDNA in 100% of analyzed stage II–IV NCSLC patients and in 50% of early-stage patients, which holds a potential for diagnostic applications. However, challenges exist in achieving sensitivity desired for the early lung cancer screening and diagnosis. A mathematical model developed to predict the ctDNA shedding rate of early-stage NSCLC[29] estimated that there would be an average of only 1.7 genome copies of ctDNA in 15 mL of blood for lung tumors with a volume of 1 cm^3. It was also shown that the presence of high cfDNA concentrations was associated with the worse clinical outcomes.[30,31] Analysis of ctDNA for genomic and epigenomic alterations, fragmentation patterns and other molecular characteristics holds a great promise for clinical applications, including early detection of cancer.[32]

Two major methodologies have been used for ctDNA analysis: targeted approach that focuses on specific genomic regions known for their role in particular cancer types that can harbor specific genetic or epigenetic variations or gene mutations, or genome-wide approach that offers a broader analysis and monitoring of the tumor genome independent of any prior data on molecular alterations.[12] Targeted approaches include polymerase chain reaction (PCR)-based methods such as routine quantitative PCR[33] or highly sensitive droplet digital PCR and BEAMing that have shown sensitivity of 0.001% to 1% in detecting somatic point mutations[34,35]. Broad screening utilizes various next-generation sequencing (NGS) methods that offer high sensitivity for detection of as low as 0.02% ctDNA mutant fractions.[28,36]

Mutation analysis of ctDNA

Detection of mutations in ctDNA has the potential to be used in early cancer detection and diagnosis, to detect MRD, to evaluate eligibility for immunotherapy, to predict disease outcome, to monitor response to therapy and to assess potential resistance to the treatment.[37] Genomic testing is recommended for all NSCLC patients and is included in National Comprehensive Cancer Network (NCCN) guidelines.[38,39] In 2016, the FDA approved the first liquid biopsy genetic test for NSCLC—Cobas EGFR Mutation Test v2 liquid biopsy test from Roche Diagnostics that detects specific mutations in the ctDNA in the blood of people with NSCLC.[40] By identifying these mutations, physicians can select the best targeted therapy that may be helpful in treating cancer. Targeted therapies work by targeting the specific mutations contributing to the cancer's growth and survival. In order to improve sensitivity of detection and increase the number of genomic alterations that can be analyzed simultaneously, several next-generation sequencing (NGS) based tests were developed. In 2021, two liquid biopsy tests for lung cancer that utilize next-generation sequencing have been approved by the FDA to detect mutations in the DNA from tumor cells in the blood. The Guardant 360 CDx panel of 55 genes is approved for use in people with NSCLC,[41] and the FoundationOne Liquid CDx test that covers 324 genes is approved for use in people with NSCLC, prostate cancer, ovarian cancer, and breast cancer.[42] These tests are being used for disease management but not for the early diagnosis. A hybrid capture-based method of targeted NGS for ctDNA detection, CAPP-Seq (Cancer Personalized Profiling by Deep Sequencing), can detect several classes of genomic alterations simultaneously, including single nucleotide variations (SNVs), copy-number variations (CNV), indels and rearrangements, with levels of detection for mutant allele fractions down to ~0.02%.[28] It was used to study drug resistance in NSCLC patients but was not tested for diagnostic applications. Further studies are needed for the possible implementation of mutation-based liquid biopsies for the early detection and diagnosis of lung cancer in clinical practice.

DNA methylation analysis of ctDNA

Epigenetic modifications, including hypermethylation of the CpG islands in the promoters of tumor suppressor genes, are frequently involved in malignant transformation and are commonly observed in tumors, including lung cancers.[43, 44] Some of the advantages of using DNA methylation signatures as diagnostic biomarkers is that they occur early during carcinogenesis, can be found in ctDNA fragments circulating in blood and are remarkably stable.[45] The discovery of biomarkers based on tumor-specific DNA methylation profiles and development of DNA methylation-based liquid biopsy tests is a promising approach to improve early detection and diagnosis of lung cancer.[46] These tests can also help prevent patient over-diagnosis and unnecessary invasive procedures when used in combination with LDCT imaging.[47]

Methylation profiles of ctDNA can be analyzed using various methods,[48] such as methylation-specific PCR (MSP),[49] real-time methylation-specific PCR,[50] MethyLight,[51] droplet digital methylation-specific PCR (ddMSP),[52] methyl-BEAMing[53] and targeted and genome-wide methylation-specific next-generation sequencing (NGS),[54, 55] to name a few. Although epigenetic changes are not necessarily unique for any one type of tumor, distinct methylation patterns can be identified in specific cancers and could be potentially useful for diagnostic purposes.

One of the early studies was conducted in 2005 to evaluate the usefulness of serum DNA methylation for early detection of lung cancer.[56] Methylation status of five tumor suppressor genes (p16 (INK4a), death associated protein kinase [*DAPK*], O-6-methylguanine-DNA methyltransferase [*MGMT*], Ras-association domain family member 1A [*RASSF1A*] and retinoic acid receptor beta [RAR-β]) in DNA isolated from serum from 200 patients undergoing bronchoscopy for abnormal findings on chest radiograph detected by lung cancer screening or surveillance was examined using methylation-specific PCR. Methylation was detected in 50.9% of stage I lung cancer patients, whereas serum protein tumor markers were positive in only 11.3% of them. These results suggested that identification of promoter methylation of tumor suppressor genes in serum DNA could be useful for early detection of lung cancer.[56] Since then, multiple other studies reported methylation signatures in single genes or gene panels as potential diagnostic biomarkers for lung cancer,[57-61] and efforts are being made to develop commercially available epigenetic-based *in vitro* diagnostic (IVD) tests.[62]

One of the discovered methylation biomarkers, short stature homeobox gene two (*SHOX2*), has demonstrated a good sensitivity and a high specificity as a biomarker for lung cancer and was developed into Conformité Européenne (CE)-*in vitro* diagnostic (IVD) marked Epi proLung BL Reflex Assay by Epigenomics AG.[63] The test utilizes quantitative methylation-specific real-time PCR for the quantitation of methylated *SHOX2* DNA in bronchial aspirates with 78% sensitivity and 96% specificity and can be used as an aid in lung cancer diagnosis.[64] *SHOX2* methylation was also assessed in circulating cf DNA obtained from blood plasma.[58] In 2017, the Epi proLung blood-based version for the lung cancer test which is based on a combination of the methylation analyses of *SHOX2* and the prostaglandin E receptor 4 gene (*PTGER4*) received the CE-IVD mark. It demonstrated significant discriminatory performance for distinguishing patients with lung cancer from subjects with no malignancy with 90% sensitivity and 73% specificity in circulating DNA from plasma samples.[65]

The development of high-throughput techniques like NGS to measure DNA methylation in cfDNA allowed the discovery of novel biomarkers and facilitated development of assays capable of genome-wide assessment of methylation profiles for diagnostic purposes.

A novel blood-based pulmonary nodule diagnostic test PulmoSeek from AnchorDx utilizes targeted methylation sequencing platform with a panel of 100 pre-selected lung cancer-specific methylation regions (features) to detect a specific methylation signature that can differentiate malignant from benign nodules.[66] The model was tested in different stages and subtypes of pulmonary nodules and demonstrated overall robust sensitivity of 93% and 99% in test and validation sets respectively with a moderate specificity of 60% and 33% in test and validation sets across different lesion locations, nodule types, and stages of lung cancer. Coupled with LDCT, the test could become a robust tool for pulmonary nodule management and lung cancer screening.

Testing for multiple cancer types simultaneously with a single multi-cancer early detection (MCED) test is a new concept for cancer screening. MCED tests use a single blood sample to detect many cancers, including lung cancer, and can be based on DNA methylation analysis of cfDNA. Galleri Multi-Cancer Test developed by Grail can identify a diversity of cancer signals with high specificity and predict the origin of the cancer signal with high accuracy across 50 cancer types.[67] In a clinical validation study, the Galleri test was able to detect lung cancer with an overall sensitivity of 78.4% and a specificity of 99.5%. Sensitivity for Stage I lung cancer was 22%.[67]

Analysis of DNA fragmentomes

Recently, a novel approach was developed to evaluate fragmentation patterns of cf DNA across the genome. It was discovered that patients with cancer had altered cfDNA fragmentation profiles compared to cfDNA profiles of healthy individuals.[68] A machine learning model incorporating genome-wide fragmentation features had sensitivities of detection ranging from 57% to >99% among the seven cancer types, including lung cancer, with 98% specificity, although the number of lung cancer samples in the initial study was small.[68] This model was further developed into a genome-wide approach for analysis of cfDNA fragmentation profiles called DNA evaluation of fragments for early interception (DELFI) and used for lung cancer detection and characterization in a prospectively collected real-world cohort study.[69] The fragmentation profiles were remarkably consistent among non-cancer subjects, including those with non-malignant lung nodules. In contrast, cancer patients displayed widespread genome-wide variation. Fragmentation features combined with clinical risk factors followed by CT imaging were able to detect 94% of patients with cancer across stages and subtypes, including 91% of stage I/II and 96% of stage III/IV, at 80% specificity. In addition, analysis of transcription factor binding sites was able to distinguish individuals with small cell lung cancer from those with non-small cell lung cancer with high accuracy (AUC = 0.98). A higher fragmentation score represented an independent prognostic indicator of survival. This approach provides promise for improvements in non-invasive detection of lung cancer.[69]

Circulating tumor cells

Circulating tumor cells (CTCs) are tumor cells that shed from the primary tumor, invade the surrounding tissue, infiltrate into peripheral blood and lymphatic vessels, travel to distant tissues, and eventually proliferate to form metastases.[70] Detection of CTCs from clinical samples can be used as a tool in cancer diagnosis and prognosis through liquid biopsy. The presence of CTC in blood of the lung cancer patients both before and after surgery was associated with an increased risk of recurrence and death compared to an absence of CTC, but significant heterogeneity was observed among the studies included in the analysis.[71]

In 2004, FDA cleared the automated CellSearch System that is the first and only clinically validated system for identification, isolation, and enumeration of CTCs from a blood test.[72] It provides an accurate and reproducible assay that can count CTCs reliably across laboratories, despite low numbers of cells and a wide range of morphologic heterogeneity. The initial studies demonstrated that the numbers of CTCs in blood from subjects without known cancer are very low and almost never exceed 1 cell per 7.5 mL of blood. In contrast, ≥2 CTCs were detected in 7.5 mL of blood in 36% of the specimens from patients with various types of carcinomas.[72] These data suggest that CTC measurement may have clinical utility in cancers of epithelial origin. Additional studies indicated that CTCs are rarely detected in non-small cell lung cancer (NSCLC).[73] CellSearch detected higher CTC counts in the pulmonary vein which is closer to the primary tumor compared with the

radial artery, implicating CTC clearance before they reach the microcirculation. Despite higher numbers, CTCs measured in the pulmonary vein were often euploid cells, indicating a benign origin, therefore, measurements of CTCs in the peripheral blood are sufficient to identify recurrence of disease.[73] However, low number of CTCs present in blood at the early cancer stages makes their application for early diagnosis of lung cancer challenging.

MicroRNA analysis

MicroRNAs (miRNAs) are small noncoding RNA gene products about 22 nt long that were first discovered in *Caenorhabditis elegans* (*C. elegans*) and are found in diverse organisms. They play key roles in regulating gene expression through base pairing to partially complementary sites in messenger RNA (mRNA), predominately in the untranslated region of the message.[74] As opposed to mRNA molecules that are generally not present in blood, cell-free miRNAs can be detected in the blood and other body fluids of cancer patients.[75, 76] They are highly stable in biological samples, which makes them convenient as potential biomarkers.[77]

Recently, a panel of 30 miRNAs was evaluated for predictive performance in distinguishing lung cancer cases from controls using a cohort of sera collected up to one year prior to diagnosis of lung cancer and matched controls.[78] The panel was developed based on review of previous reports of differential expression in lung cancer clinical specimens. The authors assessed the contributions of miRNAs for improving performance of a previously validated four-protein marker panel (4MP)[79] for distinguishing lung cancer cases from controls compared to the protein marker panel alone and identified a panel comprising three miRNAs (miR-21-5p, miR-320a-3p and miR-210-3p) for identifying individuals at high risk of developing or at early stage of disease.[78] The combination of miRNAs with a previously validated 4MP protein panel yielded significantly improved sensitivity at the highest specificity thresholds that can be used for improving lung cancer screening and detection.

Exosomes

Exosomes are small extracellular vesicles in spherical shape with a diameter of 30–100 nm which could be secreted by either normal or tumor cells.[80] Exosomes are formed by the inward budding of multivesicular bodies (MVBs) and are released from the cell into the microenvironment following the fusion of MVBs with the plasma membrane.[81] They have been found in nearly all body fluids, including blood, and can transport nucleic acids, proteins, and lipids for intercellular communication and activate signaling pathways in target cells.[82] Content of the exosomes depends not only on the cell type, but also on the origin of the cells that produced them. In cancers, exosomes may participate in growth and metastasis of tumors.[83] Exosomes can be extracted from blood by density-gradient or ultracentrifugation and analyzed for microRNAs (miRNAs), circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), DNA, proteins or other biological molecules, and therefore can be used as non-invasive biomarkers for early detection and diagnosis of cancers.[84]

In 2009, a significant difference was observed in exosome-derived miRNA levels between lung cancer patients and controls, and the similarity between the circulating exosomal miRNA and the tumor miRNA patterns suggested that circulating exosomal miRNA might be useful as a screening test for lung adenocarcinoma.[85] Later studies identified a panel of six exosomal miRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100 and miR-154-3p) capable to identify patients with malignant lung nodules with 96% sensitivity and 60% specificity,[86] however, further evaluation was needed to confirm the predictive power of those biomarkers. In recent years, various combinations of exosomal miRNAs were described as potential liquid biopsy tools for NSCLC,[87-89] but these findings are not yet developed into clinical applications.[90]

Long non-coding RNAs (LncRNAs), a novel type of RNA that can be present in exosomes, were also evaluated as potential biomarkers for lung cancer.[91-93] In a recent study, deep sequencing was performed to detect differentially expressed exosomal LncRNAs isolated from the serum of patients with NSCLC and healthy controls, and quantitative real-time polymerase chain reaction (qRT-PCR) assay was utilized to validate dysregulated LncRNAs in testing and multicentric validation cohorts.[93] Exosomal LncRNA RP5-977B1 exhibited higher levels in NSCLC than that in the healthy controls. The area under the curve (AUC) value of exosomal RP5-977B1 was 0.89 and superior to conventional protein biomarkers carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA21-1). The results suggested that exosomal RP5-977B1 might serve as a novel liquid biopsy diagnostic biomarker for lung cancer.[93]

Another class of RNAs present in exosomes and considered to be potential diagnostic biomarkers for NSCLC are circular RNAs.[94] They are more stable and highly abundant, compared to LncRNAs.[95] Analysis of tumor and normal tissue from patients with early-stage lung adenocarcinoma by a high-throughput circRNA microarray revealed that over 300 circRNAs were dysregulated in tumor samples,[96] which might offer potential targets for the early diagnosis of this disease.

Tumor-educated platelets

Platelets (also called thrombocytes) are small, disc-shaped pieces of very large cells in the bone marrow called megakaryocytes that are found in the blood and spleen. Platelets have been known for their roles in hemostasis and thrombosis, in which they rapidly bind to damaged blood vessels and prevent excessive bleeding. However, emerging evidence demonstrates that platelets are far more complex than previously considered and may have a major impact in both progression and spreading of several solid tumors, including lung cancer.[97] Platelets are known to have a dynamic, bidirectional relationship with tumors, acting beyond their role of hemostasis. Tumor-educated platelets (TEPs) are modified by the tumor in multiple ways and act as a carrier and protector of metastasis. Data so far have shown that the mRNA in TEP can be used for cancer diagnostics, with many potential applications.[98,99] RNA-sequencing data of TEPs in NSCLC patients and healthy controls identified 48 biomarker genes that could potentially facilitate early screening of NSCLC.[100] In addition, TEPs contain small nucleolar RNAs (snoRNAs) that can also serve as noninvasive biomarkers. A recent study demonstrated that small nucleolar RNA, C/D box 55 (SNORD55) was significantly decreased in TEPs from NSCLC patients, especially in early-stage patients compared with healthy controls,[101] indicating that it was capable of acting as a promising biomarker for NSCLC.

Protein biomarkers

For decades, protein biomarkers remained the main target for cancer biomarker discovery and development.[102] Many protein biomarkers are tumor-derived proteins that may be released in the circulation via shedding from the tumor and subsequently detected in blood or other samples. Biomarkers such as plasma proteins and antitumor antibodies have been investigated for early cancer detection, including lung cancer.[103] Serum and plasma proteins can be detected by immunoassays (e.g., enzyme linked immunosorbent assay [ELISA]) and mass spectrometry.

Well-established protein tumor antigens such as CEA, cancer antigen 125 (CA-125), squamous cell carcinoma antigen (SCC), CYFRA21-1 and neuron-specific enolase (NSE) were intensively studied as potential lung cancer biomarkers due to early presence in serum, however, these proteins are also associated with other tumor types, and their sensitivity for early lung cancer detection was insufficient.[104] Multiple studies report protein biomarker combinations that demonstrated improved performance, but few are being consistently used in clinical practice.[105,106]

Cellular proteins released by tumor tissues can activate the immune system and lead to the production of autoantibodies.[107] Cancer patients develop tumor-associated autoantibodies (TAABs) recognizing

self-antigens, and several tests for lung cancer detection were developed using autoantibody assays. It was demonstrated that they can offer advantages in identifying early stages of disease, and elevated autoantibody levels could be detected greater than four years prior to lung cancer diagnosis.[108] A commercially available EarlyCDT-Lung is a blood-based enzyme-linked immunosorbent assay that measures presence of autoantibodies to seven lung cancer associated antigens (p53, New York esophageal squamous cell carcinoma 1 [NY-ESO-1], cancer associated gene (CAGE), ATP-dependent RNA helicase 4-5 (GBU4-5), Sex-determining region Y-box 2 [SOX2], neuronal antigen (HuD), and melanoma-associated antigen A4 [MAGE A4]). It was developed to aid physicians in the early detection of lung cancer in high-risk populations and demonstrated a sensitivity of 41% with a specificity of 91%.[109] In a later study, the sensitivity of the test was shown to be 33% with 88% specificity.[110] So far, no evidence of the clinical impact of EarlyCDT-Lung test was identified.[111]

Combination of different biomarkers

The combination of different types of biomarkers, and their combination with other diagnostic tools can potentially improve performance of lung cancer early detection. A promising multianalyte test that can detect eight human cancer types, including lung cancer, through determination of mutations in ctDNA in combination with the levels of eight circulating protein biomarkers detected by an immunoassay (CA-125, carbohydrate antigen 19-9 [CA19-9], CEA, hepatocyte growth factor [HGF], myeloperoxidase, osteopontin [OPN], prolactin, tissue inhibitor of metal protease 1 [TIMP-1]), called CancerSEEK demonstrated specificity greater than 99% for tumor detection, however, accuracy of prediction was lowest for lung cancers.[112] The best combinations of diagnostic biomarkers for lung cancer are still to be defined.

Challenges and limitations of liquid biopsy

While application of liquid biopsy for the early detection of lung cancer holds great promise, most of the biomarkers were only evaluated in research and investigational settings, and there is still limited evidence of their clinical utility.[113-115] The most widely used samples for the liquid biopsy are peripheral blood samples that contain multiple analytes available for analysis [Table 1]. However, there is a lack of standardization for the pre-analytical steps that include sample collection, processing, storage, and isolation of the analytes. In addition, different analytes may require specific handling conditions. There are also technical challenges due to the low concentration of the analyte of interest, for example, ctDNA, compared to the background from the normal cells.[25] Additionally, clonal hematopoiesis, a process that can lead to expansion of mutations in peripheral blood cells, can lead to false-positive finding in cfDNA testing.[116] Progress in the development of sensitive technologies greatly improved detection sensitivity, but more work is needed to robustly identify analytes of interest that indicate presence of disease with low false positive rate. Many evaluated assays have limited specificity and sensitivity for lung cancer, especially in the early stages of the disease. Promising scientific observations need to be translated into robust clinically useful tests through rigorous optimization, analytical testing and by performing well-designed clinical trials with diverse cohorts in different settings. Addressing these issues would further facilitate integration of liquid biopsies in the lung cancer diagnostic workup in the routine clinical settings.

Summary and conclusions

The early diagnosis of lung cancer remains an unmet clinical need because most of the current methodologies detect cancer in advanced stages when treatment is less successful and patient prognosis is poor. Liquid biopsy presents opportunities for early screening, diagnosis, and more efficient disease management, especially when tissue samples are scarce or cannot be obtained. New biomarker discovery, and development and optimization of methods for analyte isolation and analysis from liquid biopsy are necessary to increase the diagnostic performance of the tests so that they are safe and effective for use. In addition to traditional biomarker tests, a multimodal diagnostic approach that combines multiple diagnostic methods—imaging, liquid biopsy and clinical characteristics—can be used to improve the accuracy of diagnosis. Artificial intelligence (AI) can assist in processing vast amounts of analytical and clinical data to identify the best biomarkers or their combinations. More efforts are needed to support implementation of research findings into robust diagnostic tests suitable for clinical use to realize the potential of liquid biopsy in the diagnosis and treatment of lung cancer. Further development of advanced technologies to enhance sensitivity, specificity and reliability while reducing costs of liquid biopsy is needed before it can be widely implemented in clinical practice. In the future, liquid biopsy is expected to be part of routine patient management and play a greater role in the screening, early diagnosis, treatment selection and monitoring, and prognosis evaluation of lung cancer.

Conflicts of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marina Bibikova reports a relationship with AnchorDx, Inc. that includes: employment and equity or stocks. Marina Bibikova reports a relationship with Illumina Inc that includes: employment and equity or stocks.

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