**Introduction**

Lung cancer is the most frequently diagnosed cancer and the most common cause of cancer death worldwide, accounting for an estimated 1.8 million new cases and 1.6 million deaths in 2012 (1). Approximately 85% of lung cancers are classified as non-small cell lung cancer (NSCLC), a less aggressive form of tumor than small cell lung cancer (the remaining 15% of all lung cancers). According to Surveillance, Epidemiology, and End Results (SEER) Cancer Statistics Review, the 5-year survival rate of NSCLC remains as low as 23.6% because only 19% of cases are diagnosed at localized stages when the disease is highly curable with a 59.5% 5-year survival rate (2).

An early detection of lung cancer therefore is able to reduce the mortality to a large extent. Low-dose computed tomography (LDCT) is recommended as standard screening for the high-risk population, considering a lung cancer mortality benefit of 20% demonstrated by the National Lung Screen Trial (NLST) (3). However, a high false positive rate of 96% was shown in the NLST and could give rise to increasing screening rounds, frequent radiation exposure and exaggerated anxiety, although those results were eventually proved to be benign nodules. To complement the overdiagnosis by LDCT, specific cancer biomarkers, particularly miRNAs, would contribute to a favorable laboratory examination as discussed by numerous studies (4,5).

MicroRNAs (miRNAs) are endogenous small non-coding RNAs of 18–24 nucleotides in length, acting as important regulators in post-transcriptional gene expression. More than 2,500 mature human miRNAs have been identified so far, according to the miRBase database (http://www.mirbase.org/, version 21, accessed January 2018) since the first human-encoded miRNA, let-7, was discovered in 2000. Owing to their distinct characteristics, miRNAs have been
extensively researched for the past decade for their role as potential biomarkers and novel therapeutics (6,7).

### miRNA biogenesis and function

Initially, microRNA genes are transcribed by RNA polymerase II into primary miRNAs (pri-miRNAs) in the nucleus. The pri-miRNAs are subsequently cleaved by Drosha/DGCR8 enzyme complex, and the resulting products are named as precursor miRNAs (pre-miRNAs). Exportin-5, a nuclear transport protein, then mediates the exportation of pre-miRNAs to the cytoplasm, where they are sliced by nuclease Dicer yielding miRNA duplexes, or the mature miRNAs. The strand generally with the less-thermostable 5′-terminus incorporates into RNA-induced silencing complex (RISC), composed of the Argonaute (AGO) protein family and associated proteins, while the other strand is degraded. RISC with mature miRNA is able to recognize and bind to 3′UTR of a target mRNA and prompt mRNA cleavage with perfect complementarity or translational repression with imperfect complementarity.

The expression of target proteins reduces as a consequence of mRNA degradation or translational inhibition. MiRNAs thus are able to participate in diverse physiological processes, including tissue morphogenesis, cell proliferation, differentiation, apoptosis and different signaling pathways (8). Due to the low specificity of miRNA-mRNA interaction, each mRNA can be repressed by more than one miRNA (9), and a single miRNA can regulate multiple target mRNAs (10).

In contrast, miRNA dysregulation could result in various diseases. Neurological disorders, cardiovascular diseases, monogenic disorders, autoimmune diseases and cancer have been discovered to have a relationship with miRNA disruption (11).

### Role of miRNAs in carcinogenesis and as cancer biomarkers

Both genetic and epigenetic changes through miRNA disruption engage in carcinogenesis. Genetically, miRNAs could downregulate tumor suppressive gene expression and upregulate oncogene expression (12). The same miRNA is able to have oncogenic activity in one particular cell type but a tumor-suppressive property in another based on different targets and pathogenesis (13). Under normal circumstances, miRNAs modulate the level of epigenetic regulatory proteins, such as DNA methyl transferases, histone deacetylases and components of the polycomb repressor complexes; they directly change the epigenetic status of promoter regions as well (14). Abnormal alterations of these epigenetic signatures by miRNA dysregulation are implicated in carcinogenesis.

However, first, the dysregulation of miRNAs concerns the major steps of the miRNA biogenesis as follows (15): genomic and genetic changes, such as deletions, mutations, amplifications, translocations and single nucleotide polymorphisms (SNPs); epigenetic alterations such as DNA methylation and histone acetylation; altered activity of transcription factor; and impaired expression or function of related proteins (Drosha, Dicer, Exportin-5, etc.).

Tumor tissues could be distinguished from normal tissues judging by the expression level of miRNAs. Differential expression of miRNAs is broadly revealed in lung cancer (16), colorectal cancer (17), renal cell carcinoma (18), prostate cancer (19), pancreatic cancer (20), ovarian cancer (21), etc. Moreover, specific miRNAs correspond to certain types of cancer and identify cancer tissue origins (22).

Obtaining tissue samples is much more inferior to non-invasive sampling, in terms of feasibility and safety, to detect the altered expression of miRNAs. It is observed that miRNAs are present in multiple human body fluids, such as plasma, urine, pleural fluid, colostrum, and cerebrospinal fluid (23). Additionally, it is well recognized that circulating miRNAs are correlated to tumors and are of diagnostic, prognostic, and monitoring values (24,25). Circulating miRNAs possess several advantages to distinguish themselves among cancer biomarkers. They are extraordinarily stable in body fluids, without the attack from endogenous RNases by the protection of vesicles or associated proteins (26). Both prolonged incubation at room temperature and multiple cycles of freeze-thawing exert minimal effects on miRNA amount (26). In addition, easy measurement provides sufficient dynamic data of the disease progression, response to treatments and cancer relapse, which help provide rational decision making for personalized therapies. Furthermore, circulating miRNAs overcome the problem of tumor heterogeneity because different parts of the tumor and metastatic sites all could release these pathologic signals into the circulation (27).

However, how do miRNAs gain access to the circulation? Basically, they enter via passive leakage and active secretion (24). The passive pathway, discharged from damaged and dead cells, however, plays a minor role in the
production of circulating miRNAs. The active transport is mediated by microvesicles (microparticles and exosomes) and RNA-binding proteins, that is, AGO2 (28), high-density lipoproteins (29) and nucleophosmin (30). Tumor cells more preferentially excrete larger amount of exosomes than normal cells do, which presents the dysregulated status of tumor miRNAs in the circulation (31). The research of Taylor et al. revealed the levels of eight overexpressed miRNAs were similar between cellular and exosomal miRNAs and suggested circulating tumor exosomes as diagnostic biomarkers (32). This hypothesis is supported by some studies (33-35) but repudiated by other results (28,30). In spite of the contradictions of current findings, the use of circulating biomarkers as non-invasive cancer biomarkers is well established.

miRNAs as biomarkers in NSCLC and the mechanism

To date, studies have strongly demonstrated the role of miRNAs as biomarkers in NSCLC. Overexpression of oncogenic miRNAs and decreased expression of tumor suppressive miRNAs could both be detected in NSCLC. Some of them have been confirmed to be involved in the development or progression of lung cancer, and the principal miRNAs are miR-21, miR-17-92 cluster and miR-221/222 as oncogenic miRNAs and let-7 family, miR-34 family and miR-200 family as tumor suppressive miRNAs (36).

The let-7 family was the first discovered human-encoded miRNA, of which the expression was also shown reduced in NSCLC patients indicating poor prognosis (37,38). Let-7 possesses tumor suppressive activity, inhibiting multiple oncogenes such as RAS (39), MYC (40) and HMGAA2 (41), and reduces the expression of cyclins (42). In lung cancer, chromosomal regions containing various let-7 genes were reported often deleted (43). Moreover, a frequent SNP at the let-7 complementary site 6 was validated to have an association with an increased risk for NSCLC among smokers (44).

The miR-34 family comprises miR-34a, miR-34b and miR-34c, acting as mediators of tumor suppression by P53 (45). All members of the miR-34 family are capable of repressing tumor growth and metastasis by targeting mRNAs participating in cell cycles, epithelial-mesenchymal transition (EMT), metastasis, stemness, apoptosis and senescence (46). It was observed that miR-34 genes were frequently downregulated by CpG methylation in various types of tumor or deleted as a minor cause (47). One study revealed that miR-34 synergistically with miR-15a/16 was significantly downregulated in NSCLC cell lines (48). Another study identified tissue miR-34a as an independent prognostic marker of recurrence in surgically resected NSCLC (49). Additionally, aberrant methylation of tissue miR-34 was indicated as a prognostic factor for NSCLC (50,51).

All five members of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) underwent remarkable downregulation in cells with EMT, which is regarded as a critical step in metastasis (52). EMT induced by the miRNAs was considered as a result of regulation of zinc finger E-box-binding homeobox (ZEB) transcription factors and E-cadherin (53). Loss of miR-200c expression was shown to give rise to an aggressive, invasive, and chemoresistant phenotype of NSCLC (54). However, other clinical outcomes contradict the above findings about miR-200c, as poor survival rates, not provided by previous studies, were demonstrated in NSCLC with overexpression of miR-200c (55,56). The oncogenic property of miR-200c was argued by its potential to target several tumor suppressor genes as a more dominant role than regulation of ZEB in NSCLC carcinogenesis (56).

MiR-21 is an oncogenic miRNA and overexpressed in multiple solid tumors (57), including NSCLC. MiR-21 promotes tumorigenesis through inhibition of regulators of the Ras/MEK/ERK pathway and blockage of apoptosis (58). Negative regulation on tumor suppressive genes, such as PTEN (59), MARCKS (60), PDCD4 (61) and TPM1 (62) has been reported to be part of miR-21’s oncogenic mechanism. The elevated expression of miR-21 was much higher in tumor tissues and cell lines with epidermal growth factor receptor (EGFR) mutation than those without the mutation, indicating that the EGFR signaling pathway increases the expression of miR-21 (63). To complicate the situation, downregulation of miR-21 inhibits the EGFR pathway, which implicates a feedback loop between miR-21 and EGFR (64,65). The research of Wei et al. identified plasma miR-21 as a sensitive and specific marker for early diagnosis for NSCLC and a predicative indicator for response sensitivity to platinum chemotherapy (66). Moreover, one retrospective analysis suggested the potential of miR-21 as a prognostic biomarker of early-stage lung adenocarcinoma (67).

More preferably in small cell lung cancer, the miR-17-92 cluster (miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a and miR-92a) was observed overexpressed with occasional gene amplification (68). The upregulated miR-17-92 cluster
negatively regulates E2F (69), Myc (69), HIF-1α (70) and PTEN (71) as possible routes to apply its oncogenic function.

MiR-221 and miR-222 were shown particularly increased in tissues and cell lines of invasive and TNF-related apoptosis-inducing ligand (TRAIL) resistant NSCLC (72), through PTEN and TIMP3 downregulation (73). On the other hand, a relatively new study identified the growth suppressive ability of miR-221 and miR-222 in four other cell lines, which was different from the previous reports. This interesting finding might explore the dual roles of miRNAs not only in multiple cancer types but also in various cancer cell lines within one tumor class (74).

Clinical significance of miRNA biomarkers in NSCLC

One application of miRNAs is diagnosis of NSCLC, early diagnosis in particular. Additionally, the diagnostic use will be discussed separately in the latter part of the passage.

Interestingly, miRNAs could potentially differentiate lung cancer subtypes. For example, one study found four overexpressed miRNAs (miR-205, miR-93, miR-221 and miR-30e) in squamous cell carcinoma tissue and five more highly expressed miRNAs (miR-29b, miR-29c, let-7e, miR-100 and miR-125a-5p) in adenocarcinoma tissue (75). It may help with perioperative differentiation of histologic subtypes in those NSCLCs hard to perform a biopsy.

Additionally, miRNAs have been recognized of prognostic value in NSCLC. Surgical resection remains the only curative treatment, and survival is threatened by disease recurrence, which could be reflected by the level of miRNAs. Sanfiorenzo et al. identified two plasma miRNA panels associated with poor disease-free survival in resectable NSCLC, one of adenocarcinoma (high miR-155-5p, high miR-223-3p, and low miR-126-3p) and the other of squamous cell carcinoma (high miR-20a-5p, low miR-152-3p, and low miR-199a-5p) (76). According to a 24-microRNA panel from plasma, Sestini et al. classified 84 subjects into three groups of low, intermediate and high risk, which was subsequently found to be inversely correlated with 5-year survival (88.9%, 79.5% and 40.1%, respectively) (4). Among patients who underwent surgery, 76% of the 25 high-intermediate patients showed decreased risk calculated by the miRNA panel, while the risk first lowered but rebounded afterwards in one relapsing subject.

Furthermore, miRNAs could predict response to nonsurgical therapies and the survival in NSCLC. Chen et al. enrolled 54 eligible patients of stage III or IV disease for radiotherapy and discovered four plasma miRNAs (hsa-miR-98-5p, hsa-miR-302e, hsa-miR-495-3p, and hsa-miR-613) with higher expression in responders (complete or partial response) than in non-responders (stable or progressive disease) (77). Additionally, the high miRNA level favored an objective response rate based on each cut-point. The first-line chemotherapy using cisplatin and vinorelbine was evaluated by a prospective study of biopsy tissue samples from 38 subjects (78). MiR-149 and miR-375 were discovered predictive for response and were also related to progression-free survival. Meanwhile, a prognostic score containing four miRNAs (miR-200c, miR-424, miR-29c and miR-124) was constructed and proposed to assess the prognosis in differentiating median survival times.

Except for the traditional treatments, miRNA biomarkers become involved in novel treatments as well, such as target treatment and immune therapy. One research study explored the predictive role of plasma miRNAs in first-line EGFR tyrosine kinase inhibitors (EGFR-TKIs) for advanced NSCLC patients with EGFR 19 deletion (79). Three miRNAs (miR-21, miR-27a, and miR-218) were verified with significantly high expression in the resistant group in contrast to the sensitive group. Another study found that high expression of miR-200c in tumor tissue exhibited a significant association with a higher disease control rate, longer progression-free survival and longer overall survival in advanced NSCLC patients with wild-type EGFR who received second- or third-line EGFR-TKIs, but no significant relation was found to patients with EGFR mutation (80). EMT induced by a low level of miR-200c was assumed to be the mechanism of less effective EGFR-TKIs in the wild-type EGFR population. Chen et al. showed that the expression of the miRNA-200 family in tumor tissue was associated with a level of programmed death-ligand 1 (PD-L1) (81). The regulation of PD-L1 through the miRNA-200/ZEB1 axis probably acts by targeting PD-L1 directly or suppressing EMT when high expression PD-L1 was demonstrated in the mesenchymal phenotype of NSCLC. Although lacking research evidence, miR-200 expression should serve as a promising predictor of anti-PD-1/PD-L1 therapy in NSCLC. Additional miRNAs of potentially predictive value for immune therapy include miR-34 (82) and miR-197 (83).

Media for measuring miRNAs

Sputum is rather a less studied area concerning miRNA
biomarkers than plasma/serum. Sputum samples have benefits in that they contain the respiratory epithelial cells where the prolonged molecule genetic changes can be collected and tested (84). Nevertheless, there was doubt over the stability of miRNAs for high concentration of RNase activity in the sputum. However, it was proved that the miRNAs could be detectable up to 7 days after sputum collection with resistance of endogenous miRNAs to RNase activity (85). Another issue is that not all patients are able to expectorate sputum spontaneously, particularly former smokers and cancer-free patients. To address it, Su et al. proposed the application of lung fluke for sputum collection among those people without spontaneously expectorated sputum (86). Sputum samples would still be problematic for containing inconsistent cells in various samples, making miRNA quantification less convincing.

Whole blood miRNA examination was occasionally reported of diagnostic use in NSCLC, but a new study implied that whole blood miRNA expression might lack diagnostic value for NSCLC because the miRNA candidates did not differ in case and control groups or in pre-surgical and post-surgical groups (87). They explained the results by the fact that miRNAs exist extracellularly and within blood cells, and miRNA changes in whole blood may reflect other pathologic conditions and various physiologic statuses.

The role of serum and plasma as biomarkers of NSCLC, however, has been widely investigated. Additionally, serum and plasma are both accepted as better alternatives to sputum and whole blood, with greater specificity and sensitivity (88). Differences between serum and plasma were still observed, and the study of Wang et al. recommended plasma as the choice for studying circulating miRNAs since RNA released out of the cells during the coagulation process could conceal the true miRNA information (89).

**miRNAs as biomarkers for early detection of NSCLC**

Instead of a single miRNA, miRNA panels are more usually adopted to differentiate lung cancer from healthy controls in current studies, yielding higher sensitivity and specificity (90). However, no consensus of diagnostic panels has been achieved, and several meta-analyses tried to provide a general understanding about the diagnostic potential of miRNAs in NSCLC. Wang et al. conducted a meta-analysis of non-invasive miRNAs with a total of 28 articles, involving 2,121 NSCLC patients and 1,582 healthy controls (91). Multiple miRNA assays showed a pooled sensitivity of 0.83 and specificity of 0.82, which was more accurate than single miRNA assays with a sensitivity of 0.77 and specificity of 0.71. Another meta-analysis of non-invasive miRNAs performed by Chen et al. (20 articles, involving 1,563 NSCLC patients and 1,060 healthy controls) shared similar results (92)—the performance of multiple miRNAs (sensitivity and specificity of 0.81 and 0.84, respectively) was better than that of single miRNA assays (sensitivity and specificity of 0.73 and 0.77, respectively). Although the diagnostic value is displayed by the above meta-analyses, the overall sensitivity and specificity, both approximately 0.80, do not indicate excellent performance. Fortunately, there are individual studies showing promising results, as the details described in Table 1 depict. These studies all presented different miRNA panels or diagnostic models consisting of miRNAs and radiological features that could better detect early NSCLC, with some of them showing a lower false positive rate.

In 2011, Boeri et al. reported four ratio signatures of 24 miRNAs in plasma, regarding the risk to develop lung cancer, lung cancer diagnosis, risk of aggressive lung cancer and presence of aggressive lung cancer (99). The signatures were first identified by testing the tissues in the INT-IEO cohort as a training set and subsequently validated in the MILD cohort as validation set, while the two sets were from two LDCT screening trials. In 2014, based on the previously found 24 miRNAs, the same group constructed a miRNA signature classifier (MSC) of predictive, diagnostic and prognostic value (93). From 939 participants in the MILD trial, 69 of them with lung cancer and the remaining 870 as healthy controls, an analysis was performed. Interestingly, the combination of MSC and LDCT (double-positive result) gave rise to a five-fold reduction of LDCT false-positive rate from 19.4% to 3.7%, but unfortunately there was a decrease in sensitivity to 0.69. A non-randomized single-group trial, BIOMILD, is ongoing by this group, with the estimated completion date in January 2021; it concerns plasma miRNAs as a first-line screening test for lung cancer detection (http://ClinicalTrials.gov/, Identifier: NCT02247453). The intervention of plasma assay and other radiologic tests depend on the risk of the baseline miRNA profile, and the expected primary outcome is reduction of false positive cases in lung cancer detection through plasma miRNA profiling, with overall survival as a secondary outcome.

Bianchi et al. developed a panel of 34 miRNAs from serum for detection of early-stage NSCLC among asymptomatic high-risk individuals (94). Sera from the
<table>
<thead>
<tr>
<th>MiRNAs</th>
<th>Sample source</th>
<th>Type assay</th>
<th>Clinical significance</th>
<th>Patient cohort</th>
<th>Control cohort</th>
<th>Test evaluation</th>
<th>Reference</th>
<th>More information</th>
</tr>
</thead>
<tbody>
<tr>
<td>A miRNA signature classifier (MSC) based on 24 miRNAs</td>
<td>Plasma</td>
<td>RT-qPCR</td>
<td>Combination of MSC and LDCT for reduction of LDCT false-positive rate</td>
<td>69 NSCLCs</td>
<td>870 healthy controls</td>
<td>MSC: 0.87 sensitivity; 0.81 specificity</td>
<td>(93)</td>
<td>69 NSCLCs included 37 stage I, 12 stage II–III, 19 stage IV, 1 unknown</td>
</tr>
<tr>
<td>A panel with 34 miRNAs</td>
<td>Serum</td>
<td>RT-qPCR</td>
<td>Discriminate benign nodules from malignancy</td>
<td>34 NSCLCs</td>
<td>30 healthy controls</td>
<td>0.71 sensitivity; 0.90 specificity</td>
<td>(94)</td>
<td>34 NSCLCs included 18 stage IA, 4 stage IB, 12 stage II–IV</td>
</tr>
<tr>
<td>A panel with 13 miRNAs (miR-92a-3p, miR-30b-5p, miR-191-5p, miR-484, miR-328-3p, miR-30c-5p, miR-374a-5p, let-7d-5p, miR-331-3p, miR-28a-3p, miR-148a-3p, miR-223-3p, and miR-140-5p)</td>
<td>Serum</td>
<td>RT-qPCR</td>
<td>A simplified panel to discriminate benign lesions from malignancy</td>
<td>36 lung cancers</td>
<td>972 controls</td>
<td>0.778 sensitivity; 0.748 specificity</td>
<td>(5)</td>
<td>36 lung cancers included 31 stage I, 5 stage II–III</td>
</tr>
<tr>
<td>A screening four-miRNA panel with miR-378a, miR-379, miR-139-5p, and miR-200b-5p; a diagnostic six-miRNA panel with miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p</td>
<td>Plasma</td>
<td>RT-qPCR</td>
<td>Differentiate nodules from non-nodule diseases and lung adenocarcinomas from granulomas</td>
<td>50 adenocarcinomas and 25 granulomas</td>
<td>30 healthy controls</td>
<td>The screening panel: 0.975 sensitivity; 0.720 specificity</td>
<td>(95)</td>
<td>The screening miRNA panel could discriminate nodules from non-nodule diseases first, while the diagnostic panel could further differentiate lung adenocarcinomas from granulomas</td>
</tr>
<tr>
<td>MiRNAs</td>
<td>Sample source</td>
<td>Type assay</td>
<td>Clinical significance</td>
<td>Patient cohort</td>
<td>Control cohort</td>
<td>Test evaluation</td>
<td>Reference</td>
<td>More information</td>
</tr>
<tr>
<td>--------</td>
<td>---------------</td>
<td>------------</td>
<td>-----------------------</td>
<td>----------------</td>
<td>---------------</td>
<td>----------------</td>
<td>-----------</td>
<td>------------------</td>
</tr>
<tr>
<td>A panel with miR-126, miR-210, and miR-205-5p; a classifier composed of miR-126, miR-205-5p and diameter of nodule</td>
<td>Plasma</td>
<td>Microarray dd PCR</td>
<td>A classifier to discriminate malignant from benign PNs (the same as the below two cells)</td>
<td>UMMC cohort: 69 PNs of NSCLC</td>
<td>66 benign PNs</td>
<td>The three-miRNA panel: 0.812 sensitivity; 0.864 specificity</td>
<td>(96)</td>
<td>Mayo Clinic is a commonly applied prediction model based on parameters of PN on CT images and clinical characteristics of smokers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BVAMC cohort: 63 PNs of NSCLC</td>
<td>63 benign PNs</td>
<td>The three-miRNA panel: 0.810 sensitivity; 0.857 specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>JPHTCM cohort: 49 PNs of NSCLC</td>
<td>49 benign PNs</td>
<td>The three-miRNA panel: 0.816 sensitivity; 0.857 specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A panel with seven miRNAs (has-miR-199a-3p, chr17_10932, has-miR-148a-3p, has-miR-210-3p, chr1_1402, has-miR-378d and has-miR-138-5p)</td>
<td>Tissue</td>
<td>RT-qPCR</td>
<td>Distinguish early lung adenocarcinoma from benign nodules in GGNs</td>
<td>66 adenocarcinomas</td>
<td>66 lung benign diseases</td>
<td>0.864 sensitivity; 0.606 specificity</td>
<td>(97)</td>
<td>All participants presented with GGNs</td>
</tr>
<tr>
<td>A four-miRNA panel with high sensitivity (miR-223, miR-20a, miR-448 and miR-145); a four-miRNA panel with high specificity (miR-628-3p, miR-29c, miR-210 and miR-1224)</td>
<td>Serum/plasma</td>
<td>Meta-analysis</td>
<td>A two-step screen for stage I-II NSCLC</td>
<td>1,110 NSCLC</td>
<td>1,009 controls</td>
<td>The two-step model: 0.916 sensitivity; 0.934 specificity</td>
<td>(98)</td>
<td>The highly sensitive panel decided whether to continue the highly specific panel for specific confirmation</td>
</tr>
</tbody>
</table>

RT-qPCR, reverse transcription quantitative polymerase chain reaction; NSCLC, non-small cell lung cancer; LDCT, low-dose CT; PNs, primary nodules; ddPCR, droplet digital polymerase chain reaction; GGNs, ground-glass nodules; COPD, chronic obstructive pulmonary disease.
COSMOS trial were collected and divided into a training set and a testing group. The general sensitivity and specificity was 0.71 and 0.90 in the testing set, respectively. An extra analysis was performed addressing whether the predictor discriminated benign nodules detected by LDCT without cancer development from malignancy, and the specificity was 0.79 (26 out of 30 samples). By comparing a group of sera that were sampled before and after cancer onset, a significant elevation of average risk index was detected, indicating its function as a predictor of the conversion from a normal to a cancerous state. Four years later, the same group published a modified signature to 13 miRNAs and validated the new miRNA profiling in a large population of the COMOS trial (5). The sensitivity and specificity were 0.778 and 0.748, respectively, in a validation set of 1,008 subjects. Additionally, a specific set that included 83 participants with chronic obstructive pulmonary disease (COPD), stable pulmonary nodules, benign tumors or pneumonia displayed a rate of 0.867 (72 out of 83) with miRNA negative results. The overlap of five miRNAs between this 13-miRNA panel with the previous 24-miRNA panel encourages further study of the synergism towards increasing diagnostic accuracy.

Cazzoli et al. selected two panels of miRNAs from a sum of 742 miRNAs to screen and diagnose lung adenocarcinomas (95). They first tested a set of 30 plasma samples and later reevaluated a larger group of 105 samples, both composed of lung adenocarcinomas, lung granulomas, and healthy smokers. The proposed screening miRNA signature consisted of four microRNAs—miR-378a, miR-379, miR-139-5p, and miR-200b-5p—and could discriminate nodules from non-nodule diseases, with 0.975 sensitivity and 0.720 specificity. The six-miRNA diagnostic test (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p) divided the nodule diseases into lung adenocarcinomas and granulomas and showed 0.960 sensitivity and 0.600 specificity. One concern of this study is that the nodule group was solely composed of adenocarcinomas and granulomas and ruled out other benign nodules arbitrarily, which makes the result less reliable for advanced studies and clinical practices.

Lately, an integrated classifier combined by two miRNAs and one radiological feature was introduced for diagnosis of indeterminate primary nodules (PNs) (96). From 11 identified plasma miRNAs with significantly different expression, a panel of three miRNA biomarkers (miR-126, miR-210, and miR-205-5p) was used to distinguish malignant from benign PNs in a UMMC cohort (69 PNs of NSCLC and 66 benign PNs) at the beginning, generating a sensitivity of 0.812 and a specificity of 0.864. Additionally, univariate analysis determined that a history of cancer and smoking pack-years of the patients, and the diameter, spiculation, and upper lobe location of the nodules were associated with malignancy among clinical and radiological variables. Furthermore, the researchers grouped miR-126, miR-205-5p and the diameter of the nodule for a classifier design, which yielded 0.899 sensitivity and 0.909 specificity in the UMMC cohort. To completely compare the diagnostic value of the classifier, the Mayo Clinic model, a commonly applied prediction model based on parameters of PN on CT images and clinical characteristics of smokers, was tested in the same cohort, showing 0.754 sensitivity and 0.805 specificity. The classifier was validated in two extra cohorts, the BVAMC (63 PNs of NSCLC and 63 benign PNs) and JPHTCM cohorts (49 PNs of NSCLC and 49 benign PNs), generating similar results (BVAMC: 0.889 sensitivity and 0.905 specificity; JPHTCM: 0.878 sensitivity and 0.898 specificity). The three sets together confirmed a more accurate sensitivity and specificity of the classifier than those of the three-miRNA panel or the Mayo Clinic model (all P<0.05). This study was absolutely inspiring, and with high diagnostic values of the proposed classifier, it urges that supplementary large-scale validation tests and related clinical trials should be performed.

Increasing cases of ground-glass nodules (GGNs) appearing on CT images confuse doctors and intensify patients’ concern regarding the uncertainty of the nodules. A seven-miRNA panel was identified by He et al. with the aim of distinguishing early lung adenocarcinoma from benign nodules (97). The group of He et al. initially evaluated the miRNA expression of adenocarcinoma and adjacent non-paratumor tissue in three subjects using next-generation sequencing (NGS) and the miRNAs of interest were validated in 73 lung adenocarcinomas by qRT-PCR. Out of the 23 altered miRNAs detected by NGS, seven miRNAs were confirmed to have significantly different expression (P<0.05). The panel was subsequently validated in 66 lung adenocarcinomas paired with 66 lung benign diseases, and it yielded a sensitivity of 0.864 and specificity of 0.606. One noteworthy consideration is that the miRNAs were measured from lung tissue, which is not an ideal approach for a non-invasive test, and further studies about circulating miRNAs are urgently needed. As a newly established field to differentiate GGNs, the miRNA panel has great potential, and the diagnostic specificity has room for improvement.
A very recent systematic review tried to resolve the inconsistency of proposed miRNA signatures for screening of stage I–II NSCLC (98). The included 20 studies encompassed 1,110 NSCLC patients and 1,009 controls in total. Four circulating miRNAs that were hardly influenced by hemolysis, each with high sensitivity (>0.80) and area under the curve (AUC) (>0.80), were identified as biomarkers of stage I–II NSCLC: miR-223, miR-20a, miR-448 and miR-145. Four more miRNAs—miR-628-3p, miR-29c, miR-210 and miR-1244—were proved to have high specificity (>0.90). Finally, a model of a two-step screening for stage I–II NSCLC was proposed, using the first four-miRNA panel as a sensitive test to decide whether to continue the second four-miRNA panel for a specific confirmation. This combined model, showing an estimate of more ideal sensitivity (0.916) and specificity (0.934), was assumed to be a candidate a preliminary screening test to LDCT for a cost-benefit consideration and minimal radiation exposure. For further validation of this screening model, an interventional research study with a large set is required.

Conclusions and perspectives

Currently, miRNAs have been increasingly recognized for their crucial role in tumorigenesis and have been extensively studied in the field of NSCLC, where their value as biomarkers is fully appreciated, in particular, their significance in diagnosis, prognosis and prediction of response to various treatments as described in this review. Among a great number of studies regarding the early detection of NSCLC by miRNAs, we discussed the remarkably meaningful and promising ones. Different miRNA panels and diagnostic models were proposed, with various diagnostic values reported, showing a bright future for a combined screening method for early NSCLC where the false positive rate is reduced.

However, problems yet remain. The inconsistency of miRNA panels prevents further comparison of diverse studies and decisions regarding an ideal diagnostic model. Additionally, the population of each study differs approximately, for example, in the various histological classifications of NSCLC, making the synthesis even unlikely. However, from another perspective, studies enrolling single types of NSCLC may gain more consistent results and could be conducted, supported by the literature that miRNA expression profiles could determine the histology of NSCLC (100,101). Some studies have already been performed, such as the two that only involved adenocarcinomas (95,97), but extra efforts are worth devoting to comprehensive and detailed investigations. Additionally, the combination of LDCT and miRNA assay needs more exploration for the purpose of ascertaining the imaging features that would be involved and how these two examinations could be balanced and coordinated to raise the diagnostic power. To be put into clinical practice, miRNAs still have a long way to go even if the inconsistency is solved. Once a promising model is established, large-scale clinical trials are required for validation and provision of a high level of evidence, which is the exact urgency of miRNA studies on biomarkers in NSCLC.

Acknowledgements

We would like to acknowledge professor Xianghuo He, from Fudan University Shanghai Cancer Center and Institutes of Biomedical Sciences, for his suggestions of revision.

Funding: This study was supported by the grant from Science and Technology Commission of Shanghai Municipality Medical Guidance Science&Technology Support Project (grant No. 16411966100), Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support (grant No. 20172005) and Shanghai Municipal Commission of Health and Family Planning Outstanding Academic Leaders Training Program (No. 2017BR055).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

33. Cheng L, Sharples RA, Scicluna BJ, et al. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-


60. Bandi N, Vassella E. miR-34a and miR-15a/16 are co-regulated in non-small cell lung cancer and control cell cycle progression in a synergistic and Rb-dependent manner. Mol Cancer 2011;10:55.
Cite this article as: Han Y, Li H. miRNAs as biomarkers and for the early detection of non-small cell lung cancer (NSCLC). J Thorac Dis 2018;10(5):3119-3131. doi: 10.21037/jtd.2018.05.32