Potential molecular approaches for the early diagnosis of lung cancer (Review)

CHUL HO OAK[1], DONALD WILSON[3] HU JANG LEE[4], HO-JU LIM[5] and EUN-KEE PARK[2]

Departments of [1]Internal Medicine, and [2]Medical Humanities and Social Medicine, College of Medicine, Kosin University, Busan, Republic of Korea; [3]Department of Occupational Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Kitakyushu, Japan; [4]Research Institute of Life Sciences, College of Veterinary Medicine, Gyeongsang National University, Chinju; [5]Indoor Environment and Noise Research Division, National Institute of Environmental Research, Incheon, Republic of Korea

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Abstract. Lung cancer is the leading cause of mortality from cancer among men and women worldwide. More individuals die each year of lung cancer than of colon, breast and prostate cancer combined. Despite new diagnostic techniques, the overall 5-year survival rate remains at approximately 15% and the majority of patients still present with advanced disease. Therefore, lung cancer is the most lethal cancer at present. Diagnosing and treating cancer at its early stages, ideally during the precancerous stages, could increase the 5-year survival rate by 3-4-fold, with the possibility of cure. To date, no screening method has been shown to decrease the disease-specific mortality rate. This review describes issues related to early lung cancer screening and their rationale, the management of primary cancers detected by screening and the different approaches that have been tested for cancer screening; these include imaging techniques, bronchoscopies and molecular screening, such as analysis of epigenomics using different noninvasive or invasive sources, such as blood, sputum, bronchoscopic samples and exhaled breath.

Contents

Correspondence to: Dr Donald Wilson, Department of Occupational Toxicology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan
E-mail: wilson@med.uoeh-u.ac.jp

Dr Hu Jang Lee, Research Institute of Life Sciences, College of Veterinary Medicine, Gyeongsang National University, 900 Gajwa-dong, Chinju 660-701, Republic of Korea
E-mail: hujang@gnu.ac.kr

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1. Introduction

Lung cancer is the leading cause of cancer-related mortality in the world, and 85% of cases are caused by tobacco smoke (1). Other established risk factors for lung cancer are exposure to second-hand cigarette smoke, occupational exposure to agents such as asbestos, nickel, chromium or arsenic, exposure to radiation, including radon gas in homes, and exposure to air pollution (2).

The International Agency for Research on Cancer (IARC) estimated in 2008 (3) that the crude incidence and mortality of cancer was 12.7 and 7.6 million cases, respectively, of which 56% of new cancer cases and 63% of cancer deaths occur in the less developed regions of the world. The most commonly diagnosed (N, proportion of total) cancers worldwide are lung (1.61 million, 12.7%), breast (1.38 million, 10.9%) and colorectal cancers (1.23 million, 9.7%). Furthermore, the most common causes (N, proportion of total) of cancer mortality are lung (1.38 million, 18.2%), stomach (738,000, 9.7%) and liver cancer (696,000, 9.2%).

In Korea, according to the 2008 annual report of cancer statistics (4), the number of new cancer cases was 178,816, a 7.8% increase compared to that of 2007. The crude incidence rate of cancer in 2008 was 361.9 per 100,000, a 26% increase compared to that of 2001. Stomach cancer was the most common form, while lung cancer was the 4th most common. Furthermore, lung cancer is the most frequent cancer in males over 65 years of age. The 5-year survival rate of lung cancer between 2004 and 2008 was 17.5%, an increase of 4.8% compared to that of the period between 1996 and 2000 (4).

In the United States, 75% of patients with lung cancer present symptoms of advanced incurable disease (5). Despite advances in the treatment of lung cancer, the 5-year survival rate for all stages combined is approximately 16% (6). Patients diagnosed at earlier stages inevitably have a significantly improved 5-year survival rate: 60-75% for stage I disease (7). An efficacious screening test that could result in early detection and reduced mortality would thus represent a major advance in dealing with lung cancer mortality.

Early detection would be a significant step towards reducing lung cancer incidence and mortality. However,

conventional diagnostic methods for lung cancer are unsuitable for widespread screening, as they are commonly expensive and occasionally miss tumors or invasive cancers (8-11). Computed tomography (CT) is widely used for early screening of lung cancer, although it often produces high false-positive rates (12,13). Better diagnostic methods are urgently required to improve the detection of lung cancer. Tissue and blood have been used extensively for the early detection of lung cancer (14-16). The detection of aberrant promoter methylation or metal ions in sputum has also been used (17-19). Monoclonal antibody detection, fluorescence bronchoscopy and low-dose spiral CT increase diagnostic sensitivity and improve the ability to localize early-stage lesions (20), but the screening methods are limited by the sample availability or composition variability.

Recently, biomarker discovery and their clinical use have been accelerated by the completion of the human genome project and the progress of techniques in proteomics (21). Numerous potential DNA biomarkers have been discovered as lung cancer biomarkers (22). Progress has consequently been made in early diagnosis, therapy guidance and prognosis monitoring of cancers.

In this review, we discuss various molecular biomarkers of lung cancer, and how reported individual biomarkers could be used for the early diagnosis of lung cancer.

2. Biomarkers of lung cancer

Due to the vast development of knowledge over the past several decades, different methods have been suggested to classify lung cancer biomarkers. However, these classifications should be considered in context, as identification of lung cancer biomarkers is one of the major multidisciplinary areas in the biomedical field. A schematic for the classification of biomarkers is shown in Fig. 1.

Predictive, diagnostic and prognostic biomarkers of lung cancer. Prognostic biomarkers are based on the distinguishing features between benign and malignant tumors. They could be selected based on the differentiation status of tumors, which may affect clinicians' decisions related to treatment modalities. For example, the lung metagene model predicted recurrence for individual patients significantly better than clinical prognostic factors and was consistent across all early stages of lung cancer (23,24). Such markers are also important from the point of view of predicting relapse of lung cancer.

Predictive biomarkers, occasionally referred to as response markers, are utilized exclusively in assessing the effect of administration of a specific drug. These biomarkers allow clinicians to select a set of chemotherapeutic agents that will work best for an individual patient. For example, gefitinib is useful in non-small cell lung cancer (NSCLC) showing only epidermal growth factor receptor (EGFR) mutation (25,26). Consequently, EGFR mutation is a predictive lung cancer biomarker.

Diagnostic markers may be present in any stage during cancer development (27,28). Carcinoembryonic antigen (CEA) in NSCLC is an example of a diagnostic marker present in the early stages of lung cancer. Moreover, a diagnostic cancer marker could be specific to stage, tissue, relapse, follow-up and age.

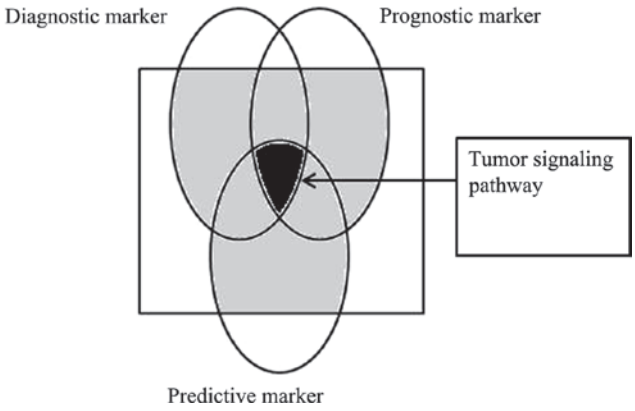

Figure 1. Representation of an ideal biomarker.

Lung cancer biomarkers on the basis of biomolecules. Lung carcinogenesis is a multi-step process resulting from the accumulation of altered molecules generated from genetic and epigenetic abnormalities of genes that are involved in cell cycle, senescence, apoptosis, repair, differentiation and cell migration controls (9,29). There are several distinct types of cancer biomarkers based on different areas: genetics, epigenetics, proteomics and metabolomics (22). Genetics-based cancer biomarkers utilize functions such as DNA arrays, polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), DNA sequencing and fluorescent *in situ* hybridization (FISH), to detect the genetic alterations occurring in the cancerous state. Recent development of epigenetic modification analyses has also improved tools for cancer biomarkers. Epigenetic modification usually occurs in the CpG island of the gene regulatory regions, which results in the downregulation of the gene expression (30,31). Proteomics includes techniques such as mass spectrometry (MS), enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, and it utilizes these tools to discover novel cancer biomarkers and validate them in clinical trials. Other than using macromolecules such as proteins and DNAs, metabolomics is concerned with the study of low molecular weight molecules or metabolites such as amino acids, peptides, lipids and carbohydrates (22,32).

DNA. Single nucleotide polymorphisms (SNPs) in many genes are major DNA markers including *XRCC1*, *ATM* and *p53* (33). Other major DNA markers include loss of heterozygosity (LOH), variation in copy number of genes, chromosomal aberrations at a gross cytogenetic level, such as translocation/fusion (BCR-ABL, PML-RARA translocation in leukemias), micro-satellite instability (MSI) and epigenetic modifications (27,34-36).

In many cases the inactivation is induced by loss of DNA or accidental chromosomal rearrangement during cell division. The most well-known, frequently-occurring abnormality is deletion of the short arm of chromosome 3 (3p), where several tumor suppressor genes (TSG) are present (37,38). Loss of chromosomal material has also been detected in metaplastic epithelium tissues of current- or ex-smokers. The loss of one allele or LOH indicates a predisposing potential to lung cancers (39,40).

Table I. Currently available protein-based biomarkers in the detection of lung cancer.

Biomarkers	Diagnosis	Therapy monitoring	Prognosis monitoring	Ontology	Details
CEA	AdenoCA, LCLC (>10 μg/l)	AdenoCA, Advanced NSCLC	AdenoCA, NSCLC	Cellular component Cell membrane: lipid anchor Immunoglobulin superfamily	In combination with CYFRA Often elevated in smokers
CYFRA21-1	NSCLC (23-70%), SCC (no., sensitivity)	Advanced NSCLC	NSCLC, SCC	Structural constitutent of cytoskeleton	Often elevated in patients with benign lung diseases
TPA	NSCLC, SCC	-	NSCLC		-
ProGRP	SCLC (47-86%) (>200 ng/l, highly suspicious) (no., sensitivity)	SCLC	-	Neuropeptide hormone activity	Increased in renal failure and certain benign lung diseases In combination with NSE
NSE	SCLC (>100 μg/l, high probability) (sensitivity for SCLC, high 74%)	SCLC	SCLC	Phosphoglycerate dehydrogenase activity Subcellular location (cytoplasm)	In combination with ProGRP May correlate with short survival Increased in inflammatory diseases
Tumor M2-pyruvate kinase	AdenoCA (sensitivty for SCLC, 50-71%)	-	AdenoCA	Pyruvate kinase activity Glycolysis Cytoplasm	Increased in multiple malignant and certain inflammatory diseases

CEA, carcinoembryonic antigen; AdenoCA, adenocarcinoma; NSCLC, non-small cell lung cancer; CYFRA 21-1, cytokeratin 19 fragment; SCC, squamous cell carcinoma; ProGRP, progastrin-releasing peptide; SCLC, small cell lung cancer; NSE, neuron-specitic enolase.

Notably, besides nuclear aberrations, alterations in mitochondrial DNA (mtDNA) molecules are suggested as clear biomarkers for lung cancers (41,42). Epigenetic modification of nucleic acids and associated proteins (histones and non-histones) are important in carcinogenesis (35,43,44). Histone de-acetylation, lysine-specific histone-H3 methylation and promoter region CpG methylation modulate transcription of tumor-suppressor genes (CDKN2A, TP53, APC, BRCA1) and DNA mismatch-repair genes (MLH1 or the O6-methyl-guanine-DNAmethyltransferase gene, MGMT). Gene silencing by CpG methylation is one of the best-characterized epigenetic modifications to date (35,41,44). The degree of methylation in sputum/serum from patients with lung cancer is directly implicated in the severity of the lesions.

RNA. Messenger RNAs (mRNAs) are promising biomarkers, and microarrays represent a powerful approach for their discovery in blood. More recent microarray studies have identified additional early detection signatures. Some of the methods used to detect cancer biomarkers at the RNA expression level include quantitative reverse transcription polymerase chain reaction (RT-qPCR), serial analysis of gene expression (SAGE), differential display, bead-based methods and microfluid card and micro-array analysis (45). A study of peripheral blood mononuclear cells (PBMCs) from lung cancer patients identified a signature of 29 genes that distinguished patients from controls (46). Panels of mRNA biomarkers for early detection have also been identified in bladder, breast and renal carcinomas (47-49). These studies suggest that blood-based mRNA signatures may be potentially useful tools for the early detection of lung cancer.

The potential use of microRNAs (miRNAs) as biomarkers for lung cancer has also been demonstrated. Several studies have identified pathognomonic or tissue-specific miRNA expression profiles in lung and other types of cancer (50-52). There is sufficient evidence indicating that miRNA expression profiles could be used to classify lung cancer, which also suggests a correlation between disease prognosis and therapeutic outcome. miRNA could act as a tumor suppressor, as well as an oncogene (53). For example, in lung cancer, let-7 is a suppressor for *RAS*. mir17 and mir21 clusters are oncogenic and modulate PTEN and TGFβ-RII (52). These observations emphasize the potential application of miRNAs as biomarkers for diagnosis, prognosis, stage, risk stratification and prediction and drug-responses in patients with cancer.

Protein markers. Several proteins are currently in use for the detection of lung cancer (Table I). An oncofetal protein, CEA and cytokeratin 19-fragment (CYFRA 21-1) proteins have been reported as potential indices for monitoring response to treatment among advanced NSCLC patients (5,54). Neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP) are also available to use as lung cancer biomarkers for SCLC (55,56). However, these protein biomarkers also usually lack lung-cancer specificity.

There are other potential lung cancer biomarker molecules that are still not available for clinical use. The potency of serum amyloid A (57,58) and haptoglobin-α2 (59,60) as lung cancer biomarkers require more clinical validation before they are approved for clinical use. Plasma granulocyte colony-stimulating factor (G-CSF) levels were significantly increased in patients with lung cancer, particularly in the advanced TNM stages (61,62). These results suggest that plasma G-CSF could be used to support the diagnostic process of lung cancer staging and as an indicator of metastasis.

Lung cancer and proteomics. Comprehensive and in-depth discovery of the disease proteome is an important issue in recent proteomic developments. Improvement in sample preparation tools will reduce the intrinsic limitations in biological samples, such as variation among individuals, differences in genetic make-up and non-specific changes (63,64).

Protein-based lung cancer biomarkers are derived from the techniques of classical 2-dimensional (2-D) fluorescence difference gel electrophoresis (DIGE), polyacrylamide gel electrophoresis (PAGE), mass spectroscopy, matrix-associated laser absorption desorption ionization time-of-flight (MALDI-TOF), surface-enhanced laser absorption desorption ionization time-of-flight (SELDITOF) and reverse phase microarray (65-67).

Quantum dots and nanoparticles are recent additions to the technologies available to assess the potential of protein molecules as cancer biomarkers (68). Quantitative proteomics have been utilized to discover biomarkers in lung cancer, such as stable isotope labeling with amino acids in cell culture (SILAC), iTRAQ and liquid chromatography-MS/MS (LC-MS/MS) (69-71).

Circulating tumor cells. Circulating tumor cells (CTCs) are indicators of cancer and have been extensively reviewed (72,73). For the early detection of cancer, one challenge is to find highly specific markers that are able to address extremely low signal-to-noise ratio. Detection of CTCs currently relies on a single marker, the epithelial cell surface epitope EpCAM. Although EpCAM is an excellent epithelial cell marker, it is not expressed on all cancer cells (74). Emerging approaches include the use of gene mutations, antibody cocktails, negative selections and filtrations on the basis of cell size or density (73).

3. Conclusion

The application of biomarkers for the early detection of all types of cancer is of significant potential value and deserves a similarly significant share of funding. However, in lung cancer, there are no sensitive and specific biomarkers such as prostate specific antigen for prostate cancer. Several biomarkers will probably have to be used together, including DNA- and RNA-based biomarkers, protein biomarkers, proteomics and CTCs.

Assay sensitivity and specificity need to be improved, techniques must be standardized and validated, and legislation on biomarkers needs to be regulated more closely. We may be confident that general progress and marked new discoveries will continue, as techniques for earlier detection are being developed, which could have major impacts. The progress of clinical medicine quite significantly depends on the testing of novel theories.

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