ABSTRACT

Lung cancer is the neoplasia with the highest incidence and mortality in men and women worldwide. Lung cancer is classified into two large histologic subgroups: small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC) according to cellular origin, molecular changes, clinical-pathological features, and response to treatments. NSCLC constitutes 80% of all lung cancer cases; nevertheless, the molecular mechanisms underlying the tumoral etiology still remain poorly understood. MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level by repressing translation or decreasing mRNA stability in numerous biological processes. In cancer, miRNAs have differential spatial and temporal expression, which is related to several clinical, biological, molecular and genomic features of tumors. miRNA
expression profiles, polymorphisms and epigenetic modification in NSCLC have been studied, and these studies have improved NSCLC diagnosis and classification, as well as provided prognostic information. In this review, we describe some of the better-characterized miRNAs in NSCLC and how they could improve lung cancer prognosis and therapy. We summarize the current understanding in expression of miRNAs and their involvement in process biology of NSCLC, as well as their potential as biomarkers for risk stratification, outcome prediction and classification of histologic subtypes, in addition to circulating biomarkers and miRNA-based NSCLC therapy.

1. INTRODUCTION

MicroRNAs (miRNAs) are single-stranded, 20–23 nucleotide–long RNA molecules that control gene expression in many cellular processes (Zhang 2009). These molecules bind through specific base-pairing to the 3′ untranslated region (3′UTR) of mRNAs to reduce their stability and translation efficiency. miRNA genes can be expressed individually or within clusters, and can be found in introns of protein-coding genes as well as within repetitive regions and transposable elements (Huang et al. 2011). In oncogenesis, miRNAs have an important role in control of gene transcription, and based on their target, might be grouped in oncomiRNAs and anti-oncomiRNAs (Ruan et al. 2009). MiRNAs have been found to be deregulated in almost all human cancers, including lung cancer; however, the molecular mechanisms in which miRNAs are involved, and that lead to the malignant phenotype have not been unraveled yet (Garzon et al. 2006). The effects of miRNAs are mediated by modification in their abundance in tumor cells; these changes may be due to aberrant expression owing to gene mutations and deletions, genomic instability and chromosomal fragile sites that generate abnormal DNA copy numbers. In addition, miRNA gene expression in cancer cells can be regulated by abnormal epigenetic modifications such as methylation of their promoter regions (Lujambio et al. 2008). MiRNAs frequently target hundreds of mRNAs, including those of genes that mediate processes in tumorigenesis, such as cell cycle regulation, cell proliferation, apoptosis, differentiation, metabolism, stress response, inflammation and invasion (Hwang et al. 2006). The identification of novel molecular markers in cancer is a high priority in order to reduce morbidity and mortality, and provide new strategies for targeted cancer therapy. In lung cancer, patient prognosis still remains poor, consequently, new molecular therapies target important pathways, such as EGFR and RAS, known to be altered in NSCLC. There are many studies that strongly support the potential of miRNAs as biomarkers. Some miRNAs are known to be intimately involved in regulation of KRAS (Johnson et al. 2005), as well as in the initiation, progression and prognosis of NSCLC. More
recently, miRNAs have been detected in peripheral blood of lung cancer patients (Fanini et al. 2011), which also makes them attractive candidates as biomarkers for noninvasive and early lung cancer diagnosis. The potential of restoring levels of aberrantly under-expressed miRNAs with miRNA mimics, or inactivating over-expressed miRNAs with miRNA inhibitors has been explored and could be the next generation of therapeutic strategies.

2. LUNG CANCER

Lung cancer remains as one of the most aggressive cancer types with nearly 1.6 million new cases worldwide each year. There were an estimated 222,520 new cases and 157,300 deaths from lung cancer in the United States in 2010 (Jemal et al. 2010). The majority of patients with lung cancer present with advanced disease; for these patients the use of systemic chemotherapy has brought above modest improvements in overall survival and quality of life, though, the survival is still low, a median survival rate of 8 to 10 months (Jemal et al. 2010). Once recurred or metastasized, the disease is essentially incurable with survival rates at 5 years of less than 5%, and this has improved only marginally during the past 25 years (Jemal et al. 2010). Lung cancer is classified in two sub-groups as non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which constitute 85 and 15% of all lung cancer cases respectively. NSCLC comprises three major histological subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. This classification has important implications for the clinical management and prognosis of the disease (Silvestri et al. 2009). Cigarette smoke is the principal risk factor for the development of this neoplasia (Mao et al. 1997), however, the cases of lung cancer unrelated to smoking are growing, suggesting environmental or genetic determinants in disease initiation and progression (Sun et al. 2007).

Epidemiologic studies showing an association between family history and an increased risk of lung cancer have provided evidence of host susceptibility. Lung-cancer susceptibility and risk is associated with rare germ-line mutations in p53, retinoblastoma, epidermal growth factor receptor (EGFR) and other genes like ERCC1 (Sato et al. 2007). Other molecular and genetic studies have shown that some molecules contribute to sporadic tumors of NSCLC, actually they are useful as predictive biomarkers. EGFR regulates important tumorigenic processes, including proliferation, apoptosis, angiogenesis, and invasion. Along with its ligands, EGFR is frequently overexpressed during the development and progression of NSCLC. EGFR gene is amplified and over-expressed in 6% of NSCLC. However, activating mutations in the EGFR kinase domain occur early in the development of adenocarcinomas that are generally unrelated to
Mutated EGFR is present in 10–15% of NSCLC tumors (Tang et al. 2005). Gene amplification and mutations in the kinase domain of C-erbB2 (HER-2/neu), a member of EGFR family, have been identified in patients with lung adenocarcinomas with a frequency of less than 5% and 5 to 10% respectively, and its overexpression is involved in ~25% of NSCLC cases (Shigematsu et al. 2005). EGFR and HER-2 kinase domain mutations have similar associations with female sex, non-smoking status, and Asian background in patients with adenocarcinoma (Pao et al. 2004).

The RAS–RAF–MEK pathway is involved in signaling downstream from EGFR leading the growth and tumor progression in NSCLC. Activating KRAS gene mutation occurs in ~30% of cases of NSCLC, mostly adenocarcinomas. KRAS mutations are localized in exon 12 (in 90% of patients) or exon 13, and they are the smoking-related G→T transversion and the nonsmoking-related G→A transition (Riely et al. 2008). KRAS mutations appear to be an early event in smoking-related lung adenocarcinoma, representing a poor prognosis in these patients. Other promising predictive markers in NSCLC are BRAF (Kobayashi et al. 2011) and the oncogenic fusion gene of EML4–ALK (Soda et al. 2007). BRAF, an effector molecule of RAS pathway, is mutated in about 2% of adenocarcinomas that do not show KRAS gene mutations. While EML4-ALK is present in 2% to 7% of NSCLC cases; essentially, this fusion gene is present in young patients with adenocarcinoma and no exposure to smoking (Brose et al. 2002). Some other molecules have been identified based on expression and genomic data, such as MYC and Cyclin D1, which are amplified and over-expressed in 2.5–10%, and 5% of NSCLC respectively, while BCL-2 over-expression is involved in ~25% of cases of NSCLC (Sato et al. 2007). Recent data have shown that gene promoter methylation is a common event in NSCLC, which contributes to oncogene over-expression or silencing of tumor suppressor genes. These epigenetic changes may be an early event in NSCLC, since the promoter region of the p16 gene is frequently methylated in smokers and premalignant lesions of lung cancer (Belinsky et al. 2006). Mutation status in EGFR and amplification in HER-2 genes correlated with good response to treatment with tyrosine kinase inhibitors (TKI e.g. gefitinib or erlotinib) (Sequist et al. 2007) and cytotoxic chemotherapy (Eberhard et al. 2005). HER-2 kinase domain mutations are associated with resistance to such TKI, but also with sensitivity to HER-2-targeted therapy (Wang et al. 2006), while KRAS mutations are correlated with poor response to treatment with TKI (Sequist et al. 2007). Modifications in PI3K-AKT-mTOR pathway have been described in NSCLC. AKT overexpression has been described in a subgroup of NSCLC tumors in conjunction with mutations or amplification of the PI3KCA gene. These genomic modifications are related to enhanced activity of PI3-K pathway, mainly in squamous cell carcinoma tumors (Rekhtman et al. 2012). On the other hand, tissues of
smoking patients show higher levels of angiogenic factors such as VEGF. VEGF expression is increased in relation to tumoral grade, which in turn, correlates with increased microvessel density, development and worse prognosis of lung cancer. Tumoral angiogenesis and angiogenic factors are regulated by hypoxia-regulated pathways such as hypoxic inductor factor (HIF) 1α and 2α or through oncogenes as EGFR, KRAS and p53 (Giatromanolaki 2001). Currently a major focus of NSCLC research has been the identification of new biomarkers and molecule targets in order to have better diagnosis, prognosis and therapeutic treatments of lung cancer patients. Gene expression signature obtained through specific gene expression profile, offer a subset of expressed genes usually associated with a specific phenotype. This kind of analysis has brought about progress in the identification of markers, mutations, and genomic signatures specific expressed in NSCLC (Branica et al. 2012). In recent years, there has been great expectation of the potential of miRNAs as possible biomarkers and molecular targets in cancer. Lung cancer tissues have demonstrated unique spatial and temporal miRNA expression patterns; and some studies have identified specific miRNA expression signatures associated with clinical outcome of NSCLC patients (Wang et al. 2012). The current understanding in expression and predicted target genes of miRNAs as well as their promising potential as circulating biomarkers suggests that miRNAs might have a potential as therapeutic targets and agents in NSCLC.

3. BIOGENESIS OF miRNAs

MiRNAs are fundamental regulators of protein abundance in human cells; therefore, they have emerged as key regulators of almost every cellular biological process (Bartel et al. 2004). MiRNA biogenesis begins in the nucleus where RNA polymerase II transcribes miRNA genes to form primary miRNA transcripts (pri-miRNAs). Like other Pol II transcripts, pri-miRNAs are 5’-capped and 3’-polyadenilated transcripts (Zhang et al. 2010). The pri-miRNAs are cleaved by the RNase III enzyme Drosha/DGCR8 complex to form precursor miRNAs (pre-miRNAs), which are hairpin-shaped RNA molecules, 70–100 base pairs (bp) in length. Then, Exportin 5 exports the pre-miRNAs from the nucleus into the cytoplasm. In the cytosol, the pre-miRNAs are cleaved by the RNase III enzyme Dicer/TRBP (transactivator RNA-binding protein) complex. The pre-miRNAs cleavage results in a small double-stranded RNA (dsRNA) duplex that contains both the mature miRNA, which targets mRNAs containing complementary sequences, and its complementary strand that will be degraded. The mature 20–25 nt long miRNA interacts with a member of the Ago (Argonaute) protein family to form a miRNA-induced silencing complex (miRISC). The miRNA guides
such protein complexes to partially complementary target sites on mRNAs. Basically miRNAs function as post-transcriptional regulators of gene expression and protein translation; it is estimated that they regulate up to 30% of all protein-coding genes (He et al. 2004). The mechanisms through which miRNAs regulate protein abundance in the cell rely upon their complementarity with protein-coding mRNAs sequence targets. MiRNAs bind to sequence-specific regulatory regions located in the 3’-UTR of mRNA targets although it has been shown that miRNAs can bind to 5’-UTR and even to the coding regions of mRNA targets. Binding of miRNA to mRNA sequences with perfect base-pairing homology induces the mRNA cleavage by Ago in the RISC, leading to inhibition of gene expression. Nevertheless, the imperfect binding to partially complementary sequences in 3’-UTR of mRNA targets, leads to repression of protein translation, this last mechanism is more commonly used by miRNAs in the cells (Pillai et al. 2007) (Fig. 1).

MiRNAs are frequently located in introns of protein-coding genes; therefore host gene promoters could control their expression, so there may be a strong correlation between mRNA and miRNA expression. However, recent reports indicate that miRNAs genes located within intronic regions

Figure 1. Schematic representation of miRNA biogenesis. MiRNA genes are transcribed in the nucleus, then, protein complex-induced nucleolytic modifications form a pre-miRNA which later is transported from the nucleus to the cytoplasm. In the cytoplasm, additional protein complexes break pre-miRNA and generate a mature miRNA, which targets sequences in mRNAs to regulate cellular proteins synthesis. MiRNAs can be released from the cell through exosomes into the bloodstream.
could have self-promoters (Golan et al. 2010). Other miRNAs are often arranged as clusters in the genome and they are transcribed as part of long non-coding RNAs to produce one pri-miRNA, which will be processed into several functional miRNAs (Bartel et al. 2004). MiRNA activity and abundance is regulated at various levels; regulation of pri-miRNA transcription, processing, target site binding and miRNA stability are among the most important (Obernosterer et al. 2006). Each cell type expresses a specific subset of miRNAs to ensure establishment and maintenance of cell type-specific mRNA profiles (Liang et al. 2007). Because of the involvement of miRNAs in the regulation of abundance of mRNAs and proteins that participate in cell mechanisms such as differentiation, cell growth, proliferation and apoptosis, it is not surprising that miRNAs have an important role in pathogenesis of cancer. 52.5% of miRNA genes are located in cancer-associated regions or fragile sites (prone to amplification, translocation or deletion) (Calin et al. 2004), besides, there are descriptions of transcriptional modifications and aberrant epigenetic events that impact in expression of miRNAs in tumors compared to normal tissues (Saito et al. 2006). These observations support the complex dual role of miRNAs, as either oncomiRNAs or anti-oncomiRNAs depending on whether they show up- or down-regulated expression in tumorigenic progression (Hwang et al. 2006). Not only do changes in expression of miRNAs in cancer have a biological implication, they also have clinical significance, which has been explored in recent years. In NSCLC cancer, there are many reports that show abnormal expression of miRNAs, that impact several modifications in the biological function of cancer cells. These observations have been the basis for the clinical significance of miRNAs in lung cancer (Wang et al. 2012).

4. GENOMIC AND EPIGENOMIC CHANGES OF miRNAs IN LUNG CANCER

4.1 Genetic Modifications of miRNAs

The genomic organization of miRNAs often dictates translational control. MiRNAs tend to be located in fragile chromosomal regions that are susceptible to translocations, microdeletions and amplifications. It has been recently reported that most known miRNAs are in regions of genomic aberration associated with cancer (Calin et al. 2004). They analyzed a panel of B-CLL samples with known deletions at 13q14 and a set of lung cancer cell lines. Mir-16a expression (located at 13q14) was low or absent in the majority of B-CLL cases. In contrast, both miR-26a (at 3p21) and miR-99a (at 21q11.2) were not expressed or were expressed at low levels in lung cancer cell lines, and this finding correlates with their location in regions of
LOH/HD in lung tumors cell lines. On the contrary, the expression of miR-16a was the same in the majority of cell lines, as they were in normal lung cells (Calin et al. 2004). In keeping with these observations, let-7 and miR-17-92 are linked to chromosomal deletions and gains respectively, indicating copy number variations as potential mechanisms for their deregulation in lung tumors (Calin et al. 2004, Hayashita et al. 2005, Rodriguez et al. 2004). Single nucleotide polymorphisms (SNPs) in pre-miRNAs could also alter miRNA processing, expression, and/or binding to target mRNA. It has been demonstrated that the hsa-mir-196a2 rs11614913 variant homozygote is associated with poor survival and susceptibility to NSCLC as well as significantly increased mature hsa-mir-196a expression; moreover, it can directly influence the target binding of hsa-mir-196a2-3p (Hu et al. 2008, Tian et al. 2009). Deletions in miRNAs have been found in lung cancer; mir-218 is deleted and down-regulated in lung squamous cell carcinoma and was found to be associated with a history of cigarette smoking (Davidson et al. 2010). Recently, a deletion of mir-101 was also found in lung cancer, associated with reduced miR-101 expression. Lung in situ carcinoma also harbored miR-101 deletions, suggesting that genomic loss may be an early event in lung cancer development (Thu et al. 2011). The influence of copy number alterations at miRNA loci in the context of drug response has also been investigated recently. For example, miR-662 was found to have a differential pattern of copy number alteration between sensitive and resistant cancer cell lines for most tested drugs. miR-662 is located on chromosomal region 16p13.3 and was found to be more frequently gained in cell lines highly resistant to AZ628, erlotinib, geldanamycin, Gö-6976, HKI-272 (Neratinib), and MK-0457. All of these drugs are TKIs, except for geldanamycin, which is an antibody that targets HSP90 (Enfield et al. 2011).

4.2 Methylation of miRNA Genes

More than 100 species of known miRNAs are embedded within or near the CpG islands of the human genome and are potentially subject to control by epigenetic alterations such as DNA methylation and histone modification. Systematic assessments of miRNA expression and epigenetic modifications among cell lines and primary tumor specimens have revealed the existence of the epigenetic regulation of miRNAs in multiple tumor types (Kunej et al. 2011, Kozaki et al. 2008, Saito et al. 2006, Watanabe 2011). The miR-34 family consists of three miRNAs (mir-34a, mir-34b and mir-34c) that are derived from two transcripts (mir-34a on chromosome 1 and mir-34b/c on chromosome 11). Mir-34s have been shown to be direct targets of p53 (Corney et al. 2007, He et al. 2007, Bommer et al. 2007). Interestingly, mir-
34a is most highly expressed in the brain, whereas mir-34b/c is most highly expressed in the lung with a low expression in the brain and no expression in any other tissues (Bommer et al. 2007), suggesting that mir-34b/c plays an important role in the p53 tumor suppressive pathway, at least in lung tissue. Lujambio et al. identified CpG island hypermethylation of miR-148, mir-34b/c, and the miR-9 family, through expression microarray analysis on DNA-demethylation drug treatment in cancer cells. These results were confirmed on a group of primary tumor samples, including colon, lung, breast, head, and neck cancer, and melanoma. When miRNA hypermethylation was evaluated with respect to the existence or not of lymph node metastasis, the presence of mir-34b/c, mir-148, and miR-9-3 CpG island hypermethylation in the primary tumor (lung, breast, melanoma) was significantly associated with those tumors that were positive for metastatic cancer cells in the corresponding lymph nodes, which highlights the importance of the in vivo role of miRNA epigenetic silencing in metastasis formation (Lujambio et al. 2008). MiR-34b hypermethylation was also recently observed in 41% of 99 primary NSCLC (Watanabe et al. 2011). In this study, the DNA methylation of miR-34b was also significantly associated with lymphatic invasion. These results suggest that miRNA methylation may be used in clinical practice as a marker to predict tumor prognosis and metastatic behavior. Recently, promoter hypermethylation of miR-34b/c was proposed as a potential prognostic factor for stage I NSCLC (Wang et al. 2011). It was also demonstrated that methylation of miR-34b/c is frequent in small-cell lung cancer (Tanaka et al. 2011). On the other hand, methylation of mir-152, mir9-3, mir124-1, mir124-2, and mir124-3 was analyzed in 96 NSCLC specimens using a combined bisulfite restriction analysis. Methylation of mir-9-3, mir124-2, and mir124-3 was individually associated with an advanced T factor independent of age, sex, and smoking habit. Moreover, the methylation of multiple miRNAs loci has been associated with a poorer progression-free survival in a univariate analysis with a median observation period of 49.5 months (Kitano et al. 2011). The silencing of tumor-suppressive miRNAs, miR-200b, miR-200c, and miR-205 was demonstrated in an in vitro premalignancy lung model consisting of 4 week-exposure of immortalized human bronchial epithelial cells to tobacco carcinogens which can induce a persistent, irreversible, and multifaceted dedifferentiation program marked by epithelial mesenchymal transition (EMT) and the emergence of stem cell-like properties (Tellez et al. 2011). The miR-200 family and miR-205 are key determinants of the epithelial phenotype by directly targeting ZEB1 and ZEB2, showing that miRNAs can indirectly regulate E-cadherin expression (Gregory et al. 2008, Park et al. 2008). So, the induction of EMT is epigenetically driven, initially by chromatin remodeling with ensuing promoter DNA methylation sustaining
stable silencing of the miR-200b, miR-200c, and miR-205 implicated in this developmental program (Tellez et al. 2011).

4.3 miRNA Expression Profiles

Specific miRNA expression profiles can improve lung cancer classification and identify new pharmacological targets. MiRNA signatures have been linked to the prognosis of clinical subsets of lung cancers (Yu et al. 2008), which can help classify this malignancy by precisely defining the miRNA expression profiles that are characteristic of different histopathologic types of lung cancer (Landi et al. 2010). Expression profiles of miRNAs can provide prognostic information in lung cancer. Specific miRNA expression patterns can suggest survival outcomes for NSCLC patients. miRNA microarray analysis have identified statistical unique profiles, which could discriminate lung cancers from noncancerous lung tissues as well as molecular signatures that differ in tumor histology. The first evidence of deregulated miRNA expression in lung cancer came from the study by Volinia et al. who identified a group of miRNAs frequently aberrantly expressed in tumor tissues with respect to the normal tissue counterpart (Volinia et al. 2006). Yanaihara et al. compared miRNA patterns of expression in tumor versus adjacent uninvolved lung tissue in 104 cases of lung cancer. They identified 43 differentially expressed miRNAs between lung tumors and adjacent uninvolved lung tissue. In addition, five miRNAs (miR-155, -17-3p, -145, -21 and let-7a-2) predicted poor prognosis among patients with lung cancer (Yanaihara et al. 2006). Moreover, the same group identified six miRNAs (hsa-mir-205, -99b, -203, -202, -102, and -204-prec) that were expressed differently in adenocarcinoma and squamous cell carcinoma (Yanaihara et al. 2006). Recent findings on human serum containing stably expressed miRNA have revealed a great potential of serum miRNA signature as a disease fingerprint to predict survival. The first significant effort in this direction came from Chen et al. By deep sequencing of pooled sera from patients with and without lung cancer, 8 miRNAs were identified as differentially expressed in comparison populations. These miRNAs then were validated in a few clinical samples by RT-PCR (Chen et al. 2008). Another exploratory study looked at the potential for microarray profiling of serum-derived miRNAs (Lodes et al. 2009). In this work, the authors demonstrate the technical feasibility of performing such profiling and demonstrate the high accuracy of predicting cancer presence versus absence by cross-validation analysis (Lodes et al. 2009). Hu et al. analyzed miRNA expression profiles in sera of 303 patients with stage I to IIIA NSCLC. They detected miRNA level alterations between patients with shorter and longer survival time. Moreover, their results revealed that four miRNAs, including miR-486,
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-30d, -1, and -499 could predict overall survival (Hu et al. 2010). Chen et al. identified, in a genome-wide serum miRNA expression study, a specific panel of 10 miRNAs that was able to distinguish NSCLC cases from controls with high sensitivity and specificity and that correlated with the stage of NSCLC. Furthermore, this 10 serum-miRNA profile could accurately classify serum samples collected up to 3 years prior to the clinical NSCLC diagnosis (Chen et al. 2012). By expression analysis of two serum miRNAs (hsa-miR-1254 and hsa-miR-574-5p), Foss et al. were able to discriminate early stage NSCLC samples from controls with a sensitivity of 82% and a specificity of 77% in a training cohort and with a sensitivity of 73% and a specificity of 71% in a validation cohort (Foss et al. 2011). Shen et al. recently identified a panel of four miRNAs, namely miR-21, -210 and -486-5p, that distinguished NSCLC patients from healthy controls with 86.22% sensitivity and 96.55% specificity (Shen et al. 2011). Furthermore, Keller et al. showed in a multicenter study that different types of cancer or non-cancer diseases could be differentiated by blood-borne miRNA profiles (Keller et al. 2011). In addition, Leidinger et al. reported miRNA signatures that differentiated blood samples of lung cancer patients from blood samples of patients with non-malignant chronic obstructive pulmonary disease with 89.2% specificity, and 91.7% sensitivity (Leidinger et al. 2011). Likewise, Boeri et al. were able to predict lung cancer in plasma samples 1–2 years prior to diagnosis using CT. For the time being, however, the source of circulating miRNAs is elusive (Boeri et al. 2011). It has been suggested that they are released due to apoptosis or active exocytosis processes (Kosaka et al. 2010). This hypothesis is supported in a study by Rabinowits et al. that showed a similarity between the circulating exosomal miRNAs and the lung tumor-derived miRNA pattern.

MiRNAs are also present in other body fluids. Yu et al. showed that miRNAs were stably present in sputum. They were able to differentiate lung adenocarcinoma patients from healthy individuals by using a panel of four sputum miRNAs, namely miR-486, -21, -200b and -375, with high sensitivity (80.6%) and a specificity of 91.7% (Yu et al. 2010). Interestingly, two of these miRNAs, miR-21 and -486, show an overlap with the study on serum by Shen et al. (2011). The same group identified three sputum miRNAs, namely miR-205, -210 and -708, that distinguished squamous cell lung carcinoma patients from healthy individuals with 73% sensitivity and 96% specificity (Xing et al. 2010). On the other hand, few researches have studied the correlations between miRNA expression and radiotherapy sensitivity of lung cancer. Shin et al. explored the alteration of miRNA profiles by ionizing radiation in A549 human non-small cell lung cancer cells and identified 12 and 18 miRNAs in 20 Gy- and 40 Gy-exposed cells respectively, that exhibited more than 2-fold changes in their expression levels (Shin et al. 2009). Their results showed that miR-22 expression was
modified in response to radiotherapy, which suggests that this miRNA could have implications in cell radio-response. At the moment, we know that miRNAs are a powerful tool for regulating the gene expression, in this context, it is logical to think that aberrant expression of miRNAs in lung cancer will result, also, in modifications of gene expression. According to their functions, a single miRNA could regulate several genes whereas several miRNAs could regulate one gene. For this reason, it has been complicated to specifically determine which genes or biological pathways could be modified as a consequence of aberrant miRNA expression. In Table 1 we summarize miRNA targets in lung cancer which have been validated in experimental models.

Table 1. Experimentally validated miRNAs in lung cancer.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression in lung cancer</th>
<th>Function</th>
<th>Target gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-1</td>
<td>Down</td>
<td>Represses proliferation and increases apoptosis</td>
<td>Met, Pim-1, FoxP1, HDAC4</td>
<td>Nasser et al. 2008</td>
</tr>
<tr>
<td>mir-7</td>
<td>Up</td>
<td>Represses apoptosis and increases proliferation</td>
<td>EGFR, Bcl-2</td>
<td>Webster et al. 2008, Xiong et al. 2011</td>
</tr>
<tr>
<td>Let-7</td>
<td>Down</td>
<td>Decreases proliferation and increases apoptosis</td>
<td>Cdk6, CDC25A, NRAS, KRAS, HMG2</td>
<td>Lee et al. 2007, Kumar et al. 2008, Johnson et al. 2007, Esquela-Kerscher et al. 2008</td>
</tr>
<tr>
<td>miR-15/16</td>
<td>Down</td>
<td>Induces Rb-dependent cell cycle arrest and apoptosis</td>
<td>Bcl-2, CCND1, CCND2, CCNE1, WNT3A</td>
<td>Cimmino et al. 2005, Bandi et al. 2009</td>
</tr>
<tr>
<td>mir-17-92</td>
<td>Up</td>
<td>Induces angiogenesis, repress apoptosis and increase proliferation</td>
<td>Tsp1, CTGF, HIF1α, PTEN, E2F1-3, BIM</td>
<td>Dews et al. 2006, Xiao et al. 2008, Sylvester et al. 2007</td>
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</table>

Table 1. contd....
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<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression in lung cancer</th>
<th>Function</th>
<th>Target gene</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-31</td>
<td>Up</td>
<td>Increases cell growth and tumorigenicity</td>
<td>LATS2, PPP2R2A</td>
<td>Liu et al. 2010</td>
</tr>
<tr>
<td>miR-93</td>
<td>Up</td>
<td>Increases proliferation</td>
<td>FUS1</td>
<td>Du et al. 2009</td>
</tr>
<tr>
<td>miR-98</td>
<td>Up</td>
<td>Increases proliferation and represses apoptosis</td>
<td>FUS1</td>
<td>Du et al. 2009</td>
</tr>
<tr>
<td>miR-101</td>
<td>Down</td>
<td>Inhibits cell proliferation and invasion</td>
<td>EZH2</td>
<td>Zhang et al. 2011</td>
</tr>
<tr>
<td>mir-128b</td>
<td>Down</td>
<td>Reduces EGFR-mediated proliferation</td>
<td>EGFR</td>
<td>Weiss et al. 2008</td>
</tr>
<tr>
<td>mir-130a</td>
<td>Down</td>
<td>Induces apoptosis and reduces migration</td>
<td>MET</td>
<td>Acunzo et al. 2012</td>
</tr>
<tr>
<td>mir-145</td>
<td>Down</td>
<td>Inhibits cell growth</td>
<td>c-myc</td>
<td>Chen et al. 2010</td>
</tr>
<tr>
<td>miR-197</td>
<td>Up</td>
<td>Increases proliferation</td>
<td>FUS1</td>
<td>Du et al. 2009</td>
</tr>
<tr>
<td>miR-183</td>
<td>Down</td>
<td>Inhibits migration and invasion</td>
<td>Ezrin</td>
<td>Wang et al. 2008</td>
</tr>
<tr>
<td>mir-210</td>
<td>Down</td>
<td>Increases apoptosis</td>
<td>SDH</td>
<td>Puisségur et al. 2011</td>
</tr>
<tr>
<td>miR-451</td>
<td>Down</td>
<td>Represses apoptosis and induces proliferation</td>
<td>RAB14</td>
<td>Wang et al. 2011</td>
</tr>
<tr>
<td>miR-494</td>
<td>Down</td>
<td>Represses cell proliferation and induces senescence</td>
<td>IGF2BP1</td>
<td>Ohdaira et al. 2012</td>
</tr>
</tbody>
</table>
5. CLINICAL SIGNIFICANCE OF miRNAs IN LUNG CANCER

Despite remarkable advances in the understanding of lung cancer over the past decades, early detection strategies, improvement in treatment strategies, better diagnosis and prognosis of NSCLC still remains deficient, which is reflected in the low treatment response rates and patient survival (Jemal et al. 2010). It is now clear that NSCLC is a highly heterogeneous cancer and that the molecular events that lead to NSCLC cancer development and progression may be dependent upon multiple biologic pathways. The advances in understanding of NSCLC biology have permitted its subdivision into various molecular subgroups; not only based on histological classification but also on the presence of molecular markers (Branica et al. 2012). Analysis of the genome and proteome of NSCLC has identified signatures for diagnosis and prognosis, as well as biological targets. Several studies in NSCLC cancer have demonstrated a deregulation of miRNA expression suggesting that they are important regulators in lung cancer pathogenesis through the modification of the expression of critical targets. In recent years, many research groups have identified individual targets and pathways of miRNAs relevant to NSCLC tumorigenesis. Studies that examine global changes in miRNA expression and the effects of individual miRNAs on lung cancer cell phenotype suggest that miRNAs are involved in NSCLC tumor development and progression and may potentially serve as biomarkers for diagnosis and prognosis (Wang et al. 2012).

5.1 miRNAs in Lung Cancer Diagnosis, Prognosis and Survival

Variability in lung cancer histology and prognosis means that better methods are needed for classifying lung tumors and stratifying patients for traditional cytotoxic therapies. A number of miRNAs are involved in lung cancer initiation, progression and prognosis. Further characterization of the highly complex miRNA regulatory networks will lead to the identification of new hallmarks of malignant growth, as well as new therapeutic targets and agents. As mentioned above, miRNA expression profiles have been useful for accurately distinguished adenocarcinomas from squamous cell carcinomas and identified the multi-step lung carcinogenesis. Changes in expression analysis of 365 miRNAs by RT-PCR have been reported during progression from hiperplasia to invasive squamous cell carcinoma (Mascaux et al. 2009). miR-15a, -32 and -34c decreased progressively whereas miR-199a and -139 were step-specific. On another novel approach to identifying miRNA signatures in lung cancer, investigators identified a 17 gene signature of genes targeted by miR-34b/34c/449 that accurately
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distinguished between NSCLC histological types, with a sensitivity of 90% for detecting lung cancer in the airway when combined with cytopathology (Liang 2008). Besides the identification of cancer type specific miRNA signatures, research is also aiming at the identification of specific miRNAs that are suited to differentiate between histological lung cancer subclasses. As treatment depends on the histological subtype, such miRNAs are likely to be useful for decision making in clinical treatment. Lebanony et al. were able to provide a highly accurate subclassification of NSCLC patients. They analyzed the expression of 141 miRNAs in adenocarcinomas versus squamous cell carcinomas and then determined that the relative levels between miRNA-205 and miR-21 can be used to distinguish the two NSCLC sub-types with 96 and 90% of sensitivity and specificity respectively. The authors propose that expression of miR-205 is a specific biomarker for squamous cell lung carcinoma, which may be useful for classification of NSCLC sub-types (Lebanony et al. 2009). Several studies have demonstrated the ability of miRNA expression for predicting the outcome of lung cancer patients, highlighting its possible diagnostic or prognostic value (Wang et al. 2012). The normal non-cancerous state of the lung is one of the tissues with the highest expression of the let-7 miRNA family (Johnson et al. 2007). Let-7 regulates the RAS pathway through repression of KRAS activity (Johnson et al. 2005), and activates mutations in KRAS that are commonly implicated in lung adenocarcinoma. Yu et al. found that expression of five miRNAs, let-7a, miRNA-221, -137, -372 and -182* which were analyzed by real time RT-PCR, had a predictive value for overall survival and relapse-free survival in 101 patients of stage I-III NSCLC. For instance, let-7 and miR-221 are related with a protective value, while, miR-137, miR-372, miR-182 and the miR-17-92 cluster were reported as a high-risk miRNAs. The miR-17-92 cluster is often over-expressed in lung cancer and has been found to promote tumor cell proliferation in these tumors (Yu et al. 2008). The poor survival time and the high relapse rates after surgery in lung cancer call for new methods to detect the disease at an early stage. Recently Võsa et al. provided evidence for miR-374 as a potential marker for early stage NSCLC (Võsa et al. 2011). As previously reviewed, Yanaihara et al. obtained expression profiles of miRNAs in an independent study of 143 resected lung tumors (Yanaihara et al. 2006). They proposed that under-expressed levels of let-7 correlated with prognosis of patients with adenocarcinoma independently of disease stage, consistent with its known tumor-suppressor role. It was reported that reduced let-7 expression was associated to significantly shorter survival in patients, regardless of disease stage (Takamizawa et al. 2004). Associated to this, Yanaihara et al. found that low expression of let-7a and high expression of the miR-155 precursor were associated with poor prognosis in NSCLC patients (Yanaihara et al. 2006). In 205 patients with stage I–IIIA of squamous cell carcinoma, Landi et al. identified a
signature of down-regulated miRNAs including let-7e, miR-34a, miR-34c–5p, miR-25, and miR-191, which were associated with poor survival in male smokers (Landi et al. 2010), while Patnaik et al. demonstrated that a miRNA expression profile could predict recurrence of the disease in patients with NSCLC in stage I who underwent radical surgery (Patnaik et al. 2010). All of these reports strongly suggested that miRNA deregulation could be a good prognostic factor in NSCLC. Moreover, recently, different groups were able to identify miRNAs that differentiated NSCLC patients with brain metastases from patients without brain metastases (Arora et al. 2011, Nasser et al. 2011). Biomarkers that allow identification of NSCLC patients with increased risk for brain metastases will be of great value for decision-making in preventive radiation treatment.

5.2 miRNAs Associated to Therapy Response

Specific miRNA profiles could predict response to particular treatment, and the variable presence of individual miRNAs during the course of treatment could also be used for monitoring therapeutic response and assessing the degree of residual disease. Raponi et al. show that reduced expression of miR-146b predicted reduced overall survival in patients undergoing radical surgery for lung squamous cell carcinoma (Raponi et al. 2009). Yanaihara et al. also reported that low levels of another miRNA, miR-155, was associated with reduced overall survival after surgical resection (Yanaihara et al. 2006). While altered expression of let-7 was observed in response to radiotherapy, forced over-expression of let-7 in lung cancer cells can sensitize them to radiotherapy in vitro (Weidhaas et al. 2007). Wang et al found that 12 miRNAs expression were significantly different between radiotherapy sensitive and resistant patients (Wang et al. 2011). According to Shin’s report, the expression of miRNA-22 was associated to radiotherapy treatment (Shin et al. 2009). Moreover, miRNAs have also shown to be predictive of response to cancer pharmacological therapy. Galluzzi et al. demonstrated that miR-630 was able to inhibit p53-regulated pro-apoptotic signaling pathways that are specifically induced by cisplatin and carboplatin (Galluzzi et al. 2010). Therapy response prediction based on miRNA expression profiles has been studied in NSCLC cell-lines resistant to apoptosis induced with TRAIL. This study reported that mir-221, -222, -100, -125 and -15b were down-regulated, while miR-9 and -96 showed up-regulation. It has been previously determined that miR-221 and miR-222 contribute to lung cancer resistance to TRAIL through silencing PTEN and TIMP3 tumor-suppressors (Garofalo et al. 2009). Recently, Voortman et al. conducted a large study on 639 patients treated with radically resected for NSCLC stage I–III, and then randomized to receive cisplatin-based adjuvant
chemistry or follow-up in order to assess the prognostic and predictive value of miRNA expression (Voortman et al. 2010). There was no significant association among chemotherapy response and survival of patients, and expression of NSCLC-relevant miRNAs as miR-34a/b/c, -21, -29b, -155 and let-7a and their association with possible targets such as TP53, EGFR and KRAS mutation status and expression of p16. Results from Voortman’s group suggested that just low expression of miR-21 could be a deleterious prognostic factor, but no single or combinatorial miRNA expression profile predicted response to adjuvant cisplatin-based chemotherapy. MiR-21 has been also found up-regulated by EGFR-activating mutations, especially in never-smokers (Seike et al. 2009). Therefore, miRNAs could affect the sensitivity to TKIs and other biological treatments that target EGFR and its downstream effectors. In vitro studies have described a possible role of miRNAs in response to anti-oncogenic treatment. MiR-7A has a role as a strong suppressor of the EGFR pathway; this miRNA is able to target EGFR, RAF1, AKT, and ERK, all of which are key players of this oncogenic network. Additional in vitro studies, have described that miR-128b can regulate EGFR in NSCLC cell lines. According to this, in a study with NSCLC tumors, it has been observed that 55% of cases shown loss of heterozygosity for miR-128b, which was correlated with enhanced clinical response to EGFR-targeted therapy with Gefitinib and survival following treatment (Weiss et al. 2008). Other miRNAs has been described as modulators of EGFR pathways. miR-145 inhibited lung cancer cell growth in patients with EGFR-activating mutations (Cho et al. 2009).

5.3 Circulating miRNAs

Currently, the identification and analysis of biomarkers in fluids as whole blood, serum, plasma or sputum is an attractive area in the study of lung cancer, because it represents a non-invasive method to establish an early and more accurate diagnosis, forecast of therapy response and to determine a prognosis of the disease. In serum from NSCLC patients has been identified a large amount of miRNAs. This finding suggested the potential of miRNAs as biomarkers in body fluids (Gilad et al. 2008). Chen et al. reported a large amount of stable miRNAs and different expression profiles in serum of patients with some kinds of tumors including lung cancer (Chen et al. 2008). Altered expression of 100 miRNAs was identified in serum of NSCLC patients compared to serum from healthy donors. Twenty-eight of them were absent while 63 new miRNAs were identified in the NSCLC patients. The same authors (Hu et al. 2010) showed over-expression of mir-23 and -225; then they report that levels of miR-486, -30d, -1 and -499 were associated with survival in a study with 303 NSCLC patients. In the
present year, Chen et al. validated their previous reports. They used serum from 400 patients and 220 controls to evaluate miRNA expression using Taqman probe-based quantitative RT-PCR. The results showed that 10 miRNAs were differentially expressed between NSCLC patients and control serum samples. This miRNA panel, comprising miR-20a, -24, -25, -145, -152, -199a-5p, -221, -222, -223 and -320, was correlated with the stage of NSCLC in younger patients with current smoking habits, and most importantly, these results suggested that this miRNA panel expression could be a tool for early NSCLC detection (Chen et al. 2012). In another report, Yanaihara et al. identified a 12 miRNA diagnostic signature of NSCLC, including 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 (Yanaihara et al. 2006). These 12 miRNAs were evaluated in the bloodstream of 27 patients with lung adenocarcinoma and 9 healthy controls. Rabinowits et al. elegantly illustrate that circulating exosomal miRNA signatures reflect those of the primary lung tumor, accurately discriminating cancer cases from controls, and thus propose this type of analysis as a potential screening tool for the early detection of lung cancer (Rabinowits et al. 2009). By using microarrays, Liu et al. analyzed the miRNA expression of six paired lung cancer and normal tissues and identified three differentially expressed miRNAs, miR-21, -141, and -200c. High expression of miR-21 and -200c in the tumor and of miR-21 in serum were associated with poor survival in NSCLC patients (Liu et al. 2012). MiRNAs also have been quantified in sputum from NSCLC patients. High levels of miR-21 in 23 sputum samples from NSCLC patients in stage I–IV and 17 controls had a much better cancer sensitivity and specificity compared to its evaluation in sputum cytology (Yu et al. 2010). All of these results suggest that miRNAs as biomarkers in body fluids could be a revolution in clinical management of patients with NSCLC. In Table 2 we summarize all the miRNAs that have been implicated in any clinical factor in lung cancer.

5.4 miRNA-based Cancer Therapy

Anticancer therapy based in miRNAs is currently under investigation. As we know, one miRNA may have several targets, which implies that it could regulate several signaling pathways. Due to promiscuity of miRNAs in the modulation of gene expression, it has been difficult to implement powerful strategies against cancer cells using miRNAs as therapy targets or as pharmacological agents, since it could result in an undesired or unspecific effect on cancer cells. However, there are some advances in miRNAs-based lung cancer therapy. An approach to the direct manipulation of miRNAs for therapeutic effect could be through knockdown using
<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Site of detection</th>
<th>Expression and diagnosis in lung cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21, miR-141, and miR-200c</td>
<td>Primary tumor</td>
<td>↑Lung cancer</td>
<td>Liu et al. 2012</td>
</tr>
<tr>
<td>hsa-miR-205</td>
<td>Primary tumor</td>
<td>↑Squamous cell lung carcinoma</td>
<td>Lebanony et al. 2009</td>
</tr>
<tr>
<td>hsa-let-7g, hsa-let-7b, hsa-let-7c, hsa-miR-29a, hsa-let-7f, hsa-let-7f, hsa-miR-98, hsa-let-7f, hsa-miR-26a, hsa-miR-30b, hsa-miR-146b-5p, hsa-miR-106b, hsa-let-7a, hsa-mir-663, hsa-mir-30d, hsa-mir-17, hsa-miR-498*, hsa-miR-26b, hsa-let-7e, hsa-mir-654-5p*, hsa-mir-181a, hsa-miR-103, hsa-miR-195, hsa-miR-191</td>
<td>Primary tumor</td>
<td>↑Adenocarcinoma vs. squamous cell lung carcinoma</td>
<td>Landi et al. 2010</td>
</tr>
<tr>
<td>hsa-miR-453*, hsa-miR-509-3p</td>
<td>Primary tumor</td>
<td>↓Adenocarcinoma vs. squamous cell lung carcinoma</td>
<td>Landi et al. 2010</td>
</tr>
<tr>
<td>hsa-miR-675, hsa-miR-93*, hsa-miR-1224-3p</td>
<td>Primary tumor</td>
<td>↑Lung cancer vs. COPD</td>
<td>Leidinger et al. 2011</td>
</tr>
<tr>
<td>hsa-miR-513b</td>
<td>Primary tumor</td>
<td>↓Lung cancer vs. COPD</td>
<td>Leidinger et al. 2011</td>
</tr>
<tr>
<td>mir-7, mir-21, mir-200b, mir-210, mir-219-1, mir-324</td>
<td>Primary tumor</td>
<td>↑Lung cancer</td>
<td>Boeri et al. 2011</td>
</tr>
<tr>
<td>miRNA(s)</td>
<td>Sample Type</td>
<td>Change</td>
<td>Stage/Prognosis</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------------</td>
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<td>------------------------------------------------------</td>
</tr>
<tr>
<td>miR-126, mir-451, mir-30a, and mir-486</td>
<td>Primary tumor</td>
<td>↑</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>miR-128b, miR-152, miR-125b, miR-205, miR-27a, miR-146a, miR-222, miR-23a, miR-24, miR-150</td>
<td>Serum</td>
<td>↑</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>miR-20a, miR-24, miR-25, miR-145, miR-132, miR-199a-5p, miR-221, miR-222, miR-223, miR-320</td>
<td>Serum</td>
<td>↑</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>hsa-miR-1254 and hsa-miR-574-5p</td>
<td>Serum</td>
<td>Early-stage NSCLC</td>
<td>Foss et al. 2011</td>
</tr>
<tr>
<td>miRNA-21, miRNA-126, miRNA-210, miRNA-486-5p</td>
<td>Serum</td>
<td>↑</td>
<td>Stage I NSCLC (lung adenocarcinomas)</td>
</tr>
<tr>
<td>hsa-miR-140-3p, hsa-miR-22</td>
<td>Serum</td>
<td>↑</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>hsa-let-7d, hsa-miR-106b, hsa-miR-98, hsa-miR-126</td>
<td>Serum</td>
<td>↓</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>miR-21, miR-375, miR-200b</td>
<td>sputum</td>
<td>↑</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>miR-486</td>
<td>sputum</td>
<td>↓</td>
<td>Lung cancer</td>
</tr>
<tr>
<td>miR-205, miR-210, miR-708</td>
<td>sputum</td>
<td>↓</td>
<td>Lung cancer</td>
</tr>
</tbody>
</table>

**Prognosis**

<table>
<thead>
<tr>
<th>miRNA(s)</th>
<th>Sample Type</th>
<th>Change</th>
<th>Stage/Prognosis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>microRNA-34b/c</td>
<td>Primary tumor</td>
<td>↓</td>
<td>Predict recurrence and poor survival (stage I NSCLC)</td>
<td>Wang et al. 2011</td>
</tr>
<tr>
<td>miR-129-5p, miR-194*, miR-631, miR-200b*, miR-585, miR-623, miR-617, miR-622, miR-638, miRPlus _27560</td>
<td>Primary tumor</td>
<td>↓</td>
<td>Predict recurrence (stage I NSCLC after resection)</td>
<td>Patnaik et al. 2010</td>
</tr>
</tbody>
</table>

*Table 2. contd...*
<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Site of detection</th>
<th>Expression and diagnosis in lung cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-24, miR-141, miR-27b, miR-16, miR-21, miR-30c, miR-106a, miR-15b, miR-23b, miR-130a</td>
<td>Primary tumor</td>
<td>↑ Predict recurrence (stage I NSCLC after resection)</td>
<td>Patnaik et al. 2010</td>
</tr>
<tr>
<td>miR-21</td>
<td>Primary tumor</td>
<td>↓ Poor survival (adjuvant chemotherapy after complete resection)</td>
<td>Voortman et al. 2010</td>
</tr>
<tr>
<td>miR-146b</td>
<td>Primary tumor</td>
<td>↑ Poor survival</td>
<td>Raponi et al. 2009</td>
</tr>
<tr>
<td>hsa-mir-155, has-mir-213</td>
<td>Primary tumor</td>
<td>↑ Poor survival</td>
<td>Yanaihara et al. 2006, Volinia et al. 2006</td>
</tr>
<tr>
<td>hsa-let-7a-2</td>
<td>Primary tumor</td>
<td>↓ Poor survival</td>
<td>Yanaihara et al. 2006, Takamizawa et al. 2004, Yu et al. 2008</td>
</tr>
<tr>
<td>hsa-miR-221</td>
<td>Primary tumor</td>
<td>↓ Poor survival</td>
<td>Yu et al. 2008</td>
</tr>
<tr>
<td>hsa-miR-137, hsa-miR-372, and hsa-miR-182*</td>
<td>Primary tumor</td>
<td>↑ Poor survival</td>
<td>Yu et al. 2008</td>
</tr>
<tr>
<td>miR-34b/c</td>
<td>Primary tumor</td>
<td>↑ Metastasis</td>
<td>Lujambio et al. 2008, Watanabe et al. 2011</td>
</tr>
<tr>
<td>hsa-miR-329-1-pre, hsa-miR-326-pre, hsa-miR-495-pre, hsa-miR-500*, hsa-miR-326, hsa-miR-370, hsa-miR-218, hsa-miR-330-3p, hsa-miR122a, hsa-miR-325, hsa-miR-489-pre, hsa-miR-599, hsa-miR-328, hsa-miR-329-2-pre, hsa-miR-346, hsa-miR-650-Pre, hsa-miR-193-b-pre, hsa-miR-103</td>
<td>Primary tumor</td>
<td>↑ Brain metastasis</td>
<td>Anora et al. 2011</td>
</tr>
<tr>
<td>hsa-miR-92a</td>
<td>Primary tumor</td>
<td>↓ Brain metastasis</td>
<td>Anora et al. 2011</td>
</tr>
<tr>
<td>miR-374a</td>
<td>Primary tumor</td>
<td>↓ Poor survival (early-stage NSCLC)</td>
<td>Voşa et al. 2011</td>
</tr>
<tr>
<td>miR-486, miR-30d</td>
<td>Serum</td>
<td>↑ Poor survival</td>
<td>Hu et al. 2010</td>
</tr>
<tr>
<td>miR-1, mir-499</td>
<td>Serum</td>
<td>↓ Poor survival</td>
<td>Hu et al. 2010</td>
</tr>
<tr>
<td>mir-27b; mir-106a, mir-19b, mir-15b mir-16, mi-21</td>
<td>Serum</td>
<td>↑ Recurrence in stage I NSCLC</td>
<td>Boeri et al. 2011</td>
</tr>
<tr>
<td>miRNA</td>
<td>Sample Type</td>
<td>Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------</td>
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<td>---------------------------------------------</td>
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</tr>
<tr>
<td>mir-221, mir-222</td>
<td>Serum</td>
<td>↑ Lung cancer aggressiveness</td>
<td>Boeri et al. 2011</td>
</tr>
<tr>
<td>mir-21</td>
<td>Serum</td>
<td>↑ Node metastasis and poor survival (advanced clinical stage of NSCLC)</td>
<td>Liu et al. 2012</td>
</tr>
</tbody>
</table>

### Therapy Response

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Sample Type</th>
<th>Effect to therapy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-22</td>
<td>Primary tumor</td>
<td>↓ Response to radiotherapy</td>
<td>Wang et al 2011</td>
</tr>
<tr>
<td>miRNA-126, miRNA-let-7a, miRNA-495, miRNA-451, miRNA-128b</td>
<td>Primary tumor</td>
<td>↑ Response to radiotherapy</td>
<td>Wang et al 2011</td>
</tr>
<tr>
<td>miRNA-130a, miRNA-106b, miRNA-19b, miRNA-22, miRNA-15b, miRNA-17-3p and miRNA-21</td>
<td>Primary tumor</td>
<td>↓ Response to radiotherapy</td>
<td>Wang et al 2011</td>
</tr>
<tr>
<td>miR-181a</td>
<td>Primary tumor</td>
<td>↓ Resistance to CDDP</td>
<td>Galluzzi et al. 2010</td>
</tr>
<tr>
<td>miR-630</td>
<td>Primary tumor</td>
<td>↑ Resistance to CDDP</td>
<td>Galluzzi et al. 2010</td>
</tr>
<tr>
<td>miR-128b</td>
<td>Primary tumor</td>
<td>↓ Response to gefitinib</td>
<td>Weiss et al. 2008</td>
</tr>
<tr>
<td>miR-221, miR-222</td>
<td>Primary tumor</td>
<td>↑ TRAIL resistance</td>
<td>Garofalo et al. 2009</td>
</tr>
<tr>
<td>miR-662</td>
<td>Primary tumor</td>
<td>↑ Resistance to AZ628, Erlotinib, Geldanamycin, G’o- 6976, HKI-272 (Neratinib), and MK-0457</td>
<td>Enfield et al. 2011</td>
</tr>
<tr>
<td>hsa-mir-10b</td>
<td>Primary tumor</td>
<td>↑ Resistance to MG-132</td>
<td>Enfield et al. 2011</td>
</tr>
<tr>
<td>hsa-mir-193b</td>
<td>Primary tumor</td>
<td>↑ Resistance to AZ628, MK-0457</td>
<td>Enfield et al. 2011</td>
</tr>
<tr>
<td>hsa-mir-328</td>
<td>Primary tumor</td>
<td>↑ Resistance to Geldanamycin</td>
<td>Enfield et al. 2011</td>
</tr>
<tr>
<td>hsa-mir-628</td>
<td>Primary tumor</td>
<td>↑ Resistance to PF-Z41066</td>
<td>Enfield et al. 2011</td>
</tr>
</tbody>
</table>

Up-regulation, Down-regulation. COPD, chronic obstructive pulmonary disease; NSCLC, non-small cell lung cancer; CDDP, cis-diamminedichloroplatinum.
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antisense oligonucleotides or through administration of synthetic miRNA analogues focused on enhancing traditional or standard cancer treatments. Several studies suggest that re-expression of specific down-regulated miRNAs in cancer cells could have a therapeutic benefit by stopping or even reversing tumor growth (Cho et al. 2012). One of most convincing evidences for miRNA replacement, as a strategy for cancer therapy is the manipulation of the tumor suppressor let-7 levels in lung cancer models (Barh et al. 2010). Loss of let-7 induces tumor formation and growth in various murine models of human lung cancer, this tumor-inducing effect appears to be carried out, at least partially, through the loss of regulation on oncogenes RAS and MYC (Johnson et al. 2005). Intratumoral injection of synthetic let-7 miRNA in a mouse model of lung cancer has been shown to reduce tumor burden (Kumar et al. 2008). Intranasal administration of let-7 in a KRAS-mutant mouse model of lung cancer effectively reduced tumor growth. Exogenous delivery of a synthetic let-7 mimic has been used to mediate remission of established NSCLC tumors in mice (Esquela-Kerscher et al. 2008).

In vitro assays of radiation-induced cell death in lung cancer cells overexpressing members of the let-7 family of miRNAs, result in increased sensitivity to radiation therapy whereas decreasing let-7 levels induce a state of radioresistance (Weidhaas et al. 2007). Despite these encouraging results, the direct application of let-7 as a therapeutic agent for cancer is premature as yet, given that details of the immunogenic and cytotoxic effect of let-7 remain to be explored. Despite advances in the study of let-7 as a therapeutic agent in lung cancer, there are some others miRNAs that have been explored. For instance, miR-29 family members have been shown to reduce tumorigenic potential in a lung cancer model (Fabbri et al. 2007). Advances in understanding the involvement of miRNAs in lung cancer biology have allowed to establish the functions of miRNAs as let-7, which is useful as basis for implementing miRNA-based lung cancer therapy with promising results. All of these works suggest that the use of miRNAs as anti-cancer agents could be a promising strategy for new treatments of NSCLC and other malignances.

6. CONCLUSIONS

Lung cancer is one of the most aggressive tumor types in worldwide. Current research focuses on study of NSCLC in order to have better diagnostic methods and therapeutic strategies. The knowledge of genomic and epigenetic modifications in NSCLC have permitted the identification of some molecules than play an important role in the carcinogenesis process of
lung cells, which implies that the known complexity of lung carcinogenesis is increasing. MiRNA functions have been associated to biological cell processes such as proliferation, DNA repair, cell death and angiogenesis. Genomic modifications and aberrant expression of miRNAs are phenomena that underlie the modified gene expression and protein translation observed in lung cancer cells. Because of their implication in lung carcinogenesis, miRNAs might represent good biomarkers for NSCLC, therefore they are emerging as intriguing and potentially powerful candidates in the arsenal to combat this kind of tumors. Analysis of miRNAs might represent a new strategy for better and more efficient diagnostic, prognostic and therapeutic approaches that decrease mortality and morbidity of lung cancer patients.

7. SUMMARY POINTS

- NSCLC is one of the tumors with the highest incidence and mortality in worldwide.
- MiRNAs are evolutionarily conserved small noncoding RNAs that negatively regulate gene expression at the post-transcriptional level.
- MiRNAs have differential spatial and temporal expression in cancer cells, which is related to several clinical, biological, molecular and genomic features of tumors.
- The study of expression profiles, polymorphisms and epigenetic modifications of miRNAs in NSCLC have permitted an understanding of the regulation of gene expression as a molecular mechanism underlying the tumoral etiology.
- MiRNAs tend to be located in fragile chromosomal regions that are susceptible to translocations, microdeletions and amplifications; moreover, they are enclosed within or near the CpG islands of the human genome and are potentially subject to control by DNA methylation.
- MiRNA expression may be used in clinical practice as a marker for lung tumor classification, metastatic behavior and survival outcome of NSCLC patients. Moreover, miRNAs themselves could represent new pharmacological targets.
- Several miRNAs as let-7, mir-34 and mir-29 have been validated as clinically significant in lung cancer.
- MiRNAs might represent good biomarkers for NSCLC, therefore they are emerging as intriguing and potentially powerful candidates in the arsenal to combat this kind of tumors.
ACKNOWLEDGEMENTS

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ABBREVIATIONS

Ago : Argonaute
bp : Base pairs
dsRNA : Double strand RNA
COPD : Chronic obstructive pulmonary disease
CDDP : Cis-diamminedichloroplatinum
EGFR : Epidermal growth factor receptor
EMT : Epithelial mesenchymal transition
HER-2 : Human Epidermal Receptor 2
HIF : Hypoxic inductor factor
miRNAs : MicroRNAs
miRISC : MiRNA-induced silencing complex
nt : Nucleotide
NSCLC : Non-small cell lung cancer
OncomiRNAs : Oncogenic miRNAs
pre-miRNAs : Precursor miRNAs
pri-miRNAs : Primary miRNA transcripts
SCLC : Small cell lung carcinoma
SNP : Single nucleotide polymorphism
TRBP : Transactivator RNA-binding protein
TKI : Tyrosine kinase inhibitor
UTR : Untranslated region

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