FEATURED NEW INVESTIGATOR

Plasma circulating microRNA-944 and microRNA-3662 as potential histologic type-specific early lung cancer biomarkers

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Altered expression of microRNAs (miRNAs) is associated with the development and invasion of cancers by regulating post-transcriptionally gene function. Possibility of detection of miRNA expression in patients' plasma or serum makes them valuable biomarkers of different neoplasms. In the present study, we investigated the potential role of miR-944 and miR-3662 expression analysis as novel lung cancer biomarkers and their lung tumor specificity in plasma samples of 90 patients with lung cancer and 85 healthy individuals using quantitative reverse transcription–polymerase chain reaction analysis. Expression of miR-944 and miR-3662 was upregulated in patients with lung cancer in comparison with healthy individuals. Receiver operating curve analysis has presented diagnostic power of analysis of both miRNA expressions for detection of patients with I and II stages of non–small cell lung cancer with area under the curve (AUC) of 0.881. Moreover, miR-944 has shown diagnostic accuracy for operable squamous cell carcinoma detection (AUC = 0.982), whereas miR-3662 has shown the diagnostic accuracy for operable adenocarcinoma (AUC = 0.926). Higher stage of lung cancer correlated with higher miRNA expressions. Our results suggest that the profile of studied miRNAs could be further evaluated and considered as potential lung cancer biomarkers. (Translational Research 2015;166:315–323)

Abbreviations: AC = adenocarcinoma; AUC = area under the curve; cDNA = complementary DNA; CT = computed tomography; LD-CT = low-dose spiral computed tomography; miRNA = microRNA; NSCLC = non-small cell lung cancer; ROC = receiver operating curve; SCC = squamous-cell carcinoma; SCLC = small-cell lung cancer

INTRODUCTION

MicroRNAs as tumor biomarkers. Despite the availability of next-generation drugs and molecularly targeted therapies, lung cancer still remains a leading cause of cancer-related deaths worldwide.[1] It is caused especially by late diagnosis of cancer, when tumor is detected in locally advanced or metastatic stages. Consequently, lung cancer patients are disqualified from surgical treatment and lose the possibility of complete recovery. Although, new molecular factors such as DNA

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AT A GLANCE COMMENTARY

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Background

Possibility of detection of microRNA (miRNA) expression in patients' blood samples make them valuable biomarkers of neoplasms. We investigated the role of miR-944 and miR-3662 expression analysis as potential histologic type-specific lung cancer biomarkers. The expression of both miRNAs has not been evaluated in blood of healthy individuals and patients with cancer before.

Translational Significance

Receiver operating curve analysis has presented the diagnostic accuracy of studied miRNAs for the detection of patients with I–II stages of non–small cell lung cancer. Moreover, miR-944 has shown the diagnostic accuracy for detection of operable squamous cell carcinoma (area under the curve 0.982), whereas miR-3662 has shown the diagnostic accuracy for operable adenocarcinoma (area under the curve 0.926). These miRNAs may be considered as lung cancer biomarkers and may be involved in the development of particular histologic subtypes of non–small cell lung cancer.

methylation, DNA hydroxymethylation, or noncoding RNA molecules are considered as early lung cancer biomarkers, there are no established criteria for their detection. Moreover, most of the studied biomarkers have limited diagnostic power and they are characterized by low sensitivity and low organ specificity. Thus, a novel approach to identify the molecular factors responsible for lung cancer development and progression should be achieved through the detection of tissue-specific agents. Moreover, development of sensitive, simple, and noninvasive (examination of blood serum or plasma) molecular methods becomes necessary.[2-4]

MicroRNA (miRNA) molecules seem to meet previously mentioned criteria. Several miRNAs are considered as neoplasm biomarkers. In cancers, the specific miRNAs have a regulatory function of tumor suppressor genes or oncogenes.[5] Individual miRNAs are responsible for regulating the expression of various genes. Therefore, miRNAs profiling in human tumors present a diagnostic challenge. However, individual genes may be targeted by different miRNAs and numerous miRNA sequences are known.[6] Thus, the role of miRNAs in human malignancies is still unknown and most studies have focused on their profiling in tumor tissues. Recognition of novel tumor-derived miRNAs in human serum or plasma as diagnostic or prognostic biomarkers seems to be justified.[7,8] Further selection of tumor-specific circulating molecules may improve early diagnosis of lung cancer. miRNA profiling could also expand the possibility of prevention of lung cancer, improving specificity of low-dose computed tomography (CT) (the differential diagnosis of small lung nodules).[9,10] A few studies have reported potential clinical and diagnostic significance of circulating miRNAs in patients with lung cancer. These molecules include miRNA-17 (miR-17), miR-21, miR-93, miR-192, miR-429, and miR-1247.[11-13] In the present study, we evaluated the potential role of miR-944 and miR-3662 as novel lung cancer biomarkers and its histologic subtype determinants.

Criteria of miR-944 and miR-3662 selection for testing. Gene coding miR-944 is located in the intron of *TP63* gene, which encodes tumor protein 63 (p63). *TP63* is significantly overexpressed in squamous types of cancer (in contrast to adenocarcinomas [ACs]), such as lung squamous cell carcinoma (SCC). Therefore, *TP63* is considered as a squamous differentiation marker, and the expression of miR-944 may correlate with *TP63* expression.[14,15] Moreover, the miR-944 may target an mRNA of *SOCS* (suppressor of cytokine signaling) family tumor suppressor genes, which are expressed in epithelial cells of lung, and thus it can promote tumor growth, proliferation, and squamous differentiation.[16] Therefore, localization and potential function of miR-944 proves its potential mechanism of acting in tumorigenesis and make it a promising marker of early squamous differentiation of lung tissue.

The role of miR-3662 in human tumors is still unclear. However, on the basis of available miRNA tools (Target Scan Human 6.2 and miRDB target predictor) and mRNA-protein tissue expression database (Human Protein Atlas) as well as literature data, we assumed the potential role of miR-3662 in lung tumorigenesis and developing of lung AC.[17-19] Firstly, we carefully selected suppressor genes that may be conservatively targeted by miR-3662. Subsequently, on the basis of mRNA-protein expression database, we evaluated the expression of selected genes in healthy lung tissue especially in pneumocytes, in which AC transformation is most typical. We selected few genes (*PTAR1*, *NPR3*, and *SEPT10*), which are cancer-related and the proteins encoded by these genes are involved in cell metabolism and structure and additionally *SIX3* gene, which expression is a promising prognostic AC marker.[20] Interestingly, these genes were highly or medium expressed in healthy pneumocytes compared with lung AC samples, where their expression was low. miR-3662