Chapter 17
Challenges and Strategies for Pulmonary Delivery of MicroRNA-Based Therapeutics

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Contents

Abstract Therapeutic options for various lung diseases, especially lung cancer, continue to expand with the development of novel therapeutic strategies. RNA interference (RNAi)-based approaches provide a promising modality for the treatment of lung diseases. One of the greatest challenges in RNAi-based therapy continues to be the method for delivering the therapeutic small interfering RNAs (siRNAs) and

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microRNAs (miRNAs) to the target cells. The advance of pulmonary delivery systems into the clinic illustrates the notion that RNAi will be a valuable modality for the treatment of lung diseases. Currently, the development of miRNA-based therapies for lung cancer is rapidly advancing with the aid of new RNAi technologies. Given the important role of miRNAs in lung carcinogenesis, increasing effort is being dedicated to the research and development of miRNA-based therapies, including the restoration of tumor suppressive miRNA function and the inhibition of oncogenic miRNAs. In this chapter, we discuss the advantages of a pulmonary drug delivery system and the strategies for miRNA-based treatment of lung cancer.

Keywords RNA interference • Small interfering RNA • MicroRNA • Lung cancer Pulmonary delivery

1 Introduction

Lung cancer is the leading cause of cancer mortality worldwide. Lung cancer can be classified into two main subtypes: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Numerous differences are found between these two subtypes, including histological type, biological behavior, prevalence, prognosis and response to therapy. NSCLC accounts for more than 80 % of all lung cancer cases. Only a small percentage of patients with NSCLC present with early stage disease. In this circumstance, surgery remains the best therapeutic option for these patients. Approximately 70 % of all newly diagnosed patients present with locally advanced or metastatic disease and require systemic chemotherapy (Ramalingam et al. 2011). However, the commonly administered chemotherapeutics provide little benefit for patients with advanced stage disease and has reached a plateau in efficacy with a median survival of 8–10 months. The poor prognosis is due to late stage disease presentation, tumor heterogeneity within histological subtypes, and our relatively limited understanding of tumor biology. Furthermore, the high frequency of drug resistance is a key contributor to the poor survival rates of lung cancer patients; improvements in survival rely on continued elucidation of the molecular mechanisms underlying lung cancer tumorigenesis and drug response. Acquiring knowledge through genomic medicine raises the possibility of unraveling the remaining mysteries of lung cancer oncogenesis and opens the door to molecular classification and risk stratification based on gene expression profiles and microRNA (miRNA) signatures.

MiRNAs are short (19–23 nucleotides in length) non-coding RNAs found in multiple organisms that regulate gene expression primarily by decreasing the levels of their target mRNAs, through binding to specific target sites in the 3′ untranslated regions (3′UTRs) of these mRNAs (Winter et al. 2009). In the human genome, transcripts of approximately 60 % of all mRNAs are estimated to be targeted by miRNAs. Accumulating evidence shows that miRNAs are grossly dysregulated in human cancers, including NSCLC, and may serve as oncogenes

or tumor suppressors (Croce 2009; Babashah and Soleimani 2011). Recent studies have not only shown that miRNAs are useful in lung cancer diagnosis but that specific miRNA profiles may also predict prognosis, drug response and disease recurrence (Yanaihara et al. 2006; Yu et al. 2008). These findings suggest that miRNAs are a promising technology for therapeutic development. In fact, given the significant role of miRNAs in multiple pathways governing lung carcinogenesis, increasing efforts are dedicated to the research and development of miRNA-based therapies, including the restoration of tumor suppressive miRNA function and the inhibition of oncogenic miRNAs (Bader et al. 2010).

The critical problems impeding the development of RNAi-based therapeutics are effective delivery to target sites, therapeutic potency, and elimination of off-target effects (Boudreau et al. 2009). The success of miRNA-based therapeutic delivery is also dependent upon uncovering a delivery route that yields efficient outcomes, is convenient, and promotes patient compliance. For this reason, direct administration of miRNA-based therapeutics to target organs is a promising approach to overcome the problems of systemic administration. Pulmonary delivery offers a new method for the treatment of various lung diseases (Fujita et al. 2013). We believe that delivery of miRNA-based therapeutics using this approach will potentially be useful in clinical practice. Here, we provide an overview of miRNAs as therapeutic targets in lung cancer and discuss the promise and limitations of pulmonary delivery strategies for miRNA-based therapeutics.

2 Role of MicroRNAs in Lung Cancer

Lung cancer biology has traditionally focused on genomic and epigenomic deregulation of protein-coding genes to identify oncogenes and tumor suppressors that are useful as diagnostic and therapeutic targets. Recently, miRNAs were also shown to up-regulate target gene expression by either directly binding to the target mRNA or indirectly repressing nonsense-mediated RNA decay (Vasudevan et al. 2007; Bruno et al. 2011). MiRNAs play an essential role in various cellular processes, such as development, proliferation and apoptosis, to ensure the cellular homeostasis of human cells. Alterations in miRNA expression are increasingly noted in relation to pathophysiological changes in cancer cells, thereby making miRNAs one of the most currently analyzed molecule types in cancer research. Numerous miRNAs are dysregulated in lung cancers, and a single miRNA can have multiple targets that are involved in different oncogenic pathways. A large body of evidence reveals that the aberrant expression of miRNAs in cancer patients can be taken advantage of innumerous ways, such as for potential use as diagnostic, clinicopathological, and/or prognostic markers and as promising therapeutic targets in lung cancer. Aberrant miRNA expression profiles provide additional insight into the clinical application of miRNA-directed therapies in lung cancer (Leidinger et al. 2011). Here, we focus on reviewing the known roles of miRNAs as regulators of cancer cell survival, drug sensitivity and tumorigenesis. These miRNAs hold great potential as targets in the treatment of lung cancer.

2.1 MiRNAs Function as Oncogenes in Lung Cancer

Many oncogenes important in controlling lung cancer tumorigenesis are targets of miRNAs. The miRNAs found in the miR-17-92 cluster (miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a, miR-92-1) are oncogenic miRNAs (oncomiRs) that reside in the amplified chromosomal region 13q31.3 (He et al. 2005). These miRNAs cooperate with c-Myc to accelerate tumor development and promote tumor angiogenesis (Dews et al. 2006). It has been reported that the miR-17-92 cluster is over-expressed in SCLC (Hayashita et al. 2005). Moreover, Ebi et al. reported that miR-17-92 over-expression is associated with retinoblastoma (RB) inactivation (Ebi et al. 2009). Collectively, these results suggest that this miRNA cluster may be a potential therapeutic target in lung cancer.

The miR-21 gene is located on chromosome 17 and was one of the first miRNAs characterized as oncogenic, with its oncogenic function established in various types of cancers (Chan et al. 2005). MiR-21 has been suggested to be an independent negative prognostic factor for the overall survival of NSCLC patients (Markou et al. 2008). MiR-21 targets tumor suppressor genes such as programmed cell death 4 (PDCD4) and phosphatase and tensin homolog deleted from chromosome 10 (PTEN) (Lu et al. 2008; Zhang et al. 2010). Furthermore, miR-21 expression is up-regulated by epidermal growth factor receptor (EGFR) signaling in lung cancer. Antisense miR-21-enhanced EGFR tyrosine kinase inhibitors induce apoptosis of lung cancer cells (Seike et al. 2009). The critical function of miR-21 in regulating lung cancer tumorigenesis makes it a promising target for developing miRNA-based therapeutics and diagnostic tools. However, because miR-21 is also dysregulated in various type of cancer, it appears to be a general oncomiR without tissue specificity (Volinia et al. 2006).

MiR-31 is another miRNA with oncogenic properties in lung cancer. The host gene encoding miR-31 is located on chromosome 9. Liu et al. showed that miR-31 functions as an oncomiR by directly repressing large tumor suppressor 2 (LATS2) and Protein phosphatase 2, regulatory subunit B, Alpha isoform (PPP2R2A) and that knockdown of miR-31 represses lung cancer cell clonal growth and *in vivo* tumorigenicity (Liu et al. 2010).

2.2 MiRNAs Function as Tumor Suppressors in Lung Cancer

Among the numerous miRNAs that function as tumor suppressors, the let-7 family is one of the most studied. Let-7 was first identified in *C. elegans* as a regulator of the timing of cell fate determination (Reinhart et al. 2000). In humans, the let-7 family is a cluster of miRNAs whose encoding genes map to various chromosomal regions that are frequently deleted in lung cancer (Calin et al. 2004). Johnson et al. (2007) showed that let-7 over-expression in the A549 cell line inhibits cell growth and reduces cell-cycle progression. In mouse models of lung cancer, over-expression of let-7g reduces tumor growth (Kumar et al. 2008), and let-7a inhibits tumor growth via suppression of v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)

and c-Myc (He et al. 2010). Furthermore, reduced let-7 gene expression in NSCLC patients correlates with poor prognosis (Yanaihara et al. 2006; Takamizawa et al. 2004). The 3′ UTR of members of the RAS GTPase family such as v-Ha-ras Harvey rat sarcoma viral oncogene homolog (HRAS), KRAS and neuroblastoma RAS viral oncogene homolog (NRAS) contains multiple putative let-7 binding sites. It has also been revealed that let-7 miRNAs negatively regulate multiple oncogenes, including MYC (Kumar et al. 2007), high mobility group AT-hook 2 (HMGA2) (Lee and Dutta 2007), B-cell leukemia/lymphoma 2 (BCL-2) (Xiong et al. 2011) and cell cycle proto-oncogenes such as cell division cycle 25A (CDC25A), cyclin-dependent kinase 6 (CDK6) and cyclin D2 (Johnson et al. 2007). These data show that let-7 miRNAs act as key tumor suppressors in regulating cell survival and proliferation in lung cancers.

The miR-34 family is another important group of miRNAs that function as tumor suppressors in many types of cancers (Hermeking 2010; Wong et al. 2011). The miR-34a gene is located on chromosome 1p36.22, and miR-34b/c are expressed from a polycistronic transcript encoded on chromosome 11q23.1. These genes are in chromosomal regions associated with fragile sites of the genome that are frequently altered in cancer (Calin et al. 2004). Structurally, miR-34 family members possess p53-binding sites, reflecting their function as tumor suppressors downstream of the p53 pathway. MiR-34a and miR-34b/c were found to be directly regulated by p53 to control apoptosis and cell cycle arrest in cancer cell lines, including lung cancer (Raver-Shapira et al. 2007; Wiggins et al. 2010). Subsequent studies demonstrated that the apoptotic function of miR-34a is mediated by the direct down-regulation of the expression of BCL-2 and sirtuin 1 (SIRT1) (Yamakuchi et al. 2008; Bommer et al. 2007). In addition, AXL (Mudduluru et al. 2011) and SNAIL1 (Kim et al. 2011) were identified as miR-34 direct targets in lung cancer cells; it is plausible that miR-34 expression inhibits lung cancer cell invasion and migration via repression of these genes. In various solid and hematological malignancies, including lung cancer, miR-34 antagonizes processes necessary for basic cancer cell viability as well as cancer stemness, metastasis and chemoresistance (Bader 2012). In the future, the utility of miR-34-directed therapeutics in the treatment of lung cancer will expand dramatically.

The anti-tumor activity of miR-143 and miR-145 in lung cancer is also well characterized. They are co-transcribed from a bicistronic gene cluster on chromosome 5 (Xin et al. 2009). MiR-143/145 have been identified as tumor suppressor in various types of cancer, including lung cancer. The restoration of miR-145 has been shown to inhibit cell growth in mouse and human lung cancer cells (Liu et al. 2009; Cho et al. 2009). It has also been reported that c-MYC, EGFR and nucleoside diphosphate linked moiety X-type motif 1 (NUDT1) are direct targets of miR-145 that regulate cell proliferation in lung cancer (Chen et al. 2010; Cho et al. 2011). Furthermore, miR-145 has also been shown to inhibit lung adenocarcinoma stem-like cell proliferation by targeting octamer-binding transcription factor 4 (OCT4) (Feng et al. 2011). Similarly, the expression of miR-143 was down-regulated in human lung tumor samples compared with normal tissues (Gao et al. 2010; Vosa et al. 2013).

Finally, miR-192 also might serve as a promising therapeutic target for lung cancer treatment. Retinoblastoma 1 (RB1) is a direct target of miR-192, and

over-expression of miR-192 results in decreased expression of RB1 mRNA and protein. Caspase-7 and poly ADP-ribose polymerase (PARP) protein were activated by miR-192 over-expression, suggesting that miR-192 induces cell apoptosis through the caspase pathway. In addition, the analysis of miRNA expression in clinical samples has revealed that miR-192 is significantly down-regulated in lung cancer tissues compared with adjacent, normal lung tissues (Feng et al. 2011).

3 MicroRNA-Based Therapies for Lung Cancer

The development of miRNA-based therapeutics represents a new strategy in cancer treatment and is growing rapidly with the help of new RNAi technologies. Compared to siRNA-based therapies, which are already in clinical trials, miRNAs are less toxic and have the potential to target multiple genes. As presented above, miRNAs are generally classified as oncomiRs or tumor suppressors, with different therapeutic approaches developed for each class. Generally, the up-regulation of miRNA expression is achieved through administration of synthetic miRNA mimics or miRNA-expressing vectors. The down-regulation of miRNA expression is achieved through administration of antisense nucleotides, often chemically modified to ensure stability and specificity. Although each approach shares similarities with other therapies, each is sufficiently distinct such that miRNA-inhibitory and replacement approaches should be viewed as separate therapeutic modalities. In view of cancer as a heterogenic disease that cannot be successfully treated via single gene targeting, miRNA-based strategies may hold the key to therapeutic success. Table 17.1 shows a summary of miRNA-based therapeutic strategies for *in vivo* models of lung cancer.

3.1 MiRNA Inhibitor-Based Therapeutics

To reduce endogenous miRNA levels, anti-miRs are typically employed. Targeting miRNAs for suppression through the use of anti-miRs is possibly the best-studied modality to date. This approach is conceptually similar to other

Table 17.1 MicroRNA-based therapeutic strategies for *in vivo* models of lung cancer

MicroRNA	Administration	Modulation strategy	Delivery technology	Reference
let-7a	Intranasal	Replacement	Adenoviruses	Esquela-Kerscher et al. (2008)
let-7b	Systemic	Replacement	Neutral liposomes	Trang et al. (2011)
let-7g	Intratracheal	Replacement	Lentiviruses	Kumar et al. (2008)
miR-7	Intratumoral	Replacement	Cationic liposomes	Rai et al. (2011)
miR-29b	Systemic	Replacement	Cationic liposomes	Wu et al. (2013)
miR-34a	Intratumoral	Replacement	Neutral liposomes	Wiggins et al. (2010)
miR-145	Intratumoral	Replacement	Polyethyleneimines	Chiou et al. (2012)
miR-150	Intratumoral	Inhibition	Cationic liposomes	Li et al. (2012)

inhibitory therapeutics that target a single gene product, such as small molecule inhibitors and siRNAs. Various methods have been employed to render anti-miR constructs more stable *in vivo* and ensure adequate tissue availability and specificity (Krutzfeldt et al. 2005). Constructs can be modified with a cholesterol-conjugated 2′-*O*-methyl group to inhibit degradation and hence improve stability. Locked nucleic acid (LNA) is an additional method of antisense oligonucleotide modification whereby the 2′ oxygen and 4′ carbon of the nucleotide is bridged with methylene to form a cyclic structure. LNA is more resistant to endogenous nucleases, less toxic, and possess a stronger affinity for the target nucleotide (Elmen et al. 2008; Wahlestedt et al. 2000). Relative to studies on miRNA mimics, studies with antisense oligonucleotides have demonstrated greater efficacy using naked oligonucleotides. Furthermore, the LNA-anti-miR compound was well tolerated in both mice and primates, as no acute or subchronic toxicities in the treated animals were detected (Elmen et al. 2008). Recent data from the first Phase IIa study in patients with chronic HCV infection treated with the LNA-modified anti-miR-122 revealed that this compound was well tolerated and provided continuing viral suppression (Janssen et al. 2013). With regard to lung cancer, anti-miR-150 delivered to lung tumor xenografts in mice caused tumor growth inhibition (Li et al. 2012). Although there are few reports using LNA-anti-miR therapeutics in lung cancer mouse models, their inhibition of miRNA function is an important and widely used approach. Currently, miRNA sponges are a novel approach to miRNA inhibition, and this technology works with multiple complementary 3′-UTR mRNA sites of a specific miRNA (Ebert et al. 2007). MiRNA sponges specifically inhibit miRNAs with a complementary heptameric seed; thus, a single sponge can inhibit an entire miRNA seed family. In fact, the development of lung metastasis in a murine breast cancer model was significantly reduced via inhibition of the MYC driven miR-9 using a miRNA sponge (Ma et al. 2010). Furthermore, the use of miRNA sponges to inhibit miR-31 in a breast cancer model resulted in a significant induction of lung metastasis (Valastyan et al. 2009). Of potential concern is the possibility that the antagonist might also non-specifically bind to other RNAs, resulting in unwanted side effects. Therefore, adequate assessment of the functional effects of miRNA inhibition is of key importance for miRNA inhibitor-based loss-of-function studies and development of miRNA therapeutics. The high potency and metabolic stability of chemically modified anti-miRs highlights the utility of anti-miRs in the development of novel RNAi therapeutic modalities based on lung cancer associated miRNAs.

3.2 MiRNA Mimic-Based Therapeutics

Tumor suppressor miRNAs are responsible for down-regulating oncogenes and are primarily expressed in cancer (Croce 2009). In this context, miRNA replacement strategies have been developed to restore normal cellular expression levels via

administration of tumor suppressor miRNA mimics (Bader et al. 2010). MiRNA mimics are synthetic RNA duplexes designed to imitate the endogenous functions of miRNAs. In addition, miRNAs may be unstable as a result of rapid degradation by endogenous nucleases or rapid elimination through renal and hepatic metabolism and extraction upon systemic administration (Bader et al. 2011). Local administration of RNAi-based therapeutics to the target cells is a promising approach to overcome the problems of systemic administration (see next section for details). Similarly, chemical modifications at specific positions or formulations with delivery vectors have been shown to improve stability. Lipid-based and polymer-based nanoparticles reduce the negative electrical charge of RNA nucleotides to promote cell uptake (Wu et al. 2011). Another strategy for efficient delivery of miRNA-based therapeutics is the use of viral vectors (Bonci et al. 2008). Indeed, adenoviral (Esquela-Kerscher et al. 2008) or lentiviral vectors (Kumar et al. 2008) can be used to transfer miRNAs to lung cancer cells. Successful delivery of miRNA-based therapeutics requires patient compliance with the intended delivery route and efficient delivery vectors. This approach has attracted much interest as it provides a novel opportunity to exploit tumor suppressors. The concept of miRNA replacement therapy is best exemplified by let-7 miRNA. Intranasal administration of a let-7 mimic into mouse models of lung cancer significantly reduced tumor growth, suggesting that miRNA replacement therapy is indeed promising (Trang et al. 2010). Based on these successful results, a clinical trial in non-small cell lung cancer using a let-7 based therapy will begin in the near future. As an additional example of the value of miRNA replacement strategies, miR-34a-based cancer therapies have powerful potential for clinical use. Both local and systemic delivery of a synthetic miR-34a mimic resulted in accumulation of miR-34a in the tumor tissue and inhibition of lung tumor growth. MiRNA therapeutics will initiate clinical trials of miR-34a mimics in 2013, making these mimics some of the first miRNA mimics to reach the clinic. Thus, the pharmacological delivery of miRNA mimics effectively inhibits tumor growth by targeting multiple genes. However, it is necessary to pay attention to any potential toxicities in normal tissues, given that therapeutic delivery of miRNA mimics can lead to an accumulation of exogenous miRNAs in normal cells. It will be important to investigate miRNA mimic-induced effects in normal cells and carefully assess the resultant toxicity before using such therapies in clinical practice.

4 Pulmonary Delivery of RNAi-Based Therapeutics

Despite the promise of miRNAs in cancer therapy, there are still hurdles to clear before clinical use, including safety, stability and successful delivery of therapeutic miRNAs to the appropriate tissue and into the appropriate cells. In general, the delivery of miRNAs can be achieved through systemic administration (via intravenous injection) or local administration (via a direct route). Conceptually, systemic delivery is an attractive option because it provides a simple route for miRNA administration to all tissues via the blood stream (Liu et al. 2007). Indeed, there have been some successful reports using systemic delivery of miRNAs in lung cancer models.

Nevertheless, this approach has more *in vivo* barriers to overcome, in addition to nuclease degradation. The delivery barriers are (i) renal clearance of molecules (<50 kDa), (ii) uptake by phagocytic immune cells, (iii) failure of molecules >5 nm in diameter to cross the capillary endothelium, (iv) limited passage through the extra-cellular matrix (polysaccharides and fibrous proteins), (v) inefficient endocytosis by target tumor cells, and (vi) inefficient endosomal release (Bader et al. 2011). Chemical modification and formulation with delivery vectors have been shown to improve stability and delivery to target tumor cells, but these alterations may attenuate the suppressive activity of oligonucleotides (Chernolovskaya and Zenkova 2010). In addition, systemic delivery of miRNAs may induce adverse events similar to those reported for other oligonucleotide-based therapies, such as aggregation and complement activation, liver toxicity and stimulation of the immune response (Kleinman et al. 2008). For these reasons, local administration of miRNAs to the target cancer cells is a promising approach to overcome the problems of systemic administration. Translation of locally administered modalities to the clinical setting is dependent upon the development of an efficient delivery system that is able to improve the pharmacokinetic and biodistribution properties of miRNAs. Thus far, locally administered modalities are available for ocular, transdermal, rectal and pulmonary delivery.

Dozens of RNAi-based therapeutics are being assessed in preclinical and clinical trials, and these studies provide further opportunities for successful results (Davidson and McCray 2011). Many of these studies are conducted using local administration to specific tissues. The lung is anatomically accessible to therapeutic drugs via the pulmonary route. Accessibility is a key requirement for successful RNAi-based *in vivo* and clinical studies, and this anatomical characteristic offers several important benefits over systemic delivery, including the use of lower doses of miRNAs, the reduction of undesirable systemic side effects, and improved miRNA stability due to reduced nuclease activity in the airways compared to serum. The local approach could potentially enhance the retention of RNAi-based therapeutics in the lungs. Because the delivery of siRNAs to the lungs is well studied using different routes and delivery strategies (Lam et al. 2012), many technologies developed for siRNAs may also be applicable to miRNAs. In most of the pulmonary RNAi-based therapy studies *in vivo*, agents were delivered intratracheally or intranasally. This approach has allowed remarkable progress in miRNA modulation in preclinical cancer models, bringing us closer to delivering on the promise of miRNAs as cancer therapeutics.

5 Strategies for Pulmonary Delivery of MicroRNA-Based Therapeutics

Pulmonary delivery approaches are very attractive because they tend to be non-invasive, locally restricted, and administered by the patient. With regard to siRNA-based therapeutics, Phase II clinical trials are underway for the treatment of respiratory syncytial virus (RSV) infection using an intranasal application of naked,

chemically modified siRNA molecules that target viral gene products (DeVincenzo et al. 2008, 2010). To date, two successful studies of pulmonary delivery of miRNA-based therapeutics for lung cancer mouse models have been reported (Kumar et al. 2008; Esquela-Kerscher et al. 2008). These studies show that pulmonary delivery of miRNA from the let-7 family reduces lung tumor formation in an orthotopic lung cancer mouse model without systemic side effects (Table 17.1). These data suggest that intranasal or intratracheal administration of miRNAs may be a potent strategy for treating lung cancer. Although there are no reports of pulmonary delivery of miRNA-inhibitors in lung cancer at present, we predict that this delivery strategy will become a valuable resource for implementing miRNA-based therapies *in vivo* and in humans.

We believe that pulmonary delivery of miRNAs has two primary advantages over systemic delivery for clinical use. First, several sophisticated inhalation devices for lung diseases are already in clinical use. Inhaled therapeutics are used routinely to treat a variety of pulmonary conditions, including asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis. Metered-dose inhalers (MDIs) and dry powder inhalers (DPIs) are the most common modes of inhaled delivery. The use of DPIs for the *in vivo* delivery of therapeutic macromolecules such as insulin (Mastrandrea and Quattrin 2006) and low-molecular-weight heparin (Bai et al. 2010) has yielded promising results. Currently, the use of spray-drying as a technique for engineering dry powder formulations of siRNA nanoparticles, which might allow the pulmonary delivery of biologically active siRNAs directly to the lung tissue, has been demonstrated (Jensen et al. 2010, 2012). Although a suitable carrier is also needed to protect miRNAs from degradation given the shear force and increased temperature of the drying process, these delivery technologies could open new avenues for pulmonary delivery of miRNAs and improve patient outcome. To make miRNA-based therapy practical in the treatment of lung cancer, we believe that the administration of inhaled miRNAs by DPIs is the best of choice of delivery strategy. Second, pulmonary delivery also offers the clinical benefit of a lower miRNA dose. The cost related to the development and application of a particular RNAi therapeutic delivery technology is undoubtedly an important factor (Dykxhoorn et al. 2006). Local administration is likely to be a more cost-efficient strategy for miRNA delivery *in vivo* and in the clinic than systemic administration. Furthermore, the advantage of pulmonary delivery is that it ensures high delivery efficiency with minimal drug loss. For this reason, pulmonary delivery of miRNAs has great potential for clinical use. However, the limitations of pulmonary delivery of miRNA-based therapeutics are important to consider. First, the pharmacokinetics of inhaled miRNAs in *in vivo* models and humans are estimated inaccurately. It is also unknown whether miRNA-based therapeutics delivered via the intrapulmonary route could also be delivered to other organs, such as the liver and kidneys. To prevent systemic side effects, the precise pharmacokinetics of miRNAs after intrapulmonary administration should be measured. Second, we also must pay attention to the pulmonary inflammatory and toxicological responses caused by the delivery vehicle. In fact, there are some reports that RNAi-based therapeutics with polyethyleneimine

(PEI) frequently cause inflammatory responses in the lungs (Beyerle et al. 2011). It has been reported that naked RNAi-therapeutic delivery possesses advantages over other delivery vectors, such as reduced toxicity and reduced inflammatory responses, as well as simple formulation (Heidel et al. 2004). However, the advantage of naked RNAi-therapeutics over delivery vectors in the treatment of lung diseases is controversial (Nielsen et al. 2010; Akinc et al. 2008). Therefore, we need to develop safer delivery technology for practical use in *in vivo* mouse models and humans.

6 Conclusions

During the past decade, miRNAs have quickly advanced from discovery to therapeutic development programs. This rapid progress reflects the importance of miRNA biology in cancer, leaving little doubt about the therapeutic potential of miRNAs in cancer treatment. Given the encouraging results of the profiled studies and preclinical testing, miRNAs are being integrated into human clinical trials. The first miRNA-targeted drug LNA-anti-miR-122 is successfully undergoing Phase II trials (Janssen et al. 2013). Accordingly, several companies are currently developing miRNA mimics or inhibitors for the treatment of cancer. The main focus in bringing miRNAs to cancer cells is the capacity of pharmacological drug delivery. The success of miRNA-based therapeutic delivery requires efficiency, convenience, and patient compliance using the delivery route. In this chapter, we showed that pulmonary delivery of miRNA-based therapeutics holds powerful potential for lung cancer treatment (Fig. 17.1). A realistic therapeutic intervention, such as inhalation, would

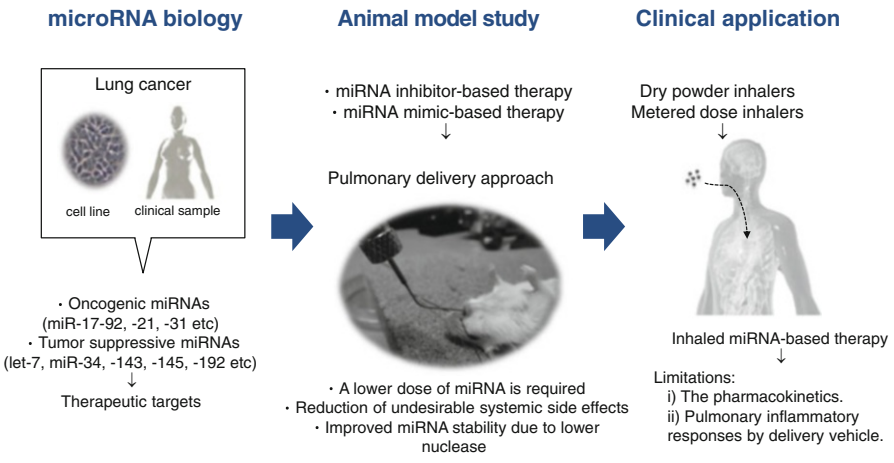

Fig. 17.1 Process for translating microRNA biology from bench to bedside in lung cancer

enhance drug delivery to the site of action and decrease systemic exposure, thereby reducing off-target effects. In the future, combining miRNA-based therapeutics with chemotherapy may potentiate the cancer treatment efficacy. Therefore, continued investigation on all fronts will be of equal importance to the eventual clinical application of miRNAs.

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