MicroRNAs as early diagnostic biomarkers for non-small cell lung cancer (Review)

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Abstract. Lung cancer (LC) is the leading cause of cancer-related death, with high incidence and mortality rate. Early diagnosis and treatment of LC are imperative to improve the 5-year survival rate for patients with LC. In recent years, miRNAs as promising biomarkers with high sensitivity and specificity for LC have been studied increasingly. In LC regulatory networks, miRNAs play crucial roles in the occurrence, development and metastasis of non-small cell lung cancer (NSCLC), such as oncogenic factors, tumor suppressors and regulators. Dysfunctional miRNAs perform tumor-suppressive or oncogenic functions in the regulation of cell proliferation, invasion, apoptosis, cell cycle disorder and angiogenesis by negatively regulating target genes. In the present review, the biological process of miRNAs was firstly summarized and recent advances in the mechanism of miRNAs involved in tumor formation and development were described. In addition, the present review concentrated on the latest findings on the miRNAs related with circulating free and extracellular vesicles in the early diagnosis of NSCLC. Additionally, the diagnostic performances of circulating free and extracellular vesicles-associated miRNAs in NSCLC were contrasted. Owing to the increased stability and wide-ranging practical applicability, miRNA may be one promising biomarker for NSCLC diagnosis.

Contents

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1. Introduction

Lung cancer (LC) is the most common cancer worldwide and the second largest cause of cancer morbidity and mortality, with 2.2 million new cases and 1.8 million deaths in 2020 (1). It is predicted that the number of incident cases of LC will reach 3.8 million by 2050 (2). LC is classified into two types based on pathological characteristics: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), with the latter including lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and large cell carcinoma (LCC) (3). NSCLC is the most common pathological type of LC, which accounts for ~85% of all cases. The mechanism of leading to LC is the apoptosis of alveolar epithelial cells mediated by asbestos via mitochondrial and p53-regulated death pathways in the human body (4). Additional disorders associated with LC include chronic obstructive pulmonary disease, tuberculosis, emphysema and interstitial lung disease (5,6).

Due to the insidious nature of NSCLC, the illness is typically diagnosed at advanced stages and the 5-year survival rate is less than 15% (7). Patients with NSCLC who receive radical surgery at an early stage can have the 5-year survival rate of 40-70% (8). Therefore, early diagnosis of NSCLC could significantly reduce patient mortality. Currently, the main clinical strategy for diagnosing NSCLC is a low-dose computed tomography (LDCT) scan. However, LDCT has certain drawbacks, such as overdiagnosis, harmful radiation exposure from repeated detections, and elevated anxiety in patients. Researchers have studied certain new biomarkers with high specificity and sensitivity for NSCLC, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), circulating tumor cells and circulating tumor DNA (9-11). Particularly, miRNAs have attracted more attention in the field of high-quality biomarkers.

miRNAs are small and evolutionarily conserved class of non-coding RNAs (ncRNAs) with a size of ~19-25 nucleotides. miRNAs are key transcriptional regulators and can affect a variety of biological functions by targeting the 3' untranslated

region (UTR) of messenger RNA (mRNA) to induce mRNA degradation and inhibit translation (12). The first miRNA, lin4, was discovered in *Caenorhabditis elegans* in 1993 (13). At present, more than 2,000 miRNAs have been identified in the human genome, which are involved in the regulation of a variety of physiological and pathological processes. Therefore, miRNAs have been widely researched as potential biomarkers and therapeutic targets (14).

Exosomes are membrane-enclosed extracellular vesicles with a diameter of 30-150 nm. In addition to proteins and lipids, exosomes also contain a rich trove of nucleic acid metabolites such as miRNAs and lncRNAs (15). Tumor cells generate exosomes that contain abundant miRNAs, and tumor-specific exosomal miRNAs vanish when tumor tissue is removed. The expression profiles of exosomal miRNAs derived from plasma or serum are significantly different between NSCLC patients and healthy controls (16).

In the present review, a variety of serum and plasma miRNAs with high specificity and sensitivity that play an important role in the early diagnosis of NSCLC were summarized. Finally, the value of exosomal miRNAs as novel biomarkers for NSCLC diagnosis was emphasized.

2. miRNA biogenesis and function

The majority of miRNA genes are located in the intron and intergenic regions of protein-coding genes (17), which are transcribed by RNA polymerase II (Pol II) and RNA polymerase III (Pol III) (18). The typical biogenesis of miRNAs involves the beginning in the nucleus and the ending in the cytoplasm (Fig. 1), and comprises three main events: cropping, export and dicing (19). miRNA is typically generated from a primary miRNA (pri-miRNA) transcript through two consecutive cutting events. The pri-miRNA is usually added with a 5' cap structure and a 3' poly (A) tail, containing one or more long hairpin structures. Because the structural characteristics of these hairpins are unique, they can be distinguished from various RNA stem ring-like structures in the nucleus. Pri-miRNA hairpins typically have an imperfect 30 bp stem with flanking single-stranded RNA fragments at the base (20). Initially, pri-miRNAs are cleaved in the nucleus by a microprocessor complex, which is composed of the RNase III enzyme Drosha, the double-stranded RNA-binding protein (RBP) DiGeorge syndrome critical region gene 8 (DGCR8) and associated proteins (21). DGCR8 recognizes the connection between the stem and the flanking single-stranded RNA of the pri-miRNA hairpin, recruits Drosha, cuts the RNA double strand, and produces a 70-bp stem-loop structure known as precursor miRNA (pre-miRNA) (22,23). Methyltransferase-like 3 (METTL3) methylates pri-miRNA, which can be recognized and processed by DGCR8. METTL3 depletion reduces DGCR8 and pri-miRNA binding, resulting in a decrease in mature miRNA and an increase in unprocessed pri-miRNA accumulation (24). Pre-miRNA is transported to the cytoplasm by exportin-5, and cleaved by the cytoplasmic RNase III enzyme Dicer to produce mature double-stranded miRNA. Then the mature double-stranded miRNA binds to Argonaute (AGO) protein and forms a miRNA-induced silencing complex (miRISC). The mature chain is kept in miRISC, while the over-guest chain is released and degraded (25). By complementary pairing with the binding site of the 3'-UTR mRNAs, miRNAs lead to target mRNA degradation and/or translation inhibition of target genes (26). Under normal physiological conditions, miRNAs regulate cell biological processes such as proliferation, differentiation, apoptosis and protein synthesis. Therefore, its disturbance is involved in the regulation of tumor development and progression. In addition, a single miRNA can regulate multiple interaction networks and translation processes by targeting multiple mRNAs, whereas an mRNA can be regulated by multiple miRNAs (27).

3. miRNAs and the pathogenesis of NSCLC

miRNAs regulate cellular processes in both physiological and pathological conditions. A miRNA can bind to one or more mRNAs, affecting the expression of oncogenes and tumor suppressor genes, which is related to the pathogenesis of NSCLC. In NSCLC, various miRNAs are upregulated or downregulated and serve as either oncogenic miRNAs or tumor suppressor miRNAs. Several significant miRNAs implicated in the development of NSCLC are listed in Table I.

miR-224. miR-224 plays a dual function in various cancer cells. It plays an oncogenic role in the formation and progression of numerous kinds of malignant cancers, including NSCLC (28), breast cancer (41) and colorectal carcinogenesis (42). Otherwise, it functions as a tumor suppressor and is downregulated in certain patients with uveal melanoma (43).

Sirtuin3 (SIRT3) is a member of the sirtuin family of NAD^+-dependent deacetylases. By controlling the acetylation of several mitochondrial proteins, it contributes to biological processes like energy metabolism and cell aging. It is also intimately associated to the formation and development of cancers (44). In comparison to paracancerous tissues and healthy controls, the expression levels of SIRT3 have significantly increased in NSCLC tissue and serum. SIRT3 may function as a tumor suppressor in NSCLC because its level was inversely related to tumor size, lymph node metastasis and TNM stage in individuals (45). miR-224 inhibits expression of SIRT3 and targets its 3'-UTR, contributing to the development of cancer. The overexpression of miR-224 may drastically reduce the degree of AMP-activated protein kinase (AMPK) phosphorylation in the co-culture model of cancer-associated fibroblasts (CAF) and NSCLC cells. Simultaneously, forced overexpression of SIRT3 may improve AMPK activation and counteract miR-224 mediated AMPK suppression. Additionally, miR-224 can stimulate the mammalian target of rapamycin/hypoxia inducible factor-1α (mTOR/HIF-1α) signal pathway to control the growth of NSCLC (28). mTOR is a serine-threonine kinase that acts as a crucial regulatory protein in typical cell physiology. The mTOR signaling pathway is crucial for regulating signals that promote cancer cell growth and survival and is a significant contributor to the development of NSCLC and other types of cancer (46). In addition, AMPK can inhibit mTOR (47). With the growth of NSCLC tumor, hypoxia aggravates the expression of HIF-1α. HIF-1α overexpression can activate downstream signaling molecules like vascular endothelial growth factor A (VEGFA) and

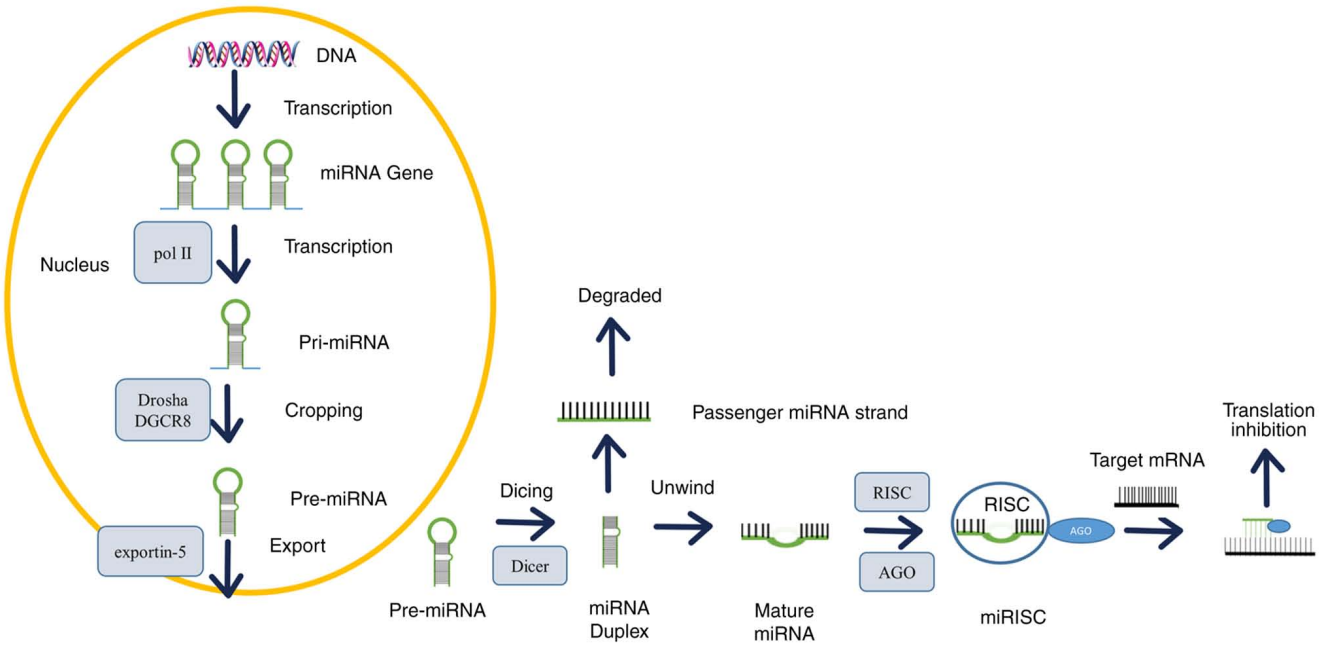

Figure 1. Schematic illustration of microRNA synthesis process.

accelerate the growth of tumors in NSCLC (48). Higher levels of HIF-1α stimulate the production of miR-224, producing the miR-224-SIRT3-AMPK-mTOR-HIF-1α positive feedback loop, which promotes tumor development, angiogenesis, and metastasis in NSCLC cells by targeting SIRT3 and inhibiting AMPK (28).

Phosphatase and tensin homolog (PTEN), as a prominent negative regulator of cell growth and phosphatidylinositol-3-kinase/v-akt murine thymoma viral oncogene homolog (PI3K/AKT) signaling pathway, plays a role as a tumor suppressor gene. Abnormal pathological features are caused by the loss of PTEN expression in numerous cancers (49). In serum-starved A549 cells, miR-224 negatively regulates PTEN through PI3K signal pathway to inhibit cell proliferation and induces apoptosis and autophagy due to the change of tumor microenvironment (50).

Angiopoietin-likeprotein1 (ANGPTL1) is another target gene of miR-224. ANGPTL1 prevents angiogenesis and the spread of cancer by acting as a tumor suppressor and an anti-angiogenic factor (51). Overexpression of snail family zinc finger 2 (SLUG) promotes tumor cell migration. By decreasing the expression of the SLUG, ANGPTL1 inhibits epithelial-mesenchymal transition (EMT) (52). The ectopic expression of miR-224 enhances NSCLC cell proliferation, migration, and lymph node metastasis by directly targeting ANGPTL1 mRNA (53).

miR-139-5p. miR-139-5p can function as tumor suppressor, which mainly regulates the translation of mRNA at the post-transcriptional level and plays an inhibitory role by mediating a variety of target genes and downstream signal pathways. The expression of miR-139-5p is decreased in multiple cancer tissues, such as pancreatic cancer, colorectal cancer and hepatocellular carcinoma. Therefore, miR-139-5p can be forcedly overexpressed in tumors to prevent the proliferation, invasion, and migration of tumor cells (54-56).

As the target gene of miR-139-5p, homeobox B2 (HOXB2) is a member of the homeobox (HOX) transcription factor family. The majority of the HOX proteins encoded by HOX gene function as transcription factors, regulating embryonic development, cell differentiation and carcinogenesis. HOXB2 is a crucial gene in the regulation of cell differentiation (57). miR-139-5p inhibits HOXB2 expression when it selectively binds to the 3'-UTR of HOXB2 and decreases tumor growth by promoting apoptosis in cells and inhibiting cell proliferation. In NSCLC cells treated with cisplatin (DDP), overexpression of miR-139-5p overcomes DDP resistance by regulating the PI3K/AKT/caspase-3 signaling pathway (31). The PI3K/AKT signaling pathway modulates diverse cellular processes, including cell proliferation. Caspase-3 is the key execution enzyme in cell survival and apoptosis (58). Therefore, overexpression of miR-139-5p inhibits cell proliferation and promotes cell apoptosis by downregulating the PI3K/AKT signaling pathway and increasing caspase-3 expression. Overexpression of miR-139-5p could attenuate paclitaxel (PTX) resistance of NSCLC cells. Integrin beta-8 (ITGB8) is an integrin family number. Integrins are located on the surface of cancer cells and promote tumor metastasis by mediating cell-to-cell adhesion and invasion. ITGB8 is typically upregulated in cancer and is associated with cancer metastasis (59). Delta/Notch-like epidermal growth factor-related receptor (DNER) is a transmembrane protein involved in the tumor development. CircDNER has been proved to function as a miRNA sponge to sequester miR-139-5p away from its target mRNA, thus reducing miRNA-mediated gene inhibition. ITGB8 is the target gene of miR-139-5p, and circDNER/miR-139-5p/ITGB8 forms a new regulatory axis. Enforced overexpression of miR-139-5p in PTX-resistant NSCLC cells reverses the tumor promoting functions of circDNER on NSCLC cell proliferation and invasion by targeting and inhibiting ITGB8, while also slows the growth of tumors and encourages the apoptosis of PTX-resistant cells (60).

Table I. Roles of miRNAs in the occurrence and progression of NSCLC.

miRNA	Target gene	Type of dysregulation	Function	(Refs.)
miR-224	Sirtuins3	Up	Promotes NSCLC cells proliferation, EMT and invasion	(28)
miR-629	FOXO1	Up	Promotes proliferation, migration and invasion in NSCLC cells	(29)
miR-654-3p	RASAL2	Down	Suppresses the viability and induce the apoptosis of NSCLC cells	(30)
miR-139-5p	HOXB2	Down	Inhibits proliferation and promotes apoptosis, enhances cisplatin sensitivity in NSCLC cells	(31)
miR-144	TLR2	Down	Inhibits NSCLC cells migration, invasion and release of inflammatory cytokines related to tumor chemical resistance	(32)
miR-195-5p	HOXA10	Down	Inhibits the growth, invasion and migration of LUAD, increases the proportion of cells in G1 phase in the cell cycle, promotes apoptosis and enhances radio sensitivity	(33)
miR-196b	AQP4	Up	Promotes the invasion and migration of LUAD cells, and the poor prognosis and low survival in patients with LUAD	(34)
miR-20a-5p	KLF9	Up	Promotes the proliferation and invasion of NSCLC cells	(35)
miR-1	Mpl	Down	Inhibits NSCLC growth and angiogenesis	(36)
miR-3157-3p	TIMP2/KLF2	Up	Promotes angiogenesis and increased vascular permeability	(37)
miR-543	MTA1	Up	Promotes proliferation and angiogenesis in NSCLC	(38)
miR-153	Jagged1/Notch1	Down	Inhibits stem cell properties and tumor growth of lung cancer cells	(39)
miR-582-3p	AXIN2, DKK3 and SFRP1	Up	Maintain stem cell-like characteristics and promote tumorigenesis, chemoresistance and relapse of NSCLC cells	(40)

miR, microRNA; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition; FOXO1, Forkhead box O1; RASAL2, ras protein activator like 2; HOXB2, homeobox B2; TLR2, toll like receptor 2; HOXA10, homeobox A10; LUAD, lung adenocarcinoma; AQP4, aquaporin-4; KLF9, Krüppel-like factor 9; Mpl, monophosphoryl lipid A; TIMP2/KLF2, Tissue inhibitor of metalloproteinases 2/Krüppel-like factor 2; MTA1, metastasis associated protein 1; AXIN2, axis formation inhibitor 2; DKK3, dikkopf-3; SFRP1, secreted frizzled-related protein 1.

Fine particulate matter (PM2.5) accelerates the development of NSCLC by suppressing the expression of miR-139-5p. High dose PM2.5 stimulation causes precancerous lesions such as bronchial epithelial dysplasia in mice, which promotes the EMT process and raises the risk of LC by lowering the level of E-cadherin protein and raising the level of vimentin protein. Meanwhile, PM2.5 regulates Notch1, the target of miR-139-5p (61). Notch1 is overexpressed in NSCLC, and that is associated with the disease's progression and poor prognosis (62). Overexpression of miR-139-5p can prevent NSCLC from developing by suppressing Notch1 expression and reversing PM2.5-induced EMT, indicating that miR-139-5p has the potential to be a therapeutic target in NSCLC.

miR-152. miR-152 is considered as a tumor suppressor and its expression is usually downregulated in different solid tumors. Tensin1 (TNS1), a member of the focal adhesion-associated proteins family, is essential for preserving normal tissue and structural stability. TNS1 is involved in cell proliferation, adhesion, migration, and regulation of signal transduction pathways (63). miR-152 could directly target and suppress the expression of TNS1. Therefore, inhibiting the expression of TNS1 could reduce metastasis and invasion of NSCLC cells (64). In addition, the core region of miR-152 is hypermethylated, and the hypermethylation level is regulated by the DNA methyltransferase 3B (DNMT3B), which leads to a reduction in miR-152 expression. Simultaneously, miR-152 directly targets and inhibits the expression of neural cell adhesion molecule 1 (NCAM1). As a highly expressed transmembrane protein in NSCLC, the NCAM1 gene may promote the proliferation and metastasis of NSCLC cells (65). This suggested that inhibiting DNMT3B can reduce the methylation level of miR-152. The overexpression of miR-152 inhibits NCAM1 and reduces NSCLC cell proliferation (66).

Fibroblast growth factor 2 (FGF2) is another target of miR-152, which is a multifunctional cytokine that expresses and influences multiple biological processes in a variety of cancers. The FGF/FGFR signaling pathway controls a number of biological functions, including cell proliferation, differentiation and migration (67). miR-152 specifically binds and inhibits the expression of FGF2 mRNA and protein, which participates in preventing NSCLC cell proliferation and migration (68).

LncRNA, a non-coding RNA, can bind to miRNA but cannot be transcribed into a protein. LncRNAs play crucial roles in a variety of biological processes, including cell proliferation, differentiation, apoptosis, and its dysregulation can lead to cancer. It is reported that lncRNA colon cancer-associated transcript 1 (CCAT1) sponge stimulates NSCLC cell growth and migration by suppressing the expression of miR-152. CCAT1 promotes EMT with the downregulation of E-cadherin (69). LncRNA prostate cancer gene expression marker-1 (PCGEM1) is correlated with lymph node metastasis and TNM stage in NSCLC. PCGEM1 targets and inhibits miR-152-3p to promote NSCLC proliferation and migration (70).

Table II. miRNA as a biomarker for early diagnosis and prognostic of NSCLC.

Up miRNA	Down miRNA	Sample type	Selection cohort	Validation cohort	Analytical technique	Normalization	Effect	(Refs.)
miR-339-3p	-	Serum	25 NSCLC 30 OLs 19 HC	117 NSCLC 113 OLs	TaqMan low-density arrays RT-qPCR	RNU6	Diagnostic biomarker for NSCLC	(71)
miR-762	-	Serum	148 NSCLC 60 HC	-	RT-qPCR	cel-miR-39	Diagnostic and prognostic biomarker for NSCLC	(72)
miR-146b miR-205 miR-29c miR-30b miR-337 miR-411	-	Serum	63 NSCLC 15 HC	65 NSCLC 17 COPD 15 HC	RT-qPCR	mean of CT of all healthy controls	Early diagnosis and prognosis biomarker for NSCLC	(74)
miR-1247-5p miR-301b-3p miR-105-5p	-	Plasma	154 NSCLC 146 HC	-	miRNA microarray RT-qPCR	U6 snRNA	Early diagnosis for NSCLC	(11)
miR-16-5p miR-92a-3p miR-451a	-	Plasma	38 NSCLC 21 HC	40 NSCLC 40 HC	Nanostring nCounter® assay RT-qPCR	The exogenous ath-miR-159	Early detection for LC	(75)
miR-210 miR-1290 miR-150 miR-21-5p	-	Plasma	40 NSCLC 20 HC	88 NSCLC 50 BLD 40 HC	RT-qPCR	U6 snRNA	Early diagnosis and prognosis for NSCLC	(76)
miR-760	miR-139-3p miR-17 miR-19a miR-26b miR-451	Plasma	220 high-risk individuals 156 AC 67 SCLC 122 SQ	203 high-risk individuals 133 AC 49 SCLC 76 SQ	miRNA microarray RT-qPCR	miR-1228	Diagnosis of LC and further discrimination of SCLC and NSCLC	(77)
miR-31-5p miR-210-3p miR-21-5p miR-486-5p	-	Sputum and plasma	76 NSCLC 72 HC	56 NSCLC 55 HC	RT-qPCR	U6 snRNA	Early detection for NSCLC	(78)
-	miR-186	Serum and exhaled breath condensate	62 NSCLC 60 HC	-	RT-qPCR	cel-miR-39	The diagnosis and severity assessment of NSCLC	(79)

Table II. Continued.

Up miRNA	Down miRNA	Sample type	Selection cohort	Validation cohort	Analytical technique	Normalization	Effect	(Refs.)
miR-21	miR-126	Serum	60 NSCLC	-	RT-qPCR	U6 snRNA	Early diagnosis for LC	(80)
miR-143	miR-155		60 HC					
miR-145	let-7a							
	miR-146							
	miR-31							
	miR-182							
	let-7g							
	miR-19b							
miR-629		Serum	166 NSCLC 70 NMLDs 100 HC	-	RT-qPCR	-	Prognostic biomarker for NSCLC	(81)

miR, microRNA; NSCLC, non-small cell lung cancer; OLs, other lung diseases; HC, healthy controls; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; RNU6, small nuclear RNA6; COPD, chronic obstructive pulmonary disease; LC, lung cancer; BLD, benign lung disease; AC, adenocarcinoma; SCLC, small cell lung carcinoma; SQ, squamous cell carcinoma; NMLDs, non-malignant lung diseases.

4. miRNAs as biomarkers for NSCLC

miRNAs have the potential to become high-quality biomarkers with the advantages of high stability, non-invasive, convenient, and efficient screening methods. In several studies, researchers used miRNA microarray or reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to analyze serum miRNA levels for patients with NSCLC, benign lung disease (BLD) and healthy subjects, to select specific miRNAs for routine examination to improve NSCLC sensitivity and specificity for early diagnosis (Table II).

In a previous study, Trakunram *et al* (71) used a TaqMan low-density array to compare the expression levels of 745 miRNAs in NSCLC, BLD and healthy subjects, and selected miR-339-3p through verification set and diagnostic evaluation. The area under the curve (AUC) of the miRNA is 0.616, indicating that it has guiding significance in the diagnosis of NSCLC. Chen *et al* (72) used RT-qPCR to profile miRNAs in 148 NSCLC patients and healthy controls. The high level of miR-762 was related to an advanced stage, poor tumor grade and positive lymph node metastasis. Combined detection of miR-762, carcinoembryonic antigen (CEA), and cytokeratin fragment antigen 21-1 (CYFRA21-1) could improve the diagnostic accuracy for NSCLC. Furthermore, miR-762 expression can be used as a predictive biomarker for gefitinib resistance, and high expression predicts poor therapeutic effect (73).

In addition, Yang *et al* (74) selected serum miRNAs for NSCLC early diagnosis, and 8 miRNAs were selected and validated by training set and validation set, ultimately obtaining the best predictive model composed of miR-146b, miR-205, miR-29c and miR-30b. The combination could be used not only in the diagnosis of NSCLC patients but also in NSCLC subtypes analysis and TNM staging. AUC of the combined training and verification sets was estimated to be 0.96 with 95.31% sensitivity and 82.98% specificity.

The researchers measured the expression of specific miRNAs in plasma, not just the serum sample. Dong *et al* (11) used a miRNA chip to examine the miRNAs in plasma from NSCLC patients and healthy volunteers. RT-qPCR was used to evaluate the expression of 11 upregulated miRNAs. Three plasma miRNAs (miR-1247-5p, miR-301b-3p and miR-105-5p) were selected and finally determined to distinguish between early NSCLC patients and healthy individuals, and their AUC are 0.769, 0.761 and 0.777, respectively. Reis *et al* (75) performed a study based on the NSCLC subtypes. They used Nanostring nCounter ®technology to evaluate the expression of miRNA in LUAD, LUSC and healthy controls, and identified a correlation between the expression of the majority of miRNAs in the two histological subtypes. A total of 12 differentially expressed miRNAs were selected for verification and the expression level of 11 miRNAs were consistent with the found set. Furthermore, 3 miRNAs (miR-16-5p, miR-92a-3p and miR-451a) with the best statistical performance were selected for pathway enrichment analysis, and it was found that the 3 miRNAs were related to the LC pathways such as epidermal growth factor receptor (EGFR) and PI3K/AKT. Concurrently, these 3 miRNAs can predict NSCLC with high specificity and sensitivity. Moreover, the researchers

selected 12 previously reported aberrantly expressed miRNAs in NSCLC. A total of 4 miRNAs (miR-210, miR-1290, miR-150 and miR-21-5p) obtained from test set and verification set could distinguish NSCLC, BLD and healthy individuals. In the study of postoperative NSCLC patients, it was found that the significantly decreased expression of the 4 miRNAs were predictors of prolonged disease-free survival. A total of 2 miRNAs (miR-210 and miR-150) could predict patient's prognosis even if their expression levels do not significantly alter as NSCLC progresses (76). Based on miRNA chip, Lu *et al* (77) identified 6 miRNAs (miR-17, miR-190b, miR-19a, miR-19b, miR-26b and miR-375). These 6 miRNAs could distinguish between LC and asymptomatic high-risk patients through screening in three stages of discovery, training and verification. Further research showed that 3 miRNAs (miR-17, miR-190b and miR-375) could accurately differentiate SCLC from NSCLC.

Previous studies have indicated that combining different fluid biopsies could improve the accuracy of NSCLC detection. For example, Liao *et al* (78) used the Taqman miRNA assay to detect the expression of 2 miRNAs (miR-31-5p and miR-210p-3p) in sputum and 3 miRNAs (miR-21-5p, miR-210-3p and miR-486-5p) in plasma. The logical regression model with limited parameters in least absolute shrinkage and selection operator was used to optimize the miRNA detection panel. The detection of 2 sputum miRNAs (miR-31-5p and miR-210-3p) and 1 plasma miRNA (miR-21-5p) in the combined model had a synergistic effect on the diagnosis of NSCLC. The combination study proved that the analysis of 2 sputum miRNA biomarkers and 1 plasma miRNA biomarker had improved performance than a single class of miRNA biomarkers. Similarly, the study by Xie *et al* (79) revealed a positive correlation between the expression of miR-186 in serum and exhaled breath condensate, and the combination of decreased miR-186 and increased IL-1β were used for the diagnosis and severity evaluation of NSCLC.

Researchers typically assessed the effectiveness of the miRNA diagnostic model for the study of early diagnosis of miRNA by using logical regression analysis and receiver operating characteristic (ROC) curve (78,80). The majority of miRNAs have been studied to distinguish early NSCLC patients from BLD patients or healthy individuals, while certain miRNAs have been studied to identify NSCLC subtypes. Previous studies (11,74) revealed that miRNAs have high specificity and sensitivity, indicating that there is considerable potential for using miRNA in the early detection of NSCLC. However, it has poor repeatability for miRNA detection. The reasons may be the heterogeneity of NSCLC patients and the regulation of tumor formation by multiple genes. The selected miRNA should be validated in large-scale NSCLC patients utilizing standard operating procedures in the future. Based on understanding the pathway of miRNA mechanism, the best miRNAs combination for NSCLC diagnosis would be found.

In addition, miRNAs can serve as prognostic biomarkers. Higher levels of the serum miR-629 have been associated with poor differentiation, lymph node metastases and advanced clinical stage in patients with NSCLC compared with those with non-malignant lung disease and healthy controls (81). miRNAs differentially expressed in serum samples provide a novel basis for predicting the prognosis of NSCLC patients.

5. Exosomal miRNAs as potential biomarkers for NSCLC

Focus has been addressed on exosomal miRNAs as potential biomarkers since they are one of the major components of exosomes and play functional roles in cell-to-cell communication. Exosomal miRNAs may be used as prognostic and diagnostic biomarkers for NSCLC (Table III).

Exosomal miRNAs in blood have been extensively studied as biomarkers for the diagnosis of NSCLC. A novel immunomagnetic separation technique was used to selectively extract exosomes from serum of patients, which is more specific than traditional ultracentrifugation, and a multiplexed array sensor is used to simultaneously detect 4 exo-miRNAs (miR-21, miR-155, miR-205 and miR-let-7b) (82). A microarray-based study found that the combination of exosomal miR-5684 and miR-125b-5p had effective diagnostic value (AUC=0.744) for patients with NSCLC. Notably, in the tumor staging studies, it was found that exosomal miR-125b-5p is highly diagnostic in distinguishing between early and late stage, lymph node metastasis and distant metastasis (83). In comparison with traditional tumor markers, the level of miR-17-5p was significantly increased in NSCLC patients compared with healthy controls, and the detection performance of miR-17-5p was superior to CEA, CYFRA21-1 and squamous cell carcinoma antigen. The combination of these 4 tumor markers outperforms a single exosomal miR-17-5p in terms of diagnostic performance, indicating that the combination of exosomal miRNA and conventional tumor markers have significant clinical utility for the diagnosis of NSCLC (84).

Exosomes are circulating membrane-enclosed vesicles that contain miRNA, RNA, lipids, and proteins. By adhering to the target cell membrane, exosomes can transport miRNA and other contents from donor cells to recipient cells, which is relevant to the diagnosis and prognosis of NSCLC patients (85). For example, an increased level of miR-1246 isolated from serum exosomes was associated with a lower survival rate in patients with NSCLC (16). Plasma exosomal miR-4448 was shown to be decreased in patients with metastatic LUAD. Exosomal miR-4448 could be used as a diagnostic marker for patients with metastatic LUAD (86). In addition, elevated levels of exosomal miR-23b-3p, miR-10b-5p and miR-21-5p were independently associated with poor overall survival in patients with NSCLC (87). Plasma exosomal miR-451a was evaluated to be a reliable biomarker for predicting recurrence and prognosis in NSCLC patients with stage I, II or III cancer (88). Similarly, plasma exosomal miR-4257 and miR-21 have been identified as biomarkers of recurrence and TNM stage in NSCLC patients (89).

6. Comparison of circulating miRNAs with exosomal miRNAs

With the further development of miRNA research, circulating miRNAs and exosomal miRNAs may become the primary research forms in the early diagnosis and prognosis of NSCLC and other cancers. Few studies have simultaneously compared

Table III. Exosomal miRNAs as diagnostic markers for NSCLC.

Up exosomal miRNA	Down exosomal miRNA	Sample type	Selection cohort	Validation cohort	Technique	Normalization	Clinical value	(Refs.)
miR-21 miR-155 miR-205 let-7b	-	Serum	7 NSCLC 1 HC	-	Conductive polymer-based exo-miRNA array sensor	-	Ultrasensitive and reproducible electrochemical detection of LC	(82)
-	miR-5684 miR-125b-5p	Serum	2 NSCLC 1 HC	330 NSCLC 312 HC	miRNA microarray RT-qPCR	U6 snRNA	Diagnostic and prognostic biomarkers for NSCLC	(83)
miR-17-5p	-	Serum	100 NSCLC 90 HC	72 NSCLC 47 HC	RT-qPCR	miR-16-5p	Early diagnostic marker for NSCLC	(84)
miR-1246	-	Serum	150 NSCLC 50 NMRD 50 HC	-	RT-qPCR	cel-miR-39	Useful diagnosis and prognosis for NSCLC	(16)
-	miR-4448	Plasma	2 NSCLC metastasis 2 NSCLC N-metastasis 2 HC	20 NSCLC metastasis 20 NSCLC N-metastasis 20 HC	miRNA microarray RT-qPCR	U6 snRNA	Diagnostic marker for metastatic adenocarcinoma	(86)
miR-23b-3p miR-10b-5p miR-21-5p	-	Plasma	10 AC 10 HC	196 NSCLC 11 non-tumor respiratory diseases 10 HC	RT-qPCR	let-7a-5p	Prognostic biomarkers for NSCLC	(87)
miR-451a	-	Plasma	6 NSCLC 3 HC	285 NSCLC 24 HC	miRNA microarray RT-qPCR	RNU6B	Biomarker for recurrence and prognosis of NSCLC	(88)
miR-21 miR-4257	-	Plasma	6 NSCLC 3 HC	195 NSCLC 30 HC	miRNA microarray RT-qPCR	miR-16a	Biomarker for the recurrence of NSCLC	(89)

miR, microRNA; NSCLC, non-small cell lung cancer; HC, healthy controls; LC, lung cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; AC, adenocarcinoma; RNU6B, mall nuclear RNA6B; NMRD, non-malignant respiratory diseases.

their detection performance. Exosomal miRNA may be more stable than circulating miRNA due to the protection of lipid bilayer. The distribution of miR-126 in the circulation of NSCLC patients at the early and advanced stages of the disease was evaluated, and it was found that miR-126 is primarily found in exosomes in the early and late stages of NSCLC. The levels of miR-126 increased in exosomes while they decreased in the exosome-free serum (90). Similarly, compared with whole plasma, the content of miR-21 in the exosomes of patients with hepatoblastoma was higher (91). The findings revealed a difference in the distribution of specific miRNAs between circulating miRNAs and exosomal miRNAs. The levels of miRNAs were higher in exosomes than serum or other bodily fluids.

Circulating miRNAs and exosomal miRNAs were distributed differentially in NSCLC patients. Several studies simultaneously investigated the changes in circulating miRNA and exosomal miRNA in cancer. In ovarian cancer, 5 miRNAs (miR-200c-3p, miR-346, miR-127-3p, miR-143-3p and miR-205-5p) were significantly upregulated in serum and exosomes (92). Wu *et al* (93) measured the expression levels of 8 serum miRNAs and their corresponding exosomal miRNAs in NSCLC, benign pulmonary lesions and healthy subjects. The AUC values of exosomal miR-146a-5p and miR-486-5p were found to be over 0.8, but the AUC values of 4 serum miRNAs (miR-21-5p, miR-141-3p, miR-222-3p and miR-486-5p) were all less than 0.8. The present study demonstrated that exosomal miRNA had an improved detection performance than circulating serum miRNA in identifying cancer samples from healthy control samples. The detection performance of the same miRNA in different studies was compared, and it was identified that the diagnostic value of exosomal miR-1246 (AUC=0.827) was greater than that of circulating plasma (AUC=0.641) (16,94). Exosomal miR-205-5p diagnostic value (AUC=0.806) was similar to that in circulating serum (AUC=0.8250) (95,96). To obtain more accurate detection results, it is necessary to detect circulating free and exosomal miRNA for one patient at the same time, and then identify which sample type is reasonable for miRNA detection.

7. Future prospects and conclusion

Numerous studies have shown that the dysregulation of miRNA is an important driver of NSCLC progression and plays crucial roles in the early diagnosis, treatment and prognosis of NSCLC (97,98). As there is a wide variety of miRNAs, there is also diversity in the roles of these miRNAs in NSCLC. The miRNAs in tissue or blood of patients with NSCLC, BLD, and healthy controls were examined using microarray, RT-qPCR, and next-generation sequencing (11,99,100). It has been found that a multitude of miRNAs have notable changes in NSCLC, suggesting that particular miRNAs can be used to diagnose NSCLC. Despite the existence of numerous studies on miRNAs, the mechanism of miRNAs in various tissue subtypes of NSCLC remains unknown due to the diversity of miRNA action mechanisms and the heterogeneity of NSCLC patients. The mechanisms of miRNAs should be studied more extensively and systematically in order to improve the use of miRNAs in clinical treatment. Additionally, the uniform operational procedure should be established for the repeatability of miRNAs detection in order to apply the miRNA detection with favorable performance for the clinical diagnosis of NSCLC.

In the present review, focus was addressed on the biological functions of miRNAs and their molecular mechanisms in the occurrence and progression of NSCLC, as well as the importance of various miRNAs in the diagnosis and prognosis of NSCLC. Although over 2,000 human miRNAs have been identified, most studies have focused on a single signaling pathway mechanism between a specific miRNA and its target gene. Future research should concentrate on the network of interactions between different miRNAs. In one word, miRNAs are well-known to exist in plasma and other bodily fluids and are one promising biomarker for NSCLC diagnosis.

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Authors' contributions

XL wrote the manuscript and revised it. QW designed and supervised the study. YW designed the tables and figure. SL edited and critically revised the article for intellectual content. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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