Minireview

MicroRNAs: novel biomarkers for lung cancer diagnosis, prediction and treatment

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Abstract

MicroRNAs (miRNAs) are short non-protein-coding RNAs that post-transcriptionally regulate mRNA expression. A large body of evidence has identified important roles for these regulators in cell proliferation, differentiation, apoptosis and metabolism, as well as activation of oncogenic and antioncogenic signals. Aberrant expression of miRNAs has been found in most human malignancies and is strongly associated with tumorigenesis, prediction, diagnosis, progress, treatment and prognosis. Thus, miRNAs may become an intriguing and promising therapeutic target for many diseases, including cancer. In addition, research into miRNAs may provide insight into the mechanisms underlying tumor occurrence, progression and metastasis. This review summarizes the current knowledge of miRNAs, their roles in lung cancer and avenues for future research.

Keywords: microRNA, lung cancer, biomarker

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Introduction

Lung cancer causes more mortality than any other malignant tumor worldwide, with an estimated 215,000 new lung cancer patients and 162,000 deaths in the USA in 2008.[1,2] Most patients are diagnosed in advanced stages due to the lack of specific biomarkers and tools for early screening and diagnosis. A number of studies have focused on improvement of medical imaging and sputum cytology analysis; however, the five-year survival rate remains low (15%).[3,4]

MicroRNA (miRNAs) are approximately 21–25 nucleotide RNAs that can recognize specified target mRNAs and regulate gene expression. They influence the transcriptional and post-transcriptional mRNAs levels by promoting the degradation of their targets and/or suppressing translation. MiRNAs regulate various biological processes, such as cellular differentiation, proliferation, metabolism, resistance and cell death.[5] They also act as tumor promoters and suppressors depending on the nucleotide sequence. Abnormal expression of miRNAs has been reported to correlate with tumorigenesis.[6,7] Therefore, the profiles of miRNA expression in the different stages of lung cancer can unveil important information in diagnosis and prognosis, and ultimately lead to individualized treatment at the molecular level.

Role of miRNAs in tumorigenesis and anticancer effects

Evidence from many studies has identified an important role of miRNAs in the regulation of the cell cycle and tumorigenesis in organs, including the lung.[8,9] For example, Takahashi *et al*.[10] found decreased expression of miR-107, miR-185 and let-7a, but increased expression of miR-31 in lung carcinoma tissues and cancer cell lines. Overexpression of miR-107 and miR-185 significantly reduced the proliferation of A549 and H1299 non-small cell lung carcinoma (NSCLC) cells. In H1299 cells, let-7a significantly reduced proliferation, but this effect was less profound in A549 cells. MiR-31 also slightly suppressed lung cancer cell growth in both cells. MiR-107 and miR-185 significantly increased the percentage of cancer cells in the G_1 phase of the cell cycle.[10] Furthermore, another report demonstrated that let-7a shared seven base sequences with the miR-16 family and induced G_1 arrest by targeting multiple cyclins and cell cycle regulators, including the miR-107 target CDK6.[11] Synthetic let-7 can cause cell cycle defects in HepG2 cells by significantly increasing the percentage of cells in G_0–G_1.[12] Thus, the mechanism by which let-7, miR-16 and miR-107 induce cell cycle arrest in the G_1 phase might be the similar. Results from these studies suggest that miRNA-based treatments may be beneficial in

treating metastatic lung cancer by repairing or normalizing abnormalities occurring in cell cycle regulation.

Much of our current knowledge regarding the relative importance of miRNAs on cell proliferation has been uncovered using inhibition or over-expression of target miRs. Inhibition of miR-107, using a synthetic inhibitor created by cloning the matching miRNA-targeted sites and inserting them into the multiple cloning sites in the luciferase reporter vectors, increased the proliferation of A549 cells, but not HeLa cells.[13] Epidermal growth factor-like domain 7 (EGFLD7) is related to cell migration and angiogenesis. Sun *et al*.[14] found that the over-expression of miR-126 resulted in an increased expression of EGFLD7 in A549 cells that inhibited cell proliferation *in vitro* and tumor growth *in vivo*. Zhong *et al*.[15] showed dose-dependent inhibition of lung cancer cell growth by miR-107, miR-126 and let-7a, suggesting that the over-expression of these miRNAs suppresses cancer. Future experiments investigating the *in vivo* effects of miRNAs in transgenic and knockouts models will offer valuable information about safety and efficacy.

MiRNA: tumor suppressor

Let-7 is an important miRNA and is highly expressed in lung tissue, as supported by *in vitro* and *in vivo* studies.[7,16] Takamizawa *et al*.[17] reported a low expression of let-7 in 40% of lung tumors and in 60% of lung cancer cell lines. The 3′ untranslated region (3′UTR) end of the RAS gene, which is highly expressed in lung tumors, has multiple let-7-binding sites. This suggests that let-7 plays a role in the down-regulation of the RAS oncogene. Studies by Johnson *et al*.[12,16] also found low let-7 expression, but high RAS expression in lung tumor models. Over-expression of let-7 inhibited cell growth, implicating let-7 in the regulation of RAS in this process. Ras-related protein 14 (RAB14) was also associated with tumorigenesis. One study showed that the level of miR-451 was correlated with differentiation, pathological stage and lymph node metastasis of NSCLC, and over-expression of miR-451 significantly inhibited RAB14 protein expression.[18] In addition, strong positive immunoreactivity of RAB14 protein was significantly associated with down-regulation of miR-451 ($P = 0.01$).[18] This result suggests that miR-451 functions as a tumor suppressor in NSCLC by targeting RAB14.

Retinoblastoma 1 (RB1) was the first identified tumor suppressor. Feng *et al*.[19] found that over-expression of miR-192 inhibited cell proliferation in A549, H460 and 95D NSCLC cells, as well as tumorigenesis in a nude mouse model probably by decreasing RB1 mRNA and protein expression and repressing RB1-30-UTR reporter activity. These results demonstrate that miR-192 is a tumor suppressor that might target the RB1 gene, thus inhibiting cell proliferation and inducing cell apoptosis in lung cancer cells.

PED/PEA-15 (a phosphoprotein enriched in diabetes and astrocytes), a 15-kDa death effector domain family member with a broad antiapoptotic function, is highly expressed in a number of human tumors including lung cancer. Incoronato *et al*.[20] identified miR-212 as a negative regulator of PED/PEA-15 expression. The ectopic expression of miR-212 increased tumor necrosis factor-related apoptosis-inducing ligand and induced cell death in NSCLC cells. *In vitro* and *in vivo* studies indicate that the expressions of PED/PEA-15 and miR-212 are inversely correlated, suggesting that miR-212 may be considered as a tumor suppressor. Regulator of G-protein signaling 17 (RGS17), which is highly expressed in lung adenocarcinoma and prostate cancer, induced tumor cell growth through induction of cyclic adenosine monophosphate response element-binding protein phosphorylation.[21] Sun *et al*.[22] showed that ectopic expression of hsa-miR-182 significantly inhibited lung cancer cell proliferation through inhibition of RGS17, suggesting that tumor suppressors might play a role in mediating the hsa-miR-182-inhibited anchorage-independent lung cell growth.

Hypoxia-inducible factor-1α (HIF-1α) is considered a key regulator of tumor angiogenesis. Cha *et al*.[23] found that miR-519c directly reduced tumor angiogenesis by binding to the HIF-1α 3′UTR. Further studies demonstrated that over-expression of miR-519c significantly inhibited the expression of the HIF-1α protein and reduced the tube formation of human umbilical vein endothelial cells. Consistent with this, over-expression of miR-519c was associated with a better prognosis in cancer patients.[23]

Fused in sarcoma 1 (FUS1) is a tumor suppressor gene located on human chromosome 3p21, and its protein expression is greatly diminished in many lung cancers.[24] This has been confirmed by studies demonstrating that over-expression of FUS1 significantly inhibits tumor growth and progression in mouse models,[25] and furthermore FUS1 knockout mice have an increased frequency of spontaneous tumors.[26] Du *et al*. showed that over-expression of miR-93, miR-98 and miR-197 reduced the expression of the FUS1 protein by interacting with the 3′UTR, which promoted lung cancer growth.[27] Epidermal growth factor receptor (EGFR) signaling has been shown to play an important role in cell migration and invasion.[28] Wang *et al*.[29] demonstrated that miR-125a-5p regulated the expression of several downstream genes involved in EGFR signaling, and antisense miR-125a-5p significantly promoted migration and invasion, suggesting that miR-125a-5p is negatively correlated with lung cancer metastasis and invasion.

Enhancer of zeste homolog 2 (EZH2), a human homolog of the drosophila polycomb group gene, plays a critical role in transcriptional regulation by chromatin remodeling, nucleosome modification and interaction with other transcription factors.[30] Increasing evidence suggests that EZH2 possesses oncogenic properties. One such study found that over-expression of EZH2 enhanced proliferation and invasion of cancer cells and promoted neoplastic transformation.[31] Reduced expression of miR-101 is associated with over-expression of EZH2 in NSCLC, and enforced expression of miR-101 or knockdown of EZH2 leads to reduced NSCLC cell proliferation and invasion and sensitized cells to paclitaxel-mediated apoptosis through inducing expression of the proapoptotic protein Bim.[32] This implies that miRNAs such as miR-100 might control the tumor-promoting effect of EZH2 in NSCLC cells.

MiRNA as oncomir

Aside from their roles as tumor suppressors, some miRNAs act as tumor promoters. For example, the miR-17-92 cluster that is located at 13q31.3, controls the expression of seven miRNAs and has been identified as a potential oncogene in a mouse model of lymphadenoma.[29] The expression of miR-17-92 is highly regulated in many solid tumors, including lung cancer,[33] and over-expression of miR-17-92 in lung cancer cells, especially in SCLC, promotes cells differentiation.[34] Although the molecular mechanisms by which miR-17-92 induce lung cancer growth remain largely unknown, miR-17-92 has been shown to target phosphatase and tensin homolog (PTEN), a tumor suppressor, in HeLa cells.[35] PTEN participates in the cell-survival signaling pathway PI3K/AKT and is usually eliminated or mutated in some sporadic and hereditary tumors including lung cancer.[36,37] Another study[38] found that miR-31 inhibits the expression of the tumor-suppressor genes large tumor suppressor 2 (LATS2) and PP2A regulatory subunit-Bα isoform (PPP2R2A) in lung cancer cells, which are highly expressed in cells with silenced miR-31, suggesting that miR-31 acts as a tumor promoter in lung cancer (Figure 1).

Recent research focused on single nucleotide polymorphisms (SNPs) and cancer uncovered that single miRNAs have the ability to regulate the expression of hundreds of mRNAs at the same time.[39] Therefore, the potential possibility of a single miRNA with abnormal function to induce cell transformation is very high and the functions of SNPs seem to be important in tumorigenesis. Chin *et al*.[40] found that the let-7 complementary site 6 variant alleles were significantly associated with increased risk for NSCLC among moderate smokers and represents a new paradigm of let-7 miRNAs in lung cancer susceptibility. Rs3134615, a miRNA-1827-binding site in MYCL1, T allele, was associated with a significant increased risk of SCLC, with the odds ratios for carrying the GT or TT genotype being 2.08 (95% confidence interval [CI]: 1.39–3.21; $P = 0.0004$) compared with the GG genotype (Figure 1).[41]

Invasion and migration are characteristics of malignant tumors including lung cancer, and miRNAs play an important role in this process. Jiang *et al*.[42] reported different expression profiles of hsa-miR-125a-3p and hsa-miR-125a-5p in NSCLC tissues and adjacent normal lung tissues, and that these miRNAs function in an opposing manner in suppressing or enhancing the migration and invasion in A549 and SPC-A-1 cell lines. This is consistent with the reverse correlations between the expression level of these miRNAs and lymph node metastasis in NSCLC. Proto-oncogene c-Crk (CRK) is an adaptor protein involved in intracellular signaling pathways, cell adhesion, proliferation and migration. Increased expression of CRK has been associated with the increased invasiveness of lung cancer.[43] Crawford *et al*.[44] found that over-expression of miR126 in lung cancer cells resulted in a decrease in CRK protein without any alteration in mRNA expression. As lung cancer cells exhibit a decrease in adhesion, migration and invasion, this suggests that CRK may be a putative target gene for miR-126. Roybal *et al*.[45] found that Flt1 was required for lung adenocarcinoma cell invasion, and metastasis, and furthermore reported that targeting Flt1 by miR-200 inhibited lung adenocarcinoma cell invasion.

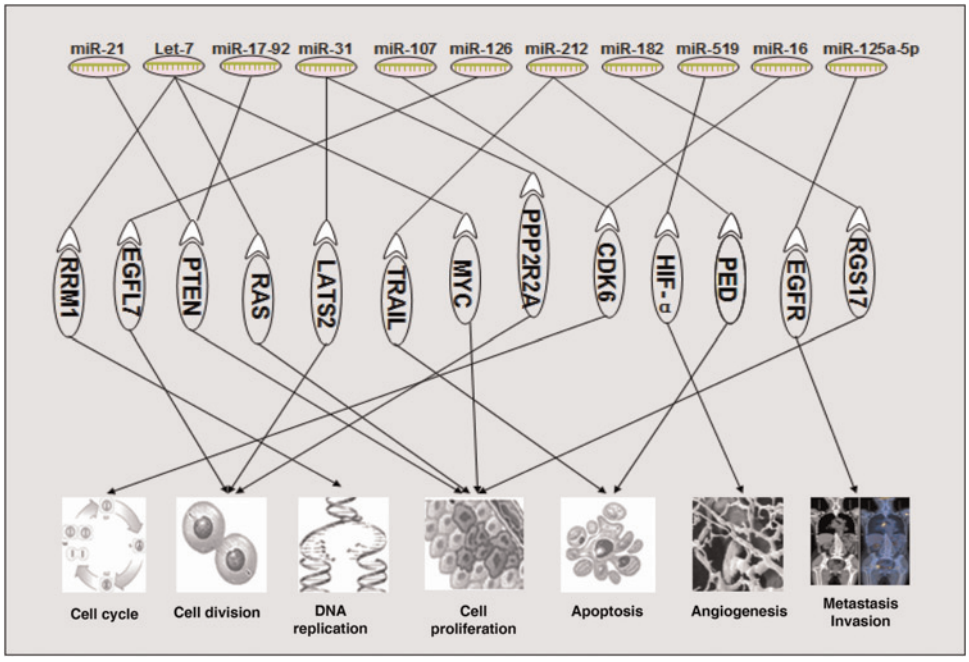

Figure 1 Targets and functions of microRNAs (miRNAs) in lung cancer. This diagram shows the antioncogenic and pro-oncogenic role of miRNAs in the regulation of cell cycle and lung cancer growth including apoptosis, angiogenesis, invasion and metastasis by targeting different or similar genes. RGS17, regulator of G-protein signaling 17; EGFR, epidermal growth factor receptor; HIF-1α, hypoxia-inducible factor-1α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; LATS2, large tumor suppressor 2l; PTEN, phosphatase and tensin homolog; EGFL7; epidermal growth factor-like domain 7. (A color version of this figure is available in the online journal)

MiRNAs as biomarkers of lung cancer

Diagnosis and prediction biomarkers

A more reliable, non-invasive diagnostic tool is important for screening and treatment of lung cancer. MiRNA expression is time- and space-specific, and the expression profiles can reflect the developmental lineage and differentiation state of tumors, and can identify poorly differentiated tumors successfully, whereas mRNA profiles are highly inaccurate when applied to the same samples.[46] Thus, the expression profiles of miRNAs have potential value for diagnosing malignant tumors. Sputum cytology analysis has been used for the diagnosis of lung cancer for decades.[47,48] Xie *et al.*[49] found that miR-21 expression in the sputum specimens was significantly higher in cancer patients (76.32 ± 9.79) compared with cancer-free individuals (62.24 ± 3.82, $P < 0.0001$). Further studies have shown that increased miR-21 expression produced 69.66% sensitivity and 100.00% specificity in the diagnosis of lung cancer by sputum cytology. Thus, examining altered miRNA expression in sputum could be a potential non-invasive approach for cancer diagnosis in the lung. Shen *et al.*[50] identified 12 miRNAs aberrantly expressed in early-stage NSCLC. Among these, five miRNAs displayed significant concordance of the expression levels in plasma and the corresponding tumor tissues (all $r > 0.850$, all $P < 0.05$) and four (miRNA-21, -126, -210 and 486-5p) yielded 86.22% sensitivity and 96.55% specificity in distinguishing NSCLC patients from healthy controls. Furthermore, the panel of miRNAs produced 73.33% sensitivity and 96.55% specificity in identifying stage I NSCLC patients. In addition, these miRNAs had higher sensitivity (91.67%) in diagnosing lung adenocarcinomas compared with squamous cell carcinomas (SCCs) (82.35%) ($P < 0.05$). For early-stage NSCLC, the expression of miR-1254 and miR-574-5p was significantly increased.[51] Compared with normal lung tissues, the top 10 deregulated miRNAs in lung tumors that were appropriate for discriminating computed tomography-detected lung cancer from normal lung tissue were miR-210, miR-21, miR-219-1,miR-7, miR-200b and miR-324, which were all up-regulated, and mir-126, mir-451, mir-30a and mir-486, which were all down-regulated.[52] Two miRNAs (mir-205 and mir-21) were reported to be accurate in distinguishing adenocarcinoma from SCC subtypes ($P \leq 0.001$). In addition, mir-518e and mir-144 were down-regulated in tumors with a faster growth rate.[52] This suggested that expression of miRNAs played a critical role in diagnosis and was also important in distinguishing NSCLC subtypes. In addition, miRNA expression profiles were also different between lung primary and metastatic tumors. Barshack *et al.*[53] reported that over-expression of hsa-miR-182 was found in lung primary tumors, whereas hsa-miR-126 was highly expressed in metastatic tumors, suggesting the distinct roles of hsa-miR-182 and hsa-miR-126 in lung primary and metastatic tumors.

Adenocarcinoma is the most common subtype of NSCLC. Yu *et al.*[54] found that the combined over-expression of miR-21, miR-486, miR-375 and miR-200b in surgical tissues and sputum were biomarkers in the prediction of lung adenocarcinoma from normal controls with 80.6% sensitivity and 91.7% specificity. The results were validated in 64 lung cancer patients and 58 cancer-free participants. Lebanony *et al.*[55] reported lower expression of hsa-mir-205 in lung adenocarcinoma, whereas higher expression was found in SCC subtypes. In addition, hsa-mir-21 and U6 were highly expressed in adenocarcinoma and SCC subtypes. This result suggested that expression of hsa-miR-205 could be a good predictor and highly accurate diagnostic biomarker of lung SCCs with high sensitivity (96%) and specificity (90%). Similar results were observed in another study.[56] In addition, Xing *et al.*[57] found that detection of miR-205, miR-210 and miR-708 expression in sputum was highly sensitive (73%) and specific (96%) in diagnosing lung cancer. Raponi *et al.*[58] demonstrated that 15 miRNAs were differentially expressed in 61 SCCs and 10 matched normal lung samples including members of the miR-17-92 cluster and its paralogues, and that miR-146b had the strongest predictive accuracy for stratifying prognostic groups. Taken together, these results suggest that miRNAs might contribute to the diagnosis of certain lung cancer subtypes.

Prognosis biomarkers

Owing to the important role that miRNAs play in oncogenesis, they have been considered promising prognosis factors as well. Studies found that let-7 is located at fragile sites, as well as in minimal regions of loss of heterozygosity, minimal regions of amplification (minimal amplicons) or common breakpoint regions.[59] To the contrary, a high expression of let-7 was found in well-differentiated tumors, whereas its target oncogenes (HMGA2 and ras) were down-regulated. Thus, let-7 may be a biomarker for poorly differentiated tumors.[60] Takamizawa *et al.*[17] showed that reduced expression of let-7 in human lung cancers was associated with decreased postoperative survival, which was independent of disease stage.

Hu *et al.*[61] studied miRNA expression patterns in patients with stage I to IIIa lung adenocarcinoma and SCC, who were treated with surgery and adjuvant chemotherapies, and found that high expression of miR-486 and miR-30d in serum correlated with shorter survival. Furthermore, they found that low expression of miR-1 and miR-499 in serum correlated with shorter survival. Further studies showed that patients carrying two or more risk miRNAs exhibited shorter survival than those carrying zero or one miRNA. Studies by Gallardo *et al.*[62] revealed that lower expression of miR-34a in tumor tissues was associated with a high recurrent risk. In combination with p53 mutations, patients might have a high probability of relapse.

Chemoresistance and survival are important factors affecting clinical treatment. Ranade *et al.*[63] found that miR-92a-2* ($P = 0.010$), miR-147($P = 0.018$) and miR-574-5 ($P = 0.039$) were significantly associated with chemoresistance in patients with SCLC. Further studies showed that higher miR-92a-2 levels were associated with shorter survival.[62] Gao *et al.*[64] found that the expression level of miR-21 was significantly higher in tumor tissues compared with adjacent normal tissues ($P = 0.026$), whereas lower expression

of miR-143 ($P = 0.000$) and miR-181a ($P = 0.000$) was observed in lung carcinoma tissues. Furthermore, low expression of miR-143 was significantly correlated with smoking status ($P = 0.026$). In addition, high miR-21 expression (hazard ratio [HR]: 5.993; 95% CI: 2.518–14.264; $P = 0.000$) and low miR-181a expression (HR: 0.328; 95% CI: 0.142–0.756; $P = 0.009$) were associated with poor survival independent of TNM staging and lymph node status. These studies by Gao *et al*.[64] suggest that miR-21, miR-143 and miR-181a might be beneficial in the diagnosis and/or prognosis of NSCLC. One study indicated that higher levels of mir-429, a member of the mir-200 family, were correlated with a worse disease-free survival, and down-regulation of mir-486-5p in plasma was associated with a poor outcome. In adenocarcinoma patients, high miR-155 expression tended to a significant negative prognostic effect on survival and was an independent prognostic factor. However, in SCC patients with lymph node metastasis, miR-155 had a positive prognostic impact on survival.[65]

Circulating miRNAs

MiRNAs are very stable in blood despite the harsh environment.[66,67] Lawrie *et al*.[68] showed that the expression of miR-21 was increased in serum from patients with diffuse large B-cell lymphoma and that higher expression was associated with longer relapse-free survival. Mitchell *et al*.[67] observed tumor-derived miRNAs in serum from a xenografted prostate carcinoma mouse model, and found over-expression of miR-141 in metastatic prostate cancers. Further studies demonstrated that expression of miR-141 in serum had favorable sensitivity and satisfactory specificity for the diagnosis of prostate cancer. Chen *et al*.[66] analyzed miR-141 in patients with NSCLC, colorectal cancer and diabetes, and found that the serum miRNA profile in cancer patients was significantly different from the healthy controls. The study also identified that eight miRNAs (such as miR-205, miR-206, miR-335, etc.) were specific for NSCLC and 14 miRNAs (such as miR-485-5p, miR-361-3p, miR-326, miR-487b, etc.) were specific for colorectal cancer.

A recent study found 63 miRNAs in blood serum from lung cancer patients, but not in healthy counterparts.[66] MiRNA phenotypes were different between serum and blood cells for lung cancer patients; however, there was no difference in healthy people.[66] Another study showed that both miR-25 and miR-223 in serum were highly expressed in NSCLC in contrast to healthy people; thus, miR-25 and miR-223 could be considered biomarkers for NSCLC.[66] Keller *et al*.[69] showed that 27 miRNAs were significantly dysregulated in blood cells of lung cancer patients compared with the controls. Using a subset of 24 miRNAs, a radial basis functional support vector system allowed for distinguishing blood cell samples of tumor patients and controls with an accuracy of 95.4% [94.9–95.9%], a specificity of 98.1% [97.3–98.8%] and a sensitivity of 92.5% [91.8–92.5%]. These findings suggest that neoplasia might lead to deregulation of miRNA expression in blood cells from cancer patients when compared with healthy individuals. A high turnover of tumor cells or/and infiltration into the circulation might be responsible for this. Rabinowits *et al*.[70] found that the average exosome concentration in the peripheral circulation was 2.85 and 0.77 mg/mL in individuals with cancer and normal people, respectively, and that the miRNA concentrations were 158.6 and 68.1 ng/mL, respectively. There were no significant differences between the levels of miRNAs in peripheral circulation and tumor tissues. However, significant differences among miRNAs were observed in total peripheral circulation and tumor tissue between tumor patients and healthy individuals. This suggests that circulating exosomal miRNAs can be used as a screening tool for lung cancer.

However, serum miRNA expression profiles have limited value due to the following reasons: (1) serum miRNA expression profiles do not directly correspond to tissue profiles. In addition, there is no direct correlation between tissue miRNA levels and serum miRNA levels due to the possible mechanisms responsible for miRNA release into the circulation (cell lysis or exosome release[71])[72] and the release of miRNAs into circulation from other tissues as a result of the cancer or other non-cancer-related conditions (Figure 2); (2) different miRNA-labeling techniques result in different expression profiles. For example, Universal Linkage System (ULS) chemical labeling (Mirus [Mirus Bio Corporation, Madison, WI, USA] and Kreatech [Kreatech Diagnostics, Amsterdam, Netherlands] kits), which only targets G residues, produces signal intensities that are directly proportional to the number of G residues in the miRNA. In contrast, labeling by incorporating modified nucleotides or label into an extended poly A tail, or by labeling with a modified primer by first strand cDNA synthesis, should produce a more even label; (3) amplification techniques such as reverse transcriptase polymerase chain reaction or T7 promoter expression, produce different miRNA expression patterns on arrays when compared with unamplified samples. This can be due to differential efficiency in the amplification process. In addition, the limited quantity of miRNAs in human serum is not sufficient for column purification methods commonly used to purify tissue miRNA.[73]

MiRNAs as new approaches and targets for lung cancer therapy

Roles of miRNAs in cancer therapy

Owing to the close relationship between high tumor miRNA expression and cell cycle control, as well as oncogenic and pro-oncogenic gene regulation, miRNAs might be considered new targets for cancer therapy. In addition, one miRNA can regulate the expression of hundreds of other mRNAs[39] and can achieve a significant therapeutic effect as 'one agent with multiple targets.' Wiggins *et al*.[74] reported that a chemical synthesized miR-34a and a lipid-based delivery vehicle administered locally or systemically, blocked tumor growth in mouse models of NSCLC with no effect on cytokine production, enzyme activity or immune response. This also provided proof of concept for the systemic delivery of synthetic tumor suppressors associated

with viral-based miRNA delivery and thus offers a better method for miRNA replacement therapy. Different miRNAs might play opposing roles in lung cancer therapy. For example, studies have shown that let-7 plays an important role in cell cycle, differentiation and apoptosis.[12] Furthermore, let-7 is considered an important molecule in the inhibition of angiogenesis and cancerization.[75] Reduced let-7 expression is directly associated with up-regulation of RAS in lung cancer, and at the same time it reacts with other mRNAs in close proximity to repress RAS and MYC translation.[16,17] Over-expression of let-7a inhibited tumor growth by suppressing the RAS and MYC genes in an athymic mouse transplantation tumor model.[76] Nasal administration of let-7 inhibited tumor growth in a K-ras-mutated lung cancer model in mice.[77] Other miRNAs also act as tumor promoters. PTEN was first discovered and identified as a tumor suppressor gene, which was reported to induce apoptosis, and control cell growth, invasion, migration and angiogenesis through interference of several signaling pathways. In cells transfected with anti-miR-21s, the level of PTEN protein was increased.[78] This largely reduced tumor cell growth and invasive characteristics, suggesting that miR-21 down-regulates the expression of PTEN, stimulating tumor growth and invasion, which might be a potential therapeutic target for NSCLC.[78] Another study suggested that miR-17, which is regulated by the STAT3 pathway, mediated MEK (MAP/ERK kinase) inhibitor resistance by suppressing BIM expression.[79] Wu *et al.*[80] reported that pre-miR-133b, a tumor suppressor, combined with cationic lipoplexes *in vitro* could target the prosurvival gene MCL-1 to regulate cell survival and sensitivity of lung cancer cells to chemotherapeutic agents. Antisense inhibition and silencing of miRNA to normalize the function of oncomirs is theoretically feasible; however, delivery of the small molecules locally and systemically is still challenging. Moreover, these methods are still in the bench stage and more work needs to be done to validate and test this *in vivo*.

MiRNAs in therapy

Weidhaas *et al.*[81] studied the role of miRNA expression related to irradiation in lung cancer cells and found that A549 cells, which contain lower let-7 levels and higher activated RAS, had significant changes in miRNAs as early as two hours after irradiation. Microarray analysis performed in a normal lung epithelial cell line and CLR2741 lung cancer cell line indicated that all members of let-7 family were altered.[16] As expected from the effects of let-7b over-expression, anti-let-7b caused significant protection from radiation.[82] Over-expression of let-7g protected A549 cells from radiation, whereas anti-let-7g increased radiosensitization in lung cancer cells.[82] MiRNAs are also involved in

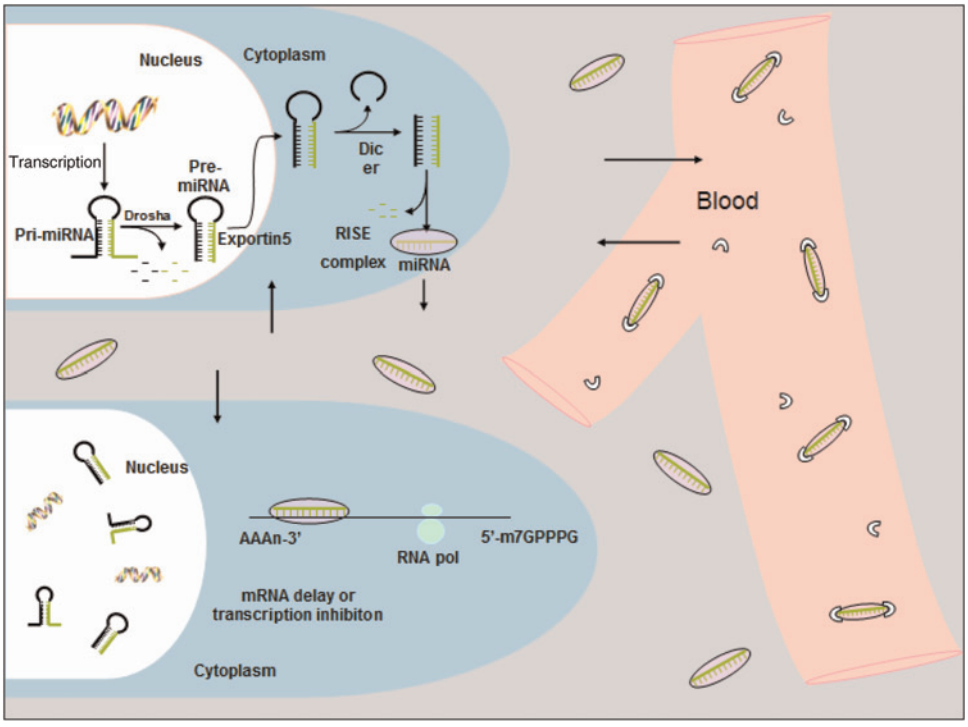

Figure 2 The potential molecular mechanisms that the tumor-related microRNAs (miRNAs) play in cells and blood. MiRNAs are packaged inside exosomes that are secreted from cells. Exosomes are membrane-bound particles abundant in plasma. In nuclei, miRNAs are transcribed by RNA polymerase II to generate the primary transcript (pri-miRNAs), which contain a hairpin-shaped structure. Then they are processed by the type III RNA endonuclease Drosha into pre-miRNAs. The pre-miRNAs are transported to cytoplasm by exportin-5, and undergo further processing by Dicer forming mature miRNA. The mature single-stranded miRNAs are assembled onto the RNA-induced silencing complex (RISC), which mediates the activity of the miRNA. The miRNA complex interacts with the complementary sites in the 3′ untranslated region of the mRNA to regulate gene expression negatively, through the inhibition of transcription. In blood, miRNAs are very stable. One possible explanation includes the protection via association with other molecules (e.g. RNA–protein complex) or modifications of the miRNAs to resist RNase activity. (A color version of this figure is available in the online journal)

lung cancer chemotherapy. In support of this, studies have indicated that pre-miR-181a and pre-miR-630 play different roles in mediating *cis*-diamminedichloroplatinum (cis-DDP)-induced cell death.[82] For example, pre-miR-630 induced cancer cell arrest in G_0–G_1 and increased the cell cycle inhibitor $p27^{kip1}$, thereby greatly diminishing the sensitivity of A549 cells in response to DDP. Ectopic overexpression of miR-451 could significantly inhibit growth, induce apoptosis of A549 cells and sensitized A549 cells to DDP possibly by increasing DDP-induced apoptosis, which might be associated with inactivation of the Akt-signaling pathway.[83] Zhong *et al*.[15] showed that the increased expression of let-7a, hsa-miR-126 and hsa-miR-145 contributed to enhanced cytotoxicity induced by gefitinib in lung cancer cells, especially for hsa-miR-126, for which the highest value of half maximal inhibitory (IC50) was significantly increased. Seike *et al*.[84] found that the expression of miR-21 was significantly higher in lung cancer with EGFR mutations from smokers compared with those that never smoked. In the non-smoker-derived lung adenocarcinoma cell line, H3255 with mutant EGFR, high levels of phosphorylated-EGFR and miR-21 were found. Antisense miR-21 enhanced AG1478 (one new agent of EGFR-tyrosine kinase inhibitors) and induced apoptosis. In the wild-type EGFR cell line H441, the antisense miR-21 not only induced apoptosis, but also showed additive effects with AG1478, suggesting that miR-21 could be a potential therapeutic target or act as a downstream effector of EGFR that can enhance the therapeutic effects of EGFR-tyrosine kinase inhibitors on the status of EGFR mutation.

Conclusions and prospectives

MiRNAs play an important role in many aspects of tumor biology and are becoming a major focus for diagnosis, treatment and prognosis of cancers. Many new technologies have been developed in this area. One study found that an artificially synthesized miRNA targeted to CXC chemokine receptor-4, one of the most prominent chemokine receptors, blocked the invasion and metastasis of breast cancer cells.[85] Ma *et al*.[86] also showed that systemic treatment of mammary tumor-bearing mice with a miR-10b antagonist that significantly decreased miR-10b and increased the target (Hoxd10) levels, reduced the growth of primary mammary tumors and suppressed the formation of lung metastases in a sequence-specific manner. This study provides a new prospective approach for the development of new anticancer agents. Some miRNAs, which act as regulators of gene expression, have enormous impact on new drug development and individualized or other target therapeutic approaches. Although lung cancer tissue specimens are difficult to obtain, examining the miRNA expression in biological fluids is more convenient. These biological fluids are suitable in screening susceptible populations, thus making miRNAs a better tool for basic and clinical researches in the near future.

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REFERENCES

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;**55**:74–108
2 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008;**58**:71–96
3 Manser RL, Irving LB, Stone C, Byrnes G, Abramson M, Campbell D. Screening for lung cancer. *Cochrane Database Syst Rev* 2004;(1):CD001991
4 Bach PB, Jett JR, Pastorino U, Tockman MS, Swensen SJ, Begg CB. Computed tomography screening and lung cancer outcomes. *JAMA* 2007;**297**:953–61
5 Ambros V. MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 2003;**113**:673–6
6 Ortholan C, Puissegur MP, Ilie M, Barbry P, Mari B, Hofman P. MicroRNAs and lung cancer: new oncogenes and tumor suppressors, new prognostic factors and potential therapeutic targets. *Curr Med Chem* 2009;**16**:1047–61
7 Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006;**9**:189–98
8 Carleton M, Cleary MA, Linsley PS. MicroRNAs and cell cycle regulation. *Cell Cycle* 2007;**6**:2127–32
9 Chivukula RR, Mendell JT. Circular reasoning: microRNAs and cell-cycle control. *Trends Biochem Sci* 2008;**33**:474–81
10 Takahashi Y, Forrest AR, Maeno E, Hashimoto T, Daub CO, Yasuda J. MiR-107 and MiR-185 can induce cell cycle arrest in human non small cell lung cancer cell lines. *PLoS ONE* 2009;**4**:e6677
11 Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M, Lim L. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol Cell Biol* 2007;**27**:2240–52
12 Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, Slack FJ. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res* 2007;**67**:7713–22
13 Cheng AM, Byrom MW, Shelton J, Ford LP. Antisense inhibition of human miRNAs and indications for an involvement of miRNA in cell growth and apoptosis. *Nucleic Acids Res* 2005;**33**:1290–7
14 Sun Y, Bai Y, Zhang F, Wang Y, Guo Y, Guo L. miR-126 inhibits non-small cell lung cancer cells proliferation by targeting EGFL7. *Biochem Biophys Res Commun* 2010;**391**:1483–9
15 Zhong M, Ma X, Sun C, Chen L. MicroRNAs reduce tumor growth and contribute to enhance cytotoxicity induced by gefitinib in non-small cell lung cancer. *Chem Biol Interact* 2010;**184**:431–8
16 Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell* 2005;**120**:635–47
17 Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 2004;**64**:3753–6
18 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

19 Feng S, Cong S, Zhang X, Bao X, Wang W, Li H, Wang Z, Wang G, Xu J, Du B, Qu D, Xiong W, Yin M, Ren X, Wang F, He J, Zhang B. MicroRNA-192 targeting retinoblastoma 1 inhibits cell proliferation and induces cell apoptosis in lung cancer cells. *Nucleic Acids Res* 2011;**39**:6669–78

20 Incoronato M, Garofalo M, Urso L, Romano G, Quintavalle C, Zanca C, Iaboni M, Nuovo G, Croce CM, Condorelli G. miR-212 increases tumor necrosis factor-related apoptosis-inducing ligand sensitivity in non-small cell lung cancer by targeting the antiapoptotic protein PED. *Cancer Res* 2010;**70**:3638–46

21 James MA, Lu Y, Liu Y, Vikis HG, You M. RGS17, an overexpressed gene in human lung and prostate cancer, induces tumor cell proliferation through the cyclic AMP-PKA-CREB pathway. *Cancer Res* 2009;**69**:2108–16

22 Sun Y, Fang R, Li C, Li L, Li F, Ye X, Chen H. Hsa-mir-182 suppresses lung tumorigenesis through down regulation of RGS17 expression in vitro. *Biochem Biophys Res Commun* 2010;**396**:501–7

23 Cha ST, Chen PS, Johansson G, Chu CY, Wang MY, Jeng YM, Yu SL, Chen JS, Chang KJ, Jee SH, Tan CT, Lin MT, Kuo ML. MicroRNA-519c suppresses hypoxia-inducible factor-1alpha expression and tumor angiogenesis. *Cancer Res* 2010;**70**:2675–85

24 Lerman MI, Minna JD. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. *Cancer Res* 2000;**60**:6116–33

25 Ji L, Nishizaki M, Gao B, Burbee D, Kondo M, Kamibayashi C, Xu K, Yen N, Atkinson EN, Fang B, Lerman MI, Roth JA, Minna JD. Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities *in vitro* and *in vivo*. *Cancer Res* 2002;**62**:2715–20

26 Ivanova AV, Ivanov SV, Pascal V, Lumsden JM, Ward JM, Morris N, Tessarolo L, Anderson SK, Lerman MI. Autoimmunity, spontaneous tumourigenesis, and IL-15 insufficiency in mice with a targeted disruption of the tumour suppressor gene Fus1. *J Pathol* 2007;**211**:591–601

27 Du L, Schageman JJ, Subauste MC, Saber B, Hammond SM, Prudkin L, Wistuba II, Ji L, Roth JA, Minna JD, Pertsemlidis A. MiR-93, miR-98, and miR-197 regulate expression of tumor suppressor gene FUS1. *Mol Cancer Res* 2009;**7**:1234–43

28 Prenzel N, Fischer OM, Streit S, Hart S, Ullrich A. The epidermal growth factor receptor family as a central element for cellular signal transduction and diversification. *Endocr Relat Cancer* 2001;**8**:11–31

29 Wang G, Mao W, Zheng S, Ye J. Epidermal growth factor receptor-regulated miR-125a-5p-a metastatic inhibitor of lung cancer. *FEBS J* 2009;**276**:5571–8

30 Simon JA, Tamkun JW. Programming off and on states in chromatin: mechanisms of polycomb and trithorax group complexes. *Curr Opin Genet Dev* 2002;**12**:210–8

31 Karanikolas BD, Figueiredo ML, Wu L. Comprehensive evaluation of the role of EZH2 in the growth, invasion, and aggression of a panel of prostate cancer cell lines. *Prostate* 2010;**70**:675–88

32 Zhang JG, Guo JF, Liu DL, Liu Q, Wang JJ. MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2. *J Thorac Oncol* 2011;**6**:671–8

33 Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 2006;**103**:2257–61

34 Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 2005;**65**:9628–32

35 Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. *Cell* 2003;**115**:787–98

36 Cully M, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006;**6**:184–92

37 Salmena L, Carracedo A, Pandolfi PP. Tenets of PTEN tumor suppression. *Cell* 2008;**133**:403–14

38 Liu X, Sempere LF, Ouyang H, Memoli VA, Andrew AS, Luo Y, Demidenko E, Korc M, Shi W, Preis M, Dragnev KH, Li H, Direnzo J, Bak M, Freemantle SJ, Kauppinen S, Dmitrovsky E. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. *J Clin Invest* 2010;**120**:1298–309

39 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;**116**:281–97

40 Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, Muller RU, Straka E, Su L, Burki EA, Crowell RE, Patel R, Kulkarni T, Homer R, Zelterman D, Kidd KK, Zhu Y, Christiani DC, Belinsky SA, Slack FJ, Weidhaas JB. 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

41 Xiong F, Wu C, Chang J, Yu D, Xu B, Yuan P, Zhai K, Xu J, Tan W, Lin D. Genetic variation in an miRNA-1827 binding site in MYCL1 alters susceptibility to small-cell lung cancer. *Cancer Res* 2011;**71**:5175–81

42 Jiang L, Huang Q, Zhang S, Zhang Q, Chang J, Qiu X, Wang E. 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

43 Wang L, Tabu K, Kimura T, Tsuda M, Linghu H, Tanino M, Kaneko S, Nishihara H, Tanaka S. Signaling adaptor protein Crk is indispensable for malignant feature of glioblastoma cell line KMG4. *Biochem Biophys Res Commun* 2007;**362**:976–81

44 Crawford M, Brawner E, Batte K, Yu L, Hunter MG, Otterson GA, Nuovo G, Marsh CB, Nana-Sinkam SP. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. *Biochem Biophys Res Commun* 2008;**373**:607–12

45 Roybal JD, Zang Y, Ahn YH, Yang Y, Gibbons DL, Baird BN, Alvarez C, Thilaganathan N, Liu DD, Saintigny P, Heymach JV, Creighton CJ, Kurie JM. miR-200 Inhibits lung adenocarcinoma cell invasion and metastasis by targeting Flt1/VEGFR1. *Mol Cancer Res* 2011;**9**:25–35

46 Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005;**435**:834–8

47 Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM. Development of carcinoma of the lung as reflected in exfoliated cells. *Cancer* 1974;**33**:256–70

48 Thunnissen FB. Sputum examination for early detection of lung cancer. *J Clin Pathol* 2003;**56**:805–10

49 Xie Y, Todd NW, Liu Z, Zhan M, Fang H, Peng H, Alattar M, Deepak J, Stass SA, Jiang F. Altered miRNA expression in sputum for diagnosis of non-small cell lung cancer. *Lung Cancer* 2010;**67**:170–6

50 Shen J, Todd NW, Zhang H, Yu L, Lingxiao X, Mei Y, Guarnera M, Liao J, Chou A, Lu CL, Jiang Z, Fang H, Katz RL, Jiang F. Plasma microRNAs as potential biomarkers for non-small-cell lung cancer. *Lab Invest* 2011;**91**:579–87

51 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

52 Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, Calabro E, Croce CM, Pastorino U, Sozzi G. 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

53 Barshack I, Lithwick-Yanai G, Afek A, Rosenblatt K, Tabibian-Keissar H, Zepeniuk M, Cohen L, Dan H, Zion O, Strenov Y, Polak-Charcon S, Perelman M. MicroRNA expression differentiates between primary lung tumors and metastases to the lung. *Pathol Res Pract* 2010;**206**: 578–84

54 Yu L, Todd NW, Xing L, Xie Y, Zhang H, Liu Z, Fang H, Zhang J, Katz RL, Jiang F. Early detection of lung adenocarcinoma in sputum by a panel of microRNA markers. *Int J Cancer* 2010;**127**:2870–8

55 Lebanony D, Benjamin H, Gilad S, Ezagouri M, Dov A, Ashkenazi K, Gefen N, Izraeli S, Rechavi G, Pass H, Nonaka D, Li J, Spector Y, Rosenfeld N, Chajut A, Cohen D, Aharonov R, Mansukhani M. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. *J Clin Oncol* 2009;**27**:2030–7

56 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

57 Xing L, Todd NW, Yu L, Fang H, Jiang F. Early detection of squamous cell lung cancer in sputum by a panel of microRNA markers. *Mod Pathol* 2010;**23**:1157–64

58 Raponi M, Dossey L, Jatkoe T, Wu X, Chen G, Fan H, Beer DG. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. *Cancer Res* 2009;**69**:5776–83

59 Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004;**101**: 2999–3004

60 Shell S, Park SM, Radjabi AR, Schickel R, Kistner EO, Jewell DA, Feig C, Lengyel E, Peter ME. Let-7 expression defines two differentiation stages of cancer. *Proc Natl Acad Sci USA* 2007;**104**:11400–5

61 Hu Z, Chen X, Zhao Y, Tian T, Jin G, Shu Y, Chen Y, Xu L, Zen K, Zhang C, Shen H. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. *J Clin Oncol* 2010;**28**:1721–6

62 Gallardo E, Navarro A, Vinolas N, Marrades RM, Diaz T, Gel B, Quera A, Bandres E, Garcia-Foncillas J, Ramirez J, Monzo M. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. *Carcinogenesis* 2009;**30**:1903–9

63 Ranade AR, Cherba D, Sridhar S, Richardson P, Webb C, Paripati A, Bowles B, Weiss GJ. MicroRNA 92a-2*: a biomarker predictive for chemoresistance and prognostic for survival in patients with small cell lung cancer. *J Thorac Oncol* 2010;**5**:1273–8

64 Gao W, Yu Y, Cao H, Shen H, Li X, Pan S, Shu Y. 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

65 Donnem T, Eklo K, Berg T, Sorbye SW, Lonvik K, Al-Saad S, Al-Shibli K, Andersen S, Stenvold H, Bremnes RM, Busund LT. Prognostic impact of MiR-155 in non-small cell lung cancer evaluated by in situ hybridization. *J Transl Med* 2011;**9**:6

66 Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008;**18**:997–1006

67 Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB, Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008;**105**:10513–8

68 Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris 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

69 Keller A, Leidinger P, Borries A, Wendschlag A, Wucherpfennig F, Scheffler M, Huwer H, Lenhof HP, Meese E. MiRNAs in lung cancer – studying complex fingerprints in patient's blood cells by microarray experiments. *BMC Cancer* 2009;**9**:353

70 Rabinowits G, Gercel-Taylor C, Day JM, Taylor DD, Kloecker GH. Exosomal microRNA: a diagnostic marker for lung cancer. *Clin Lung Cancer* 2009;**10**:42–6

71 Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol Oncol* 2008;**110**:13–21

72 Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. *Gynecol Oncol* 2009;**112**:55–9

73 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

74 Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D, Bader AG. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res* 2010;**70**:5923–30

75 Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res* 2007;**101**:59–68

76 He XY, Chen JX, Zhang Z, Li CL, Peng QL, Peng HM. The let-7a microRNA protects from growth of lung carcinoma by suppression of k-Ras and c-Myc in nude mice. *J Cancer Res Clin Oncol* 2010;**136**:1023–8

77 Grimm D, Streetz KL, Jopling CL, Storm TA, Pandey K, Davis CR, Marion P, Salazar F, Kay MA. Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature* 2006;**441**:537–41

78 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

79 Dai B, Meng J, Peyton M, Girard L, Bornmann WG, Ji L, Minna JD, Fang B, Roth JA. STAT3 mediates resistance to MEK inhibitor through microRNA miR-17. *Cancer Res* 2011;**71**:3658–68

80 Wu Y, Crawford M, Yu B, Mao Y, Nana-Sinkam SP, Lee LJ. MicroRNA delivery by cationic lipoplexes for lung cancer therapy. *Mol Pharm* 2011;**8**:1381–9

81 Weidhaas JB, Babar I, Nallur SM, Trang P, Roush S, Boehm M, Gillespie E, Slack FJ. MicroRNAs as potential agents to alter resistance to cytotoxic anticancer therapy. *Cancer Res* 2007;**67**:11111–6

82 Galluzzi L, Morselli E, Vitale I, Kepp O, Senovilla L, Criollo A, Servant N, Paccard C, Hupe P, Robert T, Ripoche H, Lazar V, Harel-Bellan A, Dessen P, Barillot E, Kroemer G. MiR-181a and miR-630 regulate cisplatin-induced cancer cell death. *Cancer Res* 2010;**70**:1793–803

83 Bian HB, Pan X, Yang JS, Wang ZX, De W. Upregulation of microRNA-451 increases cisplatin sensitivity of non-small cell lung cancer cell line (A549). *J Exp Clin Cancer Res* 2011;**30**:20

84 Seike M, Goto A, Okano T, Bowman ED, Schetter AJ, Horikawa I, Mathe EA, Jen J, Yang P, Sugimura H, Gemma A, Kudoh S, Croce CM, Harris CC. MiR-21 is an EGFR-regulated anti-apoptotic factor in lung cancer in never-smokers. *Proc Natl Acad Sci USA* 2009;**106**:12085–90

85 Liang Z, Wu H, Reddy S, Zhu A, Wang S, Blevins D, Yoon Y, Zhang Y, Shim H. Blockade of invasion and metastasis of breast cancer cells via targeting CXCR4 with an artificial microRNA. *Biochem Biophys Res Commun* 2007;**363**:542–6

86 Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, Teruya-Feldstein J, Bell GW, Weinberg RA. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. *Nat Biotechnol* 2010;**28**:341–7

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