

MINI-REVIEW

Lung Cancer Detection by Screening – Presenting Circulating miRNAs as a Promising Next Generation Biomarker Breakthrough

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Abstract

Lung cancer remains a major cause of morbidity and mortality worldwide, accounting for more deaths than any other cause. All the clinical practice guidelines recommended against routine screening for lung cancer have cited lack of robust evidence, at least until a few years back. However, the potential to screen lung cancers has received renewed interest due to superior performance of low dose CT (LD-CT) in detecting early stage cancers. The incremental costs and risks involved due to the invasive procedures in the screened population due to a high false positivity rate questions the use of LD-CT scan as a reliable community based screening tool. There is therefore an urgent need to find a less invasive and a more reliable biomarker that is crucial to increase the probability of early lung cancer detection. This can truly make a difference in lung cancer survival and at the same time be more cost and resource utilization effective. Sampling blood serum being minimally invasive, low risk and providing an easy to obtain biofluid, needs to be explored for potential biomarkers. This review discusses the use of circulatory miRNAs that have been able to discriminate lung cancer patients from disease free controls. Several studies conducted recently suggest that circulating miRNAs may have promising future applications for screening and early detection of lung cancer.

Keywords: Lung cancer screening - circulating miRNA - early stage lung cancers - plasma miRNA - serum miRNA

Asian Pacific J Cancer Prev, **14** (4), 2167-2172

Introduction

Lung cancer remains a major cause of morbidity and mortality worldwide, accounting for more deaths than any other cause. It accounted for 12.7% of total cancer cases and 18.2% of total cancer related deaths in 2008 (Jemal et al., 2011). Lung cancers, unfortunately does not become clinically apparent until it reaches an advanced stage, >75% of lung cancers are diagnosed after the disease is advanced or metastatic (Aberle et al., 2011). Despite the use of newer chemotherapeutic and targeted agents, the overall five year survival rate for patients with lung cancers has remained at <15% in western countries (Crowell et al., 2007). In developing countries such as India and neighboring countries of the Asia Pacific region, the five year survival for lung cancer is approximately only 9% (Ou et al., 2009). From a few months of median survival in the 1970s and 1980s, the survival in advanced stage lung cancers has slowly inched up, most patients with good performance status diagnosed with advanced-stage lung cancer can now expect to live to just about a year or beyond.

Lung Cancer - Indian Perspective

Lung cancer in India is a major health problem. According to the recent GLOBOCAN 2008 report, India showed 47,010 new lung cancer cases among males and 11,557 new lung cancer cases among females. The report further showed 41,865 deaths due to lung cancer among males and 10,404 deaths due to lung cancer among females. The age standardized incidence/100,000 is reported to be 10.9 for males and 2.5 for females in 2008 in India.

Cancer of the lung is the leading site of cancer according to data from four urban registries of Bhopal, Delhi, Mumbai and Chennai (National Cancer Registry Program, 2009). The fact that the disease is not rare in the rural belt too is evidenced from the rural registry of Karunagapally in Kerala wherein it is the number one cancer. The trends in cancer incidence in Chennai city and predictions for the future burden of cancer in Tamil Nadu state indicate lung cancer to be the most common cancer, surpassing cancer cervix by 2016 (Swaminathan et al., 2011).

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Our study (Krishnamurthy et al., 2011) suggested that the epidemiology of lung cancer in India is possibly changing, with close to 40% of our lung cancer patients being non-smokers. More importantly this study showed the global trend of rise in adenocarcinoma histology; this trend is in contrast to the earlier reported Indian studies of squamous carcinoma being the most common histology. A majority of lung cancer patients presenting to our thoracic oncology clinic are in advanced stages of disease with limited treatment options, mainly consisting of empirically chosen cytotoxic chemotherapeutic agents and more recently targeted therapies.

Screening for Lung Cancer

The chest radiograph remains the most commonly used imaging technique in the investigation of respiratory disease but cannot be effectively used to detect pulmonary nodules. About 50% of the nodules measuring 6-10mm can be missed owing to superimposition of the chest wall, heart and mediastinal structures (Deiderich et al., 2001). For the past 25 years, multiple large scale clinical trials have tried to validate the screening procedures in an attempt to improve the outcome of lung cancers. The screening tests include chest X-rays, Sputum Cytology, Chest CT, either alone or in combination. But none of the above procedures have helped to improve the overall mortality of lung cancer (Bach et al., 2007). Randomized screening trials conducted in the United States and Czechoslovakia failed to show reduction in cancer mortality among the smokers who were screened by sputum cytology and chest X-ray for lung cancer.

The potential to screen for lung cancer received renewed interest due to superior performance of low dose CT (LD-CT) compared to chest X ray radiography in detection of small lesions. Several recent reports from Japan, Germany, and United States have documented the ability of LD-CT to detect lung cancer at an early stage (Kaneko et al., 1996; Sone et al., 1998; 2001; Henschke et al., 2006). The National Cancer Institute funded National lung cancer screening trial (NLST) was taken up as a prospective study that screened symptom free high risk smokers using LD-CT or a standard chest x-ray. Over 53,000 people were enrolled in the NLST, 26,700 received annual LD-CT, 26,700 undergoing annual chest x-rays, with additional scans and some invasive procedures for screen detected radiographic abnormalities. The NLST study concluded that screening with the use of LD-CT reduces mortality from lung cancer by 20% when compared to chest x-ray (247 vs. 309 deaths per 100,000 person-years). This translated to the prevention of one lung cancer death for every 320 people screened with LD-CT.

The study also showed that 39% of patients had at least one abnormal CT scan among the 3 annual scans, 96% of which were determined not to be lung cancer. The benefits of screening with LD-CT would have to be balanced against the risks associated with false positives– suspicious CT scan findings that in the end prove not to be cancer-related. These incidental findings and benign pulmonary nodules warrant further invasive procedures/surgeries to assess their true clinical relevance

adding to incremental costs and risks to the screened population (Bellomi et al., 2006; Adriano et al., 2012). Although transthoracic needle biopsies are by and large safe, it is associated with a small risk of pneumothorax and hemorrhage. An observational database analysis of 15,865 transthoracic needle biopsies mostly performed in the community setting revealed a 15% incidence of pneumothorax, 7% of whom requiring a chest tube and a 1% chance of hemorrhage. Further the study quoted a 0.5% chance of dying in the hospital which could potentially increase to 4% in already hospitalized patients (Weiner et al., 2011). It is highly likely that community-based management of pulmonary nodules may result in higher complication rates from invasive procedures and surgeries.

Multiple CTs that needs be to done to monitor a suspicious lesion adds up to the cost making it prohibitively expensive for a country like India. The issues of cost effectiveness of CT based screening for lung cancer still remain unresolved (Meittinen et al., 2000; Welch et al., 2007; Welch et al., 2010). Finally, CT scan can also expose patients to potentially harmful effects of radiation that can in turn result in other cancers.

The American Lung Association (ALA) and the National Comprehensive Cancer Network (NCCN) recommend lung cancer screening using LD-CT, for high-risk people (the entry criteria for the NLST trial for all people aged 55-74 with a 30+ pack-year smoking history). The U.S. Preventive Services Task Force (USPSTF), whose recommendations are considered the most authoritative and the American Cancer Society (ACS), does not endorse the lung cancer screening recommendations and emphasize that physicians must explain to the patients, the uncertainties of the risks and benefits of getting screened with LD-CT. The issue of the use of LD-CT screening in lung cancer has thus generated more questions and debates than answers and consensus.

Looking beyond Low Dose CT Scans

With a high incidence of lung cancer in India, with its poor prognosis and survival and also with a lack of a reliable marker for early detection, prognosis and screening, it is important to make use of molecular platforms to improve strategies of diagnosis, prognostication and treatment of patients. Lethality of lung cancer is also attributed to lack of effective screening strategies for early detection at a stage when the tumour is still amenable to cure by surgery. But still there are no validated population based screening procedures available.

The frequent absence of specific symptoms of lung cancer during its early stage underlines the urgent need to develop early detection measures to identify the high risk individuals. Development of a validated cost effective screening test for lung cancer that can reliably provide an indication for early detection is a public health imperative.

There is a necessity for additional screening modalities so as to reduce the number of patients who undergo invasive procedures unnecessarily. Development of a reliable, non-invasive and cost effective confirmatory test can reduce over diagnosis and facilitate the implementation of screening CT scan procedure in the near future for early

detection of lung cancers in India.

Biomarkers for Lung Cancer

Biomarkers for lung cancer have been the paramount need of the hour as before. Molecular biomarkers can be in the form of histological markers directly associated with pathological changes, according to the stage of the disease. Serum biomarkers are even more appealing given the easy accessibility and have proteins shed, secreted, released from the tissues which can be detected in the circulation.

Disease driven proteomics based on mass spectrometry has serious technical limitations because of the complexity of the blood proteome. It spans a concentration range of at least ten orders of magnitude. It is anticipated that efficient depletion methods and multi-dimensional fractionation systems might be helpful to separate low abundance proteins and extend of the detection limit.

What are miRNAs ?

miRNAs are non-coding RNAs 19-22 nucleotides. They negatively regulate mRNA by binding sequence specifically to the complementary sites within the 3' untranslated regions (UTRs) of mRNA and inhibit their translation into polypeptides or degrade their target mRNA (Bartel et al., 2009) They also mediate activation of translation in G0/G1 phase of cell cycle (Vasudevan et al., 2007). Half of the miRNAs are located in the fragile chromosomal regions that show amplifications, deletions, translocations and their expression is frequently dysregulated in cancer (Iorio et al., 2009). Compared to the normal cells, miRNAs expression may be either down regulated or upregulated in cancer cells. miRNAs also regulate different cellular processes, e.g. apoptosis, hematopoietic cell differentiation, metabolism, neural development and metastasis (Xu et al., 2004; Kloosterman et al., 2006; Stefani et al., 2008). miRNAs can therefore have both oncogenic or tumour suppressive functions.

How and Why miRNAs Qualify as a Screening Markers

Circulating miRNAs have been proposed as attractive candidates to be used as cancer biomarkers and are ideal for screening purposes. Blood based tests for screening purposes or disease monitoring would be more suitable as they are minimally invasive, relatively have low cost and can be repeated as well. Currently however, serum based markers suitable for tumour detection are limited. Almost all of the serum markers currently in use are proteins and comprehensive approaches on proteomics have been applied. Conventional methodologies used to measure them still remain labour intensive and have been difficult for clinical diagnosis.

miRNAs are present in both serum and plasma and they are resistant to RNase A digestion, despite the fact that serum is rich in ribonucleases. Studies show that endogenous plasma miRNAs exist in a form, resistant to 4°C or 37°C incubation, freeze thaw cycles and RNase activity. The stability of the miRNAs is due to being

packaged in exosomes that are secreted within somatic cells, including cancer cells. Circulating exosomal miRNA can be a useful screening test for lung adenocarcinoma (Rabinowits et al., 2009).

Some studies also indicate that miRNAs may have a secondary structure that makes them stable or may be associated with other molecules that modify or help in their remarkable stability (Cortez et al., 2009). This aspect is very important for a clinical and diagnostic point of view.

Recent studies reveal remarkable stability of miRNAs in various clinical samples (Chen et al., 2008; Mitchell et al., 2008; Huang et al., 2010; Tsujiura et al., 2010; Xie et al., 2010; Shen et al., 2011). miRNAs can help in making a definitive pre-operative diagnosis for the malignant solitary pulmonary nodules (SPN). Plasma miRNAs with high expression of miR- 21, miR-210 and low miR-485-5p were shown as indicators of malignant SPNs compared to the healthy controls and thus had the potential to identify lung cancer among individuals with CT- detected SPNs (Shen et al., 2011).

miRNAs are unique, and highly stable and their patterns not dependent on age, race, and can be used for their potential diagnostic and prognostic utility. Evidences suggest that developing lung cancer might be associated with a specific miRNA signature even years prior to diagnosis, and this signature appears to change while the tumour is still developing. This study also suggests that most significant changes of miRNA pattern occur at a time before the time close to diagnosis possibly as a result of tumour development highlighting the use of miRNAs as valueable markers for diagnosis (Avila-Moreno et al., 2011). Moreover, they can be easily isolated from clinical samples like sputum , plasma , serum , formalin fixed paraffin embedded sections archived upto 10 years (Zheng et al., 2011).

A recent study (Boeri et al., 2011) shows that plasma levels of miR-155, miR-197, miR- 182 could serve as non-invasive biomarkers for early detection and diagnosis of lung cancer. These miRNAs were shown to be significantly elevated in plasma of the lung cancer patients compared to the cancer free control subjects by greater than 10 folds and could help discriminate the two groups.

Another study (Keller et al., 2011) has shown role of miRNAs as biomarkers for lung disease based on both lung tissues as well as plasma samples that were collected both before and at the time of disease detection from patients who were participating in a different spiral CT-screening trials with extended follow up. These patients had developed tumours with variable aggressive behaviour during the course of trials. A very salient feature of this study was miRNA profile of tumours detected in the first two years of the screening being different from the profile seen after the second year, which indicates distinct aggressive features and faster growth rate. The evidences pointed out that miRNAs are more tissue specific and role of miRNA in tissues is independent of plasma levels. This study showed a panel of 21 miRNAs belonging to major signalling pathways like cellular aging, bronchioalveolar and hematopoietic stem cell renewal, tumour recurrence in stage I lung cancer, plasma samples studied. Important

highlight of this study was identification of miRNA based signatures predicting lung cancer in plasma samples collected 1-2 years before the development of disease, thus helping in selection of high risk individuals who require CT surveillance. The results prove that specific miRNA signatures in pre-disease plasma samples can predict and discriminate the development of more aggressive disease, like in early metastatic tumours that can be frequently be undetectable by yearly spiral CT surveillance. The highlight of the study was number of deregulated miRNAs decreases with increasing time distance to diagnosis which is against the idea that extended time of sample storage can alter miRNA pattern (Hennessey et al., 2012).

A Study based on serum based biomarkers (Bianchi et al., 2011) showed a phase I/II biomarker study examining the feasibility of serum miRNAs as biomarkers for NSCLC using real time quantitative PCR. This study reports combination of two differentially expressed miRNAs miR-15b and miR-27b able to discriminate NSCLC from healthy controls with sensitivity, specificity, PPV and NPV of 100% in the training set. The study also showed that serum miRNAs have the potential to be sensitive, cost effective biomarkers for the early detection of NSCLC.

Figure 1 shows the application of miRNAs in screening the high risk group and also for disease monitoring and prognostication. Table 1 summarises the salient miRNAs that were differentially expressed between the normal and diseased states from various studies below.

miRNA Panel as a Risk Predictor Based on Score

A recent study showed circulating miRNAs, able to identify asymptomatic high risk individuals with lung cancer and distinguished malignant lesions from benign nodules shown by low dose spiral CT. The miRNA model identified patients with early stage non-small cell lung cancer in a population of asymptomatic high risk individuals with 80% accuracy. The panel with 34 miRNAs formed a risk score, assigning each patient type, a high or low risk category, belonging to both adenocarcinoma and squamous cell carcinoma types as a

multivariate risk predictor. Further validation in another independent cohort showed reproducible results with similar success rate. This test is claimed to be desirable in a clinical setting with features of distinguishing malignant lesions from benign nodules identified by LD-CT in high risk population (Foss et al., 2011). Previous Studies by Boeri et al. (2011) had an overlap of miRNAs found with the results of 34 miRNA panel. Another important aspect of this study was the specificity to lung cancer which was confirmed by screening the breast cancer cohort.

miRNA as a Triage Tool for Diagnostics

Another study (Yuxia et al., 2012) is suggestive of using these miRNAs as biomarkers as minimally invasive

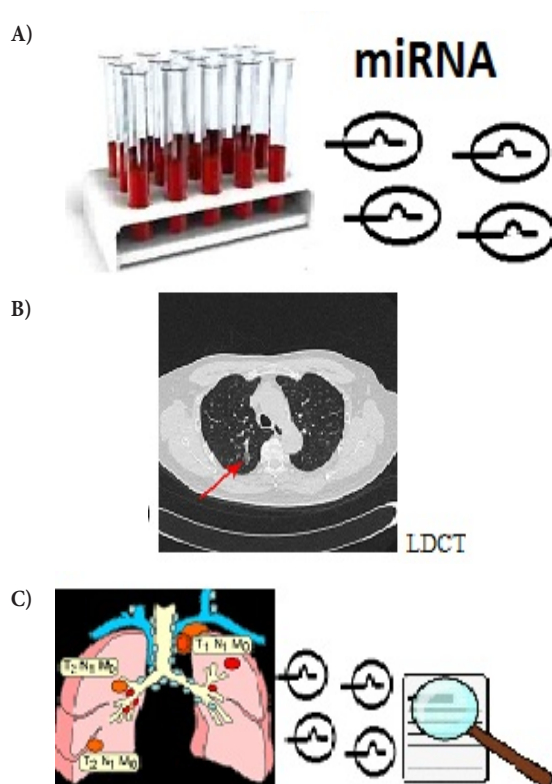


Figure 1. Applications of miRNA. A) Blood sample, B) Screening and C) Dignosis and disease monitoring

Table 1. Summarizes the Salient miRNAs that were Differentially Expressed between the Normal and Diseased States from Various Studies

Plasma miRNA	Significance	Reference
miR-155, miR-197, miR-182	Up regulated in lung cancer patients serum	Zheng et al., 2011
miR-486, miR-30d, miR-1, miR-499	Correlated to Overall Survival	Hu et al., 2010.
miR-146b, miR-221, miR-155, miR-17-5p, miR-27a, miR-106a	Significantly reduced in serum of Lung Cancer Patients	Heegaard et al., 2012
miR-92, miR-484, miR-486, miR-328, miR-191, miR-376a, miR-342, miR-331, miR-30c, miR-28, miR-98, miR-17-5p, miR-26b, miR-374, miR-30b, miR-26a, miR-142-3p, miR-103, miR-126, let-7a, let7d, let 7b, miR-32, miR-133b, miR-566, miR-432, miR-223, miR-29a, miR-148a, miR-142-5p, miR-22, miR-148-p, miR-140, miR-139	34 miRNA panel that can differentiate between tumour and normal sera	Bianchi et al., 2011
miR-1254, miR-574-5p	Triage tool	Foss et al., 2011
miR-21, miR-205, miR-30d, miR-24	Preoperative and post operative levels	Le et al., 2012
miR21, miR-210, miR-485-5p	Diagnosing solitary pulmonary nodule	Shen et al., 2011

screening and triage tools for subsequent diagnostic evaluation. MiRNAs like miR1254 and miR 574-5p were significantly increased in early stage NSCLC samples with respect to controls. Circulating miRNAs have been reported as biomarkers for stratification and prediction of prognosis in NSCLCs (Le et al., 2012) Clinical relevance of miRNA 125b was found to be significantly associated with clinical stage and was an independent prognostic indicator of poor prognosis.

miRNAs show a difference in their expression levels in pre-operative and post-operative states and can potentially be used as biomarkers for disease recurrence after surgery. A panel of miR-21, miR-205, miR-30d and miR-24 was found to be increased in the sera of lung cancer patients including the early stage cancers compared to normal volunteers. This study showed that some of the miRNAs like miR- 21 and miR-24 were significantly decreased in the post-operative patients compared to their pre-operative levels. High expressions of miR-21 and miR-30d in the pre-operative sera were independently associated with poorer survival in lung cancer patients. miR21 was shown to be several folds lower in patients with a partial response and to be a biomarker for early diagnosis of NSCLC. Plasma miR-21 could predict sensitivity to platinum based chemotherapy as well (Wei et al., 2011).

A panel of miRNAs can predict the survival and categorise the patients into higher risk and lower risk. A panel of 4 serum miRNAs (miR-486, miR-30d, miR-1, and miR-499) were shown to be significantly different between the longer survival and shorter survival groups. This miRNA signature was evaluated for distinguishing the high risk versus the low risk groups of patients by stage and histology subtypes (Hu et al., 2010). It is suggested that miRNAs could have potential therapeutic applications in the field of cancer and may play a pivotal role in future individualised management of cancers (Heneghan et al., 2010).

Conclusions

The results from various studies indicate that non-invasive circulating miRNA signatures can distinguish between malignant and benign lesions on LDCT and are able to differentiate the aggressive subgroup among the entire population of enrolled patients. The simplicity of the procedure with its relative low cost based on quantitative real time PCR can encourage population based compliance to large scale screening programs thus accelerating its application in the clinic. miRNA can help in first line screening to identify the high risk individuals who should undergo further testing like the LD-CT.

These insights can help facilitate the clinical applications in large scale and long term lung cancer screening, monitoring and also as personalised therapy. These evidences represent an important step towards clinical practice as it may reduce unnecessary surgical intervention and has the potential to serve as a non-invasive screening tool for early lung cancer diagnosis.

The future of miRNA signatures coming up as an important breakthrough in the study of early detection of lung cancer seems very promising. The miRNA diagnostic

signatures with a combination or even independent from the LDCT screening may represent a new milestone in the early diagnosis of this lethal disease.

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