Emerging Role of microRNAs as Liquid Biopsy Biomarkers in Lung Cancer: A Review

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Abstract- The emergence of patient-tailored medicine has changed all measurable disease outcomes. Among human diseases, cancers appear to be the most dangerous. Furthermore, lung cancers rank the first among human cancers in both morbidity and mortality. When lung cancer is clinically diagnosed, it is often too late for therapy. The absence of accurate and specific tools for early detection results in a poor prognosis for lung cancer. The discovery of microRNAs and their function in lung cancer offers a new mechanism for the detection of lung cancer cells. These molecules, derived from cancerous cells, circulate in the patient's blood. Recently, a revolutionary technique, *i.e.*, liquid biopsy has shown promise in discovering these circulating microRNAs molecules in body fluids, namely peripheral blood. A liquid biopsy allows the detection and isolation of circulating tumor cells, circulating nucleotides, and cellular exosome as a source of genomic and proteomic information in cancerous patients, especially in the early stages of cancer cell development. In this review, by searching various databases, including PubMed, Google Scholar, and Scopus, we explore liquid biopsy as a novel tool and the application of miRNAs in lung cancer detection in diagnostic pathology.

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Introduction

Cancer is the result of dysregulation and accumulation of mutations in tumor suppressors (negative regulators of cell proliferation) and proto-oncogenes (promoters of cell proliferation) in the genomic file of normal cells (1). One crucial approach to cancer detection is identifying the modifications and alterations in the genetic and epigenetic profile of the cells. Many cancers release mutated cells or their nucleotides (DNA and/or RNA) into the blood or other body fluids. Usually, in normal dividing cells, mutations occur at a rate of five mutations per genome per cell division. In contrast, clonal or activated proliferating cells contain tens of millions of cells with identical mutations. In cancer, these can contribute enough mutant templates to be detected in the plasma or serum (2). Cancer is one of the leading causes of death worldwide (3-5). The mortality burden of cancer has surpassed that of heart disease (6). Early detection of cancer in high-risk populations offers an opportunity for treatment with curative intent (6). Despite recent significant improvements in tumor diagnosis and treatment, cancer mortality has not decreased considerably (7). Thus, early detection remains important for improving outcomes and decreasing the recurrence and mortality of cancer patients (8). For this purpose, discovering minimally invasive methods for cancer detection has long been a central goal for early diagnosis and treatment (7-9).

Among all cancers, lung cancer appears to be one of the worst in both morbidity and mortality. The World Health Organization (WHO) has classified primary lung carcinoma into two major types: Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC), which differ in biology, therapy, and prognosis. The majority of cases are NSCLC, accounting for >80%. (9)

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Lung cancer accounts for 11.6% of all cancer-related deaths, causing approximately 1.4 million deaths every year (10). According to Trama *et al.*, in 2018, lung carcinoma was responsible for 14% of all new cases diagnosed and about 25% of all cancer deaths (11). The poor prognosis of lung cancer stems from several factors: absence of symptom in early stages, limited knowledge on lung cancer biology, variability and heterogeneity of tumors, absence of a suitable therapeutic strategy, and, in the majority of cases, clinical diagnosis of the tumor at a late stage, with many diagnosed after metastasis (12). Cigarette smoking is considered the major risk factor for lung cancer. Although any person can develop these tumors, cigarette smoking and exposure to smoke (as in passive smokers) can increase the risk of this malignancy (12). According to data, only 15-20% of smokers may develop lung cancer, while 90% of patients with lung cancer are smokers. Other relevant factors include exposure to substances such as radon (accounting for some 20,000 cases of lung cancer in the US per year, according to the United States Environmental Protection Agency [US EPA]), asbestos, arsenic, diesel exhaust and some forms of silica and chromium, as well as family history (13). The absence of obvious symptoms in the early stages of carcinoma hampers early diagnosis. Tumor biopsy and radio-imaging examination are believed to be the gold standard for detection of lung cancer, although the outcome is rather late, and thus, their application is limited by the progression of disease. In addition, traditional tumor diagnostic markers such as carcinoembryogenic antigen (CEA), CA 19-9 and some other markers usually exhibit low sensitivity (14). Surgical intervention appear to be the best treatment in early stage and chemotherapy for the advanced stages. Despite all these, patient survival is poor (15). Lung cancer is the result of many factors including deregulation of tumor cell genetics caused by many mutations accumulated in the genome profile of normal cells. Identification of driver mutation and genetic rearrangement might provide a valuable option for detection, evaluation, treatment and follow-up (16,17). For this molecular analysis, tissue biopsy has been standard, but it is not sufficient for all cases. Liquid biopsy, the newer technology of detecting circulating tumor cells, free nucleotides, and others in the blood (as described below), is rapidly introduced to clinical practice, providing possibilities to optimize cancer detection and treatment (18-22).

Liquid biopsy methods are considered a potential revolution towards achieving effective cancer management (19). They are based on blood or other body fluid tests with safer, less invasive, and more sensitive methods. They seem to be an alternative or complementary procedure for tissue biopsies. Today, blood-based liquid biopsy assessments focus on the evaluation of different biomarkers, including evaluating circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), Extracellular Vesicles (EVs) such as exosomes, oncosomes, nucleotides such as DNA, RNA, and microRNAs and tumor educated platelets (TEP) (21-23). Despite the potential advantages of individual biomarkers and techniques, each has its own disadvantages. Among the various liquid biopsy samples and circulating substances, in this review, we focus on circulating cell-free microRNAs, which represent the most promising and valuable class of molecular biomarkers for the detection of lung cancer (24). We begin by discussing the liquid biopsy method and then continue with assessing its application in detecting microRNAs biomarker for lung cancer.

Liquid biopsy

Liquid biopsy, also known as fluid biopsy or fluid phase biopsy, is a simple, noninvasive tool as an alternative to tissue biopsies for pathologic diagnosis. In this method, body fluids, mainly blood, plasma, and serum, are obtained for detailed laboratory analysis with high throughput techniques. Although tissue biopsy is the most direct method for tissue diagnosis, it is limited as it provides an incomplete representation of the entirety of the organ (14). Liquid biopsy is an old concept with a new look (25). Tumor cells in blood circulation have been reported as early as 1869 by Thomas Ashworth, who observed circulating tumor cells (CTCs) in blood during an autopsy on a patient with cancer metastasis (26). Tumor cells can be released in the blood, hemodynamic forces acting to arrest, adhesion, and extravasation of circulating tumor cells and particles. (27) The term "cell-free DNA (cfDNA)," which refers to fragmented DNA found outside cells in the blood, was first used by Mandle and Mtaise in 1948. In 1977, Leon *et al*., reported an increased concentration of circulating DNA in cancer patients (25). In clinical practice, the first interpretation of finding cancer cells using liquid biopsy as an alternative to conventional biopsy was presented in a breast cancer meeting in 2010 by Eva Lianidou *et al.*, from the University of Athens, Greece. The advent of high throughput technologies and analytic tools increased the understanding and importance of circulating molecular profiles and cancer cell products in the blood. The main objective of liquid biopsies is discovering the molecular genetic basis of neoplasia as well as non-

tumoral organ lesions (28,29). The United States Food and Drug Administration (FDA) approved the first liquid biopsy test in June 2016: the COBAS EGFR mutation test. This liquid biopsy test assesses cfDNA for the EGFR gene mutation in blood from lung cancer patients (30).

Researchers in the MD Anderson Cancer Center reported in Science Daily News that the liquid biopsy test Guardant 360 is comparable to standard tissue biopsies in detecting lung cancer biomarkers in advanced non-small cell carcinoma (31).

Many researchers have postulated that liquid biopsies represent a potential revolution in cancer diagnosis, prediction and management, and they could be used routinely in medical practice (9,32-35). Several blood-based biomolecules, such as cell-free DNA and RNAs, small and large non-coding RNAs, proteins, circulating tumor cells and extracellular vesicles such as exosome have been studied for molecular tests. Recently, a group of researchers discovered the potential of tumor educated blood platelets (TEP) as cancer biomarkers (23). TEP samples have been studied with satisfactory results in patients with different tumor types, including lung, brain, and breast cancers (33,23). In addition, liquid biopsy has been used in non-tumor diseases. Liquid biopsies have been applied to different fluids including blood, plasma, serum, sputum, bronchial washing and urine (32-36). Non-coding RNAs, both long RNAs and small microRNAs (snoRNAs), have been used as biomarkers for tumor and non-tumor conditions (37-40).

Biomarkers for lung cancer which are currently used for liquid biopsy include cell-free nucleotides (DNA-RNA and derivatives), circulating tumor cells (CTCs), tumor educated platelets (TEPs) and dissimilated tumor cell (DTCs) particles, microsomes, microvesicular bodies, and cell-free nucleotides. These particles are mostly released through apoptosis, cellular necrosis, active secretion or dissemination (16). In the lung, for the purpose of liquid biopsy, microRNAs are the altered biomarkers of choice, and have been reported in various lung tumors and non-tumoral diseases (41-42). Many liquid biopsy analyses use DNA that can be easily detected. Tumoral proliferation yields tens of millions of cells with identical mutations, which can be analyzed with liquid biopsy. The half-life of circulating cell-free DNA (cfDNA) is less than one hour, which may compromise the efficiency of biopsy analysis. In contrast, circulating microRNAs are more stable with much longer half-lives. Hence, biopsy studies are more promising using these microRNAs with long-term stability as biomarkers (43-47).

Many techniques have been reported for detecting circulating tumor nucleotides. These include Next Generation Sequencing (NSG), Digital-PCR platforms, Real-time PCR-based methods, mass-spectrometry technology, and detection of hyper-methylation of ctDNA. M.Elazezy *et al.*, have discussed some of these techniques for different biomarkers. All these techniques can include qualitative and quantitative parameters (16).

Circulating microRNAs, structure, and function

MicroRNAs (miRNAs) were discovered in *C. elegans* in 1993 (48) and were later observed in humans in 2000 (49). The first microRNAs discovered were lin-4 in 1993 and let-7 in 2000. These molecules have also been found in plants, animals, and viruses. MicroRNAs are a class of non-coding RNAs that play key roles in the regulation of gene expression. MicroRNAs genes are transcribed by RNA polymerase II as pri-microRNA; these are processed by a protein complex containing the RNase enzyme named Dorsha to form approximately 70-nucleotide precursor molecules named pre-microRNA. This precursor is transported to the cytoplasm and processed by a second RNase III enzyme called Dicer to form mature microRNA, which has approximately 22-25 nucleotides. Then, this mature molecule is incorporated into a ribonuclear particle to form the RNA-induced silencing complex (RISC), which mediates gene silencing. MicroRNAs function in mRNA silencing and regulating post-transcription of gene expression (50). Aberrant microRNA expression is seen in most cancers. It is either a driver for malignant transformation, or it is the result of dysregulation caused by the tumor. Many cellular biological processes, including differentiation, proliferation, metabolism, and apoptosis, are controlled by microRNAs (51). Until 2015, approximately 2000 miRNAs have been reported, which account for 1-3% of human genes (38). MicroRNAs (19-24 nucleotides long) regulate at least half of the human post-transcriptional gene expression. Their action is either by promoting the cleavage of target mRNA or by repressing mRNA translation (12,43,50). A single miRNA can be involved with a hundred mRNAs and one mRNA may be controlled by many miRNA. They are thought to be involved in normal cells and cancer cells phenotyping (14). In particular, they are involved in cancer cell proliferation, migration, drug resistance and cell death. Mature miRNAs are reduced in nearly all cancer cells (47). Apart from intracellular functioning of microRNAs, these molecules are abundant in body fluids, exhibiting a paracrine function. They target both the microenvironment and more distant sites in the body, facilitating cell-cell communication (38). It is well

established that miRNAs participate in all known cellular and developmental processes.

The contribution of microRNAs to the development of lung parenchyma in addition to pathogenesis of many pulmonary diseases has been recently explored. Over the last few years, researchers have shown that miRNAs not only have a fundamental role in lung development and homeostasis, but are also involved in the process of lung injuries including chronic obstructive pulmonary disease (COPD), asthma and interstitial pulmonary fibrosis (IPF) (36). Until 2018, reports on diagnostic features of miRNA markers in lung cancer have been collected by Yu Haixin *et al*., Totally, 109 circulating miRNAs were collected which were satisfactory for the diagnosis of lung cancer. Among these, 30 miRNAs were reported in at least two studies (47).

MicroRNAs frequently circulate as cf-miRNA in peripheral blood and even in bronchial washing (52) and urine (53). These molecules have long-term stability in plasma and serum, rendering them apparently suitable fluid samples for cancer detection (45). Dama *et al*., reported references of variety of circulating miRNAs to be biomarkers for lung cancer (14). The biogenesis of microRNAs is tightly conserved and controlled, and their deregulation is associated with different diseases and neoplastic performance. As signaling molecules, microRNAs influence the behavior of recipient cells. Many studies have shown the global deregulation of different miRNAs in lung cancer (36) and established that miRNAs impair cellular and developmental processes in the lung. Researchers have found that miRNAs are not only important in lung development and homeostasis, but also play a role in lung inflammation in response to various causative agents such as physical insult, radiation effects, infectious agents, allergies and others (36,41,42,51). The role of miRNAs has been reported in regulating the expression of programmed death-1(PD-1) and programmed death-ligand-1(PD-L1) immune checkpoint (54,55). The connection between deregulated miRNAs biogenesis and different pulmonary diseases and their potential therapeutic use has been explored. The researchers proposed that it is based on the restoration of the physiological miRNA signature (36,44). Search on dysregulation of different miRNAs for detection and managing, including those related to drug resistance and recurrence of lung cancer, is today's major plan in medical practice. (43,56,57,58).

Exosomes and circulating cancer biomarkers

Exosomes are small membranous cell-derived extracellular spherical vesicles. They are nano-size have a diameter of 40-100 nm and a density of1.13-1.19 g/mL (58-63). Many different human body cells can release exosomes in the blood in both physiological and pathological conditions. The most important cell types include immune cells, stem cells, and tumor cells (60,63).

Nearly all circulating biomarkers (except tumor circulating cells), including miRNAs, present in extracellular microvesicles (64,65). These vesicles are exosomes. Exosomes are cell-derived vesicles. Their origin is from the budding of endosomes. Endosomes generate intracellular multivesicular bodies (MVBs). After the fusion of MBVs to the cell membrane, the vesicle is released into the extracellular space and becomes the mature exosome. Exosomes contain molecules such as lipids, proteins, nucleic acids (RNA, miRNA, mtDNA, DNA), and other metabolites (14,66). New reports have described that tumor-released exosomes (TEX) are decorated with cancer-related molecules (61,64). These decorations influence tumor development by simulating oncogenic pathways activation, causing chemo-resistance, immune escaping, and preparing a pre-metastatic niche. Part of exosomal cargo is miRNAs which are potential biomarkers with more stability (62). Methods of isolation of exosome with the inter-individual and intra-individual variations in circulating miRNAs in exosomal cargo can be used as potential biomarkers (61-62).

Diagnostic miRNAs

A search in the literature shows numerous reports dealing with a variety of miRNA biomarkers used for the classification and diagnosis of lung cancer (67-68). Most reports address the diagnostic value of microRNAs based on their amount and stability in biological fluids. A systematic literature review by Yu H. *et al.*, up to August 2017, reported 17 studies evaluating 35 circulating miRNA markers and 19 miRNA panels in serum or plasma, which were satisfactory for evaluation. The median sensitivity and specificity were high (47). Elisa Dama and coworkers (14) listed the reported studies using detection of miRNAs as biomarkers for lung cancer. The types of miRNAs used as markers were different. It has been shown that circulating miRNAs may reveal the origin of tumor in the lung (primary or metastatic) and thus, serve as novel biomarkers for early identification and diagnosis of lung cancer (21,22). Nevertheless, there is no consensus between these studies. These disparities may be due to differences in sampling, methodology and analysis. Recently, these discrepancies have been reduced and reproducibility has improved. Bishop used miR205 for accurate

classification of non-small cell lung carcinoma (67) and Hamamoto used circulating miRNA to differentiate between lung squamous cell carcinoma and adenocarcinoma (68). Gilad and coworkers used a microRNA-based diagnostic assay for classification of the four main types of lung cancer (69). Mir-7 has been used to differentiate lung cancer from normal lung tissue (59). In addition, Kim J and coworkers reported miR-592 and miR-522 to distinguish primary lung cancer from metastasis of colon cancer to the lung (70). By quantitative reverse transcription PCR, I. Zaporozhchenko and coworkers from Novosibirsk, Russia, presented the diagnostic value as oncomir of seven plasma miRNA biomarkers (miR-21, 19b, 126, 25, 205, 183 and 125b). Four miRNAs were significantly dysregulated (miR-19b, 21, 25, 183) in lung cancer (71). In another report from Moscow, Russia, M.Wozniak identified a panel of 24 miRNAs with optimum classification performance for lung cancer (72). Circulating microRNAs have been used for molecular subtyping of lung cancer (70). Wilkerson *et al*., considered nearly 500 genes for subtyping lung adenocarcinoma. They proposed three molecular subtypes, which had distinct and peculiar characteristics and were important in management of cancer (73). E Dama *et al.,* reported some miRNA biomarkers contributing to aggressive subtype of stage one adenocarcinoma of the lung (74).

Prognostic value of miRNAs in lung cancer

It has been evident that there is molecular heterogeneity between the early stage of lung cancer and the late or recurrent stages. Many patients in stage one of cancer will express resistance to therapy and show metastasis (1). A study showed that while lung cancer was in its early stage according to miRNA findings, it had a high incidence of recurrence. This is due to the mutation heterogeneity that happens between the early stage and the recurrence (75-78). Regarding these phenomena, miRNA liquid biomarkers can predict which patient may benefit from additional or special therapies. A list of different miRNA biomarkers driving malignancy and therapeutic response presented by Jenifer Barger *et al.*, (38). In this list, miRNAs are associated with gene driver mutations of lung cancer such as EGFR, ALK, and others. Identification of microRNAs that are associated with tumor aggressiveness, metastasis, and resistance to therapy is an important research field in lung cancer. The human let-7 family of miRNAs contains 12 members, which are important in various fields of biology such as stem cell biology, development, aging, and metabolism (76). Let-7 is highly conserved across species in sequences and functions with great value in prognosis due to deregulation (76-77). Low levels of the let-7 family of miRNAs are associated with poor prognosis and have been implicated in resistance (64,65). In a meta-analysis, Xiao W *et al.,* evaluated the prognostic value of circulating microRNAs in lung cancer and found ten miRNAs contributing to the poor outcome of overall survival rate (79).

Oncogenic miRNAs (oncomiR)

OncomiR (also oncomir) is a microRNA that is associated with cancer. OncomiR causes cancer by down-regulating genes through both translational repression and messenger RNA (mRNA) destabilization mechanism (80). SM Hamond, in 2000 and 2006, reported the causative aspect of microRNAs in various human diseases, including lung cancer (81). The first link between miRNA and the growth of cancer (RNAi, oncogenic function) was reported in 2002 when Calin GA *et al.,* observed the down-regulation of miR-159 and miR-16-1 in B cell lymphoma (82). There are many reports showing the association of miRNAs with oncogenic activities. Expression of some miR correlates with poor prognosis of lung cancer. Liu *et al*., reported that the expression of serum miR-21 and tumor miR-200 in lung cancer patients is associated with a poor prognosis (83). High expression of miR-21 and miR-155 predicts recurrence and poor survival, according to a report by Yang *et al*. (84). The level of miR expression is also important for their different actions (84,85). The levels of biomarker expression may affect acquired resistance (86). Oncogenic mechanisms of miRNAs have been reported as acting on either suppressor genes or oncogenic genes. Zhang C *et al*., reported the overexpression of microRNA-411to to promote lung cancer by directly targeting the suppressor gene (87). MicroRNAs are acting as a type of tumor-suppressive control of many tumorigenic processes, including cellular proliferation and cancer metastasis. Researchers have depicted that miRNAs control all key biological processes driving malignancy; hence, they could be a target for therapeutic purposes (37). Overall, several pieces of research and many clinical trials have shown that aberration and dysregulation of miRNAs biogenesis yield different miRNAs, which can be sought using liquid biopsy methods, may dictate the response of cancer cells, could be biomarkers for either resistance or predicting the survival rate, or can be used for therapeutic targets.

Therapeutic targets for microRNAs

Regarding the oncogenic function of miRNAs, there is no doubt that based on their structure and function, microRNAs have the potential to be manipulated as cancer therapeutic agents. Many research groups across the world and many pharmaceutical companies are conducting studies to explore miRNA-based therapy and develop a new area of therapeutic miRNAs (86,88). Lu J *et al*., reviewed existing research literature on the correlation between miRNAs and therapy of NSCLC (57). However, several obstacles in cancer therapy must be overcome before miRNAs can be truly translated to clinical practice. These obstacles include the optimal methods for drug delivery, better understanding of pharmacokinetics of delivery, assessing cell specificity and minimizing side effects (37). Pan X *et al.*, and Hong L *et al*., used miR-21 in the therapy of lung cancer and reported satisfactory results (88,89). These findings are based on liquid biopsy. Many reports are available about the value of liquid biopsies for guiding cancer therapy (9,90-91).

The application of targeted miRNA therapy in lung cancer and its potential benefits have been of interest to researchers. As mentioned, microRNA-21 has been reported as a novel therapeutic target by several Chinese researchers (89-90). The initial results appear to be promising. Studies demonstrate the potential role of miR-221 and miR-222 as markers for therapeutic response to sensitized tumors. Barger JF *et al*., suggested two main strategies for using microRNAs for therapy (39), as summarized below:

1- Restoring tumor-suppressor miRNAs function: Several strategies have been suggested to restore the activity and function of miRNAs tumor suppression. These include vector delivery of miRNA oligonucleotides or applying pharmaceutical agents targeting miRNAs expression. Many studies have proposed different approaches to restore or enhance suppressor miR genes. These reports provide preclinical evidence for the potential translation of restoration techniques of miRNAs for treating lung cancer (38).
2- Blocking miRNA function: Understanding the oncogenic activities of microRNA provides possible strategies for inhibition of these activities through anti-sense oligonucleotides. These techniques include using antagomirs, locking nucleic acid miRNAs, and miRNA sponge. Some studies have demonstrated the potential blocking of miRNA function, but more studies are still needed for clinical application.

Biomarkers predictive for chemotherapy

Presently, there is not sufficient evidence predicting the results of conventional chemotherapy. Lung tumors can be intrinsically resistant or can become chemo-resistant during treatment (91). To overcome drug resistance and achieve better results with chemotherapy, the current aim of liquid biopsy research is identifying reliable miRNA biomarkers which can predict response to chemotherapy. These can be used in combination with newly discovered targeted therapy. Predictive biomarkers hold the potential to allow choosing those patients who will benefit from a particular more beneficial treatment, thus, preventing unnecessary drug exposure or toxicity (14,92). Improvement in high-throughput "omics" techniques, advanced assessment of proteomics now offers the possibility to manipulate big data for cancer heterogeneity, exploring to score and develop reliable biomarkers detectable in liquid biopsy for precision cancer medicine (93,94).

Future of liquid biopsy: Biomarkers consortium

Numerous studies have reported on liquid biopsies as a tool for identifying cancer biomarkers for diagnosis, prognosis, and treatment strategy. Procedures such as miRNA liquid biopsy will ultimately bolster the implementation of personalized therapy and clinical management of lung cancer patients. Early detection of circulating miRNAs could be a remarkable approach in this regard. Now, new sensitive assays are available for the detection of tumor nucleotides, including miRNAs. However, there remains a need for standardization of pre-analytical issues and cross-platform comparison studies (94). Liquid biopsies are being evaluated for treatment selection, monitoring disease response/resistance, and cancer diagnosis. Many studies are underway to assess the clinical utility of miRNAs in different settings. Nevertheless, pitfalls in the designing of appropriate cancer biomarkers, technical and methodical variations, and multiplicity of miRNAs have delayed their transfer to the clinical setting (95).

Researchers in the USA created a research consortium to overcome some of these issue and provide a gold standard for screening and validation of biomarkers (14). It would be desirable to launch international consortia in order to validate multi-biomarker panels of different origins. An effort towards this direction was recently undertaken by the "Biomarkers Consortium", supported by the Foundation for the National Institutes of Health (96,97).

Cancer is the result of deregulation of cell genome and

accumulation of mutations in tumor suppressors and the proto-oncogene genetic profile of normal cells. However, in these malignant tumors, many material related to tumorogenesis and destructed tumor cell release freely in the body fluids. These substances include circulating free tumor cells, nucleotides (DNA, RNA), lipids, proteins and others. This review suggests that liquid biopsy for circulating microRNAs molecules has great potential to identify biomarkers for lung cancer detection and provide candidates for cancer treatment and prediction. Lung cancers are one of the leading causes of death worldwide. The morbidity and mortality of lung cancer surpass other malignancies. Therefore, methods for early detection, treatment and prediction of lung cancer are most urgently needed. Liquid biopsy may be considered a potential revolution towards achieving effective cancer management. It is a simple noninvasive tool as an alternative to tissue biopsy for patho-oncologic diagnosis. According to many studies on these molecules, microRNAs appear to be the best candidate for early detection of lung cancer. In this method, various microRNAs from body fluids (blood, plasma, serum, urine, bronchial washing and sputum) are obtained for detailed laboratory analysis with high throughput technologies. This appears to be a promising tool for cancer treatment.

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