Clinical significance of blood-based miRNAs as biomarkers of non-small cell lung cancer (Review)

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Abstract. Non-small-cell lung cancer (NSCLC) accounts for 85% of all cases of lung cancer. However, the predicted 5-year survival rate of patients with NSCLC is only 15.9%. microRNAs (miRNAs) are single-stranded, noncoding RNA molecules that are easily detectable in blood in a non-invasive manner, with features of stability, reproducibility and consistency in blood. Therefore, miRNAs derived from blood are able to have a significant impact on NSCLC diagnosis, metastasis and targeted therapies. Compared with the clinical protein markers carcinoembryonic antigen, cytokeratin fragment 21-1 and cancer antigen-125, blood-based miRNAs also display a higher diagnostic efficacy in NSCLC. Exosomal miRNAs are identified to be easily measured and have the potential to be used as diagnostic biomarkers in NSCLC, therefore providing an alternative method of biopsy profiling. The miRNA profile in exosomes is similar to the profile in primary tumor, meaning that this feature may be a powerful tool for NSCLC clinical diagnosis and targeted therapies. The focus of the present review was the clinical significance of blood-based exosomal miRNAs in diagnosis, prognosis, metastasis and targeted therapies of NSCLC.

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1. Introduction

The mortality rate of lung cancer has increased 6.9-fold in China from 1973 to 2014 (1). In 2015, there were 4,292,000 new cancer cases and 2,814,000 incidences of cancer-associated mortality in China, with lung cancer the principal cause of this mortality (2). Non-small cell lung cancer (NSCLC) accounts for ~85% of all cases of lung cancer (3). NSCLC is a heterogeneous disease comprised of common cancer subtypes, including squamous cell carcinoma (SCC), adenocarcinoma and large cell carcinoma. Although novel therapeutics have alleviated the disease burden and enhanced the overall quality of life in patients with NSCLC, the 5-year survival rate of patients with NSCLC remains low, at 15.9% (4). The poor rate of early detection and limited efficacy of presently available therapies for advanced-stage NSCLC are the cause of the low 5-year survival rate of patients with NSCLC (5). Current diagnostic methods for NSCLC include chest radiography, sputum cytology, computed tomography (CT) or assessment of a combination of clinical protein markers of tumors. These markers include carcinoembryonic antigen (CEA), neuron-specific enolase, cytokeratin fragment 21-1 and tissue polypeptide-specific antigen; these protein markers have previously been evaluated in large-scale clinical trials (1-3). However, these current diagnostic and prognostic methods have a number of undesirable side effects, Examples of these side effects include the high erroneous diagnostic rate, the low sensitivity and specificity (5).

MicroRNAs (miRNAs/miRs) represent an appealing alternative for assessing the diagnosis and prognosis of NSCLC as they can be detected in the blood in a non-invasive manner. miRNAs are endogenous, noncoding RNA molecules ~20 nucleotides in length that have a marked influence on the biological functions of single cells and complete organisms (6). A number of prior studies have validated that miRNAs can be readily detected in the blood, and that miRNAs are released by three different mechanisms: Energy-free passive leakage from lysed cells, active release through microvesicles and active secretion in the microvesicles free form. For example,

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serum miRNAs remain stable under harsh conditions, including boiling, acidic (pH 1.0) or alkaline (pH 13.0) solutions, long-term storage and undergoing multiple freeze-thaw cycles. Furthermore, serum miRNAs in healthy subjects are also consistent, with a Pearson correlation coefficient close to 1 (6). Therefore, the present review focuses on miRNAs derived from blood samples.

There are three principal membrane vesicle types: i) Microvesicles, ii) exosome vesicles, and iii) apoptotic vesicles (7). Microvesicles, also termed shedding vesicles, are large (>200 nm diameter), dense, and include phosphatidyl-serine, integrins and CD40 ligand (8). Exosomes are spherical nano-sized vesicles, with a round cup-shaped morphology, a density of 1.13-1.19 g/ml and a diameter of 40-100 nm (9). Exosomes contain large amounts of molecular cargo, including mRNAs, miRNAs, proteins and DNA, which are protected by a lipid bilayer. In 2007, Valadi *et al* (10) identified exosomal miRNAs, which were subsequently identified to be obtained readily, had the potential to be used as diagnostic markers and could serve as an alternative for biopsy profiling. They are suitable for profiling as the miRNA profile in exosomes exhibit similarities to primary tumor profile (11); therefore, this feature may be a powerful tool for early diagnosis, prognosis, metastasis and targeted therapy in NSCLC.

2. Clinical significance of miRNAs derived from blood of NSCLC

Blood-based miRNAs and clinical diagnosis of NSCLC. According to clinical analysis of stage I to IV cases of NSCLC, the mean risk score and high risk-score rate progressively increased as the stage increased (Table I). Conversely, the false-positive rate progressively decreased (3). The Tumor-Node-Metastasis (TNM) stage (12) of patients with NSCLC was also associated with the expression level of blood-based miRNAs. A previous study demonstrated that twelve plasma miRNAs could significantly discriminate between stages I-III of patients with NSCLC and controls (13). Levels of blood-based miRNAs could also be used to distinguish early stages (stages I and II) from advanced stages (stages III and IV) of NSCLC. In the present review, blood-based miRNAs are divided into three categories: Serum-based miRNAs, plasma-based miRNAs and miRNAs derived from peripheral blood mononuclear cells (PBMCs).

Blood-based miRNAs and early stage of NSCLC. Early detection is particularly crucial for the initial stage diagnosis of patients with NSCLC who present without clinical symptoms. The properties of blood-based miRNAs could predict disease probability irrespective of whether the patient is asymptomatic or symptomatic, and also is able to distinguish benign from malignant lesions. Furthermore, miRNAs could determine the onset of the malignant disease in individual patients over time (14-16). Serum-based miRNAs had been identified differentially between patients with early-stage NSCLC and controls: hsa-miR-1254 and hsa-miR-574-5p were evidently increased in early-stage NSCLC samples (compared with the control group) in the identification and validation cohort of the study by Foss *et al* (14). The combination of miR-125a-5p, miR-25 and miR-126 could also discriminate between NSCLC

Table I. Blood-based miRNA and clinical stage of NSCLC.

Author, year	Stage	Resource	miRNA profiling	Year	Country	Cases	Controls	Sensitivity, %	Specificity, %	(Refs.)
Sanfiorenzo et al, 2013	TNM	Plasma	11 miRNAs	2013	France	52	20	81.1	82.9	(13)
Foss et al, 2011	Early	Serum	miR-1254, -574-5p	2011	USA	22	31	73	71	(14)
Wang et al, 2015		Serum	miR-125a-5p, -25, -126	2015	China	142	111	88	82.6	(15)
Bianchi et al, 2011		Serum	34 miRNA panel	2011	Italy	34	30	71	90	(16)
Chen et al, 2012		Serum	miR-20a, -24, -25, -145, -152, -199a-5p, -221, -222, 223, -320	2012	China	200	110	92.5	90	(17)
Shen et al, 2011		Plasma	miR-21, -126, -210, -486-5P	2011	USA	58	29	86.2	96.6	(18)
Ulivi et al, 2013	Advanced	PBMC	miR-328	2013	Italy	86	24	70	89	(19)
Yuxia et al, 2012		Serum	miR-125b	2012	China	193	110	78.2	66.4	(21)
Sozzi et al, 2014		Plasma	miR signature classifier	2014	Italy	69	870	87	76.9	(22)

miRNA/miR, microRNA; NSCLC, non-small cell lung cancer; TNM, Tumor-Node-Metastasis; PBMC, peripheral blood mononuclear cells.

patients and controls (15). Bianchi *et al* detected 34 miRNAs in the serum that were able to distinguish patients with early stage NSCLCs from asymptomatic high-risk individuals with 80% accuracy (16). In a retrospective study, 10-serum miRNA for the early detection of NSCLC could precisely classify serum samples which were collected 33 months ago (17). Similarly, the four miRNAs (miRNA-21, -126, -210, and 486-5p) derived from plasma were also identified as stable and reliable in discriminating patients with NSCLC from controls (18). Furthermore, using miRNAs also produced high sensitivities and specificities in validating the presence of stage I NSCLC. A further study revealed that miR-328 derived from the PBMCs was the most effective diagnostic discriminator in the group of early stage tumors (19).

Blood-based miRNAs and advanced stage of NSCLC. Patients with stage III/IV NSCLC usually have a poor prognosis: The majority of these patients have a median survival of ~1 year (20). Clinicopathological criteria, including age, histological type, TNM stage and treatment method, are frequently used prediction factors for the prognosis of NSCLC. Serum miR-125b was markedly associated with poor prognosis in NSCLC in a prior study (21). A study concerning a large number of plasma samples indicated that the miRNAs signature classifier possessed predictive, diagnostic and prognostic significance, and could reduce the false-positive rate of low-dose CT (22). Low miR-10b expression in patients with stage I-II carcinoma was a positive indicator for the clinical prognosis of NSCLC, whereas high miRNA-10b expression in stage III-IV carcinoma was a negative indicator of the clinical prognosis of NSCLC. Furthermore, the 5-year survival rate of the low miR-10b expression group was markedly higher than the high miR-10b expression group (23).

Blood-based miRNAs and clinical protein markers CEA, Cyfra21-1 and CA125 in NSCLC. Recently, serum-based miRNAs were identified to have a higher diagnostic efficacy than the clinical protein markers CEA, CA125 and Cyfra21-1 in patients with NSCLC when compared with controls. A large number of samples were used to validate the authenticity of the results obtained and reproducibility of the experiments. A total of 70 patients with stage I NSCLC and 48 controls were collected in order to analyze the expression levels of miR-29c, miR-93 and miR-429 with CEA. miR-29c, miR-429 and CEA had an area under the curve (AUC; 0.833) higher than single serum miR-29c (0.727) and miR-429 (0.723), all of which were higher than CEA (0.534) (24). Serum specimens from 112 patients with NSCLC and 104 controls were subjected to research the levels of miR-182, miR-183, miR-126 and miR-210. These four miRNAs in conjunction with CEA were further validated by the AUC of 0.965, with a high sensitivity (88.5%) and specificity (92.5%) (25). Zhou *et al* (26) screened 396 serum samples from 252 patients with NSCLC and 144 healthy individuals, finding that miR-652 together with miR-660 had a markedly higher diagnostic value than CEA and CA125 for distinguishing between patients with NSCLC/adenocarcinoma from controls in the training and test cohort. The miR652[+] miR-660[+] Cyfra21-1 model had the highest clinical value for distinguishing patients with NSCLC from controls, which displayed higher early diagnostic value

compared with the clinical value of the double miRNA models and Cyfra21-1 alone, in the training and test cohort (26). Table II surmises the findings of studies with respect to blood-based miRNAs and clinical protein markers CEA, Cyfra21-1 and CA125.

Blood-based miRNAs and metastasis of NSCLC. miRNA expression levels may be associated with distant metastasis, prognosis and TNM stage of the tumor. Metastasis is the principal cause of mortality in patients with tumors (27). A large number of miRNAs had been demonstrated to be involved in cancer metastasis. For example, studies had demonstrated that miR155, miR-222 and miR-107 serve a role in pancreatic cancer (27,28). miR-200 was involved in gastric cancer (29) and miR-133b acted in colorectal cancer (30). Therefore, an improved understanding of the role of miRNA expression in the metastasis of NSCLC may result in an improved understanding of disease development and an improvement in the detection and therapy of NSCLC.

Metastasis is a multistep procedure, in which, tumor cells lose their adhesion to the stroma, pass through the basal membrane, then move into the blood vessels, alive in the blood circulation, attached to the blood stream, extravasate and proliferation in the host organ. Tumor cells either enter a dormant state or proliferate a form that shaping from multicellular epithelial-mesenchymal transition (EMT) of NSCLC cells (31-33). miR-152 regulated the proliferation and invasion of NSCLC cells by downregulating basic fibroblast growth factor (FGF2) (34). miR-194 suppressed the proliferation, migration, invasion and metastasis by EMT of NSCLC cells that form lung metastases *in vivo* (35). miRNA-449a induced G_1 arrest, apoptosis and cellular senescence (36). An *in vitro* study demonstrated that miR-449 inhibited cell migration and invasion in NSCLC, in part by targeting c-Met (36). A total of 74 patients with NSCLC were selected for two groups (high and low expression of miRNA10b), and the low expression of miRNA10b, metastasis, with stage III-IV carcinoma was identified to indicate poor prognosis in patients with NSCLC (22).

Blood-based miRNAs and targeted therapies of NSCLC. Chemotherapy and molecular targeted therapies are generally applied either alone or in conjunction with surgery and radiotherapy to treat patients with NSCLC (Table III) (37). miRNAs may act as molecular tumor biomarkers, predicting the incidence of multidrug resistance in NSCLC. Epidermal growth factor receptor (EGFR)-mutated or anaplastic lymphoma kinase (ALK)-rearranged tumors of patients with NSCLC could be treated with tyrosine kinase inhibitors (TKIs) including, erlotinib, gefitinib, crizotinib and ceritinib (37,38). For instance, one mechanism of action of erlotinib was the regulation of miR-9-Foxo1 in NSCLC (39). It is hypothesized that miR-21 could maintain the acquired resistance of EGFR-TKI in NSCLC by downregulation of phosphatase and tensin homolog and programmed cell death 4, and activation of the phosphoinositide 3-kinase/protein kinase B pathway (40). miR-20a regulated expression of the iron exporter ferroportin in NSCLC (41). miR-512-5p and miR-373 expression increased cisplatin-induced apoptosis in lung cancer and the re-expression of miRNAs did benefit

Table II. Blood-based miRNAs and clinical protein markers CEA, Cyfra21-1 and CA125 in NSCLC.

Author, year	miRNA profiling	Year	Cases	Controls	Marker (AUC)	miRNA (AUC)	Marker/miRNA combination (AUC)	(Refs.)
Zhu *et al*, 2014	Serum miR-29c, -93, -429	2014	70	48	CEA (0.534)	miR-29c (0.727), miR-429 (0.723)	The combination of miR-29c and CEA (0.757), the combination of miR-429 and CEA (0.659), the combination of miR-29c, miR-429 and CEA (0.833)	(24)
Zhu *et al*, 2016	Serum miR-182, -183, -210, -126a	2016	112	104	CEA (0.648)	miR-182 (0.781), miR-183 (0.638), miR-210 (0.650), miR-126 (0.845)	The combination of four miRNAs and CEA (0.975)	(25)
Zhou *et al*, 2015	Serum miR-194, -652, -660	2015	252	144	Training cohort: CEA (0.745), Cyfra21-1 (0.830), CA125 (0.802) Test cohort: CEA (0.678), Cyfra21-1 (0.819), CA-125 (0.746)	Training cohort: miR-194 (0.576), miR-652 (0.819), miR-660 (0.735), Test cohort: miR-194 (0.660), miR-652 (0.819), miR-660 (0.714)	Training cohort: The combination of miR-652 and miR-660 (0.896), the combination of miR-652, miR-660 and Cyfra21-1 (0.953) Test cohort: The combination of miR-652 and miR-660 (0.858), the combination of miR-652, miR-660 and Cyfra21-1 (0.943)	(26)

miRNA, microRNA; NSCLC, non-small cell lung cancer; AUC, area under the curve; CEA, carcinoma embryonic antigen; CYFRA21-1, cytokeratin fragment.

Table III. Blood-based miRNA and targeted therapies of NSCLC.

Author, year	miRNA profiling	Year	Targeted therapies	Mechanism/description	(Refs.)
Chen et al, 2015	miR-9	2015	EGFR-targeted therapy (erlotinib)	Erlotinib is an EGFR inhibitor, miR-9 regulate FoxO1 expression is a target of erlotinib in NSCLCs	(39)
Li et al, 2014	miR-21	2014	Acquired resistance of EGFR-TKI	miR-21 is involved in acquired resistance of EGFR-TKI in NSCLC, which is mediated by downregulating PTEN and PDCD4 and activating PI3K/Akt pathway	(40)
Babu and Muckenthaler, 2016	miR-20a	2016	-	Expression of miR-20a is inversely correlated to FPN in NSCLC	(41)
Adi Harel et al, 2016	miR-512-5p and miR-373	2016	Epigenetic therapy	miR-512-5p and miR-373 augment cisplatin-induced apoptosis in lung cancer	(42)
Zhao et al, 2015	miR-17 and miR-92 families	2015	Platinum-based chemotherapy	miR-17 and miR-92 families can maintain cisplatin resistance regulated by CDKN1A and RAD21	(43)
Yamashita et al, 2015; Garofalo et al, 2008	miR-221 and 222	2015 2008	S-phase targeting drugs and TRAIL-resistant	miR-221 and miR-222 induce growth suppression in NSCLC cells. These two also modulated by p27kip1 expression and TRAIL-induced caspase mechanism	(44,45)
Lv et al, 2016	miR-155	2016	Doxorubicin-resistant	MicroRNA-155 upregulated in the doxorubicin-resistant lung cancer	(46)

miRNA, mircoRNA; NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PTEN, phosphatase and tensin homolog; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; FPN, ferroportin.

from epigenetic cancer therapy (42). The miR-17 and miR-92 families were involved in cisplatin resistance and were regulated by cyclin-dependent kinase 1A and double-strand-break repair protein RAD21 homolog in NSCLC. These major factors contributed to cisplatin resistance, potentially by suppressing DNA synthesis and the repair of DNA damage (43). miR-221 and miR-222 increased the chemosensitivity to S-phase-targeting drugs, cisplatin and gemcitabine by inducing growth suppression in NSCLC cells (44). These two miRNAs were also regulated by p27kip1 and tumor necrosis factor-related apoptosis-inducing ligand-induced caspase mechanism (45). A previous study demonstrated that miRNA-155 was upregulated in the doxorubicin-resistant lung cancer A549/dox cell line (46).

3. Clinical significance of exosomal miRNAs derived from the blood of patients with NSCLC

Blood-based exosomal miRNAs and clinical diagnosis of NSCLC. Tumor cells produce an increased number of exosomes compared with healthy organs and cells (47). There are approximately >3 billion exosomes/ml in the blood of cancer patients, which is almost twice the concentration than in the blood of healthy controls (48). Exosomal miRNAs are similar to the miRNAs of parental cancer cells, resulting in an increased amount of research into exosomal miRNAs for cancer diagnosis. A number of examples of potential clinical applications for identification of high levels of exosomal miRNAs have been identified in melanoma, glioblastoma, esophageal squamous cell carcinoma, pancreatic and prostate cancer (Table IV) (47-50).

Blood-based exosomal miRNAs and early stage of NSCLC. A number of studies have previously attempted to validate that exosomal miRNAs may be used as a biomarker for the detection, diagnosis and metastatic spread of cancer. A study involving 27 patients with lung adenocarcinoma and 9 controls were selected to validate the special 12 miRNAs; the presence of the 12 miRNAs were also mirrored in the circulating exosomes (51). Four miRNAs (miR-378a, miR-379, miR-139-5p and miR-200b-5p) were enrolled for screening tests and six miRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100 and miR-154-3p) were enrolled for diagnostic tests, all of which have demonstrated high sensitivities and specificities of patients with lung adenocarcinomas and controls (52). A prospective analysis by Rodriguez *et al* (53) regarding blood and bronchoalveolar lavage (BAL) samples derived from 30 patients with NSCLC and 75 controls demonstrated that exosomes and miRNAs levels were higher in the plasma and BAL from patients with NSCLC. Zhou *et al* (54) identified six upregulated plasma miRNAs (miR-19b3p, miR-21-5p, miR-221-3p, miR-409-3p, miR-425-5p and miR-584-5p). These miRNAs were able to distinguish between patients with lung adenocarcinoma and healthy controls, with a receiver operating characteristic curve (ROC) of 0.72, 0.74 and 0.84 for the training, testing cland the external validation stage, respectively. Additionally, levels of miR-19-3p, miR-21-5p and miR-221-3p were significantly upregulated in exosomes derived from peripheral plasma samples of patients with lung adenocarcinoma (54).

Blood-based exosomal miRNAs and advanced stage of NSCLC. The vesicle-associated miRNAs let-7f and miR30e-3p could be used to discriminate between two groups of patients for different tumor stages and therefore the surgical options available to the patients. Notably, the two miRNAs examined in NSCLC have also been associated with a poor clinical outcome (55). A follow-up survey revealed that the levels of miR-205, miR-19a, miR-19b, miR-30b and miR-20a from the plasma of patients was evidently decreased following SCC surgery (resection). Assessment of miRNA levels in patients with lung SCC revealed the presence of high levels of exosomal miRNAs expression in tumor (56). The presence of exosomal miR-23b-3p, miR-10b-5p and miR-21-5p were identified as prognostic biomarkers for patients with NSCLC. The median follow-up time was 14.40 months (range, 3.43-36.87 months), with 71 (36.22%) patients succumbing to disease by the end of the experiment (57). These data indicate that the novel biomarkers and the technology for the detection of exosomal miRNAs for NSCLC are vital to enhance the sensitivity and specificity, which may be essential for improving the overall survival of patients.

Blood-based exosomal miRNAs and metastasis of NSCLC. (Fig. 1) Extracellular vehicles (EVs) deliver nucleic acids to target cells, allowing for the exchange of genetic information between cells. EVs are also capable of altering the phenotype of neighboring cells (58). Therefore, exosomes can not only promote the biological processes of tumors, but also influence the metastatic signatures of malignant tumors. Additionally, the regulatory signatures of tumor-derived exosomes are important for shaping the tumor microenvironment (31).

Exosomes, as mediators of EMT, are involved in the migration and invasion of metastasis (31). EMT continually initiates the process of metastasis, where tumor cells lose their polarity and cell-cell junctions and acquire migratory and invasive capabilities with a low proliferation state (32). If the tumor cells reach the distant pre-metastatic niche, the reverse process occurs (33). Moreover, when EMT cells arrive at the metastatic side, these EMT cells undergo epithelial to mesenchymal transition. (31). For example, exosomal miR-23a sustained the EMT-promoting effect of transforming growth factor-β1 by suppressing E-cadherin synthesis in lung carcinoma (59,60). Tumor-derived exosomes can lead cancer cells to acquire a mesenchymal phenotype and can convert mesenchymal stem cells into cancer-associated fibroblasts following an increase in the expression of α-smooth muscle actin (61). Exosome-derived miR-302b was also demonstrated to inhibit cell proliferation and migration in lung cancer (62).

Exosomal blood-based miRNAs and targeted therapies of NSCLC. As in artificial loaders of signaling molecules, exosomes possess signatures, including biocompatibility, stability, biological barrier permeability, low immunogenicity and low toxicity (58). These features make exosomes attractive for therapeutic use. Additionally, exosomes are able to escape rapid clearance by the mononuclear phagocyte system owing to their small size (63). Xiao *et al* (64) reported that A549 cell-derived exosomes reduced the sensitivity of non-treated A549 cells to cisplatin, following cisplatin exposure. Notably, the phospholipid constituents of EV were markedly

Table IV. Blood-based exosomal miRNA and clinical diagnosis of NSCLC.

Author, year	Stage	Resource	miRNA profiling	Year	Country	Cases	Controls	(Refs.)
Rabinowits et al, 2009	Early	Plasma	12 special miRNA	2009	USA	27	9	(51)
Cazzoli et al, 2013		Plasma	Screening test: 4 microRNAs (miR-378a, miR-379, miR-139-5p and miR-200b-5p); Diagnostic test: 6 microRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100 and miR-154-3p)	2013	Italy	Screening test: 20 Diagnostic test: 80	Screening test: 10; Diagnostic test: 25	(52)
Rodriguez et al, 2014		Plasma	miR-28,29c, 141, 144, 146, 195	2014	Spain	30	75	(53)
Zhou et al, 2017		Plasma	miR-19b-3p, miR-21-5p, miR-221-3p, miR-409-3p, miR-425-5p and miR-584-5p	2017	China	18	14	(54)
Silva et al, 2011	Advanced	Plasma	5 miRNAs (let-7f, miR-20b, miR-30e-3p, miR-223 and miR-301)	2011	Spain	28	20	(55)
Aushev et al, 2013		Plasma	miR-205, -19a, -19b, -30b, and -20a	2013	France	50	6	(56)
Liu et al, 2017		Plasma	miR-23b-3p, miR-10b-5p and miR-21-5p	2017	China	196	0	(57)

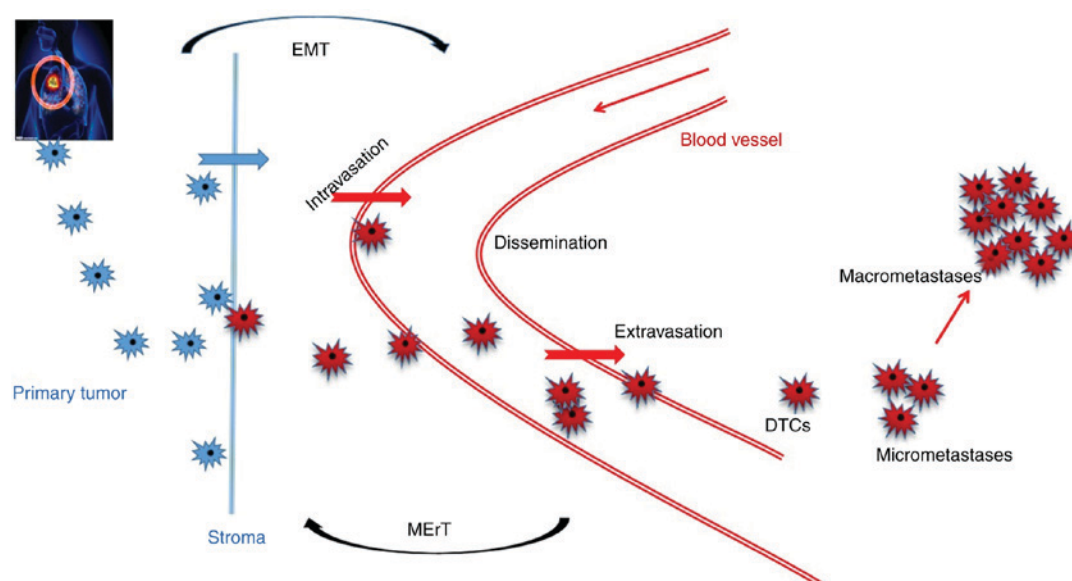miRNA, mircoRNA; NSCLC, non-small cell lung carcinoma.

Figure 1. Metastasis is a multistep procedure, in which tumor cells lose their adhesion to the stroma, pass through the basal membrane, then move into blood vessels and enter blood circulation where they then extravasate and proliferate in the host organ. Moreover, EMT cells arriving at the metastatic side need to undergo a MET. Exosomes, as mediators of EMT, are involved in the migration and invasion of metastasis. EMT continually initiates the process of metastasis. This is where tumor cells lose their polarity and cell-cell junctions, and acquire migratory and invasive capabilities with a low proliferative rate. If the tumor cells reach the distant pre-metastatic niche, the reverse process occurs.

distinguished in gefitinib-resistant NSCLC. Sophisticated mass-spectrometry-based shotgun lipidomic assays were performed for in-depth analysis. Lipid matrix-assisted laser desorption/ionization analysis revealed that EV phospholipid composition was significantly more distinct in PC9R cells compared with PC9 cells (65). There are three techniques to load an exosome with a therapeutic miRNA mimic or antagonist: Co-transfection with two plasmids, or co-transduction with two viruses; electroporation; or the transient transfection of miRNAs (66,67).

Several trials have been conducted to assess the safety of EV-based antitumor and antibacterial vaccines. This included a phase I trial that administered EVs from autologous DCs pulsed with melanoma antigen gene peptides to patients with NSCLC (68). Furthermore, a previous phase II trial was also conducted in order to evaluate interferon-γ-dendritic cell-derived exosomes loaded with major histocompatibility complex I/II-restricted cancer antigens following chemotherapeutic induction in patients with inoperable NSCLC without tumor progression (69). These results indicate that DC-derived exosomes vaccines may be safe and may promote T-cell and natural killer cell responses in patients. The extracorporeal hemofiltration of circulating EVs is a novel potential strategy, and has been previously proposed to be a therapeutic strategy for patients with cancer (70,71).

4. Clinical significance of miRNAs derived from tissue of NSCLC

In addition to blood samples, studies have demonstrated that miRNAs are also obtained from a large number of tissue specimens of patients with NSCLC and controls to validate the potential clinical significance of miRNAs in NSCLC. For example, miR-30d-5p was demonstrated to be downregulated in NSCLC tissues, with a 2-fold difference in expression between tumor tissues and the corresponding para-tumorous tissues (72). In 2006 a study describing the diagnostic miRNA signatures of NSCLC revealed that 12 specific miRNAs were overexpressed when compared with normal lung tissue (73). miRNAs extracted from tissues have been identified to be a more direct and intuitive method; however, tissue as another sample resource, are not the primary focus of the present review.

5. Conclusion

Currently, circulating tumor cells, circulating tumor DNA and exosomes, are all covered in the concept 'liquid biopsies'. A liquid biopsy is a liquid biomarker that may be easily isolated from numerous body fluids (blood, saliva, urine, ascites and pleural effusion and a tissue biopsy, a representative of the tissue from which it is obtained (74). Exosomes, as a non-invasive means of performing 'liquid biopsies', may be used for early diagnosis, obtaining prognostic information, metastasis development, real-time monitoring of tumor stages and understanding of therapeutic targets in patients with NSCLC.

The present review focuses on the clinical significance of blood-based exosomal miRNAs in the detection, metastasis and therapies of NSCLC. As a novel means of intercellular communication, exosomes can stimulate the growth, invasiveness and metastasis of NSCLC (75). Compared with the conventional methods used for clinical diagnosis of NSCLC, exosomes have numerous advantages: Exosomes contain an increased amount of genetic information, which can spread widely throughout the bodily fluids; exosomes have a relatively long circulating half-life with drugs *in vivo*; exosomes secreted by cells exhibit target selection; exosomes can enhance their cell-specific targeting function by altering their membrane; and exosomes can carry drugs *in vivo* and *in vitro* (76,77). According to recent findings in NSCLC, the significant difference in miRNAs and exosomal miRNAs derived from blood between patients with

NSCLC and controls, and the similarities between exosomal miRNAs and miRNAs all indicate that exosomal miRNAs may be useful as a biomarker for clinical diagnosis in NSCLC (76).

A number of problems surrounding the use of exosomes require addressing, which include: A lack of a reliable and cost-effective detection platforms for exosomal miRNAs in routine clinical settings; the complication of procedures, including specificity screening, detection methods suitable for the selection of the reference genes; the apparent shortcomings, which include time-consuming experiments, expensive instruments and a heavy reliance on the sample-handling skills; and a lack of research into the mechanism of action of exosomes.

Although research into exosomal miRNAs and NSCLC in its infancy, with the development of detection methods and the maturity of the research, in the future blood-based exosomal miRNAs may have prospects for application in terms of prediction, diagnosis, metastasis, prognostic evaluation and individualized treatment in lung cancer. Therefore, identifying novel therapeutic strategies and acquiring a better understanding of the biological functions of exosomal miRNAs as biomarkers in NSCLC will be of the utmost relevance in modern molecular oncology.

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Authors' contributions

JL devised the study topic. YS and LW revised the paper. MF provided additional clinical knowledge. LL wrote this manuscript.

Ethics and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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