



Exosomal microRNAs in non-small cell lung cancer

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Abstract: Lung cancer is one of the highest incidence cancer types worldwide and one with the lowest 5-year survival rate of all cancer types. Despite recent insights into lung cancer pathobiology, including novel biomarker-targeted therapies and immunotherapies, most of lung patients are diagnosed at late stages with limited and ineffective treatments. Therefore, more approaches are needed to eradicate lung cancer. In the last years, small extracellular vesicles (EVs) secreted by tumor cells have been gaining relevance. These intercellular signal mediators, called exosomes, contain a huge range of biological elements, including lipids, nucleic acids and miRNAs, among others, that carry relevant information. The role of exosomes in cancer progression is dependent on cancer type, molecular characteristics and stage. MicroRNAs molecules are a big part of the content of exosomes cargo and probably the most studied ones. Due to the regulatory role in gene expression, miRNAs may provide information of the molecular characteristics of the tumor and be also able to reprogram distant target cells. Exosomal miRNAs can modulate different biological processes in cancer such as growth, progression, invasion, angiogenesis, metastasis and drug resistance; playing a critical role in modifying the microenvironment of non-small cell lung cancer (NSCLC). Therefore, they can act by regulating tumor resistance and also be useful to monitoring the response/relapse to targeted therapies. In this work, we summarize the relevant advances on the potential role of exosomal miRNAs in NSCLC pathobiogenesis, highlighting the clinical utility of exosomal microRNAs as biomarkers for the NSCLC diagnosis, prognosis, drug resistance and therapeutic strategies.

Keywords: Exosomes; miRNAs; liquid biopsy; biomarker; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is the most frequent cause of death by cancer worldwide (1). The 5-year survival rate in lung cancer ranges from 73% (in stage IA) to 13% (in stage IV). Nevertheless, approximately 85% of lung cancer diagnosis is in advanced-disease (stage III or IV) with limited

therapeutic options. Non-small cell lung cancer (NSCLC) is one of the most frequent diagnoses, representing 85% of lung cancer cases, and it includes adenocarcinoma (ADC), squamous cell carcinoma (SCC), and large-cell carcinoma (LCC) as the main NSCLC subtypes (2). Moreover, small cell lung cancer (SCLC) represents 14–15% of all lung cancers. SCLC is classified as small cell carcinoma, when

it presents a pure histology; in other cases, it may happen that small cell carcinoma is combined with large cell carcinoma (3).

New insights into the molecular mechanisms of lung cancer have meant considerable progress in the development of new biomarkers for targeted therapies, such as therapies targeting the epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), the proto-oncogene B-Raf (BRAF), Ret Proto-Oncogene (RET), hepatocyte growth factor receptor (MET) and Neurotrophic Receptor Tyrosine Kinase 1 (NTRK), as well as for the most pioneering immunotherapies, based in checkpoint inhibitors targeting the programmed cell death 1 (PD-1) and programmed death-ligand 1 (PD-L1) pathways (4).

Despite recent advances in cancer treatments and screening methods, most lung cancer patients are still diagnosed in advanced stages, when the disease is already unresectable. Consequently, all treatment-determining diagnoses must be made in small biopsies and/or cytology-type samples. Adding these facts to the lack of a variety in effective treatments, rare 5-year survival percentages are produced (5). Taking the previous into account, the identification of new diagnostic, prognostic and predictive biomarkers is the utmost requirement to achieve effective therapeutic response in NSCLC (6). Recently, liquid biopsy has arisen as a new non-invasive approach that may provide multiple indicators in cancer, including diagnosis, prognosis, resistance to therapy and monitoring of response (7).

Liquid biopsy refers to the molecular analysis, with minimally invasive methods, used to study biomarkers in different body fluids (peripheral blood, urine, saliva, pleural liquid), being a reliable alternative to conventional tissue biopsies. The important biotechnological development in the field of detecting the components of liquid biopsy has allowed relevant clinical applications for cancer patients. The main components analysed in lung cancer liquid biopsies are circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, circulating microRNAs, circulating RNA, platelets or plasma/serum metabolites (8). Currently, liquid biopsy is not yet able to replace tissue biopsy in the daily clinical practice, although it offers additional data that cannot be obtained in any other way. For example, in cases in which patients cannot be biopsied or biopsies do not provide enough tissue for mutational analysis, liquid biopsy is the only alternative. This novel tool is already being used in clinical practice through the analysis of cfDNA-based tumor genotyping for the

management of targeted therapies in lung cancer (9,10). In patients with advanced disease, it is not feasible to obtain biopsies of every metastatic location; however, body fluids such as blood can include elements from the primary tumor and the metastases. Moreover, liquid biopsy samples can be repeatedly obtained without the risk of comorbidities and used to monitor the disease as well as early detection of response, relapse or resistance to a determined therapy (11).

For all the aforementioned reasons, the development and implementation of more sensitive and minimally invasive techniques that allow the study of specific biomarkers in body fluids at low concentration, are necessary to carry out a new approach in cancer management.

Exosomes are very small vesicles, secreted by tumor cells, capable of transferring their molecular cargo to distant cells. MicroRNAs are small non-coding RNAs often deregulated in cancer, which act as master regulators of the genome. This type of small RNA can represent a large percentage of the exosome content, acting as valuable element in the development of lung cancer. In this review we focus on the role of exosomal microRNAs in the pathobiogenesis of NSCLC.

Exosomes as new source of biomarkers in cancer

Exosomes are small extracellular vesicles (EVs) with a diameter around 40–160 nm which are produced and secreted from most eukaryotic cells and are involved in cell-to-cell communications. They are formed by a lipid bilayer structure and contain inside a wide variety of cellular components such as proteins, mRNAs, small RNAs, DNAs and lipids, among others (12).

Exosomes are produced in a process that consists of a double invagination of the plasma membrane and the formation of intracellular multivesicular bodies (MVBs), which contain intraluminal vesicles (ILVs). ILVs are finally secreted as exosomes (with a maximum size 160 nm) through MVB fusion to the plasma membrane followed by exocytosis. The amount of biogenesis of exosomes between different cells depends on their physiological and pathological states and can be found in the blood, urine, semen and other body fluids (13). In last years, knowledge about the role of exosomes in cancer has come a long way compared to research into their activity in other non-neoplastic diseases.

Different works have elucidated that exosomes are implied in many important biological processes such

as intra/extracellular communication, immune system modulation (14), cell growth and maintenance of energy pathways (15). This type of EVs can have a pleiotropic role in cancer, being associated with several hallmarks of this disease (16). Moreover, exosomes derived from cancer cells influence tumor growth and metastasis (17), immune evasion and drug resistance (18).

The role of exosomes in cancer progression is dynamic and specific to cancer type, genetics, and stage. A great number of relevant studies have been performed in exosomes from NSCLC for improving the management and knowledge of the disease. Additionally, collected results show that cancer-derived exosomal miRNAs play a key role in the activation of molecules and reprogramming of the tumor environment (19).

Exosome-derived microRNAs: a valuable tool in NSCLC study

MicroRNAs (miRNAs) are a short and endogenous type of non-coding RNAs. The majority, are transcribed from DNA sequences into primary miRNAs, then transformed into precursors, and finally forming the mature miRNAs. In most cases, miRNAs interact with the 3' untranslated region (3' UTR) of target mRNAs to induce mRNA degradation and translational repression. However, the relationship of miRNAs with other regions, including the 5' UTRs, coding sequences and promoters, have also been revealed (20-22).

MiRNAs may be secreted in extracellular fluids and transported to target cells via protein binding or within microvesicles such as exosomes (23,24). These molecules of RNA are a considerable part of the content of exosomes cargo and probably the most studied ones due to their involvement in regulation of gene expression at post-transcriptional/translational levels and modulation of cell functions in several types of cancer (25).

One of the most important aspects of exosomal miRNAs is that they represent a potential valuable tool for non-invasive early diagnosis, treatment selection and prediction of therapy resistance (26).

Exosomal miRNAs as biomarkers of relevant tumor processes (angiogenesis, EMT and metastasis) in NSCLC

The formation of new vasculature is essential for tumor development, spread and metastasis. Tumors can modulate angiogenesis through exosomes. Concretely, some studies

have shown that exosomal miRNAs stimulate angiogenesis. Liu *et al.* revealed that exosomal miR-21 leads to STAT3 activation, increasing VEGF levels and inducing malignant transformation of human bronchial epithelium (27).

Other authors demonstrated that exosomal miR-9 results in the activation of JAK/STAT pathway, promoting angiogenesis and cell migration. Moreover, Hsu *et al.* showed that exosomal miR-23a modulates tumor vasculature under normoxic and hypoxic conditions, demonstrating that lung cancer cells transfer genetic information to distant cells (28).

Exosomal miRNAs can also be involved in epithelial-to-mesenchymal transition (EMT) and metastasis processes. EMT is a process through which adherent epithelial cells acquire the ability to migrate, losing cell-to-cell adhesion and cellular polarity. Metastasis is a multifaceted process by which cells separate from the primary tumor and invade adjacent or distant tissues (29).

There seems to be different approaches of exosomal miRNA transfer during cancer metastasis. Firstly, exosomal miRNAs are transferred from invasive cells to less invasive cells promoting the metastasis. For instance, ADC cell-derived exosomal miR-494 and miR-524-3p showed capacity to target non-transformed cells and modulate pre-metastatic organ cells (17). Another example, exosomes derived from ADC cells were able to transfer to osteoclast progenitor cells and target programmed cell death 4 (PDCD4) to facilitate osteoclastogenesis through exosomal miR-21. Secondly, exosomal miRNAs can be secreted by primary tumor cells to the tumor microenvironment, contacting other cells. Fabbri *et al.* revealed that tumor-secreted miR-21 and miR-29a could activate Toll-like receptors TLR7 and TLR8 in immune cells, triggering tumor growth and metastasis (30). Furthermore, lung cancer cells can influence metastatic bone colonization via exosomal miR-192 (31). Thirdly, tumor cells can modify his behaviour because of unique miRNAs released by normal cells. For instance, exosomal miR-193a-3p, miR-210-3p and miR-5100 can be transferred from bone mesenchymal stem cells (BMSCs) to lung cancers and activate STAT3 signalling, which leads to EMT and metastasis (32).

Potential role of exosomal miRNAs as biomarkers for clinical applications

Effective therapies and early detection of lung cancer ought to be priorities and remain the most efficient approaches to reduce the number of deaths from this disease. Exosomal

microRNAs could be proposed as new non-invasive biomarkers in neoplastic disease. In this section, we review the new outcomes on exosomal miRNA involvement as diagnostic, prognostic and predictive biomarkers for NSCLC.

Exosomal miRNA for early detection and diagnosis of NSCLC

As previously mentioned, one of the main problems of lung cancer today is a late diagnosis due to the lack of efficient screening methods. Currently, a common early screening method is low-dose computed tomography. However, this type of screening presents some limitations, which include enhanced costs, high rate of benign nodule detection, over-diagnosis and radiation exposure (33).

In most cases, patients are diagnosed at advanced stages when surgery is not an option. In addition, in many of these patients, there is not even an effective therapeutic option, which leads to a high mortality rate. Therefore, early detection and diagnosis are the most effective strategies for prevention and treatment of lung cancer (34,35). For these reasons, liquid biopsy takes on a key role in order to be a source of biomarkers. Concretely, exosomes have become a valuable source of biomarkers, obtained in a minimally invasive and effective way. The study of the extensive content of molecules present in these microvesicles makes elements such as miRNAs to be postulated as tools for the early detection of this pathology (36).

Interestingly, preceding studies about the diagnostic use of miRNAs associated with lung biopsies revealed the utility of 12 particular miRNAs: miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-191, miR-192, miR-203, miR-205, miR-210, miR-212, and miR-214 (37). Afterwards, Rabinowits *et al.*, focused on these 12 miRNAs, demonstrated the resemblance of circulating exosomal miRNA and tumor-derived miRNAs profiling, suggesting that exosomal miRNAs could be used as a screening tool for detection of lung ADC (38).

It has also been demonstrated that miR-378a, miR-379, miR-139-5p, and miR-200b-5p are useful for the discrimination of nodule (lung ADCs and granulomas) and non-nodule (healthy ex-smokers) (97.5% sensitivity, 72.0% specificity). Furthermore, same study revealed that a set of 6 exosomal miRNAs derived from human plasma (miR-629, miR-30a-3p, miR-100, miR-200b-5p, miR-154-3p and miR-151a-5p) differentiated granulomas from lung ADC (96% sensitivity and 60% specificity), and a group of 4

exosomal miRNAs (miR-200b-5p, miR-378a, miR-139-5p and miR-379) distinguished nodules from non-nodules with 96.0% of sensitivity and 60.0% of specificity (39). Zhou *et al.* also found a group of exosomal miRNAs (miR-19-3p, miR-21-5p and miR-221-3p) for discriminating patients with lung ADC from healthy subjects with sensitivity and specificity rates of 67–73% and 66–80%, respectively (40).

In terms of small cell lung carcinoma (SCLC) and NSCLC histology, Poroyko *et al.* revealed that miRNAs exosomal cargo is different between patients with SCLC and NSCLC. In particular, 13 miRNAs were suitable to differentiate the two histologies. Three of them (miR-331-5p, miR-451a, miR-363-3p) were capable of distinguishing SCLC and NSCLC cases with highest rates of specificity and sensitivity. What is more, miR-203, suitable for discerning NSCLC and SCLC cases (sensitivity 80%, specificity 100%), was previously reported by Rabinowits *et al.* in blood-derived exosomes as a biomarker for lung ADC (38). These authors described 7 exosomal miRNAs (miR-451a, miR-486-5p, miR-363-3p, miR-660-5p, miR-15b-5p, miR-25-3p and miR-16-2-3p) specific to differentiate between NSCLC patients and healthy subjects (41).

Another study reported that exosomal miR-126 could be a potential diagnostic biomarker for NSCLC (42). Furthermore, it has been described a group of 3 miRNAs (miR-21, miR-205 and miR-155) for the early detection of NSCLC (43).

Focusing in the histologic heterogeneity of NSCLC, Jin *et al.* performed a remarkable study which demonstrated that four exosomal miRNAs (miR-let-7b-5p, miR-let-7e-5p, miR-23a-3p and miR-486-5p) were potential diagnostic biomarkers of stage I NSCLC patients (sensitivity 80.5% and specificity 92.31%). Besides, they also revealed that miR-181-5p, miR-30a3p, miR-30e-3p, and miR-361-5p were ADC-specific miRNAs, whereas miR-10b-5p, miR-15b-5p and miR-320b were squamous-specific with areas under the curve (AUC) of 0.936 and 0.911, respectively (44). Previously, Aushev *et al.* revealed that the levels of a specific panel of exosomal miRNAs (miR-205, miR-19a, miR-19b, miR-30b and miR-20a) decreased in the blood of patients after lung SCC surgery (45).

Exosomes are not only present in blood. Many studies have been focused on others fluids like bronchoalveolar lavage (BAL) and pleural fluid to find new diagnostic biomarkers. A study confirmed that exosomal miRNA levels are increased in plasma and BAL samples from patients with NSCLC in contrast to non-tumor patients (46).

Additionally, some authors identified 9 exosomal miRNAs in pleural fluid (miR-141-3p, miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-205-5p, miR-375, miR-483-5p, miR-429, miR-203a-3p), which were predominantly present in lung cancer over tuberculous and other benign lesions (47,48). Furthermore, exosomal miR-200 was also described in pleural effusions to distinguish ADC from patients with benign effusions (49). More recently, it has been revealed expression of miR-182 and miR-210 in malignant pleural fluid from ADC over benign pleural effusions (50) (*Table 1*).

Exosomal miRNAs as prognosis biomarkers in NSCLC

TNM stage is the most important determinant for predicting recurrence rates and survival times in NSCLC. Most of NSCLC patients are diagnosed at advanced stages leading to unfavourable prognosis (51).

An accurate prognosis prediction might reduce mortality and improve patient's survival time. In resected early-stage cases, exosomal miRNAs might be useful for detecting minimal residual disease, allowing patients to take advantage of adjuvant treatments. To accomplish this, it would be necessary the clinical validation of exosomal miRNAs on the prognosis of early-stage disease. Up to date, some exosomal circulating miRNAs have been discovered as reliable biomarkers for NSCLC prognosis. Zhang *et al.* showed that high levels of serum exosomal miR-378 correlated with positive lymph node metastasis and advanced TNM stage. In consequence, serum exosomal miR-378 might be recognized as a biomarker of poor survival in NSCLC (52).

Another group demonstrated that high serum exosomal miRNA-214 was associated with advanced disease and, therefore, it could be used as a poor survival indicator in NSCLC patients (53).

It has also been revealed that plasma exosomal miR-10b-5p, miR-23b-3p and miR-21-5p were independent prognostic biomarkers in NSCLC (54). Moreover, the prognosis of NSCLC can also be affected by miR-195 (55).

Additionally, it has been demonstrated that the expression of exosomal miR-21 and miR-4257 was significantly increased during recurrence of NSCLC patients. Subsequently, the prognostic biomarker value of these miRNAs was confirmed in an extensive cohort of patients (56).

Furthermore, in advanced NSCLC, patients with high recurrence show lower levels of exosomal miR-146a-5p

comparing with those with low recurrence (57).

Moreover, low level of exosomal let-7a-5p was recognized as poor survival biomarker in lung ADC. Interestingly, downregulation of this let-7a-5p miRNA in workers who are exposed to dust plays an important role in lung cancer development (58) (*Table 2*).

Exosomal miRNAs as predictive biomarkers in NSCLC treatments

Despite substantial advances in chemotherapy, radiotherapy, targeted therapies and immunotherapy, many patients remain refractory to these therapies or acquire resistances during treatment. Therefore, the identification of patients who will derive benefit is a priority for treatment optimization. Looking for new biomarkers to guide patient selection and provide indications of efficacy and/or toxicity is essential. Exosomal miRNAs derived from peripheral blood or plasma samples might represent reliable predictive biomarkers for optimal treatment selection of patients with NSCLC.

Regarding radiotherapy treatment, exosomal miR-29a-3p and miR-150-5p were recognized as biomarkers of dose exposure, that could be used as predictive biomarkers of response and/or toxicity (59). Moreover, it has been shown that the presence in serum of exosomal miR-208a can affect the radiosensitivity of lung tumor cells by targeting p21 (60).

With respect to chemotherapy treatments, Yuwen *et al.* revealed that serum exosomal miR-146a-5p could be used as a biomarker for improving predictions of the efficacy of Cisplatin in NSCLC and monitoring of resistance to therapy (57). Some authors have also demonstrated exosomal miR-425-3p as a potential biomarker for predicting the clinical response to platinum-based-chemotherapy in patients with NSCLC (61). Furthermore, plasma levels of miR-32 are correlated with the efficacy of platinum-based-chemotherapy and prognosis in NSCLC (62). Recently, serum levels of exosomal miR-222-3p have been suggested to have a prognostic value for gemcitabine sensitivity in NSCLC (63).

In reference to targeted-therapies, exosomal plasma levels of miR-221-3p and miR-222-3p were linked to the response to Osimertinib in EGFR-mutated NSCLC patients (64).

More studies are needed to clarify the role of exosomal miRNAs in the regulation of tumors treated with TKIs-targeted therapies.

As for the most recent treatment strategies, plasma

Table 1 Main studies focused on exosomal miRNAs used as diagnostic biomarkers in NSCLC

Exosomal miRNAs	Source	Clinical value	Reference
miR-378a, miR-379, miR-139-5p, and miR-200b-5p	Plasma	Diagnostic biomarkers to differentiate adenocarcinoma and healthy subjects	(39)
miR-19-3p, miR-21-5p and miR-221-3p	Plasma	Diagnostic biomarkers for lung adenocarcinoma	(40)
miR-331-5p, miR-451a, miR-363-3p	Plasma	Diagnostic biomarkers for distinguishing SCLC from NSCLC	(41)
miR-451a, miR-486-5p, miR-363-3p, miR-660-5p, miR-15b-5p, miR-25-3p and miR-16-2-3p	Plasma	Diagnostic biomarkers to differentiate adenocarcinoma from healthy subjects	(41)
miR-126	Plasma	Diagnostic biomarker for NSCLC	(42)
miR-21, miR-205 and miR-155	Plasma	Diagnostic biomarkers for early-stage NSCLC	(43)
miR-let-7b-5p, miR-let-7e-5p, miR-23a-3p and miR-486-5p	Plasma	Diagnostic biomarkers of stage I NSCLC patients	(44)
miR-181-5p, miR-30a3p, miR-30e-3p and miR-361-5p	Plasma	Diagnostic biomarkers for lung adenocarcinoma	(44)
miR-10b-5p, miR-15b-5p and miR-320b	Plasma	Diagnostic biomarkers for SCC	(44)
miR-205, miR-19a, miR-19b, miR-30b and miR-20a	Plasma	Diagnostic biomarkers for SCC after surgery	(45)
miR-205-5p, miR-483-5p, miR-375, miR-200c-3p, miR-429, miR-200b-3p, miR-200a-3p, miR-203a-3p and miR-141-3p	Pleural fluid	Diagnostic biomarkers for discriminating lung adenocarcinoma over tuberculosis and other benign lesions	(47,48)
miR-200	Pleural fluid	Diagnostic biomarker for discriminating lung adenocarcinoma from benign effusions	(49)
miR-182 and miR-210	Pleural fluid	Diagnostic biomarkers for distinguishing lung adenocarcinoma from benign effusions	(50)

exosomal miR-320d, miR-320c and miR-320b have been identified as potential biomarkers for predicting the efficacy of immunotherapy in advanced NSCLC. Furthermore, in cases when the T-cell suppressor miR-125b-5p is downregulated during the treatment, patients achieve increased T-cell function and show an excellent response to immunotherapy (65) (Figure 1, Table 2).

Exosomal miRNAs as drug resistance biomarkers in NSCLC

The comprehension of drug resistance mechanisms is one of the main objectives to reduce the poor prognosis in lung cancer. Development of drug resistance to standardized chemotherapy as well as to targeted therapies, like tyrosine kinase inhibitors, or immunotherapy is one of the main problems in cancer (66-68). Accordingly, the study of the mechanisms of resistance potentially regulated by exosomal

microRNAs will help to improve cancer treatments.

Wu *et al.* reported a significant higher expression of exosomal miR-96 in lung cancer compared to control lung tissues. This microRNA modifies the chemotherapeutic sensitivity by downregulating the drug resistance-related gene lime domain only protein 7 (LMO7). In consequence, by targeting LMO7, circulating exosomal microRNA-96 promotes cell proliferation, migration and drug resistance (69).

Yuwen *et al.* demonstrated that higher serum exosomal levels of miR-146a-5p predicts increased therapeutic effect of Cisplatin in NSCLC patients, possibly by inhibiting autophagy-related protein 2 (ATG2) to suppress autophagy (57). Additionally, exosomal miR-512 and miR-373 have also been associated with high sensitivity to Cisplatin and suppression of tumor progression (70).

On the other hand, exosomal miRNAs can also be involved in resistance mechanisms to TKIs. In 2018, Jing

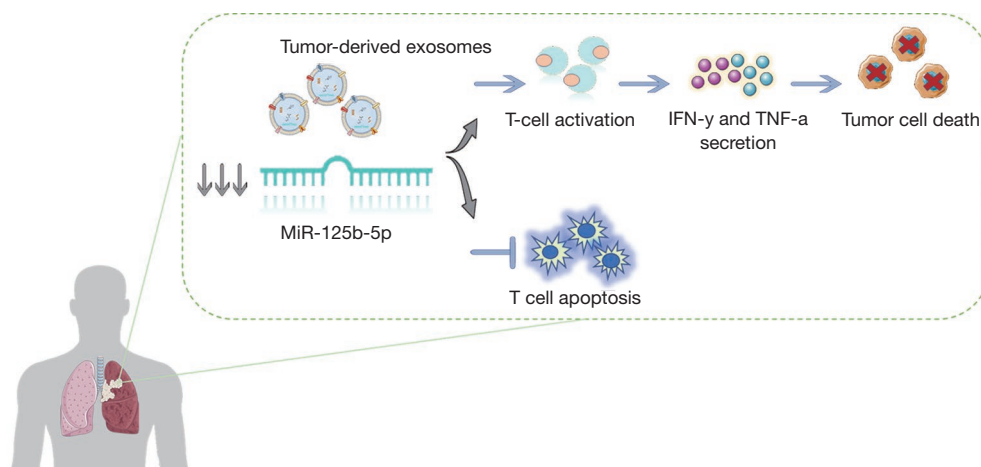


Figure 1 Diagram of the potential regulation process of miR-125-5p on T-cells.

Table 2 Main studies focused on exosomal miRNAs used as prognostic and predictive biomarkers in NSCLC

Exosomal miRNAs	Source	Clinical value	Reference
miR-378	Serum	Biomarker associated with poor survival in NSCLC	(52)
miRNA-214	Serum	Biomarker associated with advance disease and poor survival indicator in NSCLC patients	(53)
miR-23b-3p, miR-10b-5p and miR-21-5p	Plasma	Independent prognostic biomarker of NSCLC	(54)
miR-21 and miR-4257	Plasma	Prognostic biomarkers in NSCLC	(56)
miR-146a-5p	Serum	Predictive biomarker of the efficacy of Cisplatin in NSCLC and drug resistance	(57)
let-7a-5p	Serum	Biomarker associated with poor survival in lung adenocarcinoma	(58)
miR-29a-3p and miR-150-5p	Plasma	Biomarkers used to predict response or toxicity in radiotherapy treatment in NSCLC	(59)
miR-208a	Serum	Predictive biomarker of radiosensitivity in lung cancer	(60)
miR-425-3p	Serum	Predictive biomarker of the clinical response to platinum-based-chemotherapy in patients with NSCLC	(61)
miR-32	Plasma	Predictive biomarker associated with the efficacy of platinum-based-chemotherapy and prognosis in NSCLC	(62)
miR-222-3p	Serum	Potential prognostic biomarker of Gemcitabine sensitivity in NSCLC	(63)
miR-221-3p and miR- 222-3p	Plasma	Predictive biomarkers associated with the response to Osimertinib in EGFR-mutated NSCLC	(64)
miR-320d, miR-320c, miR-320b and miR-125b-5p	Plasma	Predictive biomarker of the efficacy of immunotherapy in advance NSCLC	(65)

et al. reported that exosomal transfer of miR-21 from Gefitinib-resistant H827R cells to Gefitinib-sensitive HCC827 cells activated AKT signalling, leading to Gefitinib resistance (71).

Same year, another group also revealed a significant

higher expression of miR-214 in Gefitinib-resistant PC9GR cells and their derived exosomes than in Gefitinib-sensitive PC9 cells and exosomes. The authors reported that sensitive cells acquired resistance to Gefitinib when exosomal miR-214 was transferred from PC9GR cells

to PC9 cells. Nevertheless, when PC9GR cells were transfected with a miR-214 antagomir, cells became sensitive to Gefitinib (72).

Accordingly, we can conclude that exosomal miRNAs can provide information on the molecular characteristics of the cells by which they were secreted and be endowed with the capacity to reprogram distant target cells. Moreover, exosomal miRNAs can regulate tumor resistance and represent a potential tool for monitoring the response/relapse of anti-neoplastic therapies.

Exosomal miRNAs as immune-oncology biomarkers in NSCLC microenvironment

The delivering of exosomal miRNAs by tumor and stromal cells in the microenvironment has been confirmed to be associated with tumor initiation, relapse, progression and metastasis. Exosomes can promote tumor progression by transferring immunosuppressive factors such as exosomal miRNAs, which can modulate the function of immune cells such as T-lymphocytes, dendritic cells (DCs) and natural killer (NK) cells (73).

Some exosomal miRNAs, such as miR-183, are TGF- β -inducible and can silence tumor-associated NK cells by targeting and suppressing other activating proteins (74).

Furthermore, the susceptibility to the lysis by cytotoxic T cells is regulated by hypoxia inducible miR-210. Hypoxic tumor-derived exosomes can negatively regulate NK cell function by a mechanism which involves TGF- β and miR-23a (75).

Another example of this immunomodulation in lung cancer cells is exosomal mir-21/29a, that is able to initiate tumor growth and metastasis through activation of TLR7 and TLR8 on immune cells (30). Yin *et al.* also confirmed communication between lung cancer cells and CD4+ lymphocytes through exosomal mir-214, which efficiently decreased PTEN expression, promoting regulatory T cell (Treg) expansion and tumor growth (76).

Additionally, lung cancer cell invasion has been described to be stimulated by exosomal transfer of miR-223 from platelets, through suppression of erythrocyte membrane protein band 4.1 like 3 (EPB41L3) (77). Finally, exosomal miR-193a-3p, miR-210-3p and miR-5100 from bone marrow-derived mesenchymal stem cells (BMSCs) are also reported to promote lung tumor cell invasion via activation of STAT3 signalling-induced EMT (32).

Conclusions

The discovery of relevant functions of exosomes has been a milestone in the searching of biomarkers in liquid biopsy. MicroRNAs, abundant elements in the exosome cargo, participate in the proliferation, metastasis, EMT, angiogenesis and immunomodulation processes in lung cancer. Furthermore, current researches have demonstrated the relevant use of exosomal miRNAs in the study of lung cancer, providing a significant advance in the clinical management. Due to their great potential, exosomal miRNAs can be used as an excellent non-invasive tool for early diagnosis, prognosis and prediction of treatment success or drug resistances in this pathology.

However, there are still challenges to be achieved. Firstly, because of the heterogeneity in size (different subtypes of vesicles), standardized methodologies must be established for isolation, characterization and study of exosomes cargo. Secondly, a complete comprehension of exosomal miRNA-mediated immunomodulation or drug resistance mechanisms is needed. Therefore, further studies are required to determine specific components and mechanisms of the exosomal miRNA to understand their role in cancer pathogenesis.

In conclusion, exosomal miRNAs have a considerable potential for the optimal management of NSCLC patients. Regardless multiple and recent technical and scientific developments in the field of liquid biopsy, the ultimate goal is to implement a useful and standardized methodology with high sensitivity/specificity for the isolation and analysis of exosomes and their content from minimally invasive samples, which might allow improvements in the prognosis, prediction and monitoring of the disease in the clinical practice.

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Footnote

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