MicroRNAs: A novel potential biomarker for diagnosis and therapy in patients with non-small cell lung cancer

Qun Zhou^{1*} | Shao-Xin Huang^{2*} | Feng Zhang^{1*} | Shu-Jun Li^{3*} | Cong Liu¹ | Yong-Yong Xi¹ | Liang Wang¹ | Xin Wang² | Qi-Qiang He⁴ | Cheng-Cao Sun¹ | De-Jia Li¹

¹Department of Occupational and Environmental Health, School of Public Health, Wuhan University, Wuhan, Hubei 430071, China

²Department of Social Medicine and Public Health, School of Basic Medical Science, Jiujiang University, Jiujiang, Jiangxi, China

³Wuhan Hospital for the Prevention and Treatment of Occupational Diseases, Wuhan, 430022, Hubei, China

⁴Department of School of Public Health, Wuhan University, Wuhan, Hubei 430071, China

Correspondence

De-Jia Li and Cheng-Cao Sun, Department of Occupational and Environmental Health, School of Public Health, Wuhan University, Wuhan, Hubei 430071, China.
Emails: lodjlwhu@sina.com; chengcaosun@whu.edu.cn

Funding information

This study is funded by the National Natural Science Foundation of China (No. 81360447), the National Natural Science Foundation of China (No.81372973), the National Natural Science Foundation of China (No. 81660535), the Fundamental Research Funds for the Central Universities (No. 2015305020202) to Cheng-Cao Sun and the National Postdoctoral Program for Innovative Talents (No. BX201700178) to Cheng-Cao Sun

Abstract

Background: Lung cancer is still one of the most serious causes of cancer-related deaths all over the world. MicroRNAs (miRNAs) are defined as small non-coding RNAs which could play a pivotal role in post-transcriptional regulation of gene expression. Increasing evidence demonstrated dysregulation of miRNA expression associates with the development and progression of NSCLC.

Aims: To emphasize a variety of tissue-specific miRNAs, circulating miRNAs and miRNA-derived exosomes could be used as potential diagnostic and therapeutic biomarkers in NSCLC patients.

Materials & Methods: In the current review, we paid attention to the significant discoveries of preclinical and clinical studies, which performed on tissue-specific miRNA, circulating miRNA and exosomal miRNA. The related studies were obtained through a systematic search of Pubmed, Web of Science, Embase.

Results: A variety of tissue-specific miRNAs and circulating miRNAs with high sensitivity and specificity which could be used as potential diagnostic and therapeutic biomarkers in NSCLC patients. In addition, we emphasize that the miRNA-derived exosomes become novel diagnostic biomarkers potentially in these patients with NSCLC.

Conclusion: MiRNAs have emerged as non-coding RNAs, which have potential to be candidates for the diagnosis and therapy of NSCLC.

Abbreviations: NSCLC, Non-small cell lung cancer; SCC, squamous cell carcinoma; ZEB1, zinc finger E-box-binding homeobox 1; TGFBR2, transforming growth factor beta receptor 2; BMPR2, bone morphogenetic protein receptor type 2; CDK4, cyclin-dependent kinase 4; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; DNMT1, DNA methyltransferase 1; PTEN, tensin homologue deleted on chromosome 10; HOXA9, homeobox A9; FoxO1, forkhead box O1; AKT, serine/threonine kinase 1; CYP1B1, cytochrome P450 family 1 subfamily B member 1; MAP3K2, mitogen-activated protein kinase kinase kinase 2; STAT3, signal transducer and activator of transcription 3; ITGB1, integrin subunit beta 1; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; VIM, vimentin; CX3CR1, chemokine (C-X3-C motif) receptor 1; FYCO1, FYVE and coiled-coil domain containing 1; E2F2, E2F transcription factor 2; TCGA, The Cancer Genome Atlas; MT1G, metallothionein 1G; TICs, tumour-initiating cells; qPCR, quantitative polymerase chain reaction; MSCs, mesenchymal stem cells; HSP70, heat shock protein 70; MCP-1, macrophage cationic peptide 1; TLR2, toll-like receptor 2; TLR7, toll-like receptor 7; TLR8, toll-like receptor 8.

*These authors contributed equally to this work

1 | BACKGROUND

Lung cancer is one of the most harmful diseases which associates with cancer deaths around the world, with more than 220 000 new diagnoses and nearly 158 000 deaths occurring in the United States in 2016.¹ Non-small cell lung cancer occupied nearly 85% of lung cancer cases; it was composed primarily of squamous cell carcinoma (SCC) and adenocarcinoma.² In spite of advances in the diagnosis and current molecular targeted therapies, the overall 5-year survival rate is only 18%.¹ Consequently, there is an urgent assignment to investigate the potential molecular mechanisms of lung tumorigenesis and identify novel therapeutic targets, which can enhance the survival probability for patients with lung cancer.

MicroRNAs (miRNAs) are defined as small non-coding RNAs with a length of about 20-25 nucleotides, which exert pivotal effects in various biological processes.³ miRNAs can regulate gene expression at post-transcriptional level through 3'-untranslated region (3'UTR) pairing with target gene mRNAs.⁴ miRNAs regulate various targets which have critical roles in a wide spectrum of biological processes, including tumorigenesis and development, cell proliferation, metastasis, invasion and apoptosis.⁵ In addition, miRNA participated in almost all of the essential signalling pathways, including the expression of many important tumour-associated genes such as oncogenes and tumour suppressors.⁶ The identification of alteration in miRNA levels is associated with various human cancers including NSCLC.⁷ A large number of studies have shown that the identification of various risk factors and biomarkers could help to better comprehend the cellular and molecular alterations involved in the cancer progress including NSCLC. In addition, it would be a better monitor of NSCLC patients for therapy by using a powerful diagnostic and prognostic biomarker.⁸ miRNAs have appeared to be novel diagnostic, therapeutic and prognostic biomarkers in NSCLC patients among various recommended biomarkers such as DNAs, RNAs, proteins, epigenetic changes and glycoproteins.⁹ Several lines of evidence have confirmed that miRNAs were related to molecular pathological changes and signalling cascades of lung cancer.¹⁰ Additionally, dysregulation of these molecules could be associated with inhibition and/or activation of several cellular and molecular therapy targets, which have functions in tumorigenesis and development of NSCLC.¹¹ miRNAs were considered to be novel candidates for the diagnosis and therapy of NSCLC, as it could be detected with non-invasive and comparatively easy assay methods.

Moreover, the discovery of miRNA in exosomes opened an attractive world that they could play an important role in diagnosing patients with NSCLC as significant biomarkers.¹² Exosomes were drivers as small vesicles (50-150 nm) of endocytic initially, which released from many different cell types, such as tumour cells, stem cells, mesenchymal cells, thrombocytes and some normal cells.¹³ There is emerging evidence that suggest that vesicles could carry various cargos such as DNA, mRNAs, miRNAs and proteins. It has been reported that secretion of exosomes could result in alteration in the behaviour of cancer cells or tumours.^{14,15} As a proof concept, exosomes might be applied as significant diagnostic and therapeutic biomarkers in NSCLC patients. In this paper, we summarize a variety of tissue-specific miRNAs and

circulating miRNAs with high sensitivity and specificity which could be used as potential diagnostic and therapeutic biomarkers in NSCLC patients. In addition, we emphasize that the miRNA-derived exosomes become novel diagnostic biomarkers potentially in these patients with NSCLC.

2 | FEATURES OF MIRNA

The miRNAs are endogenous small (19-22 nucleotides), non-coding RNAs which play pivotal roles in various biological processes.³ The miRNA genes are typically transcribed from RNA polymerase II into long primary transcripts, which are up to several kilobases (pri-miRNA).¹⁶ Then they undergo a further step that processes it into shorter hairpin sequences of approximately 70 nucleotides (pre-miRNA) by the nuclear microprocessor complex formed by the RNase III Drosha¹⁷ and DiGeorge syndrome critical region gene 8 (DGCR8).¹⁸ Next, these pre-miRNA are transported into the cytoplasm carried out by RNase III Dicer to form a double-stranded RNA.¹⁹ Generally, one of the chains is chosen to act as the mature miRNA through the Argonaut proteins to enter the RNA-induced silencing complex (RISC), while the remaining complementary chain is degraded. The miRNA recognizes target genes based on sequence complementarity, primarily to the 3'-UTR of the target mRNAs. Moreover, miRNA modulates the translation of proteins through binding to their target mRNA.^{4,20-22} So miRNA mediates translational repression or degradation of mRNAs depending on the degree of homology to their 3'-UTR target sequence, and miRNA could regulate gene expression emerged in multiple signalling participating in carcinoma growth, dissemination, metastatic and even rejection to therapy.²³ Accordingly, dysregulated miRNA will contribute to a variety of pathological events, including cancer initiation and progression.

3 | MECHANISMS OF MIRNA IN CANCER

It is essential to maintain normal cellular homeostasis through controlling of miRNA levels precisely. During the past years, it is a critical task to determine the mechanisms of how the miRNA was dysregulated in cancer.

Several miRNA genes are located in cancer-related genomic regions or in fragile sites; miRNA is highly susceptible to genomic alterations of solid tumours and haematological malignancies.²⁴ Calin et al²⁵ suggested that the loss or down-regulation of miR-15a and miR-16-1 located at a 30-kb region on chromosome 13q14 is caused by frequent deletions or down-regulations of B-cell chronic lymphocytic leukaemia. Follow-up studies demonstrated that a large number of miRNA genes are in cancer-related genomic regions or in fragile sites. For example, there is a loss of heterozygosity region of 17p13 aligning with the hsa-miR-22 cluster in lung cancer.²⁶

Aberrant epigenetic changes such as DNA hypermethylation, extensive genomic DNA hypomethylation and alteration of histone modification patterns appear frequently in cancer cells.²⁷ Actually,

epigenetic modifications represent a widespread mechanism associated with the changes of miRNA expression in cancer.²⁸ For example, miR-145 was demonstrated to be induced after DNA methylation in patients with lung adenocarcinoma.²⁹ As for histone modifications of miRNA, Seol et al³⁰ suggested that miR-373 was silenced by histone modification in lung cancer cells and it functioned as a tumour suppressor through down-regulating IRAK2 and LAMP1. In addition, deregulation of the miR-101 could increase the expression of EZH2 during cancer progression, which contributed to the epigenetic silencing of target genes and regulated the metastasis of cancer cells.³¹

Most miRNA are transcribed by the RNA polymerase II; moreover, numerous PolII-related factors are involved in this process, which exert critical effect regulating miRNA genes transcription in cells.¹⁶ It is the first reported that miR-17/92 cluster mediated transcriptional activation through the c-Myc oncogene. c-Myc increases the expression of the miR-17/92 cluster, which is able to balance the apoptotic activity of E2F1.³² miR-34 family was identified to be transcriptionally regulated by the fundamental tumour suppressor p53 and played pivotal roles controlling p53-mediated cell cycle arrest and apoptosis.³³

In brief, dysregulation of miRNA in cancer can occur at genetic and epigenetic, transcriptional and post-transcriptional levels via miRNA genomic localization, epigenetic changes such as DNA methylation and histone modifications.

4 | MIRNA FUNCTION IN CANCER

The development of cancer is a multi-step process that involves multiple alterations in oncogenes and tumour suppressor genes over a period of time, generally over several years. Cancer-associated miRNA have been known as 'onco-miRs', which play critical roles as oncogenes and/or tumour suppressors. A variety of studies suggested that

miRNA would play a critical role as tumour suppressors or oncogenes. Valuably, they modulate almost all cell functions including apoptosis, proliferation, cell cycle, differentiation, stem cell maintenance and metabolism.²³ Selected functional miRNAs and their targets in NSCLC are summarized in Figure 1.

4.1 | Tumour suppressor

It is reported that the miR-34 family was down-regulated in cancer tissues compared with normal tissues, and decreased expression of miR-34a was associated with a worse prognosis of cancer.³⁴ Bommer et al³⁵ confirmed that miR-34 expression was significantly decreased in NSCLC and their data also suggested that the miR-34 family might be the key effectors of p53 tumour suppressor to inhibit NSCLC cells growth. Young Hun Kim et al³⁶ identified the combined effect of miR-34b/c methylation on the prognosis of NSCLC. These results suggest that methylation of the miR-34 families may serve as an inhibitor of tumour metastasis and as prognostic biomarkers for patients with NSCLC.

The miRNA encoded by the let-7 family (including 12 human homologues) are considered to be tumour inhibitors because they map to brittle sites associated with lung, breast, urothelial and cervical cancers.²⁶ It has been shown that the inhibition of oncogenes, such as rat sarcoma (RAS), myelocytomatosis oncogene (Myc), the high mobility group AThook 2 (HMGA2), cyclin D2, cyclin-dependent kinase 6 (CDK6) and cell division cycle 25A (CDC25A) in cellular growth and proliferation, led to suppression of cancer cell proliferation and cell cycle distribution.³⁷ Takamizawa et al³⁸ observed that the expression of let-7 frequently decreased in lung cancer both in vitro and in vivo; in addition, the down-regulated let-7 was significantly associated with poor post-operative survival, independently with the state of disease.

FIGURE 1 Dysregulated miRNA participating in biological processes of lung cancer. The schematic representation depicting a variety of miRNA would emerge in cell proliferation, migration, invasion and apoptosis when down-regulated or up-regulated. It is known to us that miRNA play tumour suppressor and/or oncogenic roles in NSCLC by negatively modulating the target genes which exert tumour suppressor and/or oncogenic effect involved in cell proliferation, metastasis, invasion and apoptosis

miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) are demonstrated to be down-regulated in human cancer cells and tumours due to aberrant epigenetic gene silencing. In addition, these miRNAs exert pivotal effect inhibiting epithelial-to-mesenchymal transition (EMT), tumour cell metastasis and invasion through targeting key mRNAs involved in EMT.³⁹ In lung cancer cells, miR-200c overexpression led to reduced expression of zinc finger E-box-binding homeobox 1 (ZEB1) and increased expression of E-cadherin.⁴⁰

4.2 | Oncogenic miRNA

Apart from their roles as tumour suppressors, some miRNAs function as tumour promoters by targeting tumour suppressor genes have been proved to having important regulatory effects on cell proliferation in a variety of human cancers, including lung cancer.⁴¹ Members of this cluster could directly target the tumour suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which participated in the cell-survival signalling pathway phosphoinositide 3-kinase (PI3K)/Akt in lung cancer.⁴² Genetic mutation of p53 and inhibition of the miR-17-92 cluster are synthetic lethal in NSCLC due to up-regulation of vitamin D signalling.⁴³

miR-155 gene was found to be one of the miRNA most consistently in several solid tumours, where it was considered to be a marker of poor prognosis.⁴⁴⁻⁵⁰ The results suggested that miR-21 and miR-155 could promote the cell growth of NSCLC through down-regulating SOCS1, SOCS6 and PTEN.⁵¹

5 | CLINICAL APPLICATIONS OF MIRNA IN NSCLC

Early diagnosis of NSCLC is important for prognosis and can lead to reducing mortality rate in patients.⁵² After describing these examples of how miRNA target specific pathways that are important for NSCLC, an important and compelling application is to concern miRNA to be used as biomarkers in the clinic. The discovery of sensitive and specific biomarkers for NSCLC will improve current treatment of malignant disease and early detection of cancer.

Another important task is how to detect the miRNA in panels of different haematological and solid cancer types. Quantitative real-time reverse transcription PCR (qRT-PCR) is a quantitative gold standard for gene expression and it is one of the most common methods for detecting low levels of miRNA with high sensitivity and specificity.^{53,54} Recently, enzymatic luminescence miRNA assay, for example, quantitative bioluminescence assay, has been proposed as suitable for clinical diagnostic. This assay is designed for rapid and sensitive miRNA expression analysis based on isothermal rolling circle amplification.⁵⁵ Over the years, hybridization based-methods have improved such as in situ hybridization (ISH) with locked nucleic acid probes.⁵⁶ Microarray method⁵⁷ is widely used to screen large numbers of miRNAs simultaneously. Moreover, the appearance of deep-sequencing methods consider to be a new star which depend on next-generation sequencing

machines that can process millions of sequence reads in parallel in just a few days, has driven to detect most of the miRNA.⁵⁸ Circulating miRNA, which presented in serum, plasma, urine, saliva and other human body fluids, is often detected by high-throughput profiling techniques, sequencing and miRNA microarray combined with qRT-PCR.⁵⁹⁻⁶¹

Shen et al⁶² identified that miR-145 was down-regulated in poorly differentiated NSCLC specimens; moreover, the relative expression levels of miR-145 in stage II and III were significantly lower than those in stage I. In terms of miR-126, it was associated with tumour stage and lymph nodes metastasis, suggesting that miR-126 might play a key role in the progression and metastasis of NSCLC.⁶³ Stenvold et al⁶⁴ also found that miR-182 has tumour suppressor properties in stage II and in SCC patients, suggesting miR-182 tended to be a favourable prognostic factor in the total NSCLC cohorts. In addition, the expression of miR-451 in NSCLC tissues was also associated with poor degree of tumour differentiation, advanced pathological stage, lymph node metastasis and poor prognosis.⁶⁵ These clinical data suggested that miRNA could act as pivotal part for the detection of novel diagnostic, prognostic and therapeutic biomarkers in patients with NSCLC.

5.1 | Tissue-specific miRNA as diagnostic, prognostic and therapeutic biomarkers in NSCLC

miRNAs appeared to become a novel valuable diagnostic candidates, and would be more advanced than other various biomarkers, which are employed for the diagnosis of NSCLC in early stages.⁶⁶ It has been shown that significantly different miRNA expression profiles emerged in human tumours,⁷ and the dysregulated expression patterns of miRNA were detected with high sensitivity and specificity.⁶⁷ Moreover, miRNA has an advantage with high stability.⁶⁸ These evidences speculated that miRNA may have potential to play a crucial role in cancer.

Recently, there were a large number of studies identifying up-regulation or down-regulation of a variety of tissue-specific miRNAs in NSCLC.⁶⁹ Surgical resection remains the most effective treatment of early-stage NSCLC.³⁶ Up to this point, many patients with NSCLC still develop tumour metastasis and recurrence after receiving pulmonary resection procedure, due to lack of promising biomarkers for the diagnosis, prognosis, and therapy of lung cancer.⁷⁰ Gao et al⁷¹ identified a 7-miRNA signature, including miR-139, miR-326, miR-944, miR-190, miR-183, miR-182 and miR-101, as potential outcome predictor for SCC patients from clinical characteristics of Kaplan-Meier and ROC curves. miR-139 was suppressed frequently in primary NSCLCs; in addition, H3K27me3-mediated silencing of miR-139 significantly enhances invasiveness and lymph node metastasis of NSCLC.⁷² Mao et al⁷³ showed that miR-187-5p is down-regulated in NSCLC, which plays pivotal roles in promoting the growth and metastasis of NSCLC cells by directly targeting the oncogenic CYP1B1 gene.

miRNA appeared appropriately to become valuable therapeutic targets reversing cancer phenotypes or sensitizing tumours to chemotherapy. It is the fact that each miRNA simultaneously targets various genes, and a single miRNA is potential to regulate the activity of a

FIGURE 2 miRNA involved in epithelial-to-mesenchymal transition (EMT) in NSCLC. The schematic representation depicting EMT is a pivotal process to promote tumour cells. miR-134 inhibits EMT evidenced by up-regulation of E-cadherin expression and down-regulation of vimentin and integrin subunit beta 1 (ITGB1) expression. miR-206 inhibits EMT through decreased expression of c-Met and Bcl2 in lung cancer cells. Slug is a potent trigger for EMT, and miR-124 inhibits EMT by down-regulating Slug protein. FHIT inhibits EMT through transcriptional repression of EMT-related genes. A FHIT: Fragile histidine triad-activated miRNA, miR-30c, inhibits EMT via targeting metastasis-related genes (MTDH) and high mobility group A2 (HMGA2)

molecular signalling pathway, or coordinately regulate multiple targets in different pathways.^{23,74} Several lines of evidences identified that molecular/cellular targets could mediate tumorigenesis and development such as angiogenesis, apoptosis, survival, cell cycle and differentiation.⁷⁴ Therefore, in a global sense, dysregulation of miRNA would make some changes in the behaviour of cells and have a tendency to cancerous conditions. A variety of dysregulated miRNAs, which could act as potential diagnostic and therapeutic biomarkers in NSCLC patients, including let-7c,⁷⁵ miR-138,⁷⁶⁻⁷⁸ miR-145,⁷⁹ miR-183,⁸⁰ miR-29 family,⁸¹ miR-34a,⁸² miR-34c-3p,⁸³ miR-101-3p,⁸⁴ miR-129,⁸⁵ miR-200b,⁸⁶ miR-212,⁸⁷ miR-218,⁸⁸ miR-449a⁸⁹ and miR-451⁶⁵ were down-regulated, while miR-21/155,⁵¹ miR-25,⁹⁰ miR-31,⁹¹ miR-221/222,⁹² miR-224,⁹³ miR-191,⁹⁴ miR-494,⁹⁵ miR-19a⁹⁶ and miR-346⁹⁷ were up-regulated in NSCLC samples. These results indicated that the different expression profile of miRNA could be applied as diagnostic and therapeutic biomarkers in patients with NSCLC.⁹⁸

Huang et al⁹⁹ indicated that miR-186 played a key role as a tumour suppressor in NSCLC cells and its overexpression could inhibit cell proliferation, invasion, migration and induce cell apoptosis of NSCLC cells via targeting mitogen-activated protein kinase kinase 2 (MAP3K2). Hence, miR-186 might represent a novel therapeutic candidate in patients with NSCLC. Mitogen-activated protein kinase kinase 2 is one of great potential targets for NSCLC and plays critical roles in the tumorigenesis and development of NSCLC.⁹⁹

In other studies, Sun et al¹⁰⁰ confirmed that miR-9600 could be used as tumour suppressor and a negative controller for NSCLC patients to inhibit metastasis through signal transducer and activator of transcription 3 (STAT3) signalling pathway. In addition, miR-9600 is deregulated in NSCLC lung tissues and cell lines, which would be a favourable factor for prognosis. It has been observed that miR-9600 is capable to repress cell motility, metastasis, invasive and cell proliferation in vitro. This molecule exerts its effect via downstream genes expression of the STAT3 signalling pathway, which was relative to cell

cycle. miR-9600 could also have a certain function on the protein expression of MMP-2, MMP-7 and MMP-9 in A549 and SPC-A-1 cells. These results suggested that miR-9600 could play an important role as potential therapeutic biomarkers in NSCLC patients.¹⁰⁰

Integrin subunit beta 1 (ITGB1) is an essential subgroup of the integrin family, which plays an important role in regulating cell-extracellular matrix (ECM) adhesion and signalling, affecting various of cellular processes, including cell proliferation, apoptosis, metastasis, invasion and survival.¹⁰¹ It has been observed that positive expression or activation of ITGB1 signalling was associated with poor prognosis in lung cancer.¹⁰² Epithelial-mesenchymal transition is a pivotal process that promotes tumour cells to obtain a more migratory and invasive mesenchymal phenotype, during which tumour cells exert down-regulation of the expression of epithelial proteins such as E-cadherin, while up-regulation of the expression of mesenchymal proteins such as N-cadherin and vimentin (VIM; Figure 2).¹⁰³ Qin et al¹⁰⁴ demonstrated that miR-134 down-regulated ITGB1 expression and inhibited EMT, resulting in suppressed migration and invasion of NSCLC both in vitro and in vivo. These researches indicated that miR-134 might be applied as a novel therapeutic biomarkers in patients with NSCLC.¹⁰⁴ Table 1 illustrates a large number of miRNAs and their targets that are involved in lung cancer pathogenesis and could be used as diagnostic, prognostic and therapeutic biomarkers in this disease.

6 | CIRCULATING MIRNAS AS DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC BIOMARKERS IN NSCLC

A large number of studies have suggested circulating miRNA was known as a miRNA released into bloodstream.¹⁰⁵ Chen et al and Mitchell et al^{106,107} revealed that large amounts of circulating miRNA were detected stably in serum and plasma, which could be used as

TABLE 1 Tissue-specific miRNAs up-/down-regulated in lung cancer

miRNA	Expression	Target genes or pathways	Function of NSCLC	References
miR-139	Down-regulation	PDE2A	Enhance invasiveness and metastasis	72
miR-187-5p	Down-regulation	CYP1B1	Promote growth and metastasis	73
Let-7c	Down-regulation	ITGB3, MAP4K3	Suppress metastasis and invasion	75
miR-138	Down-regulation	Sirt1, GIT1, SEMA4C, EZH2	Suppresses the proliferation, metastasis	76-78
miR-145	Down-regulation	FSCN1	Inhibits migration and invasion	79
miR-183	Down-regulation	FoxO1	Promotes growth	80
miR-29 family	Down-regulation	WIF-1	Inhibits proliferation and induce apoptosis	81
miR-34a	Down-regulation	TGF β R2	Inhibits proliferation and promotes apoptosis	82
miR-34c	Down-regulation	PAC1/MAPK	Suppresses proliferation and invasion	83
miR-101-3p	Down-regulation	MALAT-1	Inhibits growth and metastasis	84
miR-129	Down-regulation	MMP9	Prevent the metastasis	85
miR-200b	Down-regulation	FSCN1	Inhibits migration and invasion	86
miR-212	Down-regulation	SOX4/EMT	Inhibited cell migration, invasion and EMT	87
miR-218	Down-regulation	HMGB1	Suppresses cell migration and invasion	88
miR-449a	Down-regulation	c-Met	Inhibits migration and invasion	89
miR-451	Down-regulation	RAB14	Inhibits proliferation and promotes apoptosis	65
miR-9600	Down-regulation	STAT3	Inhibits proliferation, migration, invasion and promotes apoptosis	100
miR-186	Down-regulation	MAP3K2	Inhibit cell proliferation, invasion, migration and induce apoptosis	99
miR-134	Down-regulation	ITGB1	Suppress migration and invasion	104
miR-21/155	Up-regulation	SOCS1, SOCS6, PTEN	Promote cell growth	51
miR-25	Up-regulation	FBXW7	Promotes cell proliferation and motility	90
miR-31	Up-regulation	ABCB9	Inhibits apoptosis	91
miR-221/222	Up-regulation	EMT	Inhibits cell growth and apoptosis	92
miR-224	Up-regulation	RASSF8: RAS association domain family 8	Promotes cell proliferation	93
miR-191	Up-regulation	NIFA: transcription factor, nuclear factor 1 α	Promotes cell proliferation and migration	94
miR-494	Up-regulation	BIM: also called BCL2-like 11	Promotes cell proliferation and Inhibits apoptosis	95
miR-346	Up-regulation	XPC, ERK, E-cadherin	Promotes cell growth and metastasis, and suppresses cell apoptosis	97
miR-19a	Up-regulation	SOCS1	Promotes cell growth metastasis and inhibits invasion	96

ITGB1, integrin subunit beta 1; MAP3K2, mitogen-activated protein kinase kinase kinase 2; PTEN, tensin homologue deleted on chromosome 10; STAT3, signal transducer and activator of transcription 3; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FoxO1, forkhead box O1

non-invasive serological markers of tumours concurrently in 2008. Circulating miRNA could be a novel attractive option for the diagnosis and treatment of several diseases including lung cancer. Multiple lines of evidence has revealed that dysregulation of circulating miRNA could be used as potential diagnostic and therapeutic biomarkers in NSCLC.¹⁰⁸⁻¹¹⁶

Circulating miR-422a may provide a potential target for therapeutic approaches in management of lung cancer.¹¹⁷ Wu et al¹¹⁷ suggested that miR-422a would play the most crucial diagnostic roles in

lymphatic metastasis through miRNA microarray (AUC, 0.744; 95% CI, 0.570-0.918). They conducted a group of 51 lung cancers and found that miR-422a could act as a potential diagnose in the lung cancer patients with AUC (0.880; 95% CI, 0.787-0.972). In addition, they suggested that a series of target genes, such as CX3CR1, FYCO1, E2F2, exerted their unique effects contributing to the biological processes of transport, apoptosis and protein phosphorylation significantly through analysing GEO: The Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>) and The Cancer Genome Atlas (TCGA). These

findings highlight that circulating miR-422a could emerge as a non-invasive biomarker with sufficient accuracy in predicting lymph node metastasis in lung cancer patients.

Nadal et al¹¹⁸ identified circulating miRNA expression profile in NSCLC patients. They revealed some differentially expressed miRNAs, such as miR-141, miR-193b, miR-301 and miR-200b in the serum of NSCLC patients. Their results indicated that the expression of these miRNAs could have high specificity for detecting the NSCLC patients. These findings suggested that these circulating miRNA could exert effects as attractive option to diagnose patients with NSCLC.¹¹⁸

Dinh et al¹¹⁹ showed from an unbiased screen that miR-29a and miR-150 were decreased in the circulation of NSCLC patients undergoing thoracic RT: thoracic radiotherapy. Franchina et al¹²⁰ investigated that miR-22, miR-24, and miR-34a were found up-regulated ($P < .05$) in NSCLC patients vs healthy controls. In addition, these up-regulated miR-22, miR-24 and miR-34a in lung cancer patients could be represented as significant biomarkers in the follow-up of early-stage NSCLC and for innovative approaches to early diagnosis in healthy heavy smokers. Ma et al measured serum levels of miR-125b in NSCLC and determined the relationship of miR-125b expression to

TABLE 2 Circulating miRNAs up-/down-regulated in lung cancer

miRNA	Expression	Materials	Stages	Samples	References
miR-422a	Up-regulation	Serum	I-IV	77	117
miR-22	Up-regulation	Blood	III-IV	54	120
miR-24	Up-regulation	Blood	III-IV	54	120
miR-34a	Up-regulation	Blood	III-IV	54	120
miR-125b	Up-regulation	Serum	I-IV	193	121
miR-1246	Up-regulation	Serum	I- III	143	122
miR-1290	Up-regulation	Serum	I- III	143	122
Let-7c	Down-regulation	Plasma	I-IV	120	123
miR-152	Down-regulation	Plasma	I-IV	120	123
miR-574-5p	Up-regulation	Serum	I-IV	75	109
miR-874	Up-regulation	Serum	I-IV	75	109
miR-21	Up-regulation	Serum	I-II	85	110
miR-126	Down-regulation	Serum	I-II	85	110,111
Let-7a	Down-regulation	Serum	I-II	85	110
miR-125b	Up-regulation	Serum	I-IV	100	112
miR-200b	Up-regulation	Serum	I-IV	100	112
miR-34b	Up-regulation	Serum	I-IV	100	112
miR-203	Up-regulation	Serum	I-IV	100	112
miR-205	Up-regulation	Serum	I-IV	100	112
miR-429	Up-regulation	Serum	I-IV	100	112
miR-448	Up-regulation	Plasma	I-IV	90	113
miR-4478	Up-regulation	Plasma	I-IV	90	113
miR-506	Down-regulation	Plasma	I-IV	90	113
miR-182	Up-regulation	Serum	I-III	112	111
miR-183	Up-regulation	Serum	I-III	112	111
miR-210	Up-regulation	Serum	I-III	112	111
miR-15b-5p	Down-regulation	Serum	I-III	164	114
miR-16-5p	Up-regulation	Serum	I-III	164	114
miR-20a-5p	Up-regulation	Serum	I-III	164	114
miR-324-3p	Up-regulation	Plasma	I	395	115
miR-1285	Down-regulation	Plasma	I	395	115
miR-98-5p	Up-regulation	Plasma	III-IV	15	116
miR-302e	Up-regulation	Plasma	III-IV	15	116
miR-495-3p	Up-regulation	Plasma	III-IV	15	116
miR-613	Up-regulation	Plasma	III-IV	15	116
miR-1246	Up-regulation	Serum	I-III	59	122
miR-1290	Up-regulation	Serum	I-III	59	122

diagnosis, stratification and patient prognosis. They observed that circulating miR-125b levels can distinguish patients with NSCLC from healthy controls. The concentration of circulating miR-125b may be a valuable blood biomarker for NSCLC to screen, which could represent a potential biomarker for disease progression.¹²¹

In other studies, Zhang et al¹²² confirmed that miR-1246 and miR-1290 could drive tumour initiation and cancer progression, due to a high specificity with the tumour-initiating cell (TIC) in NSCLC. Significantly, they suggested that miR-1246 and miR-1290 played pivotal roles in regulating the spontaneous metastasis of primary tumour cells. Metallothionein 1G (MT1G) is a great gene that represses tumour. Moreover, it could mediate the function of lung cancer cells through targeting both miR-1246 and miR-1290 together. It has been observed that miR-1246 and miR-1290, which were enriched in TICs, exerted great effects in regulating tumour growth and metastasis through the repression of metallothioneins, especially MT1G. These results highlight that miR-1246 and miR-1290 might be a valuable therapeutic modality for NSCLC patients by directly targeting MT1G.¹²²

Dou et al¹²³ used plasma miRNA for the diagnosis of NSCLC in the early stages. They assessed miRNA expression in 120 NSCLC patients and 360 healthy controls. They have observed down-regulated expression of let-7c and miR-152 in plasma in NSCLC. These findings identified that circulating let-7c and miR-152 could play an important role in detecting NSCLC with an advantage of non-invasive and valuable diagnosis. In addition, in order to determine the effects of let-7c and miR-152 expression as a significant potential biomarker for NSCLC, they have screened a large number of plasma samples. Table 2 illustrates a number of circulating miRNA which might be applied as diagnostic and therapeutic biomarkers for lung cancer patients.

7 | EXOSOMAL MIRNA AS DIAGNOSTIC BIOMARKERS IN NSCLC

Exosomes were microvesicles containing 5'-nucleotidase activity released from different types of cells such as tumour cells, stem cells and some normal cells, which could play a crucial role in contributing to intercellular communication.^{13,124} Exosomes can take along a series of different functional molecules such as DNAs, mRNAs, miRNAs and proteins to recipient cells. Hence, exosomes and their bioactive

cargos altered the recipient cells' behaviour differently such as re-programming the recipient cells through transferring particular message to these cells in the microenvironment.¹²⁵ A wide range number of studies have indicated that exosomes released from cancer cells play great roles in tumour progression in several diseases such as cancer.^{14,15,126} Several lines of studies have identified that exosomal miRNA performed different expression profiles between lung cancer patients and controls, suggesting exosomal miRNA might be as a novel screening modality for patients with lung cancer.¹²⁷⁻¹³⁰ Thus, it has opened the window to a new world of possibilities to diagnose NSCLC in early stage.

Munagala et al¹²⁵ evaluated miRNA expressed profile of lung cancer and normal bronchial epithelial cells as well as in exosomes, which secreted from these cells in culture media. The results identified that miR-132, miR-155, miR-21, miR-331-5p and miR-483-5p were significantly up-regulated in lung cancer cells and exosomes, while let-7e, miR-26a, miR-193b, miR-345, miR-16 and miR-423-5p were down-regulated, suggesting exosomal miRNA can be requisition as valuable biomarkers for diagnosis in lung cancer.¹²⁵

Liu et al¹³¹ assessed exosomal miRNA as novel biomarker for early diagnosis and prognosis in lung cancer patients through quantitative polymerase chain reaction (qPCR) array panel. Their results indicated that the expression of exosomal miR-23b-3p, miR-10b-5p and miR-21-5p were associated with the prognostic significance of NSCLC. Moreover, the expression of exosomal miR-21-5p and miR-10b-5p were up-regulated, while miR-23b-3p was observed down-regulated in NSCLC patients compared with the control group. As a consequence, the expression of exosomal miR-23b-3p, miR-10b-5p and miR-21-5p provided a significantly survival prediction for NSCLC and suggested these exosomal miRNA could be used as prognostic biomarkers independently.¹³¹

Li et al¹³² indicated that exosomes released from lung cancer mesenchymal stem cells (MSCs) could carry various cargos such as some proteins and RNAs which could be used as biomarkers in lung cancer cells. They showed that exosomal proteins, including CD63, heat shock protein 70 (HSP70), P65 and macrophage cationic peptide 1 (MCP-1), are useful biomarkers for the identification of lung cancer cells. Moreover, they detected other cargos such as mRNA (TLR2, TLR7 and TLR8) and miRNA (miR-142-5p, miR-203, miR-21 and miR-29a) in exosomes released from lung cancer MSCs. In addition, they identified that lung cancer cell-derived exosomes could educate

TABLE 3 Exosomal miRNA biomarkers in lung cancer

miRNAs	Detection method	Samples (human/cell lines)	Stages	References
miR-29a, miR-150	qRT-PCR	5	III	119
miR-182, miR-185, miR-21, miR-127, miR-142, miR-155, miR-138, miR-125-5p, let-7e, miR-193b, miR-16, miR-26a, miR-345, miR-423-5p,	qRT-PCR	H1299		125
miR-1228-3p, miR-30b, miR-30c, miR-103, miR-122, miR-195, miR-203, miR-221, miR-222	qRT-PCR	12		128
miR-197-5p, miR-4443, miR-642a-3p, miR-27b-3p, miR-100-5p	qRT-PCR	A549		129
miR-98, miR-133b, miR-138, miR-181a, miR-200c	qRT-PCR	A549		130
miR-23b-3p, miR-10b-5p, miR-21-5p	qRT-PCR	196	I-IV	131

naive MSCs into a novel kind of pro-inflammatory MSCs (P-MSCs) by activating TLR2/NF- κ B signalling through exosomal surface HSP70. Finally, these results confirmed that exosomal RNAs and miRNAs, and proteins might exert effects as potential diagnosis biomarkers in lung cancer patient.¹³² Table 3 indicates a number of exosomal miRNA released from lung cancer cell.

8 | CONCLUSION AND PERSPECTIVE

Non-small cell lung cancer is the most common serious cancer and a leading cause of cancer-related death worldwide. Early diagnosis of NSCLC is a prerequisite for proper management and increasing survival. Several lines of evidence have indicated that NSCLC patients have a poor prognosis, due to the lack of simple, reliable and non-invasive diagnostic tools for the early stage. At present, according to the clinical stage of lung cancer treatment options, surgical treatment has been recognized as the preferred method of treatment for lung cancer. But lung cancer patients transfer rate and mortality rate has not been significantly improved due to lack of effective diagnostic tools in the early stage. miRNAs have emerged as non-coding RNAs, which have potential to be candidates for the diagnosis and therapy of NSCLC. Moreover, exosomal biomarkers such as miRNA, proteins and mRNAs could open new horizons for the diagnosis of NSCLC.

9 | AUTHORS' CONTRIBUTIONS

Q Z, S-X H and F Z drafted the manuscript. C-C S revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ORCID

De-Jia Li <http://orcid.org/0000-0001-5332-8171>

Shu-Jun Li <http://orcid.org/0000-0003-1978-9833>

REFERENCES

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:7-30.
- Molina JR, Yang P, Cassivi SD, Schild SE, Adjei AA. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc*. 2008;83:584-594.
- Mallory AC, Vaucheret H. MicroRNAs: something important between the genes. *Curr Opin Plant Biol*. 2004;7:120-125.
- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004;116:281-297.
- Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer*. 2006;94:776-780.
- Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell*. 2003;113:673-676.
- Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nature reviews. Cancer*. 2006;6:857-866.
- Thakur MK, Gadgeel SM. Predictive and prognostic biomarkers in non-small cell lung cancer. *Semin Resp Crit Care*. 2016;37:760-770.
- Coleman WB, Tsongalis GJ, Phong Trang MPH, Joanne B. Weidhaas M. MicroRNAs and Cancer. In: Coleman WB, Tsongalis GJ, eds. *The Molecular Basis of Human Cancer*. New York: Springer, 2017:277-286.
- Backes C, Ludwig N, Leidinger P, et al. Paired proteomics, transcriptomics and miRNomics in non-small cell lung cancers: known and novel signaling cascades. *Oncotarget*. 2016;7:71514-71525.
- Zang H, Wang W, Fan S. The role of microRNAs in resistance to targeted treatments of non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2017;79:227-231.
- Taverna S, Giallombardo M, Gil-Bazo I, et al. Exosomes isolation and characterization in serum is feasible in non-small cell lung cancer patients: critical analysis of evidence and potential role in clinical practice. *Oncotarget*. 2016;7:28748-28760.
- Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol*. 2002;2:569-579.
- Kahlert C, Melo SA, Protapopov A, et al. Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem*. 2014;289:3869-3875.
- Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9:654-659.
- Lee Y, Kim M, Han J, et al. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J*. 2004;23:4051-4060.
- Lee Y, Ahn C, Han J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003;425:415-419.
- Denli AM, Tops BBJ, Plasterk RHA, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. *Nature*. 2004;432:231-235.
- Hutvagner G, Zamore PD. A microRNA in a multiple-turnover RNAi enzyme complex. *Science (New York, N.Y.)*. 2002;297:2056-2060.
- Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nucleic acid mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature*. 2000;404:293-296.
- Diederichs S, Haber DA. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. *Cell*. 2007;131:1097-1108.
- Chendrimada TP, Gregory RI, Kumaraswamy E, et al. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*. 2005;436:740-744.
- Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. *Nature reviews. Drug Discov*. 2010;9:775-789.
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*. 2004;429:457-463.
- Calin GA, Dumitru CD, Shimizu M et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA*. 2002;99:15524-15529.
- Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA*. 2004;101:2999-3004.
- Lujambio A, Ropero S, Ballestar E, et al. Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. *Cancer Res*. 2007;67:1424-1429.

28. Lopez-Serra P, Esteller M. DNA methylation-associated silencing of tumor-suppressor microRNAs in cancer. *Oncogene*. 2012;31:1609-1622.
29. Xia W, Chen Q, Wang J, Mao Q, Dong G. DNA methylation mediated silencing of microRNA-145 is a potential prognostic marker in patients with lung adenocarcinoma. *Sci Rep*. 2015;5:e16901.
30. Seol HS, Akiyama Y, Shimada S, et al. Epigenetic silencing of microRNA-373 to epithelial-mesenchymal transition in non-small cell lung cancer through IRAK2 and LAMP1 axes. *Cancer Lett*. 2014;353:232-241.
31. Varambally S, Cao Q, Mani R, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science (New York, N.Y.)*. 2008;322:1695-1699.
32. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*. 2005;435:839-843.
33. He L, He X, Lim LP, et al. A microRNA component of the p53 tumour suppressor network. *Nature*. 2007;447:1130-1134.
34. Gallardo E, Navarro A, Vinolas N, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis*. 2009;30:1903-1909.
35. Bommer GT, Gerin I, Feng Y, et al. p53-mediated activation of miRNA34 candidate tumor-suppressor genes. *Curr Biol*. 2007;17:1298-1307.
36. Kim YH, Lee WK, Lee EB, Son JW, Kim DS, Park JY. Combined Effect of Metastasis-Related MicroRNA, miR-34 and miR-124 Family, Methylation on Prognosis of Non-Small-Cell Lung Cancer. *Clin Lung Cancer*. 2017;18:e13-e20.
37. Johnson CD, Esquela-Kerscher A, Stefani G, et al. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res*. 2007;67:7713-7722.
38. Takamizawa J, Konishi H, Yanagisawa K, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res*. 2004;64:3753-3756.
39. Mongroo PS, Rustgi AK. The role of the miR-200 family in epithelial-mesenchymal transition. *Cancer Biol Ther*. 2010;10:219-222.
40. Hurteau GJ, Carlson JA, Spivack SD, Brock GJ. Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Res*. 2007;67:7972-7976.
41. Mendell JT. miRiad roles for the miR-17-92 cluster in development and disease. *Cell*. 2008;133:217-222.
42. Lewis BP, Shih I, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell*. 2003;115:787-798.
43. Borkowski R, Du L, Zhao Z, et al. Genetic mutation of p53 and suppression of the miR-17 approximately 92 cluster are synthetic lethal in non-small cell lung cancer due to upregulation of vitamin D Signaling. *Cancer Res*. 2015;75:666-675.
44. Volinia S, Calin GA, Liu C, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA*. 2006;103:2257-2261.
45. Calin GA, Ferracin M, Cimmino A, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*. 2005;353:1793-1801.
46. Kluiver J, Poppema S, de Jong D, et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. *J Pathol*. 2005;207:243-249.
47. Metzler M, Wilda M, Busch K, Viehmann S, Borkhardt A. High expression of precursor microRNA-155/BIC RNA in children with Burkitt lymphoma. *Genes Chromosomes Cancer*. 2004;39:167-169.
48. Iorio MV, Ferracin M, Liu C, et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*. 2005;65:7065-7070.
49. Garzon R, Volinia S, Liu C, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood*. 2008;111:3183-3189.
50. Yanaihara N, Caplen N, Bowman E et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*. 2000;9:189-198.
51. Xue X, Liu Y, Wang Y, et al. MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN. *Oncotarget*. 2016;7:84508-84519.
52. Zhang Y, Yang D, Weng L, Wang L. Early lung cancer diagnosis by biosensors. *Int J Mol Sci*. 2013;14:15479-15509.
53. Jiang J, Lee EJ, Gusev Y, Schmittgen TD. Real-time expression profiling of microRNA precursors in human cancer cell lines. *Nucleic Acids Res*. 2005;33:5394-5403.
54. Schmittgen TD, Lee EJ, Jiang J et al. Real-time PCR quantification of precursor and mature microRNA. *Methods (San Diego, Calif.)*. 2008;44:31-38.
55. Sun Y, Gregory KJ, Chen NG, Golovlev V. Rapid and direct microRNA quantification by an enzymatic luminescence assay. *Anal Biochem*. 2012;429:11-17.
56. McDougall JK, Dunn AR, Jones KW. In situ hybridization of adenovirus RNA and DNA. *Nature*. 1972;236:346-348.
57. Liu C, Calin GA, Volinia S, Croce CM. MicroRNA expression profiling using microarrays. *Nat Protoc*. 2008;3:563-578.
58. Friedlander MR, Chen W, Adamidi C, et al. Discovering microRNAs from deep sequencing data using miRDeep. *Nat Biotechnol*. 2008;26:407-415.
59. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods (San Diego, Calif.)*. 2010;50:298-301.
60. Michael A, Bajracharya SD, Yuen PST, et al. Exosomes from human saliva as a source of microRNA biomarkers. *Oral Dis*. 2010;16:34-38.
61. Hu Z, Chen X, Zhao Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. *J Clin Oncol*. 2010;28:1721-1726.
62. Shen H, Shen J, Wang L et al. Low miR-145 expression level is associated with poor pathological differentiation and poor prognosis in non-small cell lung cancer. *Biomed Pharmacol*. 2015; 69:301-305.
63. Chen S, Wang T, Tian Y, Zheng Y. Down-regulation of microRNA-126 and microRNA-133b acts as novel predictor biomarkers in progression and metastasis of non small cell lung cancer. *Int J Clin Exp Pathol*. 2015;8:14983-14988.
64. Stenvold H, Donnem T, Andersen S, Al-Saad S, Busund LT, Bremnes RM. Stage and tissue-specific prognostic impact of miR-182 in NSCLC. *BMC Cancer*. 2014;14:138.
65. Wang R, Wang Z, Yang J, Pan X, De W, Chen L. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). *Oncogene*. 2011;30:2644-2658.
66. Zandberga E, Kozirovskis V, Abols A, Andrejeva D, Purkalne G, Line A. Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. *Genes Chromosomes Cancer*. 2013;52:356-369.
67. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005;435:834-838.
68. Li J, Smyth P, Flavin R, et al. Comparison of miRNA expression patterns using total RNA extracted from matched samples of formalin-fixed paraffin-embedded (FFPE) cells and snap frozen cells. *BMC Biotechnol*. 2007;7:36.
69. Florczuk M, Szepechinski A, Chorostowska-Wynimko J. miRNAs as Biomarkers and Therapeutic Targets in Non-Small Cell Lung Cancer: Current Perspectives. *Target Oncol*. 2017;12:179-200.
70. Coello MC, Luketich JD, Litle VR, Godfrey TE. Prognostic significance of micrometastasis in non-small-cell lung cancer. *Clin Lung Cancer*. 2004;5:214-225.

71. Gao X, Wu Y, Yu W, Li H. Identification of a seven-miRNA signature as prognostic biomarker for lung squamous cell carcinoma. *Oncotarget*. 2016;7:81670-81679.
72. Watanabe K, Amano Y, Ishikawa R, et al. Histone methylation-mediated silencing of miR-139 enhances invasion of non-small-cell lung cancer. *Cancer Med*. 2015;4:1573-1582.
73. Mao M, Wu Z, Chen J. MicroRNA-187-5p suppresses cancer cell progression in non-small cell lung cancer (NSCLC) through down-regulation of CYP1B1. *Biochem Biophys Res Commun*. 2016;478:649-655.
74. Kasinski AL, Slack FJ. Epigenetics and genetics. MicroRNAs en route to the clinic: progress in validating and targeting microRNAs for cancer therapy. *Nat Rev Cancer*. 2011;11:849-864.
75. Zhao B, Han H, Chen J, et al. MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. *Cancer Lett*. 2014;342:43-51.
76. Ye Z, Fang B, Pan J, et al. miR-138 suppresses the proliferation, metastasis and autophagy of non-small cell lung cancer by targeting Sirt1. *Oncol Rep*. 2017;37:3244-3252.
77. Zhang H, Zhang H, Zhao M, et al. MiR-138 inhibits tumor growth through repression of EZH2 in non-small cell lung cancer. *Cell Physiol Biochem*. 2013;31:56-65.
78. Li J, Wang Q, Wen R, et al. MiR-138 inhibits cell proliferation and reverses epithelial-mesenchymal transition in non-small cell lung cancer cells by targeting GIT1 and SEMA4C. *J Cell Mol Med*. 2015;19:2793-2805.
79. Zhang Y, Yang X, Wu H, Zhou W, Liu Z. MicroRNA-145 inhibits migration and invasion via inhibition of fascin 1 protein expression in non-small-cell lung cancer cells. *Mol Med Rep*. 2015;12:6193-6198.
80. Zhang L, Quan H, Wang S, Li X, Che X. MiR-183 promotes growth of non-small cell lung cancer cells through FoxO1 inhibition. *Tumour Biol*. 2015;36:8121-8126.
81. Tan M, Wu J, Cai Y. Suppression of Wnt signaling by the miR-29 family is mediated by demethylation of WIF-1 in non-small-cell lung cancer. *Biochem Biophys Res Commun*. 2013;438:673-679.
82. Ma Z, Hou P, Li Y, et al. MicroRNA-34a inhibits the proliferation and promotes the apoptosis of non-small cell lung cancer H1299 cell line by targeting TGFbeta2. *Tumour Biol*. 2015;36:2481-2490.
83. Zhou Y, Xu Y, Qiao C. MiR-34c-3p suppresses the proliferation and invasion of non-small cell lung cancer (NSCLC) by inhibiting PAC1/ MAPK pathway. *Int J Clin Exp Pathol*. 2015;8:6312-6322.
84. Zhang X, He X, Liu Y et al. MiR-101-3p inhibits the growth and metastasis of non-small cell lung cancer through blocking PI3K/AKT signal pathway by targeting MALAT-1. *Biomed Pharmacother* 2017; 93:1065-1073.
85. Li J, Wang H, Ke H, Ni S. MiR-129 regulates MMP9 to control metastasis of non-small cell lung cancer. *Tumour Biol*. 2015;36:5785-5790.
86. Xiao P, Liu W, Zhou H. miR-200b inhibits migration and invasion in non-small cell lung cancer cells via targeting FSCN1. *Mol Med Rep*. 2016;14:1835-1840.
87. Tang T, Huan L, Zhang S, et al. MicroRNA-212 functions as a tumor-suppressor in human non-small cell lung cancer by targeting SOX4. *Oncol Rep*. 2017;38:2243-2250.
88. Zhang C, Ge S, Hu C, Yang N, Zhang J. MiRNA-218, a new regulator of HMGB1, suppresses cell migration and invasion in non-small cell lung cancer. *Acta Biochem Biophys Sin*. 2013;45:1055-1061.
89. Luo W, Huang B, Li Z, et al. MicroRNA-449a is downregulated in non-small cell lung cancer and inhibits migration and invasion by targeting c-Met. *PLOS ONE*. 2013;8:e64759.
90. Xiang J, Hang J, Che J, Li H. MiR-25 is up-regulated in non-small cell lung cancer and promotes cell proliferation and motility by targeting FBXW7. *Int J Clin Exp Pathol*. 2015;8:9147-9153.
91. Dong Z, Zhong Z, Yang L, Wang S, Gong Z. MicroRNA-31 inhibits cisplatin-induced apoptosis in non-small cell lung cancer cells by regulating the drug transporter ABCB9. *Cancer Lett*. 2014;343:249-257.
92. Yamashita R, Sato M, Kakumu T, et al. Growth inhibitory effects of miR-221 and miR-222 in non-small cell lung cancer cells. *Cancer Med*. 2015;4:551-564.
93. Wang L, Liu W, Zhang Y, Huang X. The miR-224 promotes non-small cell lung cancer cell proliferation by directly targeting RASSF8. *Eur Rev Med Pharmacol*. 2017;21:3223-3231.
94. Zhao J, Qiao C, Ding Z, et al. A novel pathway in NSCLC cells: miR191, targeting NFIA, is induced by chronic hypoxia, and promotes cell proliferation and migration. *Mol Med Rep*. 2017;15:1319-1325.
95. Romano G, Acunzo M, Garofalo M, et al. MiR-494 is regulated by ERK1/2 and modulates TRAIL-induced apoptosis in non-small-cell lung cancer through BIM down-regulation. *Proc Natl Acad Sci USA*. 2012;109:16570-16575.
96. Wang X, Chen Z. MicroRNA-19a functions as an oncogenic microRNA in non-small cell lung cancer by targeting the suppressor of cytokine signaling 1 and mediating STAT3 activation. *Int J Mol Med*. 2015;35:839-846.
97. Sun C, Li S, Yuan Z, Li D. MicroRNA-346 facilitates cell growth and metastasis, and suppresses cell apoptosis in human non-small cell lung cancer by regulation of XPC/ERK/Snail/E-cadherin pathway. *Aging*. 2016;8:2509-2524.
98. Hashemi ZS, Khalili S, Forouzandeh MM, Sadroddiny E. Lung cancer and miRNAs: a possible remedy for anti-metastatic, therapeutic and diagnostic applications. *Expert Rev Respir Med*. 2017;11:147-157.
99. Huang T, She K, Peng G, et al. MicroRNA-186 suppresses cell proliferation and metastasis through targeting MAP3K2 in non-small cell lung cancer. *Int J Oncol*. 2016;49:1437-1444.
100. Sun C, Li S, Zhang F, et al. The Novel miR-9600 Suppresses Tumor Progression and Promotes Paclitaxel Sensitivity in Non-small-cell Lung Cancer Through Altering STAT3 Expression. *Mol Ther Nucleic Acids*. 2016;5:e387.
101. Desgrosellier JS, Cheresh DA. Integrins in cancer: biological implications and therapeutic opportunities. *Nat Rev Cancer*. 2010;10:9-22.
102. Oshita F, Kameda Y, Hamanaka N, et al. High expression of integrin beta1 and p53 is a greater poor prognostic factor than clinical stage in small-cell lung cancer. *Am J Clin Oncol*. 2004;27:215-219.
103. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol*. 2014;15:178-196.
104. Qin Q, Wei F, Zhang J, Li B. miR-134 suppresses the migration and invasion of nonsmall cell lung cancer by targeting ITGB1. *Oncol Rep*. 2017;37:823-830.
105. Hou J, Meng F, Chan LW, Cho WC, Wong SC. Circulating Plasma MicroRNAs As Diagnostic Markers for NSCLC. *Front Genet*. 2016;7:193.
106. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res*. 2008;18:997-1006.
107. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA*. 2008;105:10513-10518.
108. Zhao Y, Song Y, Yao L, Song G, Teng C. Circulating microRNAs: Promising Biomarkers Involved in Several Cancers and Other Diseases. *DNA Cell Biol*. 2017;36:77-94.
109. Zhou R, Zhou X, Yin Z, et al. MicroRNA-574-5p promotes metastasis of non-small cell lung cancer by targeting PTPRU. *Sci Rep-UK*. 2016;6:e35714.
110. Campomenosi P, Gini E, Noonan DM, et al. A comparison between quantitative PCR and droplet digital PCR technologies for circulating microRNA quantification in human lung cancer. *BMC Biotechnol*. 2016;16:60.
111. Zhu W, Zhou K, Zha Y, et al. Diagnostic Value of Serum miR-182, miR-183, miR-210, and miR-126 Levels in Patients with Early-Stage Non-Small Cell Lung Cancer. *PLOS ONE*. 2016;11:e153046.
112. Halvorsen AR, S MB, Leblanc M, Holm AM, Bolstad N. A unique set of 6 circulating microRNAs for early detection of non-small cell lung cancer. *Oncotarget* 2016;7(24):37250-37259.

113. Powrozek T, Krawczyk P, Kowalski DM, et al. Application of plasma circulating microRNA-448, 506, 4316, and 4478 analysis for non-invasive diagnosis of lung cancer. *Tumour Biol.* 2016;37:2049-2055.
114. Fan L, Qi H, Teng J, et al. Identification of serum miRNAs by nano-quantum dots microarray as diagnostic biomarkers for early detection of non-small cell lung cancer. *Tumour Biol.* 2016;37:7777-7784.
115. Gao X, Wang Y, Zhao H, et al. Plasma miR-324-3p and miR-1285 as diagnostic and prognostic biomarkers for early stage lung squamous cell carcinoma. *Oncotarget.* 2016;7:59664-59675.
116. Chen X, Xu Y, Liao X, et al. Plasma miRNAs in predicting radiosensitivity in non-small cell lung cancer. *Tumour Biol.* 2016;37:11927-11936.
117. Wu L, Hu B, Zhao B, et al. Circulating microRNA-422a is associated with lymphatic metastasis in lung cancer. *Oncotarget.* 2017;8:42173-42188.
118. Nadal E, Truini A, Nakata A, et al. A Novel Serum 4-microRNA Signature for Lung Cancer Detection. *Sci Rep.* 2015;5:12464.
119. Dinh TT, Fendler W, Chalubinska-Fendler J et al. Circulating miR-29a and miR-150 correlate with delivered dose during thoracic radiation therapy for non-small cell lung cancer. *Radiation Oncol (London, England)* 2016; 11:61.
120. Franchina T, Amodeo V, Bronte G, et al. Circulating miR-22, miR-24 and miR-34a as novel predictive biomarkers to pemetrexed-based chemotherapy in advanced non-small cell lung cancer. *J Cell Physiol.* 2014;229:97-99.
121. Yuxia M, Zhennan T, Wei Z. Circulating miR-125b is a novel biomarker for screening non-small-cell lung cancer and predicts poor prognosis. *J Cancer Res Clin.* 2012;138:2045-2050.
122. Zhang WC, Chin TM, Yang H, et al. Tumour-initiating cell-specific miR-1246 and miR-1290 expression converge to promote non-small cell lung cancer progression. *Nat Commun.* 2016;7:11702.
123. Dou H, Wang Y, Su G, Zhao S. Decreased plasma let-7c and miR-152 as noninvasive biomarker for non-small-cell lung cancer. *Int J Clin Exp Med.* 2015;8:9291-9298.
124. Trams EG, Lauter CJ, Salem NJ, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim Biophys Acta.* 1981;645:63-70.
125. Munagala R, Aqil F, Gupta RC. Exosomal miRNAs as biomarkers of recurrent lung cancer. *Tumour Biol.* 2016;37:10703-10714.
126. Reclusa P, Sirera R, Araujo A, et al. Exosomes genetic cargo in lung cancer: a truly Pandora's box. *Transl Lung Cancer Res.* 2016;5:483-491.
127. Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer.* 2009;10:42-46.
128. Giallombardo M, Chacartegui Borrás J, Castiglia M, et al. Exosomal miRNA Analysis in Non-small Cell Lung Cancer (NSCLC) Patients' Plasma Through qPCR: A Feasible Liquid Biopsy Tool. *J Vis Exp.* 2016;111:e53900.
129. Qin X, Yu S, Xu X, Shen B, Feng J. Comparative analysis of microRNA expression profiles between A549, A549/DDP and their respective exosomes. *Oncotarget.* 2017;8(26):42125-42135.
130. Xiao X, Yu S, Li S, et al. Exosomes: decreased sensitivity of lung cancer A549 cells to cisplatin. *PLOS ONE.* 2014;9:e89534.
131. Liu Q, Yu Z, Yuan S, et al. Circulating exosomal microRNAs as prognostic biomarkers for non-small-cell lung cancer. *Oncotarget.* 2017;8:13048-13058.
132. Li X, Wang S, Zhu R, Li H, Han Q, Zhao RC. Lung tumor exosomes induce a pro-inflammatory phenotype in mesenchymal stem cells via NFkappaB-TLR signaling pathway. *J Hematol Oncol.* 2016;9:42.

How to cite this article: Zhou Q, Huang S-X, Zhang F, et al. MicroRNAs: A novel potential biomarker for diagnosis and therapy in patients with non-small cell lung cancer. *Cell Prolif.* 2017;50:e12394. <https://doi.org/10.1111/cpr.12394>