Chapter 5
miRNA Targeted Therapy in Lung Cancer

Aamir Ahmad, Kevin R. Ginnebaugh, Yiwei Li, Bin Bao, Shirish M. Gadgeel, and Fazlul H. Sarkar

1 Introduction

Lung cancer is the leading cause of cancer-associated deaths [1] with non-small-cell lung cancer (NSCLC) being the most prevalent histological cancer subtype worldwide [2]. Lung cancer is often associated with poor prognosis because many patients are diagnosed at an advanced stage when surgery is no longer an option. In order to reduce the high mortality rate, new predictive and prognostic biomarkers need to be validated in lung cancer models. The individual components of various signaling pathways have long been tested for their use as biomarkers and/or therapeutic targets with limited success. Research in recent years has proposed microRNAs (miRNAs) as molecules with enormous potential as therapeutic targets.

The miRNAs are small (19–24 nucleotides) non-coding RNA molecules that down-regulate gene expression by interacting with sequences located mostly in the 3′ untranslated region (UTR) region of multiple target mRNAs, resulting in either translational repression or degradation of mRNAs. The miRNA-mediated regulation of oncogenes/tumor suppressor genes is now widely accepted as a key step in

A. Ahmad (✉) • K.R. Ginnebaugh • Y. Li • B. Bao
Department of Pathology, Karmanos Cancer Institute, Wayne State University School of Medicine, 707 Hudson Webber Cancer Research Center, Detroit, MI 48201, USA
e-mail: ahmada@karmanos.org

S.M. Gadgeel
Department of Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, 4100 John R, 4 Hudson Webber Cancer Research Center, Detroit, MI 48201, USA

F.H. Sarkar
Department of Pathology, Karmanos Cancer Institute, Wayne State University School of Medicine, 707 Hudson Webber Cancer Research Center, Detroit, MI 48201, USA

Department of Oncology, Karmanos Cancer Institute, Wayne State University School of Medicine, 4100 John R, 4 Hudson Webber Cancer Research Center, Detroit, MI 48201, USA
e-mail: fsarkar@med.wayne.edu

F.H. Sarkar (ed.), *MicroRNA Targeted Cancer Therapy*,
DOI 10.1007/978-3-319-05134-5_5,

the progression of human malignancies. The miRNAs are believed to play a regulatory role in almost every aspect of tumor progression. They are known to regulate proliferation, migration, invasion, angiogenesis, metastasis as well as relapse of human cancers. As such they offer an attractive target for therapy of human cancers. While the importance of miRNA-mediated regulation has been demonstrated in virtually every known cancer type, we will limit our discussion in this chapter to lung cancer. Without going into the details of individual miRNAs that have been associated with lung cancer cells' proliferation and other *in vitro* characteristics, we will discuss the most recent literature that has demonstrated the possible use of miRNAs in lung cancer therapy. The focus of this chapter will be on the studies with clinical implications. We will start with a discussion of reports on the regulation of resistance phenotype by miRNAs and then look at numerous recent reports that have tied miRNAs with some diagnostic/prognostic importance in clinical lung cancers.

2 Resistance to Therapies

Despite significant progress made in cancer research in recent years, the mortality rate for lung cancer has largely remained unchanged. A major factor that has contributed to this is acquired resistance to conventional and targeted therapies [3]. Simply speaking, this refers to the ability of lung cancers to turn refractory/resistant to very therapeutic regimes to which they responded initially. Accumulating evidence has connected miRNAs to the phenomenon of drug resistance [4]. In the context of targeted therapy, the topic of resistance is very important. Any future targeted therapy needs to carefully evaluate the possible mechanisms that can lead to resistance against it. In the next few sub-sections, we will detail the role of miRNAs in acquired resistance against chemotherapy as well as radiation therapy, the two major types of therapies used for treatment of cancers.

2.1 Drug Resistance

We start our discussion on resistance to therapies with resistance against chemotherapy. A number of chemotherapeutic drugs have been approved for the treatment of lung cancers. These drugs often meet the same fate when used for prolonged periods and the tumors that acquire resistance against chemotherapy are most often very aggressive and very difficult to manage.

2.1.1 Cisplatin

Cisplatin belongs to the class of platinum-containing anticancer drugs. The miRNAs are now well known to influence the phenomenon of drug resistance against cisplatin. In one study that focused on understanding the mechanism of cisplatin resistance, a role of let-7c was discovered [5]. It was observed that the expression of let-7c miRNA is reduced in A549-derived cells that are resistant to cisplatin. Re-expression of let-7c levels led to alterations in the sensitivity of cells to cisplatin, suggesting a specific role of this miRNA in determining resistance to cisplatin. In this context, ABCC2 and Bcl-XL were identified as targets of this miRNA. It might be important to point out that let-7 family of miRNAs are known regulators of EMT (epithelial-to-mesenchymal transition) wherein their high expression is largely associated with a less aggressive and epithelial phenotype. This study reported lower levels of let-7c in cisplatin resistant cells which means that the resistant cells might be exhibiting a mesenchymal phenotype. These results, therefore, indicate a possible role of EMT in drug resistance. Although there are numerous reports that connect EMT to drug resistance, this report was one of the first connecting let-7c to cisplatin resistance in a lung cancer model. Similar to the effects of let-7c, another report [6] implicated a similar activity of miR-503. This miRNA was also observed to be down-regulated in cisplatin resistant A549 cells. Though this study did not identify a single target of miR-503 leading to cisplatin resistance mediated effects, a number of drug resistance related factors such as MDR1, MRP1, ERCC1, survivin and bcl-2 were reported to be down-regulated significantly with the over-expression of miR-503. Qiu et al. also reported a down-regulated miR-503 in A549 cells that were resistant to cisplatin [7]. They identified Bcl-2 as a target of miR-503. Ectopic expression of miR-503 reduced the levels of its target Bcl-2 and resulted in re-sensitization of cisplatin-resistant A549 cells to cisplatin. Recently, miR-101 over-expression has also been shown to sensitize A549 cells to cisplatin with increased apoptosis through activation of caspase 3 [8].

Another miRNA involved in cisplatin resistance in lung cancer cells is miR-135 [9]. The miR-135a/b were observed to be expressed at relatively low levels in A549 cells that were resistant to cisplatin. To confirm the role of miR-135a/b in cisplatin resistance, these miRNAs were over-expressed in cisplatin resistant cells which led to the reversal of cisplatin resistance. This study identified MCL1 as a direct target of miR-135a/b and, therefore, levels of MCL1 were high in the cisplatin resistance cells which went down with the over-expression of miR-135a/b. The miR-98 is yet another miRNA implicated in cisplatin resistance [10]. This miRNA was identified for its role in cisplatin resistance in a study that looked at differential expression of miRNAs in cisplatin resistant A549-derived cells vs. cisplatin sensitive parental A549 cells. Based on whether the expression was increased at least two-folds or halved, 14 miRNAs were listed to be up-regulated and 8 miRNAs were down-regulated in cisplatin resistance cells, compared to parental cells. The miR-98 was found to be down-regulated three-folds in the resistant cells while its

target gene HMGA2 was up-regulated. Increased expression of miR-98 in cells led to increase sensitivity to cisplatin, thus confirming its role in cisplatin resistance.

With the knowledge that copper-transporting p-type adenosine triphosphatase A (ATP7A) is involved in the resistance to cisplatin, Song et al. [11] looked for miRNA(s) that can target ATP7A which, in turn, may play a role in cisplatin resistance. The miR-495 was identified as one such miRNA that could target ATP7A. ATP7A is induced in cisplatin-resistant cells while miR-495 was found to be down-regulated in the same cells. Up-regulation of miR-495 reduced the levels of ATP7A and sensitized cells to cisplatin. Increased miR-495 levels resulted in accumulation of drug inside the cells which can explain enhanced sensitivity to cisplatin. Conversely, low levels of miR-495 (and high levels of ATP7A) resulted in lower intracellular levels of cisplatin. A role of miR-31 has also been suggested in resistance to cisplatin [12]. Endogenous expression of miR-31 was observed to be up-regulated in cells resistant to cisplatin and its down-regulation led to the sensitization of cells to cisplatin. Interestingly, ABCB9, the drug-resistance associated gene, was identified as a target gene.

2.1.2 Docetaxel (Taxotere)

Docetaxel is a chemotherapeutic agent that interferes with mitosis, and as such targets the relatively fast dividing cancer cells. Similar to its involvement in resistance to cisplatin, let-7 has been shown to be involved in resistance to docetaxel as well [13]. The lung cancer cells, SPC-A1 that were engineered to be resistant to docetaxel, were found to express low endogenous levels of let-7c. Ectopic over-expression of let-7c once again sensitized these cells to docetaxel. Interestingly, resistance to docetaxel was accompanied by resistance to radiations as well, and thus ectopic over-expression of let-7c sensitized the cells to radiation therapy. As expected, increased expression of let-7c reversed EMT and the metastatic potential of cells as well. This study identified Bcl-xL as a molecular target of let-7c and deregulations of Bcl-xL levels were shown to be enough to abrogate let-7c effects. This study successfully demonstrated a similar underlying molecular mechanism for chemo- and radio-resistance that involved modulation by a miRNA.

2.1.3 EGFR-TKI

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) are routinely used in clinical setting for the treatment of lung cancer. However, like most of the other conventional drugs, lung cancer patients often develop resistance to EGFR-TKIs which remains a major concern. EMT, in particular, has been linked to the development of resistance to EGFR-TKIs. Kitamura and coworkers [14] looked for a correlation between EMT-inducing miRNAs and resistance to EGFR-TKI gefitinib. TGF-β1 was used as an inducer of EMT and it was observed to induce the expression of several miRNAs. Mechanistic studies revealed an

important role of miR-134 and miR-487b in the induction of EMT and resistance to gefitinib. Knockdown of these two miRNAs reversed EMT as well as TGF-β1-induced resistance to gefitinib.

Investigations in our own laboratory have revealed an important role of hedgehog signaling in TGF-β1-induced EMT, and resistance of NSCLC cells to EGFR-TKI erlotinib [15]. We observed reduced levels of miR-200 and let-7 families of miRNAs in TGF-β1-treated A549 cells that were resistant to erlotinib. From the two families of miRNAs, we chose to focus on miR-200b and let-7c because these were the two most differentially expressed miRNAs in our study. Over-expression of these two miRNAs significantly reversed EMT and also re-sensitized cells to erlotinib. Inhibition of hedgehog signaling pathway, by use of pharmacological inhibitor GDC-0449 as well as the use of specific siRNA, also had similar effects, i.e. reversal of EMT and sensitization to erlotinib. Our results, thus, confirmed a complex relationship between hedgehog signaling, EMT and erlotinib resistance, which involved regulation by EMT-modulatory miRNAs.

2.1.4 Pemetrexed

Pemetrexed is another drug used for the treatment of NSCLC. Its structure is similar to folic acid and, therefore, it belongs to the class of anticancer drugs called "folate antimetabolites". It has been approved for use against locally advanced and metastatic NSCLC in combination with cisplatin. Franchina et al. [16] evaluated the expression levels of miR-22, miR-24 and miR-34a in blood samples of NSCLC patients treated with pemetrexed for a possible correlation with clinical outcome. Of the three miRNAs tested, miR-22 was reported as the most promising miRNA because its levels were significantly high in patients that developed progressive disease. Thus, miR-22 can be a marker for predicting progressive disease in patients being treated with pemetrexed.

2.2 Radioresistance

Resistance to radiotherapy is a major clinical problem. Advanced stage tumors often exhibit resistance to radiation therapy. To understand the molecular basis of such radioresistance, Zhang and coworkers [17] developed a radioresistant derivative of NSCLC cell line A549. This was achieved by subjecting A549 cells to 2Gy/day X-rays amounting to a total of 68 Gy. Western blot analysis revealed an up-regulated TRIB2 in the cells that were consistent with resistance to radiation. As a regulator, miR-511 was observed to be down-regulated in the resistant cells. Based on these observations, it was hypothesized that miR-511 expression is lost in cells that acquire resistance to radiation. To prove this hypothesis, miR-511 was

over-expressed in the resistant cells which led to a decrease in the levels of TRIB2 with simultaneous inhibition of cell growth and also increased apoptosis. An involvement of Bax was also suggested based on the observation that resistant cells had low levels of Bax and these levels were restored with over-expression of miR-511. This study suggested that miR-511 can be targeted in lung cancer patients for the reversal of radioresistance. Another study by Grosso et al. [18] found a similar role of miR-201 in the radioresistance. The logic behind the study on miR-201 was based on the connection of this miRNA with hypoxia-inducible factor (HIF)-1. In this work, A549 cells were stably transfected with miR-210 and it was observed that these cells had significantly stabilized HIF-1. As a result, miR-210-expressing cells were similarly radioresistant under normoxic conditions as the un-transfected cells under hypoxia. Furthermore, under hypoxic conditions, miR-210 overexpressing cells were able to tolerate radiations up to 10 Gy and showed significantly reduced apoptosis upon exposure to radiation. It was suggested that specific targeting of miR-210 might be an option for sensitization of radioresistant lung cancers to radiation treatment.

Liu and co-workers [19] exposed isogenic lung cancer cells CL1-0 and CL1-5 to 10 Gy radiation and looked at the differential expression of miRNAs in an attempt to list the miRNAs that play a role in sensitivity to radiation. Of the 26 miRNAs, miR-449a was found to be down-regulated in cells after 24 h of exposure to radiation. Based on this observation, this miRNA was further evaluated for testing the hypothesis that down-regulation of this miRNA might be a mechanism for resistance to radiation. To prove this hypothesis, miR-449a was over-expressed in CL1-0 cells which resulted in increased apoptosis leading to sensitization of cells to radiation, thus confirming a role of miR-449a in radiosensitization of lung cancer cells. Wang et al. [20] observed increased levels of miR-21 in NSCLC samples which was correlated with increased metastasis and poor prognosis. With the down-regulation of miR-21, sensitization to radiation was observed and there was clear evidence of increased degree of apoptosis. These observations connected miR-21 as a molecular determinant of sensitivity of lung cancer cells to radiation therapy.

The process of EMT has been linked to resistance to chemotherapy as discussed earlier. In the sections above, we have discussed the role of EMT-influencing let-7 family of miRNAs in determining resistance to chemotherapy. We pointed out that let-7 miRNAs, being negative regulators of EMT, are down-regulated in resistant cells. Another family of miRNAs which functions similarly to negatively regulate EMT is the miR-200 family. In a study [21] that tried to connect miR-200c with resistance to radiation therapy, it was observed that ectopic miR-200c over-expression resulted in sensitization of lung cancer cells (A549 cells) to radiation. Over-expression of miR-200c would normally result in the reversal of EMT and can explain the reversal of resistance to therapy. This work also evaluated VEGF-VEGFR2 pathway as a target of miR-200c. Down-regulation of miR-200c in resistant cells abrogated the repression of pro-oncogenic VEGFR2 and activated pro-survival and angiogenic mechanisms.

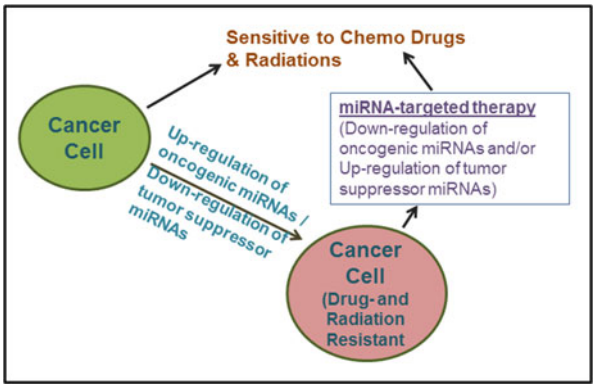

Fig. 5.1 miRNAs in Drug and Radiation Resistance. Oncogenic miRNAs are up-regulated while tumor suppressor miRNAs are frequently down-regulated leading to resistance against chemotherapeutic drugs and/or radiations. Emerging evidence has suggested that a miRNA-based therapy that involves reversal of such miRNA events can reverse resistance against drugs and radiations leading to re-sensitization of cancer cells

In the last few decades, there has been a realization that non-toxic natural agents, the 'nutraceuticals', can be potent anticancer agents by virtue of their pleiotropic roles, including their ability to modulate resistance to radiation therapy [22]. Such activity of flavonoid compounds rhamnetin and cirsiliol was recently described in NSCLC cells H1299 and H460 [23]. It was determined that the resistance of NSCLC cells to radiation involved up-regulation of Notch signaling. Since rhamnetin and cirsiliol could down-regulate Notch-1, it was suggested that such inhibition of Notch-1 by these natural compounds could result in re-sensitization of cells to radiation. This was found to be true and the underlying mechanism was found to be an up-regulation of miR-34a. Notch-1 is a target of miR-34a and up-regulation of miR-34a resulted in the down-regulation of Notch-1, resulting in the reversal of resistance to radiation therapy.

Based on the reports discussed so far, it is evident that miRNAs play interesting roles in determining resistance to therapies (Fig. 5.1). These effects are summarized in Table 5.1. It is interesting to note that many studies describe the same cell type, for example, A549 NSCLC cells that have been made resistant to a specific chemotherapeutic drug. In spite of working with essentially the same model system, individual reports have mostly implicated a novel miRNA for the observed effect, which suggests the complexity with miRNAs research. It appears that multiple miRNAs might be involved in the regulation of similar patho-physiological responses, and thus more detailed studies will be needed to better understand these regulatory mechanisms.

Table 5.1 miRNAs involved in regulation of resistance to chemotherapy/radiation therapy

miRNA	Expression	Effect	References
miR-21	High	Radioresistance	[20]
miR-31	High	Cisplatin Resistance	[12]
miR-34a	Low	Radioresistance	[23]
miR-92b	High	Cisplatin Resistance	[24]
miR-98	Down	Cisplatin Resistance	[10]
miR-134	High	EGFR-TKI Resistance	[14]
miR-135a/b	Down	Cisplatin Resistance	[9]
miR-200b	Down	EGFR-TKI Resistance	[15]
miR-200c	Down	Radioresistance	[21]
miR-210	High	Radioresistance	[18]
miR-449a	Down	Radioresistance	[19]
miR-487b	High	EGFR-TKI Resistance	[14]
miR-495	Down	Cisplatin Resistance	[11]
miR-503	Down	Cisplatin Resistance	[6, 7]
miR-511	Down	Radioresistance	[17]
Let-7c	Down	Cisplatin Resistance	[5]
Let-7c	Down	Docetaxel Resistance	[13]
Let-7c	Down	Radioresistance	[13]
Let-7c	Down	EGFR-TKI Resistance	[15]

3 miRNA in Diagnosis and Prognosis of Lung Cancer

The miRNAs have largely been studied for their therapeutic potential. In addition to possible use of miRNAs in the therapy of lung cancer, their use as diagnostic, prognostic and predictive biomarkers has also been advocated [24–29]. For example, miR-34c was found to be inversely correlated with histology and has been suggested as a quantitative biomarker which parallels histologic response in formalin-fixed biopsies [30]. The miRNAs can also distinguish between current and former smokers, and miR-375 has been found to be up-regulated in the current smokers [30]. The advantage of using miRNAs for prediction, diagnosis and prognosis is that this approach is non-invasive. Many cancers are now believed to have a "miRNA fingerprint", which, in simple terms, refers to the expression pattern (up-regulation/down-regulation) of certain specific miRNAs in the biological fluids within the physiological systems which can be exploited for diagnosis and/or prognosis in the clinical setting. In addition to the traditional screening of blood/serum/plasma/tissue specimens described in the following subsections, it is worth mentioning that recent literature has pointed to a possible use of exosome-derived miRNAs as biomarkers of lung cancer diagnosis [31].

3.1 miRNAs in Serum/Plasma/Circulation

Most of the procedures in practice for the detection of human cancers, including lung cancer, are invasive. However, recent work with miRNAs has opened up the

possibility of using these tiny molecules as non-invasive diagnostic tools for reliable detection of lung cancer. Tang et al. explored three miRNAs – miR-21, miR-145 and miR-155 for their possible role in predicting onset of lung cancer [32]. The expression levels of these miRNAs were evaluated in 62 lung cancer patients and 60 smokers. Of the three miRNAs, plasma levels of miR-145 were down-regulated while those of miR-21 and miR-155 were up-regulated in lung cancer patients, compared to the healthy smokers. In a study in Egyptian population [33] which looked at serum levels of some of these miRNAs and a few additional miRNAs (miR-21, miR-155, miR-182 and miR-197), the findings on miR-21 and miR-155 were confirmed i.e. these miRNAs were found to be expressed at higher levels in lung cancer patients' sera. Additionally, miR-182 and miR-197 levels were also found to be high in lung cancer patients [33]. The prognostic value of miR-21 and miR-155 has been confirmed in a meta-analysis by Wang et al. [34]. This meta-analysis of 19 studies concluded that high levels of miR-21 and miR-155 are indeed reliable prognostic biomarkers for NSCLC progression as well as increased risk of lymphoid cells infiltration.

The recovery of miRNAs from circulation has opened a new field with the possibility of the use of blood-based miRNAs in diagnosis and/or assessing progression of lung cancer [35, 36]. In one such study [37], expression of 12 plasma miRNAs, miR-20a-5p, miR-24-3p, miR-25-3p, miR-126-3p, miR-145-5p, miR-152-3p, miR-155-5p, miR-191-5p, miR-223-3p, miR-199a-5p, miR-296-5p, and let-7f-5p allowed significant discrimination between controls and NSCLC patients with an accuracy of 82.1 %. A six-plasma miRNA panel was found to distinguish between NSCLC patients and chronic obstructive pulmonary disease patients. A three-miRNA plasma signature was found to be significantly associated with a higher risk for progression in adenocarcinoma patients and a separate three-miRNA plasma panel significantly predicted survival of squamous cell carcinoma patients. In a study [38] that aimed at identifying diagnostically relevant miRNAs which can distinguish between benign pleural effusion and lung carcinoma-associated malignant pleural effusion, miR-198 was reported to be significantly down-regulated in lung carcinoma-associated malignant pleural effusions.

Clinical management of patients with lung cancer offers a lot of challenges, one of which is the metastatic and recurrent disease. It is desirable that the physicians have an access to a 'molecular signature' which can predict the high vs low risk of disease recurrence [39]. A number of molecular factors have been evaluated for their ability to predict the recurrence of lung cancer and in recent years miRNAs have been the target of many such investigations. One such study reported high levels of circulating miR-142-3p and miR-29b in serum of patients with early relapse of lung adenocarcinoma [40]. This study compared patients with and without recurrence 24 months post-surgical intervention. With the identification of these two miRNAs at high levels in the serum of patients with recurrence, it might be possible for the physicians to put a patient at high risk of cancer relapse incase the levels of these miRNAs are found to be elevated in regular follow-up studies after surgery. In a report by Markou et al. [41], worst disease free interval was correlated with low tissue levels of miR-10a while high expression of miR-30e-

5p was correlated with shortened overall survival. There is evidence supporting an influence of single-nucleotide polymorphism within miRNA processing on the prognosis of lung cancer patients [42, 43]. Such genetic variations can lead to altered regulation of target genes by miRNAs although this field need more in-depth investigations.

3.2 miRNAs in Tissue Specimens

Formalin-fixed paraffin-embedded tissue specimens are invaluable sources for investigation. Lin et al. found high expression of miR-19a in NSCLC tissues, compared to non-malignant cancerous tissues [44]. These high levels were also confirmed in serum of the patients. High miR-19a levels were correlated with TNM stage, increased metastases and poor survival. In another study aimed at identifying miRNA that might be involved in lymph node metastasis, miR-31 was found to be up-regulated in lung cancer tissue, compared to normal tissue [45]. This miRNA was distinctly up-regulated in patients with lymph node metastases, compared to those without lymph node metastases. It was no surprise that reduced expression of miR-31 was found to be correlated with excellent survival. In a very similar study [46], miR-9 was found to be up-regulated in lung cancer tissues, compared to adjacent normal tissues. Furthermore, up-regulation of miR-9 was correlated with advanced stage tumors and lymph node metastases. The miR-92b is another miRNA that is expressed at higher levels in lung cancer tissues, compared to matched adjacent normal tissues [47]. Through the regulation of its well-known tumor suppressor target PTEN, miR-92b was shown to regulate response to cisplatin. High levels of miR-92b and concomitant low PTEN levels resulted in cisplatin resistance.

Epigenetic events can also help predict prognosis. As an example, hypermethylation of miR-886 promoter leading to the loss of miR-886-3p expression has been linked to poor outcome prediction in small cell lung cancer [48]. Similarly, hypermethylation of miR-148a coding region resulting in the reduced expression of this miRNA has been linked to poor prognosis of NSCLC with increased metastasis, shortened disease-free survival and reduced overall survival [49].

3.3 miRNAs in Sputum

Shen et al. [50] performed an analysis for the possible use of miRNAs as biomarkers in the sputum. The analyses were done using qRT-PCR in 64 lung cancer patients and 73 cancer-free smokers. Of the short-listed 12 miRNAs, miR-31 and miR-210 were found to be the best predictors of lung cancer. When combined, these two miRNAs resulted in 65.2 % sensitivity and 89.7 % specificity. When combined

Table 5.2 miRNAs implicated in diagnosis and prognosis of lung cancers

miRNA	Source	Diagnosis/ prognosis	Observation	References
miR-9	Tissue	Prognosis	High levels correlate with poor prognosis	[47]
miR-10a	Tissue	Prognosis	Low levels correlate with worst disease free interval	[42]
miR-19a	Serum, Tissue	Prognosis	High levels correlate with poor survival	[45]
miR-21	Plasma, Serum	Diagnosis	High in lung cancer patients	[33–35]
miR-22	Blood	Prognosis	High levels in patients with progressive disease	[16]
miR-29b	Serum	Prognosis	High in recurrent disease	[41]
miR-30e-5p	Tissue	Prognosis	High levels correlate with poor overall survival	[42]
miR-31	Tissue	Prognosis	High in lymph node metastases	[46]
miR-142-3p	Serum	Prognosis	High in recurrent disease	[41]
miR-145	Plasma	Diagnosis	Low in lung cancer patients	[33]
miR-148a	Tissue	Prognosis	Low levels correlate with poor survival	[49]
miR-155	Plasma, Serum	Diagnosis	High in lung cancer patients	[33–35]
miR-182	Serum	Diagnosis	High in lung cancer patients	[34]
miR-197	Serum	Diagnosis	High in lung cancer patients	[34]
miR-198	Cell-free circulation	Diagnosis	Low in malignant pleural effusion	[39]
miR-886	Tissue	Prognosis	Low levels associated with poor survival	[48]

with the standard diagnostic tool – computed tomography, these two miRNAs increased the sensitivity of computed tomography.

The literature discussed in this section outlined the remarkable progress with regards to the use of miRNAs as biomarkers for prediction, diagnosis and prognosis. The information is summarized in Table 5.2. This information suggests that miRNAs can be invaluable tool for monitoring the onset and progression of lung cancer. While this information is encouraging, it is to be noted that all this information has emerged within the last 1 year and novel information is trickling in very fast. It might be some time before a complete picture emerges and when it does many of these important miRNAs will become the target for lung cancer therapy.

4 Targeted Delivery of miRNAs – Challenges and Progress

The discussion so far attests to the fact that miRNAs have great potential as regulators of cellular signaling affecting key physiological functions that determine cancer cells' behavior such as drug resistance, metastasis and tumor aggressiveness.

Therefore, targeting of miRNAs for therapeutic purposes sounds a reasonable approach moving forward although the proof-of-concept has been documented in pre-clinical setting. However, accomplishing such targeting *in vivo* is very challenging. One of the first challenges associated with *in vivo* injecting of miRNAs is that these tiny molecules are highly unstable or not bioavailable especially in circulation for executing their function. They are degraded very rapidly in the systemic circulation and it is highly unlikely they can reach their target tumor cells under normal conditions which is in part due to their binding to large molecular weight proteins. Another challenge is that they are negatively charged which leads to a poor uptake by cancer cells. Therefore, to target miRNAs to their intended targets, the very first step is to afford them protection. In a study performed in breast cancer model [51], we achieved an *in vivo* effect of miRNA through the use of locked nucleic acid (LNA)-modified oligonucleotide (miR-200b in this study) that was delivered intravenously to mice three times a week for a total of 5 weeks. Such persistent dosing was suitable to observe the effects of miRNA regulation on its target genes and the phenotype *in vivo* in an experimental assay of pulmonary metastases. Prior to our study, Chen et al. [52], reported a liposome-polycation-hyaluronic acid nanoparticle formulation which was modified with tumor-targeting single-chain antibody fragment. This was used for systemic delivery of not only miRNAs but also siRNA. Similar to our study where we studied experimental pulmonary metastases of breast cancer cells [51], this study also looked into pulmonary metastasis where the tumor was induced by murine B16F10 melanoma cells.

Not much progress has been made in the targeted delivery of miRNAs but there are some preliminary reports which are encouraging. As discussed above, let-7 miRNA is a promising target for therapy of lung cancer. It is involved in EMT and the drug resistance phenotype. Also, since it is a tumor suppressor miRNA and it is usually down-regulated in metastatic, drug resistant cancers, its delivery to tumor cells *in vivo* can possibly be a good strategy to inhibit metastasis and/or sensitize drug resistant cells to conventional therapeutics. Recently, let-7a was packaged in a novel liposomal preparation – DOTAP (N-(1-(2.3-dioleoyloxy)propyl)-N,N, N-trimethyl ammonium)/Cholesterol/DSPE (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-(cyanur(polyethylene glycol) – 2000)) – PEG (polyethylene glycol)-cyanur liposomal nanoparticles (LNP) [53]. DOTAP and DSPE-PEG are cationic lipids that contain cyanuric groups. The ligand of EphA2, ephrin A1, was conjugated on surface of liposomal nanoparticles to specifically target lung cancer cells that express EphA2 receptor. Such transfections in lung cancer cells were found to be very effective and the expected effects on cellular functions and expression of target genes were observed. This study only looked at lung cancer cells-based assays. The next important step would be to demonstrate the efficacy of formulation *in vivo*. Another important tool that can help translation studies is the development of more appropriate *in vivo* model systems. Towards this end, a metastatic fluorescent Lewis lung carcinoma mouse model has been described [54] that can potentially help identify/screen novel miRNAs involved in malignant progression.

5 Conclusions

The research on miRNAs in lung cancer has come a long way in recent years, more so in the past 1 year that we have discussed here. A lot of information is rapidly emerging which will take some time for more robust analysis and independent verifications. Meanwhile, the treatment of lung cancer in the clinical setting is steadily moving towards using approaches that are much more 'individualized'. This approach is based on the realization that each and every tumor presented in the clinical setting has its individuality. While there are major classes for grouping most cancers, including lung cancers, it is also evident that every single tumor also has its unique characteristics even though a tumor mass is composed of highly heterogeneous population of cancer cells. This heterogeneity is in part responsible for treatment failure using conventional therapeutics including targeted therapy. Personalized treatment of human lung cancers is an emerging field and it is envisioned that miRNAs have their own unique niche in such personalized therapy for the management of lung cancer patients [55]. On a similar note, understanding the epigenetic changes that accompany the onset of carcinogenesis is crucial [56]. In recent years, a number of agents that can target epigenetic changes have been investigated in translation studies. It is interesting to note an important role that miRNAs are increasingly being realized to play in the epigenetic regulation of lung cancer [48, 57–60]. Based on these evidences, it appears that miRNAs hold a lot of promise in advancing the research that focuses on finding a cure for lung cancer.

References

1. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer Statistics, 2014. CA Cancer J Clin 64:9–29
2. Kumar MS, Armenteros-Monterroso E, East P, Chakravorty P, Matthews N, Winslow MM, Downward J (2014) HMGA2 functions as a competing endogenous RNA to promote lung cancer progression. Nature 505:212–217
3. Tang J, Salama R, Gadgeel SM, Sarkar FH, Ahmad A (2013) Erlotinib resistance in lung cancer: current progress and future perspectives. Front Pharmacol 4:15
4. Maftouh M, Avan A, Galvani E, Peters GJ, Giovannetti E (2013) Molecular mechanisms underlying the role of microRNAs in resistance to epidermal growth factor receptor-targeted agents and novel therapeutic strategies for treatment of non-small-cell lung cancer. Criti Rev Oncog 18:317–326
5. Zhan M, Qu Q, Wang G, Zhou H (2013) Let-7c sensitizes acquired cisplatin-resistant A549 cells by targeting ABCC2 and Bcl-XL. Pharmazie 68:955–961
6. Wu Y, Guo L, Liu J, Liu R, Liu M, Chen J (2014) The reversing and molecular mechanisms of miR-503 on the drug-resistance to cisplatin in A549/DDP Cells. Zhongguo fei ai za zhi = Chin J Lung Cancer 17:1–7
7. Qiu T, Zhou L, Wang T, Xu J, Wang J, Chen W, Zhou X, Huang Z, Zhu W, Shu Y, Liu P (2013) miR-503 regulates the resistance of non-small cell lung cancer cells to cisplatin by targeting Bcl-2. Int J Mol Med 32:593–598

8. Yin J, Wang M, Jin C, Qi Q (2014) miR-101 sensitizes A549 NSCLC cell line to CDDP by activating caspase 3-dependent apoptosis. Oncol Lett 7:461–465
9. Zhou L, Qiu T, Xu J, Wang T, Wang J, Zhou X, Huang Z, Zhu W, Shu Y, Liu P (2013) miR-135a/b modulate cisplatin resistance of human lung cancer cell line by targeting MCL1. Pathol Oncol Res POR 19:677–683
10. Xiang Q, Tang H, Yu J, Yin J, Yang X, Lei X (2013) MicroRNA-98 sensitizes cisplatin-resistant human lung adenocarcinoma cells by up-regulation of HMGA2. Pharmazie 68:274–281
11. Song L, Li Y, Li W, Wu S, Li Z (2013) MiR-495 enhances the sensitivity of non-small cell lung cancer cells to platinum by modulation of copper-transporting P-type adenosine triphosphatase A (ATP7A). J Cell Biochem. doi:10.1002/jcb.24665
12. Dong Z, Zhong Z, Yang L, Wang S, Gong Z (2014) MicroRNA-31 inhibits cisplatin-induced apoptosis in non-small cell lung cancer cells by regulating the drug transporter ABCB9. Cancer Lett 343:249–257
13. Cui SY, Huang JY, Chen YT, Song HZ, Feng B, Huang GC, Wang R, Chen LB, De W (2013) Let-7c governs the acquisition of chemo- or radioresistance and epithelial-to-mesenchymal transition phenotypes in docetaxel-resistant lung adenocarcinoma. Mol Cancer Res MCR 11:699–713
14. Kitamura K, Seike M, Okano T, Matsuda K, Miyanaga A, Mizutani H, Noro R, Minegishi Y, Kubota K, Gemma A (2014) MiR-134/487b/655 cluster regulates TGF-beta-induced epithelial-mesenchymal transition and drug resistance to gefitinib by targeting MAGI2 in lung adenocarcinoma cells. Mol Cancer Ther 13:444–453
15. Ahmad A, Maitah MY, Ginnebaugh KR, Li Y, Bao B, Gadgeel SM, Sarkar FH (2013) Inhibition of Hedgehog signaling sensitizes NSCLC cells to standard therapies through modulation of EMT-regulating miRNAs. J Hematol Oncol 6:77
16. Franchina T, Amodeo V, Bronte G, Savio G, Ricciardi GR, Picciotto M, Russo A, Giordano A, Adamo V (2014) 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 229:97–99
17. Zhang HH, Pang M, Dong W, Xin JX, Li YJ, Zhang ZC, Yu L, Wang PY, Li BS, Xie SY (2014) miR-511 induces the apoptosis of radioresistant lung adenocarcinoma cells by triggering BAX. Oncol Rep 31:1473–1479
18. Grosso S, Doyen J, Parks SK, Bertero T, Paye A, Cardinaud B, Gounon P, Lacas-Gervais S, Noel A, Pouyssegur J, Barbry P, Mazure NM, Mari B (2013) MiR-210 promotes a hypoxic phenotype and increases radioresistance in human lung cancer cell lines. Cell Death Dis 4:e544
19. Liu YJ, Lin YF, Chen YF, Luo EC, Sher YP, Tsai MH, Chuang EY, Lai LC (2013) MicroRNA-449a enhances radiosensitivity in CL1-0 lung adenocarcinoma cells. PLoS One 8:e62383
20. Wang XC, Wang W, Zhang ZB, Zhao J, Tan XG, Luo JC (2013) Overexpression of miRNA-21 promotes radiation-resistance of non-small cell lung cancer. Radiat Oncol (London, England) 8:146
21. Shi L, Zhang S, Wu H, Zhang L, Dai X, Hu J, Xue J, Liu T, Liang Y, Wu G (2013) MiR-200c Increases the Radiosensitivity of Non-Small-Cell Lung Cancer Cell Line A549 by Targeting VEGF-VEGFR2 Pathway. PLoS One 8:e78344
22. Ahmad A, Biersack B, Li Y, Bao B, Kong D, Ali S, Banerjee S, Sarkar FH (2013) Perspectives on the role of isoflavones in prostate cancer. AAPS J 15:991–1000
23. Kang J, Kim E, Kim W, Seong KM, Youn H, Kim JW, Kim J, Youn B (2013) Rhamnetin and cirsiliol induce radiosensitization and inhibition of epithelial-mesenchymal transition (EMT) by miR-34a-mediated suppression of Notch-1 expression in non-small cell lung cancer cell lines. J Biol Chem 288:27343–27357
24. Vannini I, Fanini F, Fabbri M (2013) MicroRNAs as lung cancer biomarkers and key players in lung carcinogenesis. Clin Biochem 46:918–925

25. Wang Y, Zhang X, Liu L, Li H, Yu J, Wang C, Ren X (2013) Clinical Implication of MicroRNA for Lung Cancer. Cancer Biother Radiopharm 28:261–267
26. Rani S, Gately K, Crown J, O'Byrne K, O'Driscoll L (2013) Global analysis of serum microRNAs as potential biomarkers for lung adenocarcinoma. Cancer Biol Ther 14
27. Huang Y, Hu Q, Deng Z, Hang Y, Wang J, Wang K (2013) MicroRNAs in body fluids as biomarkers for non-small cell lung cancer: a systematic review. Technol Cancer Res Treat. doi:10.7785/tcrt.2012.500377
28. Mozzoni P, Banda I, Goldoni M, Corradi M, Tiseo M, Acampa O, Balestra V, Ampollini L, Casalini A, Carbognani P, Mutti A (2013) Plasma and EBC microRNAs as early biomarkers of non-small-cell lung cancer. Biomarkers 18:679–686
29. Zhang H, Su Y, Xu F, Kong J, Yu H, Qian B (2013) Circulating MicroRNAs in relation to EGFR status and survival of lung adenocarcinoma in female non-smokers. PLoS One 8: e81408
30. Mascaux C, Feser WJ, Lewis MT, Baron AE, Coldren CD, Merrick DT, Kennedy TC, Eckelberger JI, Rozeboom LM, Franklin WA, Minna JD, Bunn PA, Miller YE, Keith RL, Hirsch FR (2013) Endobronchial miRNAs as biomarkers in lung cancer chemoprevention. Cancer Prev Res (Phila) 6:100–108
31. Cazzoli R, Buttitta F, Di Nicola M, Malatesta S, Marchetti A, Rom WN, Pass HI (2013) MicroRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. J Thorac Oncol 8:1156–1162
32. Tang D, Shen Y, Wang M, Yang R, Wang Z, Sui A, Jiao W, Wang Y (2013) Identification of plasma microRNAs as novel noninvasive biomarkers for early detection of lung cancer. Eur J Cancer Prev 22:540–548
33. Abd-El-Fattah AA, Sadik NA, Shaker OG, Aboulftouh ML (2013) Differential microRNAs expression in serum of patients with lung cancer, pulmonary tuberculosis, and pneumonia. Cell Biochem Biophys 67:875–884
34. Wang Y, Li J, Tong L, Zhang J, Zhai A, Xu K, Wei L, Chu M (2013) The prognostic value of miR-21 and miR-155 in non-small-cell lung cancer: a meta-analysis. Jpn J Clin Oncol 43:813–820
35. Zandberga E, Kozirovskis V, Abols A, Andrejeva D, Purkalne G, Line A (2013) Cell-free microRNAs as diagnostic, prognostic, and predictive biomarkers for lung cancer. Genes Chromosomes Cancer 52:356–369
36. Ramshankar V, Krishnamurthy A (2013) Lung cancer detection by screening – presenting circulating miRNAs as a promising next generation biomarker breakthrough. Asian Pac J Cancer Prev 14:2167–2172
37. Sanfiorenzo C, Ilie MI, Belaid A, Barlesi F, Mouroux J, Marquette CH, Brest P, Hofman P (2013) Two panels of plasma microRNAs as non-invasive biomarkers for prediction of recurrence in resectable NSCLC. PLoS One 8:e54596
38. Han HS, Yun J, Lim SN, Han JH, Lee KH, Kim ST, Kang MH, Son SM, Lee YM, Choi SY, Yun SJ, Kim WJ, Lee OJ (2013) Downregulation of cell-free miR-198 as a diagnostic biomarker for lung adenocarcinoma-associated malignant pleural effusion. Int J Cancer 133:645–652
39. Ahmad A (2013) Pathways to breast cancer recurrence. ISRN Oncol 2013:290568
40. Kaduthanam S, Gade S, Meister M, Brase JC, Johannes M, Dienemann H, Warth A, Schnabel PA, Herth FJ, Sultmann H, Muley T, Kuner R (2013) Serum miR-142-3p is associated with early relapse in operable lung adenocarcinoma patients. Lung Cancer 80:223–227
41. Markou A, Sourvinou I, Vorkas PA, Yousef GM, Lianidou E (2013) Clinical evaluation of microRNA expression profiling in non small cell lung cancer. Lung Cancer 81:388–396
42. Pu X, Roth JA, Hildebrandt MA, Ye Y, Wei H, Minna JD, Lippman SM, Wu X (2013) MicroRNA-related genetic variants associated with clinical outcomes in early-stage non-small cell lung cancer patients. Cancer Res 73:1867–1875
43. Cheng M, Yang L, Yang R, Yang X, Deng J, Yu B, Huang D, Zhang S, Wang H, Qiu F, Zhou Y, Lu J (2013) A microRNA-135a/b binding polymorphism in CD133 confers decreased

risk and favorable prognosis of lung cancer in Chinese by reducing CD133 expression. Carcinogenesis 34:2292–2299
44. Lin Q, Chen T, Lin Q, Lin G, Lin J, Chen G, Guo L (2013) Serum miR-19a expression correlates with worse prognosis of patients with non-small cell lung cancer. J Surg Oncol 107:767–771
45. Meng W, Ye Z, Cui R, Perry J, Dedousi-Huebner V, Huebner A, Wang Y, Li B, Volinia S, Nakanishi H, Kim T, Suh SS, Ayers LW, Ross P, Croce CM, Chakravarti A, Jin VX, Lautenschlaeger T (2013) MicroRNA-31 predicts the presence of lymph node metastases and survival in patients with lung adenocarcinoma. Clin Cancer Res 19:5423–5433
46. Xu T, Liu X, Han L, Shen H, Liu L, Shu Y (2013) Up-regulation of miR-9 expression as a poor prognostic biomarker in patients with non-small cell lung cancer. Clin Transl Oncol (in press). doi:10.1007/s12094-013-1106-1
47. Li Y, Li L, Guan Y, Liu X, Meng Q, Guo Q (2013) MiR-92b regulates the cell growth, cisplatin chemosensitivity of A549 non small cell lung cancer cell line and target PTEN. Biochem Biophys Res Commun 440:604–610
48. Cao J, Song Y, Bi N, Shen J, Liu W, Fan J, Sun G, Tong T, He J, Shi Y, Zhang X, Lu N, He Y, Zhang H, Ma K, Luo X, Lv L, Deng H, Cheng J, Zhu J, Wang L, Zhan Q (2013) DNA methylation-mediated repression of miR-886-3p predicts poor outcome of human small cell lung cancer. Cancer Res 73:3326–3335
49. Chen Y, Min L, Zhang X, Hu S, Wang B, Liu W, Wang R, Gu X, Shen W, Lv H, Zou J, Chen Y, Xu X, Chen L (2013) Decreased miRNA-148a is associated with lymph node metastasis and poor clinical outcomes and functions as a suppressor of tumor metastasis in non-small cell lung cancer. Oncol Rep 30:1832–1840
50. Shen J, Liao J, Guarnera MA, Fang H, Cai L, Stass SA, Jiang F (2014) Analysis of MicroRNAs in Sputum to Improve Computed Tomography for Lung Cancer Diagnosis. J Thor Oncol 9:33–40
51. Ahmad A, Aboukameel A, Kong D, Wang Z, Sethi S, Chen W, Sarkar FH, Raz A (2011) Phosphoglucose isomerase/autocrine motility factor mediates epithelial-mesenchymal transition regulated by miR-200 in breast cancer cells. Cancer Res 71:3400–3409
52. Chen Y, Zhu X, Zhang X, Liu B, Huang L (2010) Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. Mol Ther 18:1650–1656
53. Lee HY, Mohammed KA, Kaye F, Sharma P, Moudgil BM, Clapp WL, Nasreen N (2013) Targeted delivery of let-7a microRNA encapsulated ephrin-A1 conjugated liposomal nanoparticles inhibit tumor growth in lung cancer. Int J Nanomed 8:4481–4494
54. Rask L, Fregil M, Hogdall E, Mitchelmore C, Eriksen J (2013) Development of a metastatic fluorescent Lewis lung carcinoma mouse model: identification of mRNAs and microRNAs involved in tumor invasion. Gene 517:72–81
55. Tufman A, Tian F, Huber RM (2013) Can MicroRNAs improve the management of lung cancer patients? A clinician's perspective. Theranostics 3:953–963
56. Ahmad A, Li Y, Bao B, Kong D, Sarkar FH (2014) Epigenetic regulation of miRNA-cancer stem cells nexus by nutraceuticals. Mol Nutr Food Res 58:79–86
57. Wang J, Yang B, Han L, Li X, Tao H, Zhang S, Hu Y (2013) Demethylation of miR-9-3 and miR-193a Genes Suppresses Proliferation and Promotes Apoptosis in Non-Small Cell Lung Cancer Cell Lines. Cell Physiol Biochem 32:1707–1719
58. Watanabe K, Takai D (2013) Disruption of the expression and function of microRNAs in lung cancer as a result of epigenetic changes. Front Genet 4:275
59. Lin CW, Chang YL, Chang YC, Lin JC, Chen CC, Pan SH, Wu CT, Chen HY, Yang SC, Hong TM, Yang PC (2013) MicroRNA-135b promotes lung cancer metastasis by regulating multiple targets in the Hippo pathway and LZTS1. Nat Commun 4:1877
60. Cai J, Fang L, Huang Y, Li R, Yuan J, Yang Y, Zhu X, Chen B, Wu J, Li M (2013) miR-205 targets PTEN and PHLPP2 to augment AKT signaling and drive malignant phenotypes in non-small cell lung cancer. Cancer Res 73:5402–5415