Clin Chem Lab Med 2011;49(10):xxx-xxx © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.661

Review

Prognostic, therapeutic and diagnostic potential of microRNAs in non-small cell lung cancer

Athina Markou[1], Yu Liang[2,*] and Evi Lianidou[1,*]

[1] Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Athens, Greece
[2] Genomic Assays – R&D, Life Technologies, Foster City, CA, USA

Abstract

Non-small cell lung carcinomas (NSCLC) account for about 80% of lung cancers and their remarkable heterogeneity manifests in histology, pathogenesis, prognosis, and response to treatments. Recent advances in molecular characterization help stratifying NSCLC patients for their potential benefit from targeting therapies. However, the fundamental mechanisms underlying the tumoral heterogeneity remain poorly understood. Expression profiling of many microRNAs (miRNAs) in various normal and disease tissues demonstrated unique spatial and temporal expression patterns and some miRNAs have been functionally characterized as oncogenes or tumor suppressor genes. Genome-wide screening identified specific miRNA expression signatures associated with clinical outcome of NSCLC patients. A group of miRNAs that has enriched expression in normal lung was found down regulated in NSCLC and may function as tumor suppressor genes. In this review we: a) summarize the current understanding of the critical role that miRNAs play in normal cell functions and in disease biology especially in lung cancer tumorigenesis, b) highlight their potential as biomarkers for lung cancer risk stratification, outcome prediction and classification of histologic subtypes, c) critically assess current knowledge on lung-enriched miRNAs and expression of their predicted target genes in NSCLC and d) evaluate their potential as circulating biomarkers and therapeutic targets in lung cancer.

Keywords: diagnosis; microRNAs; non-small cell lung cancer; prognosis; tumor biomarkers.

Introduction

Lung cancer is the leading cause of cancer-related death in both males and females worldwide. There are an estimated 222,520 new cases and 157,300 deaths from lung cancer in the United States in 2010 (1). Despite years of research, the prognosis for patients with lung cancer remains dismal. Lung cancers are classified according to the histological types and this classification has important implications for the clinical management and prognosis of the disease (2). There are two main histological groups of lung cancer including non-small-cell lung cancer (NSCLC, 85%) and small cell lung cancer (SCLC, 15%). Non-small cell lung carcinomas (NSCLC) comprises three major histological subtypes: adenocarcinoma (AD), squamous cell carcinoma (SCC), and large cell carcinoma (LCC) (3).

Standard treatment strategies include surgical resection followed by radiation and/or chemotherapy. Chemotherapy is usually palliative rather than curative due to resistance (4) so more effective systemic therapy is in urgent need. The disease is usually diagnosed at advanced stages when the prognosis is poor. When the disease is at earlier stages, the clinical behavior of each histological subtype appears to be different. In a retrospective study on 1119 completely resected stages I and II NSCLC patients, five-year survival is between 30% and 54% in general, but the AD patients had a significantly better survival than the non-AD patients in stage I, whereas the SCC patients had a better survival than the non-SCC patients in stage II (5).

Over the past decade, it has become evident that subsets of lung cancer, particularly those with AD histology, can be defined at the molecular level by mutations. Because the presence or absence of such mutations can heavily influence treatment outcomes in cases of targeted therapy, genetically informed lung cancer medicine that involves the prospective genotyping of lung cancers is becoming a new standard of care. The use of tyrosine kinase inhibitors to target the epidermal growth factor receptor (EGFR) in patients with NSCLC is effective but limited by the emergence of drug-resistance mutations. The identification of mutations in the EGFR has changed how clinicians approach certain groups of individuals with lung cancer. Mutations in the EGFR tyrosine kinase in NSCLCs can cause oncogenic transformation and change the level of sensitivity to tyrosine kinase inhibitors, such as gefitinib and erlotinib (6, 7) while KRAS mutations are negative predictors of radiographic response to these EGFR inhibitors. In a recent study it was shown that first-line gefitinib treatment for patients with advanced NSCLC who were selected on the basis of EGFR mutations improved progression-free survival, with acceptable toxicity as compared with standard chemotherapy (8).

Various molecular factors have been evaluated as prognosis biomarkers including markers of nucleotide excision

*Corresponding authors: Yu Liang, Genomic Assays – R&D, Life Technologies, Foster City, CA 94404, USA
E-mail: Yu.Liang@lifetech.com
Evi Lianidou, Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, 15771, Athens, Greece
E-mail: lianidou@chem.uoa.gr
Received March 17, 2011; accepted June 2, 2011

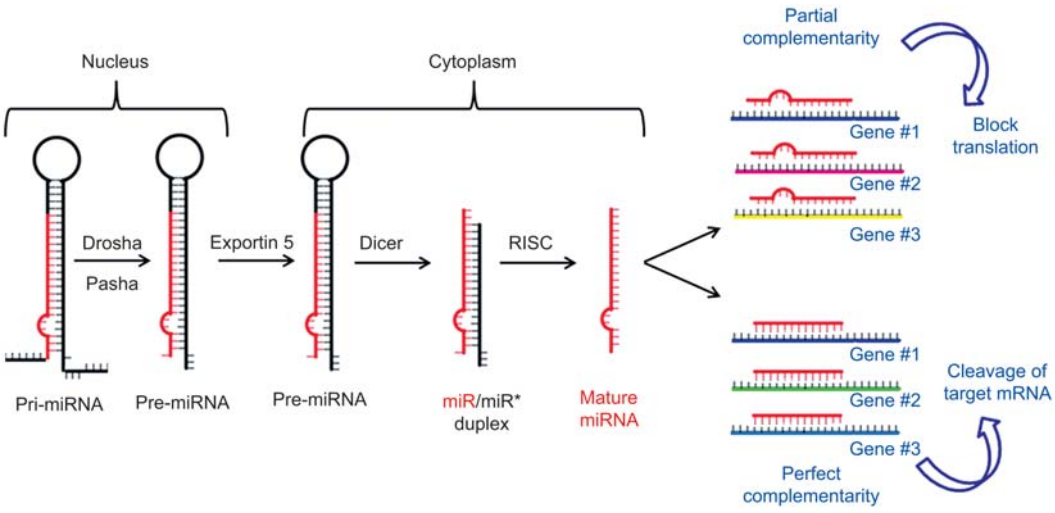

Figure 1 miRNAs biogenesis and way of action.

repair pathway (ERCC1, RRM1, BRCA1), tumor proliferation, cellular adhesion and cellular growth (RAS, RB and EGFR) or apoptosis (TP53 and BCL-2) which are involved in NSCLC carcinogenesis (9–11). Genome and proteome analyses have demonstrated that focusing on molecular heterogeneity within lung cancers may be a viable approach towards the development of novel therapeutics.

Recent emerging evidence suggests that microRNAs (miRNAs) have the potential to regulate translation in a cell cycle-dependent manner, which opens new horizons in advancing our understanding of cancer at the molecular level (12). Changes in the miRNA expression level have been detected in many human tumor types, and recent studies have demonstrated the critical roles of miRNAs in cancer pathogenesis. In this review we: a) summarize the current understanding of the critical role that miRNAs play in normal cell functions and in disease biology especially in lung cancer tumorigenesis, b) highlight their potential as biomarkers for lung cancer risk stratification, outcome prediction and classification of histologic subtypes, c) critically assess current knowledge on lung-enriched miRNAs and expression of their predicted target genes in NSCLC and d) evaluate their potential as circulating biomarkers and therapeutic targets in lung cancer.

Critical roles of microRNAs in normal cell functions and in disease biology

MicroRNAs (miRNAs) are small non-coding, 18 to 25 nucleotide-long, naturally occurring RNA molecules that post-transcriptionally modulate gene expression (13) that were first identified in *C. elegans* (14). miRNAs by binding to the 3′-untranslated region (3'-UTR) of target miRNAs can cause translational repression (15) or degradation (16). There are currently at least 1000 loci encoding miRNAs in humans (17). Some miRNAs may have as many as a few thousand targets and bioinformatics data indicate that miRNAs have the potential to regulate at least 20%–30% of human genes. The biogenesis of miRNA is a multistep process beginning in the nucleus and culminating in the cytoplasm and involves numerous enzymes and accessory proteins. Within the nucleus, a long primary (pri)-miRNA transcript ranging from hundreds to thousands of nucleotides in length is transcribed by RNA polymerase II (18) and the processing of this pri-miRNA to a smaller stem loop, of approximately 70-nucleotide precursor (pre)-miRNA molecules is facilitated by RNAse III endonuclease (19). This pre-miRNA is subsequently processed in the cytoplasm to form the final active form of mature miRNA (Figure 1).

miRNAs are involved in a myriad of biological processes, including proliferation, apoptosis, metabolism, differentiation, and epithelial-mesenchymal-transition (EMT). Examples include miR-273 and the miRNA encoded by lys-6 are involved in patterning the *C. elegans* nervous system (20, 21), miR-181 in the differentiation of mammalian pancreatic cell development and the regulation of insulin secretion (22), miR-1 that is involved in mammalian heart development (23), miR-375 which regulates pancreatic insulin secretion (22), miR-181 which influences the differentiation of hematopoietic cells toward the B-cell lineage (24), and miR-430 which is regulated for zebra fish brain development (25). MicroRNAs are also involved in stem cell division and development, and as Hatfield et al. have shown the miRNA pathway might be part of a mechanism that makes stem cells insensitive to environmental signals that normally stop the cell cycle at the G1/S transition (26). Further characterization of miRNAs might reveal other gene regulators that coordinate proper organ formation, embryonic patterning and body growth, and might also provide insight into the mechanisms of human diseases, such as cancer. Just as miRNAs are important in the normal functioning of cells, a dysfunction of the miRNA regulation system would result in disruption of normal cell functions and cause diseases as well.

Changes in the miRNA expression level have been detected in many human tumor types, and recent studies have dem-

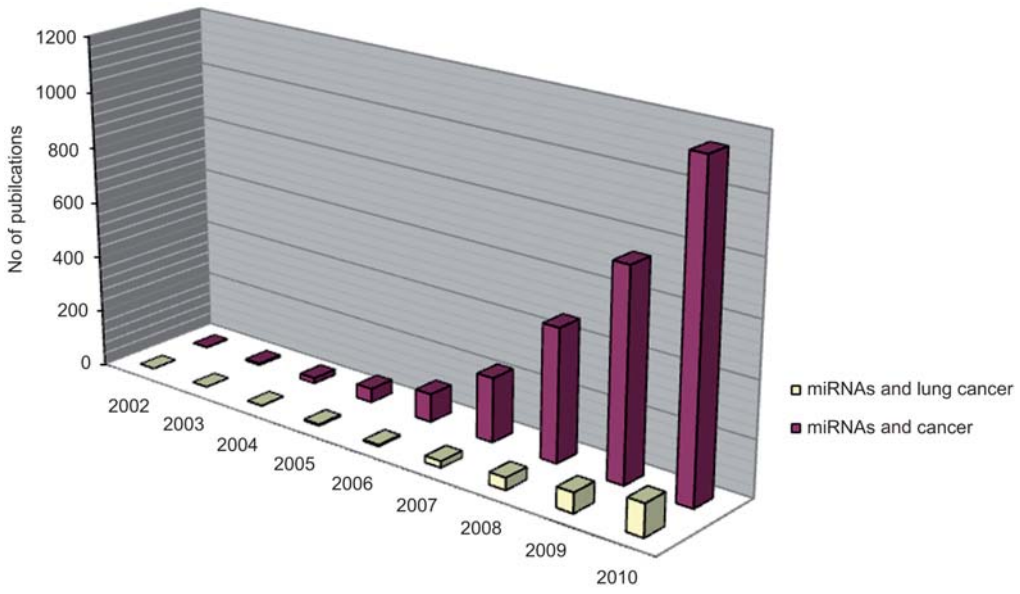

Figure 2 Publications on miRNAs in cancer and NSCLC (PubMed, keywords: microRNAs, cancer, non-small cell lung cancer).

onstrated the critical roles of miRNAs in cancer pathogenesis (27–30). Calin et al. first showed that miR-15a and miR-16-1, located in the fragile chromosomal region 13q14, were frequently under-expressed in patients with chronic lymphocytic leukemia (CLL) (31), and they further determined that both miRNAs are likely to function as tumor suppressors in CLL. Hayashita et al. found that miR-17-92 was markedly overexpressed in lung cancer, especially in SCLC (32). Chang et al. showed that miR-34a is frequently absent in pancreatic cancer cells (33). MiR-21 is overexpressed in six types of cancer including breast, lung, gastric and prostate (34) and let-7 miRNA family negatively regulates the RAS oncogenes and down regulation of let-7 expression is a characteristic of NSCLC (35). At the present time the main mechanism that underlies changes in the function of miRNAs in cancer cells seems to be aberrant gene expression, characterized by abnormal levels of expression for mature and/or precursor miRNAs compared to the corresponding normal tissues.

miRNA profiling in most types of tumors has shown significant different miRNA profiles when compared to normal cells from the same tissue. A systematic analysis of 334 leukemias and solid cancers has shown that miRNA-expression profiles can classify human cancers according to the lineage and differentiation state of the tumors; in particular, miRNA expression in tumors was found globally lower than in corresponding normal tissues (36). Another signature of up-regulated 21 miRNAs common to at least three tumor types was described by Volinia et al. (34). Because of the limited sample size and experimental expense, the statistical power of individual research projects is not sufficient to yield a robust conclusion. However, collected microarray datasets of expression profiles provide opportunities to compile the information of individual studies. A recent meta-analysis of miRNA expression microarray datasets from 28 published tumor studies has comprised 33 comparisons and nearly 4000 tumor and corresponding non-tumoral samples and reported 52 miRNAs as common signatures that are deregulated in tumors. According to this study, in addition to the commonly altered miRNAs, five solid cancers displayed specific tissue patterns of altered miRNAs as well. This meta-analysis also revealed some novel tumor-related miRNAs, such as miR-144, miR-130b, miR-132, miR-154, miR-192, and miR-345 (37).

Tellez et al. have studied the role of EMT and epigenetic silencing through DNA methylation of tumor suppressive microRNAs, such as miR-200b, miR-200c, and miR-205, which were implicated in the dedifferentiation program in primary lung tumors after exposure to tobacco carcinogens (38). Melo et al. suggest that a cancer-specific mechanism guides the subcellular distribution of miRNA precursors and prevent them from being processed to the active mature miRNA. Controlling the miRNA biosynthesis pathway is emerging as an important mechanism in defining the spatiotemporal pattern of miRNA expression in cancer cells (39).

miRNAs as biomarkers in NSCLC

Recent data from multiple studies strongly support the potential of microRNAs as biomarkers in NSCLC. There is increasing evidence in the recent literature (Figure 2) that altered microRNA expression is associated with tumor progression and survival in lung cancer patients.

The let-7 family

The let-7 family is a cluster of miRNAs whose genes map to different chromosomal regions that are frequently deleted in lung cancer (40). Reduced let-7 gene expression in NSCLC patients has been correlated with poor prognosis (41, 42),

suggesting a role of this miRNAs cluster as tumor suppressors. In addition, let-7 can negatively regulate multiple oncogenes, including RAS (35), MYC (43), HMGA2 (44) and cyclins (45). A single nucleotide polymorphism in let-7 complementary site 6 of the KRAS mRNA 3′-UTR has been found significantly associated with an increased risk of NSCLC (46). High expression of miR-155 and low expression of let-7a-2 were strongly associated with poor survival of patients with lung AD (42). Using quantitative RT-PCR-based analysis, poor prognosis was shown associated with reduced let-7 and miR-221 expression and increased levels of miR-137, miR-372 and miR-182 (47). Epigenetic factors regulate miRNAs expression as well. For example, let-7a-3 is found heavily methylated in normal lung cells, but hypomethylated and expressed in a subset of lung AD (48).

miR-17-92, miR-126, miR-125a and miR-206

miR-17-92 acts as an oncogene and may be a potential therapeutic target in lung cancer. miR-17-92 overexpression is associated with retinoblastoma (RB) inactivation (49). miR-17-92 is also involved in regulation of angiogenesis. Vascular Endothelial Growth Factor (VEGF) induces miR-17-92 expression in endothelial cells (50). Disruption of miR-17-92 clusters was shown to cause lethal abnormalities, including lung hypoplasia, ventricular septal defects and inhibition of B cell development (51). The 3'-UTR of VEGF mRNA has a binding site for miR-126; expression of miR-126 was down regulated in eight lung cancer cell lines, and may alter lung cancer cell invasive capacity and growth by targeting Crk (52, 53). miR-126 overexpression was also detected in metastatic vs. primary tumor in a study using miRNA microarray in formalin-fixed paraffin-embedded (FFPE) tissues (54). Recently, miR-125a and miR-206 have been shown to associate with invasive and metastatic capabilities of various lung cancer cell lines (55, 56).

The miR-29 family

Among the reported down-regulated miRNAs in lung cancer, the miR-29 family (29a, 29b, and 29c) has intriguing complementarities to the 3'-UTRs of DNA methyltransferase (DNMT)-3A and -3B, two key enzymes involved in DNA methylation that are frequently up regulated in lung cancer and associated with poor prognosis. It was found that the expression of miR-29s is inversely correlated to DNMT3A and -3B in lung cancer tissues, and that miR-29s directly target both DNMT-3A and -3B. Enforced expression of miR-29s in lung cancer cell lines restores normal patterns of DNA methylation, induces re-expression of methylation-silenced tumor suppressor genes and inhibits tumorigenicity in vitro and in vivo. These findings support a role of miR-29s in the epigenetic normalization of NSCLC, providing a rationale for the development of miRNA-based strategies for the treatment of lung cancer (57).

miR-21

miR-21 is overexpressed in several solid malignancies including breast and lung cancer (58). Inhibition of miR-21 in a breast cancer cell line has a subtle effect on cell growth both in vitro and in vivo but significantly reduces invasion and lung metastasis in animals (59). Overexpression of mature miR-21 was shown to be an independent negative prognostic factor for overall survival in NSCLC patients (60). Changes in the expression of mature miR-21 are more remarkable in the presence of EGFR mutations and miR-21 post-transcriptionally down-regulates the expression of the tumor suppressor PTEN and subsequently stimulates growth and invasion in NSCLC (61). Aberrantly increased expression of miR-21, which is enhanced further by the activated EGFR signaling pathway, plays a significant role in lung carcinogenesis in never-smokers, as well as in smokers, and is a potential therapeutic target in both EGFR-mutant and wild-type cases (62). There seems to be an intricate balance between EGFR and miR-7 under tissue-specific context, in that EGFR promotes lung tumorigenesis by activating miR-7 expression (63), whereas miR-7 suppressed EGFR expression and functions in glioblastoma as a tumor suppressor gene (64). Finally, miR-21 drives tumorigenesis through inhibition of negative regulators of the RAS/MEK/ERK pathway and inhibition of apoptosis (65). Very recently Saito et al. have shown that increased miR-21 expression is associated with disease progression and survival in stage I lung cancer. This suggests that expression of miR-21 may contribute to lung carcinogenesis and serve as a therapeutic target or early stage prognostic biomarker for lung adenocarcinoma (66).

The miR-34 family

This family is composed of miR-34a, miR-34b, and miR-34c that are part of the p53 network and their expression is directly induced by p53 in response to DNA damage or oncogenic stress (67). MiR-34a is lost or down-regulated in many tumors (68, 69), and in vitro miR-34a overexpression leads to decreased proliferation and activation of apoptosis in multiple tumor cell types (70–72), indicating a role for miR-34a as a tumor suppressor gene. The miR-34 family is down-regulated in NSCLC when compared to normal tissues and NSCLC patients with low miR-34a expression have a higher risk of relapse (73).

miR-221, miR-222 and miR-210

miR-221 and miR-222 are overexpressed in aggressive NSCLC by targeting PTEN and TIMP3 tumor suppressors, induce TRAIL assistance and enhance cellular migration through the activation of the AKT pathway and matrix metalloproteinases (74), making these two miRNAs promising therapeutic targets or diagnostic tools for TRAIL resistance in NSCLC (75). miR-210 is overexpressed in late stages of lung cancer and mediated mitochondrial alterations associated with modulating activity of hypoxia-inducible factor-1 (76).

miR-451

Very recently, Wang R et al. analyzed the miRNA expression profiles in NSCLC by use of a miRNA microarray platform

and identified 40 differentially expressed miRNAs. They showed that miRNA (miR)-451 was the most downregulated in NSCLC tissues. The expression level of miR-451 was found to be significantly correlated with tumor differentiation, pathological stage and lymph-node metastasis. Moreover, low miR-451 expression level was also correlated with shorter overall survival of NSCLC patients ($P < 0.001$). Their findings suggest that miR-451 regulates survival of NSCLC cells partially through the downregulation of RAB14. They propose that targeting with the miR-451/RAB14 interaction might serve as a novel therapeutic application to treat NSCLC patients (77).

A recent study by Voortman et al., determined whether expression levels of a panel of biologically relevant microRNAs can be used as prognostic or predictive biomarkers in patients who participated in the International Adjuvant Lung Cancer Trial (IALT), the largest randomized study conducted to date of adjuvant chemotherapy in patients with radically resected non-small cell lung carcinoma (NSCLC). Expression of miR-21, miR-29b, miR-34a/b/c, miR-155, and let-7a was determined by quantitative real-time PCR in FFPE tumor specimens from 639 IALT patients. No significant association was found between any of the tested microRNAs and survival, with the exception of miR-21 for which a deleterious prognostic effect of lowered expression was suggested. Otherwise, no single or combinatorial microRNA expression profile predicted response to adjuvant cisplatin-based chemotherapy. Results indicated that the microRNA expression patterns examined were neither predictive nor prognostic in a large patient cohort with radically resected NSCLC, randomized to receive adjuvant cisplatin-based chemotherapy vs. follow-up only (78).

Despite undergoing curative resection, nearly a third of patients with stage I NSCLC die of recurrent disease. There are no reliable clinical or molecular predictors of relapse in patients with resected stage I NSCLC. Identifying patients at

Table 1 Prognostic significance of miRNAS in NSCLC tissues.

Tested miRNAs	Patients	Cancer type	Differently expressed miRNAs	Prognostic significance	References
Technology used: microarray platform					
All human miRNAs	363	Breast, lung, stomach, colon cancer	Up-regulated: miR-21, miR-17-5p, miR-191	Not evaluated	(34)
	177	Normal tissues	Down-regulated: miR-128, miR-155		
All human miRNAs	104	Pairs of NSCLC	Up-regulated: miR-155	OS ($p = 0.006$)	(42)
			Down-Regulated: let-7a-2	OS ($p = 0.033$)	
700 mature miRNAs	33	Primary lung	Up-regulated: miR-182	Not evaluated	(54)
	43	Metastatic lung	Up-regulated: miR-152		
All human miRNAs	62	Squamous carcinoma	miR-205 as biomarker of squamous carcinoma	Not evaluated	(84)
	60	Adenoma carcinoma			
713 mature miRNAs	125	Squamous carcinoma	Down-regulated: miR-29a, let-7b	Yes	(85)
	165	Adenoma carcinoma	Up-regulated: miR-21, miR-26a		
All human miRNAs	8	Pairs of NSCLC	Down-regulated: miR-181a, miR-143	OS (miR-181a: $p = 0.050$ and miR-143: $p = 0.386$)	(80)
			Up-regulated: miR-21	OS ($p = 0.002$)	
All human miRNAs	23	Pairs of NSCLC	Up-regulated: miR-451	OS ($p < 0.001$)	(77)
Technology used: real-time RT-qPCR					
let-a	143	NSCLC tissues	Down-regulation	OS ($p = 0.0003$)	(42)
157 mature human microRNAs	112	NSCLC tissues	miR-221, let-7a, miR-137, miR-372, miR-182	OS ($p = 0.026$) DFI ($p = 0.024$)	(47)
miR-21 and miR-205	48	Pairs of NSCLC	Up-regulated: miR-21	OS ($p = 0.027$)	(60)
miR-21	20	Pairs of NSCLC	Overexpression		(61)
miR-34 family	70	Pairs of NSCLC	Down-regulation	DFI ($p = 0.039$)	(73)
miR-17, miR-21,	317	NSCLC tissues	Overexpression: miR-21	Yes	(66)
miR-21 and miR-205	25	Adenoma carcinoma	Down-regulated: miR-205	Not evaluated	(88)
	24	Squamous carcinoma			
	1	Adenocarcinoma			
let-7a, miR-7, miR-21, miR-155, miR-221	46	Pairs of NSCLC	Down-regulation: miR-221	OS ($p = 0.0036$)	(79)

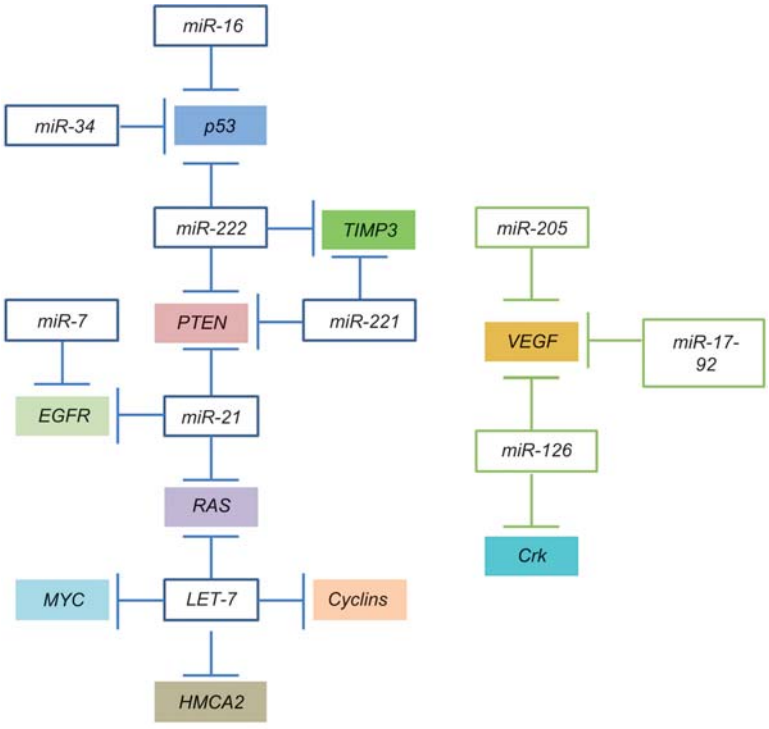

Figure 3 Network of the most studied so far lung-enriched miRNAs and their predicted target genes in NSCLC.

risk for relapse after surgical resection is one of the important challenges today. In an exploratory study, Duncavage et al. determined whether the expression of six miRNAs (let-7a, miR-7, miR-21, miR-155, miR-210, and miR-221) were associated with tumor recurrence in patients with resected stage I NSCLC and according to their findings, and if confirmed in prospective studies, miRNA expression in resected NSCLC could potentially identify patients at high risk of relapse after surgery (79).

Gao et al. explored the global expression profile of miRNAs in NSCLC and its potential relevance to clinic-pathological characteristics and prognosis. By using LNA miRNA microarrays in eight surgically removed lung carcinoma tissues and their corresponding normal lung tissues they selected miR-21, miR-143 and miR-181a for further study in another 47 paired samples by qRT-PCR using Taqman miRNA assays. Their data indicate the potential of miR-21, miR-143 and miR-181a as novel diagnostic or prognostic biomarkers for NSCLC (80).

In Table 1 we summarize the prognostic significance of miRNAS as evaluated so far in NSCLC tissues, while in Figure 3 we outline the network of the most studied so far lung-enriched miRNAs and their predicted target genes in NSCLC.

miRNAs in classification of histologic subtypes of NSCLC

Accurate classification of NSCLC is of paramount clinical relevance, as novel chemotherapeutic agents show different efficacy in AD compared with SCC. Cyto- and histomorphology may sometimes be insufficient for this distinction and immunohistochemistry may improve diagnostic accuracy. AD and SCC are two major histologic subtypes in NSCLC that present unique histopathological characteristics at distinctive preferential anatomical locations, and yet are classified together in traditional diagnosis, sharing similar staging system and treatment in clinical management. However, precise molecular mechanisms that differentiate the histopathology and characterize the tumor initiation and progression in these two subtypes are not completely understood. Recent clinical trials have revealed that histologic subtypes of NSCLC respond differently to certain treatments. For example, bevacizumab plus platinum-based chemotherapy have been approved in unresectable, locally advanced, recurrent or metastatic non-SCC lung cancer (81). Advanced-stage SCC patients tend to have more severe side effects from treatment with bevacizumab. Pemetrexed treatment following platinum-based chemotherapy in locally advanced or metastatic AD and LCC had superior outcome when compared to patients with squamous histology (82).

Global miRNA expression profiling demonstrated differential expression of 6 miRNAs (miR-205, miR-99b, miR-203, miR-202, miR-102, and pre-mir-204) between AD and SCC (42). Among these 6 miRNAs, miR-205 was confirmed using FFPE samples or preoperative biopsies in later studies (83, 84). A separate investigation did not reproduce this finding but rather identified a larger panel of 34 miRNAs that were differentially expressed between AD and SCC, with most of them up-regulated in AD, and among the top 5 miRNAs (miR-181a, miR-191, miR-107, miR-103, and let-

7b) there was a significant association with the expression of their predicted target genes (85).

One important feature of miRNA expression is the lineage-specific pattern demonstrating in maintenance of the stemness, embryonic development, and tissue differentiation. Expression profiles of miRNAs was able to distinguish tumors derived from different tissue origins, with greater performance compared to mRNA expression profiles even in poorly-differentiated samples (36). A group of 48 miRNAs was able to accurately predict the tissue origin of cancers of unknown primary origin in most cases (86). This can explain the observation of significant differential miRNA expression between lung cancer histological groups (87), or even between histologic subtypes within the NSCLC group (85). Very recently Del Vescovo et al. have reported that the relative quantification of miR-205 and miR-21 seems to be a promising diagnostic tool and that the measurement of miR-205 may be another tool for the distinction between AD and SCC (88).

Lung-enriched miRNAs and expression of their predicted target genes in NSCLC

It has been shown that many tissue-specific factors have reduced expression in cancers derived from the tissues in which these factors are specifically expressed (89, 90). The same pattern of changes in expression has been observed in miRNAs as well. Typical examples are decreased expression of miR-122 (liver), miR-345 (pancreas), and miR-124 (brain), in liver cancer (91), pancreatic cancer (92), and gliomas (93), respectively. A prior genome-wide expression profiling of 345 miRNAs in 40 normal human tissue types revealed that miR-34b, miR-34c, and miR-449 are enriched in only a few tissues including lung and trachea, while the rest of tissues examined had no or barely detectable levels of expression (94). Reduced expression of miR-34b and miR-34c has been previously shown in lung cancer cell lines (95) and primary tumor specimens (36), which was later confirmed by several independent investigations (85, 96, 97). Both miR-34b and miR-34c are p53-induced genes and part of the p53 tumor suppressor network (98), and miR-34c did show growth suppressive activity in murine and human lung cancer (96). Reduced expression of miR-34c together with another 4 miRNAs (miR-25, miR-191, let-7e, and miR-34a) also correlated with poor overall survival of SCC patients (85).

Functional roles of a miRNA should be determined by its target genes. In a proof-of-principle study (99), the predicted miR-34b/34c/449 target genes from the most frequent ''gene ontology'' term could classify histologic subtypes from a Stanford lung cancer dataset (100), especially between AD and SCC/SCLC. This list of genes was further reduced to a minimal signature of 17 genes for validation of classifying AD vs. SCC in total nine published lung cancer gene expression datasets with average 87% and 82% of accuracy to AD and SCC, respectively (99). Among these 17 genes and the original list from the developmental processes category, transforming growth factor (TGF)-β signaling pathway is particularly enriched, underscoring possible roles for TGF-β pathway genes in lung cancer tumorigenesis in general and their differential functionalities in AD and SCC.

Circulating miRNAs as lung cancer biomarkers

The identification of tumor biomarkers that detect the presence of disease using non-invasive diagnostic procedures is a key part of cancer research. As already stated above, the control of gene expression by miRNAs influences many cellular processes and the presence of miRNAs is mainly for the regulation of cancer-associated genes in tissues.

Very recently the extraction and reliable determination of cell-free miRNAs, circulating in body fluids like plasma and serum has already been shown in several studies and comprises a very promising novel circulating biomarker. Circulating miRNA profiles have now been associated with a range of different tumor types (101–104), diseases, such as stroke and heart disease (105, 106) as well as altered physiological states, such as pregnancy (107). For lung cancer, it was shown that serum miRNAs are promising prognostic biomarkers. Hu et al. demonstrated that 11 serum miRNAs were found to be altered more than five-fold between longer-survival and shorter-survival groups, and the levels of four miRNAs (miR-486, miR-30d, miR-1 and miR-499) were significantly associated with overall survival (108).

Futhermore, serum miRNA profiles among different cancer types including NSCLC have been analyzed. When Chen et al. investigated the expression profile of miRNAs in various patients and compared it with that of normal subjects, several miRNAs were found to be significantly differentially expressed among these two groups. Concerning NSCLC, 28 miRNAs were missing and 63 new miRNAs were detected after the comparison between healthy subjects and lung cancer patients (109). Another group has studied the expression levels of the 22 miRNAs selected in the study of Chen et al., in 18 malignant and 12 benign effusions and after discarding nine lowly expressed miRNAs, a panel of 13 miRNAs were measured in 30 samples and found that miR-24, miR-26a and miR-30d were expressed differently between the two groups (110). Finally, very recently Foss et al. showed that miR-1254 and miR-574-5p were significantly increased in plasma of early-stage NSCLC patients with respect to the control volunteers suggesting that theses two miRNAs can be used as serum-based minimally invasive biomarkers (111). Shen et al. validated expressions of the miRNAs in paired lung tumor tissues and plasma specimens from 28 stage I NSCLC patients by real-time quantitative reverse transcription PCR, and then evaluated the diagnostic value of plasma miRNAs in a cohort of 58 NSCLC patients and 29 healthy individuals. According to their findings, altered expressions of miRNAs in plasma would provide potential blood-based biomarkers for the clinical laboratory (112).

Very recently, Boeri et al. explored miRNA expression profiles of lung tumors, normal lung tissues and plasma samples from cases with variable prognosis identified in a completed spiral-CT screening trial with extensive follow-up.

Table 2 Detection of circulating miRNAS in NSCLC.

Tested miRNAs	No of samples	Plasma/serum	Techniques	Significantly expressed	Normalization	References
All human miRNAs	11	Healthy donors	Microarrays	miR-1254, miR-574-5p	External control: cel-miR-39	(111)
	11	NSCLC			Internal control: U6	
miR-21, 126, 145, 139, 182,	29	Healthy donors	RT-qPCR	Up-regulated: miR-21, miR-210	External control: cel-miR-238	(112)
200b, 205, 210, 375, 429, 486-5p, and 708	58	NSCLC		Down-regulated: miR-126, miR-486-5p	Internal control: miR-16 and U6	
365 human mature miRNAs	20	Healthy donors	RT-qPCR (Taqman low-density arrays)	Down-regulated: miR-30e-3p, let-7f		(115)
	28	NSCLC				
All human miRNAs	11	NSCLC (pool)	Solexa sequencing and RT-qPCR for Validation	Up-regulated: miR-25, miR-223	Directly normalized to total RNA	(109)
	10	Healthy male donors				
	11	Healthy female donors				
All human miRNAs	30	Patients with longer survival	Solexa sequencing	Up-regulated: miR-486, miR-30d,	External control: miR-168 for plant	(108)
	30	Patients with shorter survival		Down-regulated: miR-1, miR-499		
miR-20a, miR-21, miR-22, miR-24, miR-25, miR-26a, miR-26b, miR-27a, miR-27b, miR-29a, miR-30d,	18	Malignant effusions	RT-qPCR	Down-regulated: miR-24, miR-26a and miR-30d	External control: ath-miR-156a	(110)
miR-145,miR-146a, miR-152,miR-199a, miR-200c, miR-221, miR-222, miR-223, miR-320, miR-375, miR-382	12	Benign effusions				

According to their data, miRNA expression patterns significantly distinguished: (i) tumors from normal lung tissues, (ii) tumor histology and growth rate, (iii) clinical outcome, and (iv) year of lung cancer CT detection. According to this study, miRNA profiles in normal lung tissues also displayed remarkable associations with clinical features, suggesting the influence of a permissive microenvironment for tumor development. It is impressive that miRNA expression analyses in plasma samples collected 1–2 years before the onset of disease, at the time of CT detection and in disease-free smokers enrolled in the screening trial, resulted in the generation of miRNA signatures with strong predictive, diagnostic, and prognostic potential (area under the ROC curve ≥ 0.85). These signatures were validated in an independent cohort from a second randomized spiral-CT trial. These results indicate a role for miRNAs in lung tissues and plasma as molecular predictors of lung cancer development and aggressiveness and have a strong clinical implication both for lung cancer management and in the clinical laboratory (113).

By using a microarray platform that enables the simultaneous analysis of all human microRNAs by either fluorescent or electrochemical signals, Lodes et al. have shown that sufficient miRNAs are present in one milliliter of serum to detect miRNA expression patterns, without the need for amplification techniques. According to their findings these expression patterns could correctly discriminate between normal and cancer patient samples (114). Silva et al. analyzed 365 human miRNAs in the plasma from 28 NSCLC patients and 20 controls. They selected five miRNAs (let-7f, miR-20b, miR-30e-3p, miR-223 and miR-301) and validated them independently by real-time PCR in plasma from 78 NSCLC and 48 controls and correlated with pathologic parameters and survival. They found that let-7f, miR-20b and miR-30e-3p were decreased in plasma vesicles of NSCLC patients, and that let-7f and miR-30e-3p levels could distinguish between two groups of patients for stage of disease and therefore possibility of surgery. Plasma levels of miR-30e-3p and let-7f were associated with short disease-free survival and overall survival, respectively. NSCLC patients and healthy controls differ in vesicle-related miRNAs in plasma. Levels of let-7f and miR-30e-3p in NSCLC patients are associated with poor outcome (115). Moreover, very recently it

was shown by Yu et al. that miRNAs in sputum can be used as highly sensitive and specific non-invasive markers for early detection of lung adenocarcinoma (116).

In conclusion plasma miRNAs obtained by non-invasive methods could serve as circulating tumor biomarkers of discriminating and prognostic value in NSCLC. However, there is still a lot of work to be done before the establishment of miRNAs as biomarkers in the clinical laboratory, especially towards the standardization of analytical methodologies used, the inclusion of internal and external controls in each assay, and the consensus towards normalization of these results. In Table 2 we summarize findings presented so far on the detection of circulating miRNAS in plasma and serum of NSCLC patients.

miRNAs as therapeutic targets in lung cancer

Many recent findings implicate that miRNAs could play an important role for the design of innovative therapies for NSCLC. Numerous studies have documented the implications of miRNAs in nearly every carcinogenesis process of lung cancer, including tumor development, apoptosis, invasion and metastasis, as well as anti-cancer drug resistance. Forced expression or suppression of specific miRNAs can regulate the biological alteration during carcinogenesis, underscoring the therapeutic potential of miRNAs in lung cancer. Recent reviews have shown that some key microRNAs can modulate the lung cancer carcinogenesis process, and discuss the perspectives of microRNAs as therapeutic targets for lung cancer (117–119). By exploiting the unique characteristics of miRNAs, clinicians can come ever closer to achieving the goal of individualized cancer treatment.

Esquela-Kerscher et al. have shown that the let-7 microRNA directly represses cancer growth in the lung (120). They found that let-7 inhibits the growth of multiple human lung cancer cell lines in culture, as well as the growth of lung cancer cell xenografts in immunodeficient mice. These findings provide direct evidence that let-7 acts as a tumor suppressor gene in the lung and indicate that this miRNA may be useful as a novel therapeutic agent in lung cancer. Weiss et al. investigated if the loss of microRNA-128b, that is a putative regulator of EGFR, correlated with response to targeted EGFR inhibition (121). Loss of microRNA-128b would be equivalent to losing a tumor suppressor gene because it would allow increased expression of EGFR. They found that microRNA-128b loss of heterozygocity (LOH) was frequent in tumor samples and correlated significantly with clinical response and survival following administration of gefitinib.

Tumor suppressor miRNAs provide a new opportunity to treat cancer. This approach, miRNA replacement therapy is based on the concept that the reintroduction of miRNAs depleted in cancer cells reactivates cellular pathways that drive a therapeutic response. Wiggins et al. described the development of a therapeutic formulation using chemically synthesized miR-34a and a lipid-based delivery vehicle that blocks tumor growth in mouse models of NSCLC. Their data provide proof of concept for the systemic delivery of a synthetic tumor suppressor mimic, obviating obstacles associated with viral-based miRNA delivery and facilitating a rapid route for miRNA replacement therapy into the clinic (122). Paxillin (PXN) gene mutations are associated with lung adenocarcinoma progression and PXN is known to be a target gene of microRNA-218 (miR-218). Wu et al. have shown that miR-218 expression in lung tumors was negatively associated with PXN expression and that PXN and miR-218 might independently predict overall survival and regression free survival, respectively, in NSCLC (123). Their findings suggest that PXN overexpression induced by miR-218 suppression is an independent predictor of survival and relapse in NSCLC, highlighting PXN as a potential therapeutic target to improve clinical outcomes in this disease.

Recent results by Chen et al. demonstrate that miR-145 inhibits proliferation of NSCLC cells through c-Myc and suggest that increasing miR-145 expression may provide a novel approach for the treatment of NSCLC (124). Frezzetti et al. have very recently shown that a LNA directed against miR-21 slows down tumor growth in mice (125). Consistently, a search for mRNAs downregulated by miR-21 shows an enrichment for mRNAs encoding cell cycle checkpoints regulators, suggesting an important role for miR-21 in oncogenic RAS-induced cell proliferation.

Identification of miRNA targets is a critical step to design novel therapies and interrogate molecular mechanisms underlying miRNA signatures, but two major hurdles still exist. First, correct prediction of miRNA target genes through computation algorithms is still a major challenge. It has been shown that the union of miRNA target genes predicted by three computational algorithms (miRanda, PicTar, and TargetScan) is one of the strategies that give the highest sensitivity (126), but such sensitivity will be undoubtedly compromised by a large list of false-positive prediction and hence appropriate data filtering would be required. Secondly, as the number of published miRNA expression profiles in lung cancer starts to grow, it appears that the miRNA signatures from different groups are non-overlapping. This interestingly coincides with the lack of consensus in mRNA signatures from at least two dozens of gene expression profiling datasets in lung cancer that have been published so far. In a report using three most prominent lung adenocarcinoma gene expression profiles (127), no common gene signature was found, although the gene expression profile in each of the three datasets can reproducibly stratify the adenocarcinoma into three subtypes. This could be caused by the heterogeneity in histologic subtypes and ethnic origins of lung cancer, platform-to-platform discrepancies, batch effect and sample preparation within the same platform, and even the statistic methods being used (128). All these highlight the importance of meta-analysis of multiple datasets crossing different platforms for either *in silico* marker identification, or experimental validation of any gene signature using samples from independent sources.

We believe that in the near future all these difficulties, concerning identification and verification of critical miRNA targets and lack of safe and specific delivery system will be

overcome and miRNAs will be established as therapeutic targets in cancer.

Conclusions

In conclusion, miRNAs have the potential to serve both as biomarkers and therapeutic agents in cancer, by personalizing diagnosis and therapy (129). There is increasing evidence that altered microRNA expression is associated with tumor progression and survival in lung cancer patients. Recent data from multiple studies strongly support the potential of microRNAs as biomarkers in NSCLC. Expression profiles of miRNAs were able to distinguish tumors derived from different tissue origins enabling classification of histologic subtypes of NSCLC. Especially cell-free miRNAs, circulating in body fluids like plasma and serum comprise today very promising novel tumor biomarkers that will play a critical role in the clinical laboratory in the near future. miRNAs could play an important role for the design of innovative therapies for NSCLC and by exploiting the unique characteristics of miRNAs, clinicians can come ever closer to achieving the goal of individualized cancer treatment.

However, there is still a lot of work to be done before the establishment of miRNAs as biomarkers in the clinical laboratory, especially towards the standardization of analytical methodologies used, the inclusion of internal and external controls in each assay, and the consensus towards normalization of these results.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. American Cancer Society. Cancer facts and figures 2010. http://www.cancer.org/acs/groups/content/@nho/documents/document/acspc-024113.pdf 2010.
2. Feinstein AR, Gelfman NA, Yesner R. Observer variability in the histopathologic diagnosis of lung cancer. Am Rev Respir Dis 1970;101:671–84.
3. Travis WD, Travis LB, Devesa SS. Lung cancer. Cancer 1995;75:191–202.
4. Clegg A, Scott DA, Hewitson P, Sidhu M, Waugh N. Clinical and cost effectiveness of paclitaxel, docetaxel, gemcitabine, and vinorelbine in non-small cell lung cancer: a systematic review. Thorax 2002;57:20–8.
5. Okamoto T, Maruyama R, Suemitsu R, Aoki Y, Wataya H, Kojo M, et al. Prognostic value of the histological subtype in completely resected non-small cell lung cancer. Interact Cardiovasc Thorac Surg 2006;5:362–6.
6. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–39.
7. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497–500.
8. Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, et al. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. N Engl J Med 2010;362:2380–8.
9. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer: a decade of progress. Chest 2002;122:1037–57.
10. Zheng Z, Chen T, Li X, Haura E, Sharma A, Bepler G. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007;356:800–8.
11. Coate LE, John T, Tsao MS, Shepherd FA. Molecular predictive and prognostic markers in non-small-cell lung cancer. Lancet Oncol 2009;10:1001–10.
12. Baffa R, Fassan M, Volinia S, O'Hara B, Liu CG, Palazzo JP, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol 2009;219:214–21.
13. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
14. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;75:843–54.
15. Tang G, Reinhart BJ, Bartel DP, Zamore PD. A biochemical framework for RNA silencing in plants. Genes Dev 2003;17:49–63.
16. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, et al. Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 2005;122:553–63.
17. Griffiths-Jones S. miRBase: microRNA sequences and annotation. Curr Protoc Bioinformatics, Chapter 12, Unit 12 19 11-10, John Wiley and Sons, March 2010.
18. Bartel DP, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet 2004;5:396–400.
19. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U. Nuclear export of microRNA precursors. Science 2004;303:95–8.
20. Chang S, Johnston RJ, Frokjaer-Jensen C, Lockery S, Hobert O. MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode. Nature 2004;430:785–9.
21. Johnston RJ, Hobert O. A microRNA controlling left/right neuronal asymmetry in Caenorhabditis elegans. Nature 2003;426:845–9.
22. Poy, MN, Eliasson, L., Krutzfeldt, J, Kuwajima, S, Ma, X, Macdonald, PE, et al. A pancreatic islet-specific microRNA regulates insulin secretion. Nature 2004;432:226–30.
23. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 2005;436:214–20.
24. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. Science 2004;303:83–6.
25. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, et al. MicroRNAs regulate brain morphogenesis in zebrafish. Science 2005;308:833–8.
26. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H. Stem cell division is regulated by the microRNA pathway. Nature 2005;435:974–8.

27. He L, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network – another piece in the tumour-suppression puzzle. Nat Rev Cancer 2007;7:819–22.
28. Esquela-Kerscher A, Slack FJ. Oncomirs – microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259–69.
29. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006;6:857–66.
30. Hammond SM. MicroRNAs as oncogenes. Curr Opin Genet Dev 2006;16:4–9.
31. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 2002;99:15524–9.
32. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005;65:9628–32.
33. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 2007;26:745–52.
34. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103: 2257–61.
35. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–47.
36. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–38.
37. Wang, W, Peng, B, Wang, D, Ma, X, Jiang, D, Zhao, J, et al. Human tumor MicroRNA signatures derived from large-scale oligonucleotide microarray datasets. Int J Cancer 2010. [Epub ahead of print.]
38. Tellez CS, Juri DE, Do K, Bernauer AM, Thomas CL, Damiani LA, et al. EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. Cancer Res 2011;71:3087–97.
39. Melo SA, Esteller M. A precursor microRNA in a cancer cell nucleus: get me out of here! Cell Cycle 2011;10:922–5.
40. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 2004;101:2999–3004.
41. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004;64:3753–6.
42. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006;9:189–98.
43. Sampson VB, Rong NH, Han J, Yang Q, Aris V, Soteropoulos P, et al. MicroRNA let-7a down-regulates MYC and reverts MYC-induced growth in Burkitt lymphoma cells. Cancer Res 2007;67:9762–70.
44. Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 2007;21:1025–30.
45. Johnson, CD, Esquela-Kerscher, A, Stefani, G, Byrom, M, Kelnar, K, Ovcharenko, D, et al. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 2007; 67:7713–22.
46. Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. Cancer Res 2008;68:8535–40.
47. Yu SL, Chen HY, Chang GC, Chen CY, Chen HW, Singh S, et al. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell 2008;13:48–57.
48. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M, et al. The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res 2007;67:1419–23.
49. Ebi H, Sato T, Sugito N, Hosono Y, Yatabe Y, Matsuyama Y, et al. Counterbalance between RB inactivation and miR-17-92 overexpression in reactive oxygen species and DNA damage induction in lung cancers. Oncogene 2009;28:3371–9.
50. Suarez Y, Fernandez-Hernando C, Yu J, Gerber SA, Harrison KD, Pober JS, et al. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc Natl Acad Sci USA 2008;105:14082–7.
51. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. Cell 2008;132:875–86.
52. Fish JE, Santoro MM, Morton SU, Yu S, Yeh RF, Wythe JD, et al. miR-126 regulates angiogenic signaling and vascular integrity. Dev Cell 2008;15:272–84.
53. Liu B, Peng XC, Zheng XL, Wang J, Qin YW. MiR-126 restoration down-regulate VEGF and inhibit the growth of lung cancer cell lines in vitro and in vivo. Lung Cancer 2009;66: 169–75.
54. Barshack I, Lithwick-Yanai G, Afek A, Rosenblatt K, Tabibian-Keissar H, Zepeniuk M, et al. MicroRNA expression differentiates between primary lung tumors and metastases to the lung. Pathol Res Pract 2010;206:578–84.
55. Wang X, Ling C, Bai Y, Zhao J. MicroRNA-206 Is associated with invasion and metastasis of lung cancer. Anat Rec (Hoboken) 2011;294:88–92.
56. Jiang L, Huang Q, Zhang S, Zhang Q, Chang J, Qiu X, et al. Hsa-miR-125a-3p and hsa-miR-125a-5p are downregulated in non-small cell lung cancer and have inverse effects on invasion and migration of lung cancer cells. BMC Cancer 2010;10:318.
57. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA 2007;104:15805–10.
58. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005;65:6029–33.
59. Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res 2008;18:350–9.
60. Markou A, Tsaroucha EG, Kaklamanis L, Fotinou, M, Georgoulias V, Lianidou ES. Prognostic value of mature microRNA-21 and microRNA-205 overexpression in non-small cell lung cancer by quantitative real-time RT-PCR. Clin Chem 2008;54: 1696–704.
61. Zhang JG, Wang JJ, Zhao F, Liu Q, Jiang K, Yang GH. MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chim Acta 2010;411:846–52.
62. Seike, M, Goto, A, Okano, T, Bowman, ED, Schetter, AJ, Horikawa, I, et al. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. Proc Natl Acad Sci USA 2009;106:12085–12090.

63. Chou YT, Lin HH, Lien YC, Wang YH, Hong CF, Kao YR, et al. EGFR promotes lung tumorigenesis by activating miR-7 through a Ras/ERK/Myc pathway that targets the Ets2 transcriptional repressor ERF. Cancer Res 2010;70:8822–31.
64. Kefas B, Godlewski J, Comeau L, Li Y, Abounader R, Hawkinson M, et al. microRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. Cancer Res 2008;68:3566–72.
65. Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E, et al. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. Cancer Cell 2010;18: 282–93.
66. Saito M, Schetter AJ, Mollerup S, Kohno T, Skaug V, Bowman ED, et al. The association of microRNA expression with prognosis and progression in early stage, non small cell lung adenocarcinoma: a retrospective analysis of three cohorts. Clin Cancer Res 2011;17:1875–82.
67. Hermeking H. The miR-34 family in cancer and apoptosis. Cell Death Differ 2010;17:193–9.
68. Yan D, Zhou X, Chen X, Hu DN, Dong XD, Wang J, et al. MicroRNA-34a inhibits uveal melanoma cell proliferation and migration through downregulation of c-Met. Invest Ophthalmol Vis Sci 2009;50:1559–65.
69. Tarasov V, Jung P, Verdoodt B, Lodygin D, Epanchintsev A, Menssen A, et al. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. Cell Cycle 2007; 6:1586–93.
70. Cole KA, Attiyeh EF, Mosse YP, Laquaglia, MJ, Diskin SJ, Brodeur GM, et al. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. Mol Cancer Res 2008;6:735–42.
71. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, et al. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. Cancer Res 2009;69:7569–76.
72. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. Proc Natl Acad Sci USA 2007;104:15472–7.
73. Gallardo E, Navarro A, Vinolas N, Marrades RM, Diaz T, Gel B, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. Carcinogenesis 2009;30:1903–9.
74. Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Ngankeu A, et al. miR-221 and 222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. Cancer Cell 2009;16:498–509.
75. Garofalo M, Quintavalle C, Di Leva G, Zanca C, Romano G, Taccioli C, et al. MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. Oncogene 2008;27:3845–55.
76. Puissegur MP, Mazure NM, Bertero T, Pradelli L, Grosso S, Robbe-Sermesant K, et al. miR-210 is overexpressed in late stages of lung cancer and mediates mitochondrial alterations associated with modulation of HIF-1 activity. Cell Death Differ 2011;18:465–78.
77. Wang R, Wang ZX, Yang JS, Pan X, De W, Chen LB. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). Oncogene 2011;30:2644–58.
78. Voortman J, Goto A, Mendiboure J, Sohn JJ, Schetter AJ, Saito M, et al. MicroRNA expression and clinical outcomes in patients treated with adjuvant chemotherapy after complete resection of non-small cell lung carcinoma. Cancer Res 2010; 70:8288–98.
79. Duncavage E, Goodgame B, Sezhiyan A, Govindan R, Pfeifer J. Use of microRNA expression levels to predict outcomes in resected stage I non-small cell lung cancer. J Thorac Oncol 2010;5:1755–63.
80. Gao W, Yu Y, Cao H, Shen H, Li X, Pan S, et al. Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. Biomed Pharmacother 2010;64:399–408.
81. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V, et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for non-squamous non-small-cell lung cancer: AVAil. J Clin Oncol 2009;27:1227–34.
82. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol 2008;26:3543–51.
83. Bishop JA, Benjamin H, Cholakh H, Chajut A, Clark DP, Westra WH. Accurate classification of non-small cell lung carcinoma using a novel microRNA-based approach. Clin Cancer Res 2010;16:610–9.
84. Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, et al. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. J Clin Oncol 2009;27:2030–7.
85. Landi MT, Zhao Y, Rotunno M, Koshiol J, Liu H, Bergen AW, et al. MicroRNA expression differentiates histology and predicts survival of lung cancer. Clin Cancer Res 2010;16:430–41.
86. Rosenfeld N, Aharonov R, Meiri E, Rosenwald S, Spector Y, Zepeniuk M, et al. MicroRNAs accurately identify cancer tissue origin. Nat Biotechnol 2008;26:462–9.
87. Du L, Schageman JJ, Irnov Girard L, Hammond SM, Minna JD, Gazdar AF, et al. MicroRNA expression distinguishes SCLC from NSCLC lung tumor cells and suggests a possible pathological relationship between SCLCs and NSCLCs. J Exp Clin Cancer Res 2010;29:75.
88. Del Vescovo V, Cantaloni C, Cucino A, Girlando S, Silvestri M, Bragantini E, et al. miR-205 Expression levels in nonsmall cell lung cancer do not always distinguish adenocarcinomas from squamous cell carcinomas. Am J Surg Pathol 2011;35: 268–75.
89. Axelsen JB, Lotem J, Sachs L, Domany E. Genes overexpressed in different human solid cancers exhibit different tissue-specific expression profiles. Proc Natl Acad Sci USA 2007;104: 13122–7.
90. Ge X, Yamamoto S, Tsutsumi S, Midorikawa Y, Ihara S, Wang SM. et al. Interpreting expression profiles of cancers by genome-wide survey of breadth of expression in normal tissues. Genomics 2005;86:127–41.
91. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009;28:3526–36.
92. Lee EJ, Gusev Y, Jiang J, Nuovo GJ, Lerner MR, Frankel WL, et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer 2007; 120:1046–54.
93. Silber J, Lim DA, Petritsch C, Persson AI, Maunakea AK, Yu M, et al. miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. BMC Med 2008;6:14.

94. Liang Y, Ridzon D, Wong L, Chen C. Characterization of microRNA expression profiles in normal human tissues. BMC Genomics 2007;8:166.
95. Gaur A, Jewell DA, Liang Y, Ridzon D, Moore JH, Chen C, et al. Characterization of microRNA expression levels and their biological correlates in human cancer cell lines. Cancer Res 2007;67:2456–68.
96. Liu X, Sempere LF, Galimberti F, Freemantle SJ, Black C, Dragnev KH, et al. Uncovering growth-suppressive MicroRNAs in lung cancer. Clin Cancer Res 2009;15:1177–83.
97. Mascaux C, Laes JF, Anthoine G, Haller A, Ninane V, Burny A, et al. Evolution of microRNA expression during human bronchial squamous carcinogenesis. Eur Respir J 2009;33: 352–9.
98. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature 2007;447:1130–4.
99. Liang Y. An expression meta-analysis of predicted microRNA targets identifies a diagnostic signature for lung cancer. BMC Med Genomics 2008;1:61.
100. Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, et al. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci USA 2001;98:13784–9.
101. Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008;141:672–5.
102. Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13–21.
103. Huang Z, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. Int J Cancer 2010;127:118–26.
104. Kanemaru H, Fukushima S, Yamashita J, Honda N, Oyama R, Kakimoto A, et al. The circulating microRNA-221 level in patients with malignant melanoma as a new tumor marker. J Dermatol Sci 2011;61:187–93.
105. Niu Z, Li A, Zhang SX, Schwartz RJ. Serum response factor micromanaging cardiogenesis. Curr Opin Cell Biol 2007;19: 618–27.
106. Niu Z, Iyer D, Conway SJ, Martin JF, Ivey K, Srivastava D, et al. Serum response factor orchestrates nascent sarcomerogenesis and silences the biomineralization gene program in the heart. Proc Natl Acad Sci USA 2008;105:17824–9.
107. Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, et al. Serum microRNAs are promising novel biomarkers. PLoS One 2008;3:e3148.
108. Hu Z, Chen X, Zhao Y, Tian T, Jin G, Shu Y, et al. Serum microRNA signatures identified in a genome-wide serum microRNA xpression profiling predict survival of non-small-cell lung cancer. J Clin Oncol 2010;28:1721–6.
109. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997–1006.
110. Xie L, Chen X, Wang L, Qian X, Wang T, Wei J, et al. Cell-free miRNAs may indicate diagnosis and docetaxel sensitivity of tumor cells in malignant effusions. BMC Cancer 2010;10: 591.
111. Foss KM, Sima C, Ugolini D, Neri M, Allen KE, Weiss GJ. miR-1254 and miR-574-5p: serum-based microRNA biomarkers for early-stage non-small cell lung cancer. J Thorac Oncol 2011;6:482–8.
112. Shen J, Todd NW, Zhang H, Yu L, Lingxiao X, Mei Y, et al. Plasma microRNAs as potential biomarkers for non-small-cell lung cancer. Lab Invest. 2011;91:579–87.
113. Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, et al. MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. Proc Natl Acad Sci USA 2011;108:3713–8.
114. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One 2009;4:e6229.
115. Silva J, García V, Zaballos A, Provencio M, Lombardía L, Almonacid L, et al. Vesicle-related microRNAs in plasma of nonsmall cell lung cancer patients and correlation with survival. Eur Respir J 2011;37:617–23.
116. Yu L, Todd NW, Xing L, Xie Y, Zhang H, Liu Z, et al. Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers. Int J Cancer 2010;127:2870–8.
117. Cho WC. MicroRNAs as therapeutic targets for lung cancer. Expert Opin Ther Targets 2010;14:1005–8.
118. Heneghan HM, Miller N, Kerin MJ. MiRNAs as biomarkers and therapeutic targets in cancer. Curr Opin Pharmacol 2010; 10:543–50.
119. Du L, Pertsemlidis A. microRNAs and lung cancer: tumors and 22-mers. Cancer Metastasis Rev 2010;29:109–22.
120. Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, et al. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. Cell Cycle 2008;7:759–64.
121. Weiss GJ, Bemis LT, Nakajima E, Sugita M, Birks DK, Robinson WA, et al. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Ann Oncol 2008;19: 1053–9.
122. Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res 2010;70:5923–30.
123. Wu DW, Cheng YW, Wang J, Chen CY, Lee H. Paxillin predicts survival and relapse in non-small cell lung cancer by microRNA-218 targeting. Cancer Res 2010;70:10392–401.
124. Chen Z, Zeng H, Guo Y, Liu P, Pan H, Deng A, et al. miRNA-145 inhibits non-small cell lung cancer cell proliferation by targeting c-Myc. J Exp Clin Cancer Res 2010;29:151.
125. Frezzetti D, De Menna M, Zoppoli P, Guerra C, Ferraro A, Bello AM, et al. Upregulation of miR-21 by Ras in vivo and its role in tumor growth. Oncogene 2011;30:275–86.
126. Sethupathy P, Megraw M, Hatzigeorgiou AG. A guide through present computational approaches for the identification of mammalian microRNA targets. Nat Methods 2006;3:881–6.
127. Hayes DN, Monti S, Parmigiani G, Gilks CB, Naoki K, Bhattacharjee A, et al. Gene expression profiling reveals reproducible human lung adenocarcinoma subtypes in multiple independent patient cohorts. J Clin Oncol 2006;24:5079–90.
128. Boutros PC, Lau SK, Pintilie M, Liu N, Shepherd FA, Der SD, et al. Prognostic gene signatures for non-small-cell lung cancer. Proc Natl Acad Sci USA 2009;106:2824–8.
129. Nana-Sinkam SP, Fabbri M, Croce CM. MicroRNAs in cancer: personalizing diagnosis and therapy. Ann NY Acad Sci 2010;1210:25–33.