Biomarkers for the lung cancer diagnosis and their advances in proteomics

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Over a last decade, intense interest has been focused on biomarker discovery and their clinical uses. This interest is accelerated by the completion of human genome project and the progress of techniques in proteomics. Especially, cancer biomarker discovery is eminent in this field due to its anticipated critical role in early diagnosis, therapy guidance, and prognosis monitoring of cancers. Among cancers, lung cancer, one of the top three major cancers, is the one showing the highest mortality because of failure in early diagnosis. Numerous potential DNA biomarkers such as hypermethylation of the promoters and mutations in K-ras, p53, and protein biomarkers; carcinoembryonic antigen (CEA), CYFRA21-1, plasma kallikrein B1 (KLKB1), Neuron-specific enolase, etc. have been discovered as lung cancer biomarkers. Despite extensive studies thus far, few are turned out to be useful in clinic. Even those used in clinic do not show enough sensitivity, specificity and reproducibility for general use. This review describes what the cancer biomarkers are for, various types of lung cancer biomarkers discovered at present and predicted future advance in lung cancer biomarker discovery with proteomics technology. [BMB reports 2008; 41(9): 615-625]

Cancer biomarkers

Biomarkers are referred to every means of tools for quantifiable measurements of biological homeostasis, which distinguish what is abnormal from what is normal (1). Thus, the simplest definition of a biomarker in more applicable means is a molecule that indicates an alteration in physiology from normal. A more practical definition of a biomarker would require clinical utility of this molecule (2). Cancer biomarkers give good guidance on many areas of cancer biology. They are not only useful in early diagnosis of cancers but also provide important information in cancer therapy such as, verification of cancer staging, response to therapy, therefore, guidance on therapy, and clinical end points or surrogate end points. Cancer biomarkers contribute to the advances in understanding the cancer staging. Currently, established convention of cancer staging is the anatomically based TNM (tumor, node, metastases) system, developed in France in the 1940s by Pierre Denoix. Cancer biomarkers will provide more knowledge in many areas of biology, which the TNM system cannot.

The clinical information given by cancer biomarkers is significant in selection of appropriate treatment leading to personalized cancer therapy. Currently, a number of cancer therapies are carried out based on specific cancer biomarkers. Chronic myelogenous leukemia (CML) treatment with ‘imatinib mesylate’ in BCR-ABL translocation patients (3), ‘rituximab’ for CD20 positive lymphoma patients (4), and ‘trastuzumab’ for HER2/neu positive breast cancer patients are the examples of biomarkers of drug targets (5), which are therefore therapy directing. These indicate the anticipated benefits of biomarkers also as the guidance on therapy and identification of new targets in cancer treatment.

Types of cancer biomarkers

There are several distinct types of cancer biomarkers based on different areas: genetics, epigenetics, proteomics, metabolomics, imaging technology, and general physical techniques. Genetics-based cancer biomarkers utilize DNA arrays, polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR), DNA sequencing, fluorescent in situ hybridization (FISH) etc. to detect the genetic alterations occurring in the cancerous state. On the other hand, recent development of epigenetic modification analysis also provides tools as cancer biomarkers. Epigenetic modification usually occurs in CpG island of the gene regulatory regions, which results in the down-regulation of the gene expression. These alterations can evade the cells from their normal cell cycle control, and thus result in cancer cells formation (6, 7). Proteomics techniques include mass spectrometry (MS), ELISA, and immunohistochemistry etc., and utilize 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. The metabolome is believed to represent only about 2,500 small mole-
cules and may provide an insight to cancer biomarkers. Broadly used imaging techniques such as Positron Emission Tomography (PET), Computed Tomographic (CT) scans and Magnetic Resonance Imaging (MRI) are still major means of cancer diagnosis and have distinct ability to localize the cancer that molecular based biomarkers cannot.

**Application of cancer biomarkers in cancer diagnosis and therapy**

Unlike uniformity of long-established TNM system, cancer biomarkers are considered to be more suitable to the heterogeneous nature of cancer. In the case of lung cancer, although categorized in small cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC) by histological character, there can be many other criteria dividing subtypes of lung cancers for example, EGFR mutation-induced lung cancers. These sub-cATEGORIES cannot be determined without invasive biopsied tissue test and therefore cancer type specific biomarkers will be useful in more accurate cancer diagnosis. Thus, possibility of accurate diagnosis of cancers by biomarkers is expected to bring some benefits in molecular based cancer patient care. First, with potential to predict possibility to progress into cancerous status, biomarkers can prevent cancer in person who has high risk (1). Cancer biomarkers are expected to not only predict the predisposed factors but also diagnose cancer patients at early of their stage. This will greatly increase the chances to treat cancer and reduce the mortality. Second biomarkers will be guidance to cancer therapy. Some biomarkers change in expression levels responding to treatment. This change will indicate the response to therapy and will tell the important clinical endpoint or surrogate endpoint. In other aspects of biomarker contribution to the cancer therapy is the drug target discovery. In other words, tissue derived biomarkers can be used for potential drug targets as well as imaging diagnostic biomarkers. Aminopeptidase-p and Annexin A1 discovered in lung endothelial surfaces of mouse lung cancer model (8) and coatamer protein complex, subunit gamma (COPG), thymopoietin (TMPO) and peroxiredoxin 4 (PRDA 4) found by our group from endothelial cells mouse lung cancer model (8) and coatomer protein complex, and Annexin A1 discovered in lung endothelial surfaces of smoker or exsmokers. The loss of one allele of inactivation of p16, p15, glutathione transferase 1, O(6)-methylguanine-DNA methyltransferse (O6-MGMT), tissue inhibitor of metalloprotease (TIMP)-3 and death associated protein (DAP)-kinase following the hypermethylation of their promoter regions cannot be determined without invasive biopsied tissue test and therefore cancer type specific biomarkers will be useful in more accurate cancer diagnosis. Thus, possibility of accurate diagnosis of cancers by biomarkers is expected to bring some benefits in molecular based cancer patient care. First, with potential to predict possibility to progress into cancerous status, biomarkers can prevent cancer in person who has high risk (1). Cancer biomarkers are expected to not only predict the predisposed factors but also diagnose cancer patients at early of their stage. This will greatly increase the chances to treat cancer and reduce the mortality. Second biomarkers will be guidance to cancer therapy. Some biomarkers change in expression levels responding to treatment. This change will indicate the response to therapy and will tell the important clinical endpoint or surrogate endpoint. In other aspects of biomarker contribution to the cancer therapy is the drug target discovery. In other words, tissue derived biomarkers can be used for potential drug targets as well as imaging diagnostic biomarkers. Aminopeptidase-p and Annexin A1 discovered in lung endothelial surfaces of mouse lung cancer model (8) and coatamer protein complex, subunit gamma (COPG), thymopoietin (TMPO) and peroxiredoxin 4 (PRDA 4) found by our group from endothelial cells in human lung cancer tissues are good examples of biomarkers role in drug targets (9). Finally, in case of molecular based cancer biomarkers, they will help to find new drug target molecules for personalized cancer therapy.

**Lung cancer and its biomarkers at present**

Lung cancer is one of the most prevalently occurring and the most life-threatening neoplasia in most part of the world. It has an incidence of 1.2 million people in worldwide, and accounted for about 25% of all cancer deaths (10, 11). In Korea, the incidence and the mortality associated with lung cancer are predicted to steadily increase. This is largely attributed to the diagnosis at late stage. Some ongoing diagnostic tools at clinics include CT scans, bronchoscopy and sputum analysis, none of which turns out to be effective in early diagnosis of lung cancer. As early detection as IA stage of lung cancer can raise the 5-year survival to 80%, comparing to 15% of overall non-small cell lung cancer (NSCLC) (12). Therefore, discovery of novel lung cancer specific biomarker is emerging as an important platform toward early detection and targeted therapy.

**DNA-based lung cancer biomarkers**

Cancers are thought to arise by genetic alteration, environmental factors and combined both. Although fewer than 10% of cancers are considered to be linked to Mendelian inheritance of genetic traits (13), genetics are closely related to the nature of the cancer. Following the development in genomics, fundamental advances in DNA- or RNA-based cancer biomarkers have been brought into clinical uses. Similar to other cancers, lung carcinogenesis is also multi-step process resulting from the accumulation of altered molecules generated from genetic and epigenetic abnormalities of genes which are involved in cell cycle, senescence, apoptosis, repair, differentiation, and cell migration controls (14-16). Uncontrolled cell growth is derived from either oncogene activation or tumor suppressor gene (TSG) inactivation, thus, genetics-based cancer biomarkers are closely related to these genes (14, 17). Considering that the close connection exists between genetic changes and malignant transformation of lung cancer, it is obvious that genomics would also provide potential lung cancer biomarkers.

**A. Chromosomal changes**

Inactivation of tumor suppressor genes during the cell division is one of the key factors that drive clonal cells of cancer into uncontrolled growth, migration and metastasis (18). In many cases the inactivation is induced by loss of DNA or chromosomal rearrangement accidently happening during cellular division. Most well-known frequently occurring abnormality is deletion of the short arm of chromosome 3 (3p) where several TSG are present (Table 1) (19-22). Loss of chromosomal material has also been reported to be detected in metaplastic epithelium tissues of smoker or exsmokers. The loss of one allele or loss of heterozygosity (LOH) indicates predisposing potentials to lung cancers, too (23, 24).

**B. Gene hypermethylation**

Altered hypermethylation, methylation of the cytosine phosphate guanosine rich regions (CpG islands) of various promoter regions, is a representative epigenetic change in the cell and may cause gene silencing. As an alternative mechanism for inactivating TSGs, hypermethylation is generally discovered in most tumors, including lung cancer (15, 23-31). Thus, certain methylation status in the genes can be biomarkers in lung cancers especially in TSGs. For example, the hypermethylation of inactivation of p16, p15, glutathione transferase 1, O(6)-methylguanine-DNA methyltransferse (O6-MGMT), tissue inhibitor of metalloprotease (TIMP)-3 and death associated protein (DAP)-kinase following the hypermethylation of their promoter

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regions are well-known in lung cancers (Table 1) (7).

C. Genetic change of oncogenes
In an opposite action to previous gene silencing, activation of genes involved the growth factors, their receptors, their messengers or cell cycle activators by mutations also play key roles in carcinogenesis and cancerization. Mutation of ras, a second messenger delivering proliferation signal to nucleus, is discovered to be also involved in lung cancer. Most ras mutation discovered in lung cancer patients appears on codon 12 of K-ras and is known to be related to precancerous state of lung cancers (32, 33). p53-retinoblastoma gene (Rb) pathway can result in genotoxic stress once activated. p53 mutation loses its control of Rb phosphorylation and G1 arrest leading to uncontrolled growth of tumor cells (34-36). Thus, mutations or alterations of protooncogene, which cause hyperactivation of cell cycle, can be good biomarkers in lung cancers (Table 1).

Protein biomarkers
Despite the significant advances in genomics- and genetics-based biomarker discovery, there is still no novel cancer specific biomarker in clinical uses. DNA-based biomarkers, described previously, are known to have potential as a lung cancer biomarker but no biomarkers have adequate sensitivity, specificity and reproducibility. This is due to the fact that mRNA levels are not always linked directly to levels of proteins, the molecules that actually biologically do functions (41). Currently, human genome is known to contain 20,488 genes (42). Proteins, however, give much more varieties due to alternative splice variants, protease cleavages, and post-translational modifications such as glycosylation, phosphorylation, ubiquitination, methylation, acetylation and so on. This means protein biomarkers can be more specific to cancer type and status.

Protein lung cancer biomarkers can be classified by the source of the proteins into three categories: serum biomarkers, tissue biomarkers, and sputum biomarkers (Fig. 1) (43). In sputum, cancer cells apart from cancer sites are major protein sources. In biopsied lung tissue, not only cancer cells but also other molecules involved in self-defense of human body such as, immune cells, cytokines and derivatives from immune or inflammatory response can be found. In blood, however, more potential biomarkers exist and this include the biomarkers found in biopsied cancer tissue and many circulating protein fragments generated in the diseased tissue microenvironment or by circulating proteins and cells derived from the diseased tissue. Because the ultimate goal of biomarker is specific, early and non-invasive diagnosis and post-therapy monitoring of cancer, blood has been thought as an appropriate biological material. Thus, many biomarker discoveries are carried out with blood-based strategies (44, 45) and most of lung cancer biomarkers listed below and already studied are mostly serum biomarkers.

A. Lung cancer protein biomarkers: currently available
Some of lung cancer biomarkers are clinically available although the use of those is not recommended in clinical diagnosis (Table 2). An oncofetal protein, carcinoembryonic antigen (CEA) concentration is elevated in lung cancer patients especially in adenocarcinoma and large cell lung cancer (LCLC) (46-48). CEA alone has limit to diagnosis and is preferably used in combination with CYFRA. As a therapy response monitoring indicator, CEA is used for advanced NSCLC and adenocarcinoma (49, 50). However, CEA and CYFRA have been also found to be associated with other types of cancers such as colorectal cancers (51, 52). Thus they are not thought to be lung cancer-specific biomarkers, but more general cancer biomarkers.

CYFRA 21-1 proteins are indicator of increased level of cytokeratin 19 fragments that implies the presence of lung cancer (46, 47, 53, 54) and has in potential monitoring treatment response of advanced NSCLC patients. Unlike CYFRA21-1, tissue polypeptide antigen (TPA) measures cytokeratin 8, 18 and 19 detecting NSCLC (55). Progastrin-releasing peptide (ProGRP), quite stable protein, is produced by the neuroendocrine tissues of the gastrointestinal and respiratory tracts and is also an available lung cancer biomarker for SCLC (47, 54, 56). Neuron-specific enolase (NSE) is a glycolytic enzyme produced in central and peripheral neurons (57). However, NSE is also increased in the use of those is not recommended in clinical diagnosis (Table 2). An oncofetal protein, carcinoembryonic antigen (CEA) concentration is elevated in lung cancer patients especially in adenocarcinoma and large cell lung cancer (LCLC) (46-48). CEA alone has limit to diagnosis and is preferably used in combination with CYFRA. As a therapy response monitoring indicator, CEA is used for advanced NSCLC and adenocarcinoma (49, 50). However, CEA and CYFRA have been also found to be associated with other types of cancers such as colorectal cancers (51, 52). Thus they are not thought to be lung cancer-specific biomarkers, but more general cancer biomarkers.

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Epidermal growth factor receptor (EGFR) mutation is novel biomarker for the diagnosis of EGFR mutation induced NSCLC that is, strong predictive biomarker for EGFR-targeted protein tyrosine kinase inhibitors (58). As consequence of this biomarker discovery, molecules that target this pathway (e.g., erlotinib, gefitinib, cetuximab, panitumumab) are currently used for specific cancer therapy purpose (59).

B. Lung cancer protein biomarkers: potential
There are also many potential lung cancer biomarker molecules, although not yet available in clinical usage as those listed above (Table 3). The potency of serum amyloid A (95), haptoglobin-alpha2 (96), and a fragment of apolipoprotein A-1 (97) for lung cancer biomarker is mentioned in some studies and need more clinical validation to be available in clinics. In the process of serum glycoprotein analysis in lung cancer patients, our group has identified plasma kallikrein B1 (KLKB1) fragment as potentially useful biomarker in diagnosis of lung adenocarcinoma (98). KLKB1 has homology to proteins of the serine protease-trypsin family and is related to surface-dependent procoagulation, fibrinolysis, kinin generation, and inflammation. It has also homology to KLK3, of which protein product is known as prostate specific antigen (PSA). About 18 kDa fragment of KLKB1, possibly containing H4 domain of it, showed especially high de-

Fig. 1. Schematic workflow of lung cancer biomarker discovery. Biomaterials from three for protein biomarker discovery, lung tissues, sputum, and body fluids, are usually used. In sputum, cancer cells apart from cancer sites are supposed to be detected. In biopsied lung tissues, not only cancer cells but also other molecules involved in self-defense mechanism of human body such as, immune cells, cytokines and derivatives from immune or inflammation responses can be found. In body fluids, although pleural fluids, ascite, urine etc can be used, blood is most commonly used in biomarker studies for its advantages of easy access and routine blood chemistry measurements in the patients. In blood, however, more potential biomarkers exist and this includes many biomarkers found in biopsied cancer tissues and many circulating protein fragments generated in the diseased tissue microenvironment or by circulating proteins derived from the diseased tissues. After proper preparation each protein can be analyzed by similar procedures of the identification, verification, and validation. For protein identification, prepared samples are analyzed by mass spectrometry. LC-ESI-MS/MS and MALDI-TOF/MS are most commonly used platforms. Potential biomarkers, elevated or decreased in their expression levels, are confirmed by Western blot, ELISA or by recent development of multiple reaction monitoring.
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Diagnosis Therapy monitoring Prognosis monitoring Ontology Details References

CEA AdenoCA, LCLC (>10 μg/L) AdenoCA, Advanced NSCLC AdenoCA, NSCLC Cellular component of cell membrane: lipid anchor and immunoglobulin superfamily. Use in combination with CYFRA. Often elevated in smokers. (46-50, 60-71)

NSCLC, SCC (Sensitivity for NSCLC varies between 23 and 70%) Advanced NSCLC NSCLC, SCC Structural constituent of cytoskeleton Often elevated in patients with benign lung diseases. (46-50, 53-55, 60-62, 72-83)

CYFRA21-1 AdenoCA, Advanced NSCLC - - Use in combination with CYFRA. (46-50, 60-71)

TPA NSCLC, SCC - NSCLC - Increased in renal failure and some benign lung diseases. (46, 53, 55)

ProGRP NSCLC, SCC (>200 ng/L = Highly suspicious) SCLC SCLC Phosphoglycerate dehydrogenase activity Use in combination with ProGRP. May correlate with short survival. Increased in inflammatory diseases (60, 66, 72, 74, 77, 78, 83, 86, 91-94)

SCLC (50-71%) SCLC - Pyruvate kinase activity Increased in multiple malignant diseases and some inflammatory diseases (85)

Table 2. Protein-based biomarkers in detection of lung cancer: currently available

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Therapy monitoring</th>
<th>Prognosis monitoring</th>
<th>Ontology</th>
<th>Details</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>AdenoCA, LCLC (&gt;10 μg/L)</td>
<td>AdenoCA, Advanced NSCLC</td>
<td>AdenoCA, NSCLC</td>
<td>Cellular component of cell membrane: lipid anchor and immunoglobulin superfamily. Use in combination with CYFRA. Often elevated in smokers.</td>
<td>(46-50, 60-71)</td>
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<tr>
<td>NSCLC, SCC (Sensitivity for NSCLC varies between 23 and 70%)</td>
<td>Advanced NSCLC</td>
<td>NSCLC, SCC</td>
<td>Structural constituent of cytoskeleton</td>
<td>Often elevated in patients with benign lung diseases.</td>
<td>(46-50, 53-55, 60-62, 72-83)</td>
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<tr>
<td>CYFRA21-1</td>
<td>AdenoCA, Advanced NSCLC</td>
<td>-</td>
<td>-</td>
<td>Use in combination with CYFRA.</td>
<td>(46-50, 60-71)</td>
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<tr>
<td>TPA</td>
<td>NSCLC, SCC</td>
<td>-</td>
<td>NSCLC</td>
<td>-</td>
<td>Increased in renal failure and some benign lung diseases.</td>
</tr>
<tr>
<td>ProGRP</td>
<td>NSCLC, SCC (&gt;200 ng/L = Highly suspicious)</td>
<td>SCLC</td>
<td>SCLC</td>
<td>Phosphoglycerate dehydrogenase activity</td>
<td>Use in combination with ProGRP. May correlate with short survival. Increased in inflammatory diseases</td>
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<tr>
<td>SCLC (50-71%)</td>
<td>SCLC</td>
<td>-</td>
<td>Pyruvate kinase activity</td>
<td>Increased in multiple malignant diseases and some inflammatory diseases</td>
<td>(85)</td>
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CEA = carcinoembryonic antigen, CYFRA 21-1 = cytokeratin 19 fragment, TPA = tissue polypeptide antigen, ProGRP = progastrin-releasing peptide, NSE = neuron-specific enolase, AdenoCA = adenocarcinoma, SCC = squamous cell carcinoma, SCLC = small cell lung cancer, NSCLC = non-small cell lung cancer

Table 3. Protein-based biomarkers for the detection of lung cancer: potential

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Ontology</th>
<th>Details</th>
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<tbody>
<tr>
<td>Serum amyloid A</td>
<td>Lipid transporter activity</td>
<td>Elevated to 62.4 μg/ml (2 μg/ml in healthy control)</td>
<td>(95, 101)</td>
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<td></td>
<td>Acute phase response</td>
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<td></td>
<td>Immune cell chemotaxis</td>
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<td></td>
<td>Extracellular region</td>
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<td></td>
<td>Serine-type endopeptidase activity</td>
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<td>Defense response</td>
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<td></td>
<td>Proteolysis</td>
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<tr>
<td></td>
<td>Extracellular region</td>
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<tr>
<td>Haptoglobin-α 2</td>
<td>AdenoCA</td>
<td>Lipid transporter activity</td>
<td>(96)</td>
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<td></td>
<td>Lipase inhibitor activity</td>
<td>Apolipoprotein A-1 fragment: downregulated in cancer patients</td>
<td>(96)</td>
</tr>
<tr>
<td></td>
<td>Cholesterol efflux, homeostasis, metabolic</td>
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<td></td>
<td>process, transport</td>
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<td>Extracellular region</td>
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<tr>
<td>APOA1</td>
<td>AdenoCA</td>
<td>Peptidase activity</td>
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<td>KLKB1</td>
<td>AdenoCA</td>
<td>Peptidase activity</td>
<td>(98)</td>
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<td>Proteolysis</td>
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Proteomics and lung cancer biomarkers in advance

Proteomic technology at present

Following the completion of human genome project, proteomics has emerged as a new area of study. Proteomics means large-scale characterization of proteins including more complicated features; isoforms, modifications, interactions, functional structures, etc. (102). Dramatic progress of proteomic technologies such as, protein separation, quantification, and identification makes it possible to search proteins more intensive and to understand their functions deeper (103, 104). Therefore systemic overview of whole expressed proteins became possible and this is linked to improvement of cancer therapy in diagnosis, treatment and prognosis monitoring.

Proteomics has approached in cancer biomarker field in two aspects; one is profiling the whole protein expressions and the other is identification of specific individual protein which is potential as a biomarker. Former enables systemic overview of neoplasia and ultimately the improvement in cancer diagnosis and therapy and the latter brings discovery of new cancer specific biomarker and early diagnosis (57, 105). Proteomics employs mass spectrometry, protein microarrays, and electrophoresis as analytical tools to identify proteins (106). Microarrays using multiple capture antibodies are efficient method to detect the presence of numerous proteins rapidly, and electrophoresis is particularly useful in separating proteins by molecular weight and/or pKa. The major techniques that fundamentally supported the discovery of cancer biomarkers was mass spectrometry which can determine precise mass and charge of protein, thus identity of the actual precursor proteins.

In the sense of limitation of current proteomics technology, mainly mass spectrometry-based, it is generally accepted that subproteome analysis is important to look for proteomes deeper and more specific low abundant proteins. This is mainly due to the high dynamic range, more than $10^{13}$ order magnitude, of proteins present in the serum; highest proteins of albumin about 40 mg/ml to Interleukin-6, one of lowest proteins, about 10 pg/ml (107). Thus, before searching biomarker proteins in the body fluids, proteomes in the body fluids need to be separated by its characteristics; such as by glycoproteome or phosphoproteome enrichments, ion charges, hydrophobicity or hydrophilicity by SCX/SAX, reverse phase or normal phase chromatography, or by their molecular weights etc. Glycoproteome analysis in the body fluids has great advantages in the area of cancer biomarker discovery. About half of the serum proteins are known to be glycosylated and the glycosylation status on glycoproteins, their degrees and forms, is also altered by certain disease conditions including cancers. Also, various known biomarkers are found to be glycoproteins; such as breast cancer biomarkers CA-125 and ErbB.

Proteomic technology ahead and biomarkers in the future

Since cancer biomarker discovery has started extensively with the progress of proteomic technology, many protein molecules have been indicated as potential cancer biomarkers. There are several hurdles that have been acted as major causes of cancer biomarker unsuccessfulness. First, lack of analytic reagent, especially antibodies for validation of mass-spectrometry data or for clinical tests had great hindrance. Lack of commercially available specific antibodies makes it much slower to validate the cancer biomarker candidates through immunoblot analysis or enzyme-linked immunoborbent assays (ELISAs). Second, most of the previously published studies had technical limitations; insufficient sensitivity, inability to detect low abundant proteins etc.

Proteomic techniques are gradually improving in sample preparation steps and also analysis steps. Improvement in sample preparation tools will reduce the intrinsic limitations in biological samples, such as variation among individuals, differences in genetic make-up, and nonspecific changes (108) and progress in analytic techniques will increase sensitivity to detect low abundance or low molecular weight protein molecules. Recently, remarkable new orthogonal MS-based clinical assay (multiple-reaction-monitoring, MRM-MS (109, 110) is expected to accelerate the discovery of cancer biomarkers, the verification of the biomarkers, and also their clinical translations. Modification of this tool using isotope-coded antibody capture
technology (111) or by multi-dimensional fractionation (112), the sensitivity will increase to ng/ml levels that can make it possible to diagnose direct biological samples targeting specific cancer biomarkers.

Conclusion

There has been great advance in the discovery and development of lung cancer biomarkers in company with the development of genomics and proteomics technologies. In cancer therapy, especially in case of lung cancer, diagnosis at early stage is crucial for effective treatment. Although, the currently available or potential lung cancer biomarkers are not sensitive or specific enough to be used clinically in the diagnosis, stratification, prognosis, or drug responses, we can envision that this will be greatly improved in the future. For the development of useful lung cancer biomarkers, we think three aspects need to be considered. First, we need to analyze currently available or potential biomarkers in a large set of clinical samples including other cancer types and other diseases conditions, especially inflammatory diseases. This will reveal the usefulness of these biomarkers. Second, with further improvements of technology, we need to look for more specific and low abundant lung cancer biomarkers focused on specific subtypes of lung cancers. Third, one specific biomarker may not be enough to predict or monitor lung cancers, thus, several good biomarkers need to be combined, with quantitative information, to be really useful in the clinical purposes.

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