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**Mini Review**

# 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 hypermethylations 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-

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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 coatomer 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 closelely 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 accidentally 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 herterozygosity (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, 25-31). Thus, certain methylation status in the genes can be biomakers 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

**Table 1.** Gene-based biomarkers in detection of lung cancer: potential

| Groups | Types of genes | |
|---|---|---|
| Chromosomal changes | Deletion of the short arm of chromosome 3 (3p) (21) | 27-88% in circulating DNA of lung cancer patients |
| Hypermethylation | Serine protease family member-trypsinogen IV (PRSS3) (22) | - |
| | Tissue inhibitor of metalloproteinase (TIMP)-3 (37) | - |
| | Death associated protein (DAP)-kinase (38) | - |
| | P16, FHIT (39) | Associated with an increased risk of lung cancer recurrence after therapy |
| Genetic changes | K-ras (40) | 20-30% in circulating DNA of lung cancer patients |
| | P53 (40) | 27% in circulating DNA of lung cancer patients |

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 fragements 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 nueroendocrine 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 SCLC and its elevated level shows great potential to be a monitoring tool for post-therapy. Again, these protein biomarkers usually lack their lung-cancer specificity in terms of their usages as biomarkers.

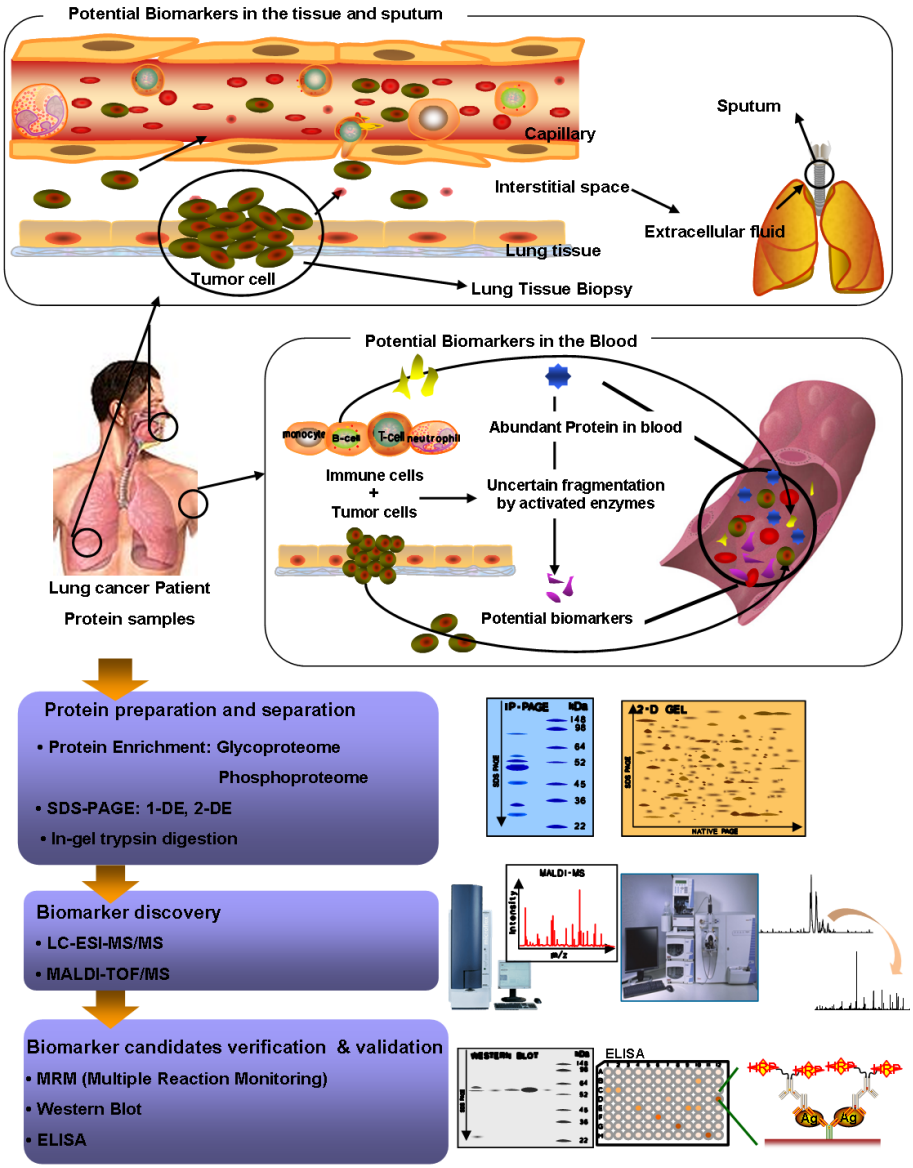


**Fig. 1.** Schematic workflow of lung cancer biomarker discovery. Biomaterials from three for protein biomarker discovery, lung tissues, sputum, and body fluids, are usually used. In sputum, cancer cells apart from cancer sites are supposed to be detected. In biopsied lung tissues, not only cancer cells 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.

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 consequent 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-

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

| | Diagnosis | Therapy monitoring | Prognosis monitoring | Ontology | Details | References |
|---|---|---|---|---|---|---|
| CEA | AdenoCA, LCLC (>10 ug/L) | AdenoCA, Advanced NSCLC | AdenoCA, NSCLC | Cellular component<br>Cell membrane: lipid anchor<br>Immunoglobulin superfamily | Use in combination with CYFRA.<br>Often elevated in smokers. | (46-50, 60-71) |
| CYFRA21-1 | NSCLC, SCC (Sensitivity for NSCLC varies between 23 and 70%) | Advanced NSCLC | NSCLC, SCC | Structural constitutent of cytoskeleton | Often elevated in patients with benign lung diseases. | (46-50, 53-55, 60-62, 72-83) |
| TPA | NSCLC, SCC | - | NSCLC | | - | (46, 53, 55) |
| ProGRP | SCLC (>200 ng/L=Highly suspicious) (Sensitivities for SCLC range 47-86%) | SCLC | - | Neuropeptide hormone activity | Increased in renal failure and some benign lung diseases.<br>Use in combination with NSE | (48, 54, 56, 84-90) |
| NSE | SCLC (>100 ug/L=High probability) (Sensitivities for SCLC as high as 74%) | SCLC | SCLC | Phosphoglycerate dehydrogenase activity<br>Subcellular location (cytoplasm) | Use in combination with ProGRP<br>May correlates with short survival<br>Increased in inflammatory diseases | (60, 66, 72, 74, 77, 78, 83, 86, 91-94) |
| Tumor M2-pyruvate kinase | AdenoCA (Sensitivities for SCLC range 50-71%) | - | AdenoCA | Pyruvate kinase activity<br>Glycolysis<br>Cytoplasm | Increased in multiple malignant diseases and some inflammatory diseases | (85) |

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

| | Diagnosis | Ontology | Details | Reference |
|---|---|---|---|---|
| Serum amyloid A | Lung cancer | Lipid transporter activity<br>Acute phase response<br>Immune cell chemotaxis<br>Extracellular region | Elevated to 62.4 μg/ml (2 μg/ml in healthy control) | (95, 101) |
| Haptoglobin-α 2 | AdenoCA | Serine-type endopeptidase activity<br>Defense response<br>Proteolysis<br>Extracellular region | - | (96) |
| APOA1 | AdenoCA | Lipid transporter activity<br>Lipase inhibitor activity<br>Cholesterol efflux, homeostasis, metabolic process, transport<br>Extracellular region | Apolipoprotein A-1 fragment: downregulated in cancer pateients | (96) |
| KLKB1 | AdenoCA | Peptidase activity<br>Proteolysis | 17-18 kDa fragment of plasma kallikrein B1 | (98) |

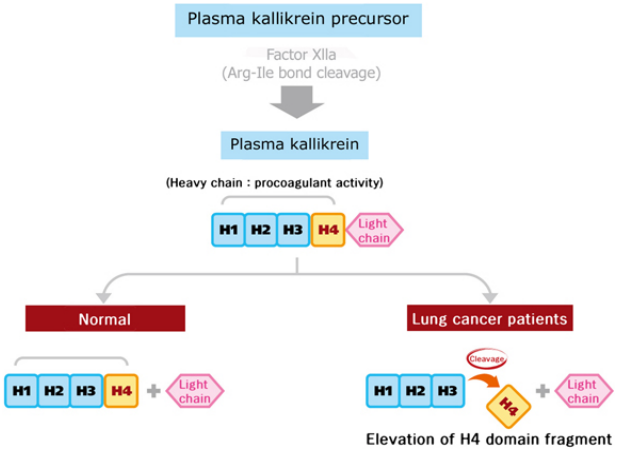


**Fig. 2.** A diagram showing the generation of plasma kallikrein (KLKB1) fragment. A KLKB1 has homology to proteins of serine protease-trypsin family and by the action of Factor XIIa, KLKB1 are cleaved into two main chains: a heavy chain containing four domains with procoagulant activity and a light chain, active catalytic domain, with trypsin-like activity. A fragment including H4 domain of KLKB1 is produced in the sera of lung cancer patients, which can be a potential biomarker, whereas it is not or minimally produced in normal population.

tection in the lung adenocarcinoma (Fig. 2). The finding of KLKB1 fragment supports the recent ideas of serum peptidomes as great diagnostic potential biomarkers in cancer diagnosis (99), and this low molecular weight protein fragment also implies that that there will be much more potent lung cancer biomarkers derived from cancer-type-specific enzymatic breakdowns (100).

## 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 potent 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 $10^{11}$ 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 immunaborbent 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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## REFERENCES

1. Dalton, W.S. and Friend, S.H. (2006) Cancer biomarkers–an invitation to the table. *Science* **312**, 1165-1168.
2. Fung, E.T., Wright, G.L., Jr. and Dalmasso, E.A. (2000) Proteomic strategies for biomarker identification: progress and challenges. *Curr. Opin. Mol. Ther.* **2**, 643-650.
3. Druker, B.J. (2003) Imatinib mesylate in the treatment of chronic myeloid leukaemia. *Expert Opin. Pharmacother.* **4**, 963-971.
4. Leget, G.A. and Czuczman, M.S. (1998) Use of rituximab, the new FDA-approved antibody. *Curr. Opin. Oncol.* **10**, 548-551.
5. Arteaga, C.L., Moulder, S.L. and Yakes, F.M. (2002) HER (erbB) tyrosine kinase inhibitors in the treatment of breast cancer. *Semin. Oncol.* **29**, 4-10.
6. Baylin, S.B. (2005) DNA methylation and gene silencing in cancer. *Nat. Clin. Pract. Oncol.* **2 Suppl 1**, S4-11.
7. Belinsky, S.A. (2004) Gene-promoter hypermethylation as a biomarker in lung cancer. *Nat. Rev. Cancer* **4**, 707-717.
8. Oh, P., Li, Y., Yu, J., Durr, E., Krasinska, K.M., Carver, L.A., Testa, J.E. and Schnitzer, J.E. (2004) Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. *Nature* **429**, 629-635.
9. Park, H.J., Kim, B.G., Lee, S.J., Heo, S.H., Kim, J.Y., Kwon, T.H., Lee, E.B., Ryoo, H.M. and Cho, J.Y. (2008) Proteomic profiling of endothelial cells in human lung cancer. *J. Proteome Res.* **7**, 1138-1150.
10. Granville, C.A. and Dennis, P.A. (2005) An overview of lung cancer genomics and proteomics. *Am. J. Respir. Cell Mol. Biol.* **32**, 169-176.
11. Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., Smigal, C. and Thun, M.J. (2006) Cancer statistics, 2006. *CA. Cancer J. Clin.* **56**, 106-130.
12. Mulshine, J.L. and Sullivan, D.C. (2005) Clinical practice. Lung cancer screening. *N. Engl. J. Med.* **352**, 2714-2720.
13. Ludwig, J.A. and Weinstein, J.N. (2005) Biomarkers in cancer staging, prognosis and treatment selection. *Nat. Rev. Cancer* **5**, 845-856.
14. Brambilla, C., Fievet, F., Jeanmart, M., de Fraipont, F., Lantuejoul, S., Frappat, V., Ferretti, G., Brichon, P.Y. and Moro-Sibilot, D. (2003) Early detection of lung cancer: role of biomarkers. *Eur. Respir J. Suppl.* **39**, 36s-44s.
15. Chung, G.T., Sundaresan, V., Hasleton, P., Rudd, R., Taylor, R. and Rabbitts, P.H. (1995) Sequential molecular genetic changes in lung cancer development. *Oncogene* **11**, 2591-2598.
16. Kishimoto, Y., Sugio, K., Hung, J.Y., Virmani, A.K., McIntire, D.D., Minna, J.D. and Gazdar, A.F. (1995) Allele-specific loss in chromosome 9p loci in preneoplastic lesions accompanying non-small-cell lung cancers. *J. Natl. Cancer Inst.* **87**, 1224-1229.
17. Wood, L.D., Parsons, D.W., Jones, S., Lin, J., Sjoblom, T., Leary, R.J., Shen, D., Boca, S.M., Barber, T., Ptak J., Silliman, N., Szabo, S., Dezso, Z., Ustyanksky, V., Nikolskaya, T., Nikolsky, Y., Karchin, R., Wilson, P.A., Kaminker, J.S., Zhang, Z., Croshaw, R., Willis, J., Dawson, D., Shipitsin, M., Willson, J.K., Sukumar, S., Polyak, K., Park, B.H., Pethiyagoda, C.L., Pant, P.V., Ballinger, D.G., Sparks, A.B., Hartigan, J., Smith, D.R., Suh, E., Papadopoulos, N., Buckhaults, P., Markowitz, S.D., Parmigiani, G., Kinzler, K.W., Velculescu, V.E. and Vogelstein, B. (2007) The genomic landscapes of human breast and colorectal cancers. *Science* **318**, 1108-1113.
18. Wistuba, II, Lam, S., Behrens, C., Virmani, A.K., Fong, K.M., LeRiche, J., Samet, J.M., Srivastava, S., Minna, J.D. and Gazdar, A.F. (1997) Molecular damage in the bronchial epithelium of current and former smokers. *J. Natl. Cancer Inst.* **89**, 1366-1373.
19. Senchenko, V.N., Liu, J., Loginov, W., Bazov, I., Angeloni, D., Seryogin, Y., Ermilova, V., Kazubskaya, T., Garkavtseva, R., Zabarovska, V.I., Kashuba, V.I., Kisselev, L.L., Minna, J.D., Lerman, M.I., Klein, G., Braga, E.A. and Zabarovsky, E.R. (2004) Discovery of frequent homozygous deletions in chromosome 3p21.3 LUCA and AP20 regions in renal, lung and breast carcinomas. *Oncogene* **23**, 5719-5728.
20. Wiest, J.S., Franklin, W.A., Drabkin, H., Gemmill, R., Sidransky, D. and Anderson, M.W. (1997) Genetic markers

for early detection of lung cancer and outcome measures for response to chemoprevention. *J. Cell Biochem. Suppl.* **28-29**, 64-73.
21. Zabarovsky, E.R., Lerman, M.I. and Minna, J.D. (2002) Tumor suppressor genes on chromosome 3p involved in the pathogenesis of lung and other cancers. *Oncogene* **21**, 6915-6935.
22. Xue, X., Zhu, Y.M. and Woll, P.J. (2006) Circulating DNA and lung cancer. *Ann. N.Y. Acad. Sci.* **1075**, 154-164.
23. Mao, L., Lee, J.S., Kurie, J.M., Fan, Y.H., Lippman, S.M., Lee, J.J., Ro, J.Y., Broxson, A., Yu, R., Morice, R.C., Kemp, B.L., Khuri, F.R., Walsh, G.L., Hittelman, W.N. and Hong, W.K. (1997) Clonal genetic alterations in the lungs of current and former smokers. *J. Natl. Cancer Inst.* **89**, 857-862.
24. Wistuba, II, Behrens, C., Milchgrub, S., Bryant, D., Hung, J., Minna, J.D. and Gazdar, A.F. (1999) Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* **18**, 643-650.
25. Belinsky, S.A., Nikula, K.J., Palmisano, W.A., Michels, R., Saccomanno, G., Gabrielson, E., Baylin, S.B. and Herman, J.G. (1998) Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 11891-11896.
26. Chaussade, L., Eymin, B., Brambilla, E. and Gazzeri, S. (2001) Expression of p15 and p15.5 products in neuroendocrine lung tumours: relationship with p15(INK4b) methylation status. *Oncogene* **20**, 6587-6596.
27. Esteller, M., Sanchez-Cespedes, M., Rosell, R., Sidransky, D., Baylin, S.B. and Herman, J.G. (1999) Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res.* **59**, 67-70.
28. Kurakawa, E., Shimamoto, T., Utsumi, K., Hirano, T., Kato, H. and Ohyashiki, K. (2001) Hypermethylation of p16 (INK4a) and p15(INK4b) genes in non-small cell lung cancer. *Int. J. Oncol.* **19**, 277-281.
29. Palmisano, W.A. Divine, K.K., Saccomanno, G., Gilliland, F.D., Baylin, S.B., Herman, J.G. and Belinsky, S.A. (2000) Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res.* **60**, 5954-5958.
30. Virmani, A.K., Rathi, A., Zochbauer-Muller, S., Sacchi, N., Fukuyama, Y., Bryant, D., Maitra, A., Heda, S., Fong, K.M., Thunnissen, F., Minna, J.D. and Gazdar, A.F. (2000) Promoter methylation and silencing of the retinoic acid receptor-beta gene in lung carcinomas. *J. Natl. Cancer Inst.* **92**, 1303-1307.
31. Zochbauer-Muller, S., Fong, K.M., Virmani, A.K., Geradts, J., Gazdar, A.F. and Minna, J.D. (2001) Aberrant promoter methylation of multiple genes in non-small cell lung cancers. *Cancer Res.* **61**, 249-255.
32. Rodenhuis, S. and Slebos, R.J. (1992) Clinical significance of ras oncogene activation in human lung cancer. *Cancer Res.* **52**, 2665s-2669s.
33. Sugio, K., Ishida, T., Yokoyama, H., Inoue, T., Sugimachi, K. and Sasazuki, T. (1992) ras gene mutations as a prognostic marker in adenocarcinoma of the human lung without lymph node metastasis. *Cancer Res.* **52**, 2903-2906.
34. Brambilla, E., Gazzeri, S., Lantuejoul, S., Coll, J.L., Moro, D. Negoescu, A. and Brambilla, C. (1998) p53 mutant immunophenotype and deregulation of p53 transcription pathway (Bcl2, Bax and Waf1) in precursor bronchial lesions of lung cancer. *Clin Cancer Res.* **4**, 1609-1618.
35. Gazzeri, S., Brambilla, E., Caron de Fromentel, C., Gouyer, V., Moro, D., Perron, P., Berger, F. and Brambilla, C. (1994) p53 genetic abnormalities and myc activation in human lung carcinoma. *Int. J. Cancer* **58**, 24-32.
36. Levine, A.J. (1997) p53, the cellular gatekeeper for growth and division. *Cell.* **88**, 323-331.
37. Yanagawa, N., Tamura, G., Oizumi, H., Kanauchi, N., Endoh, M,. Sadahiro, M. and Motoyama, T. (2007) Promoter hypermethylation of RASSF1A and RUNX3 genes as an independent prognostic prediction marker in surgically resected non-small cell lung cancers. *Lung Cancer* **58**, 131-138.
38. Wistuba, II, Gazdar, A.F. and Minna, J.D. (2001) Molecular genetics of small cell lung carcinoma. *Semin. Oncol.* **28**, 3-13.
39. Kim, J.S., Kim, J.W., Han, J., Shim, Y.M., Park, J. and Kim, D.H. (2006) Cohypermethylation of p16, and FHIT promoters as a prognostic factor of recurrence in surgically resected stage I non-small cell lung cancer. *Cancer Res.* **66**, 4049-4054.
40. Aviel-Ronen, S., Blackhall, F.H., Shepherd, F.A. and Tsao, M.S. (2006) K-ras mutations in non-small-cell lung carcinoma: a review. *Clin Lung Cancer* **8**, 30-38.
41. Simpson, R.J., Bernhard, O.K., Greening, D.W. and Moritz, R.L. (2008) Proteomics-driven cancer biomarker discovery: looking to the future. *Curr. Opin. Chem. Biol.* **12**, 72-77.
42. Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., Gocayne, J.D., Amanatides, P., Ballew, R.M., Huson, D.H., Wortman, J.R., Zhang, Q., Kodira, C.D., Zheng, X.H., Chen, L., Skupski, M., Subramanian, G., Thomas, P.D., Zhang, J., Gabor Miklos, G.L., Nelson, C., Broder, S., Clark, A.G., Nadeau, J., McKusick, V.A., Zinder,N., Levine, A.J., Roberts, R.J., Simon, M., Slayman, C., Hunkapiller, M., Bolanos, R., Delcher, A., Dew, I., Fasulo, D., Flanigan, M., Florea, L., Halpern, A., Hannenhalli, S., Kravitz, S., Levy, S., Mobarry, C., Reinert, K., Remington, K., Abu-Threideh, J., Beasley, E., Biddick, K., Bonazzi, V., Brandon, R., Cargill, M., Chandramouliswaran, I., Charlab, R., Chaturvedi, K., Deng, Z., Di Francesco, V., Dunn, P., Eilbeck, K., Evangelista, C., Gabrielian, A.E., Gan, W., Ge, W., Gong, F., Gu, Z., Guan, P., Heiman, T.J., Higgins, M.E., Ji, R.R., Ke, Z., Ketchum, K.A., Lai, Z., Lei, Y., Li, Z., Li, J., Liang, Y., Lin, X., Lu, F., Merkulov, G.V., Milshina, N., Moore, H.M., Naik, A.K., Narayan, V.A., Neelam, B., Nusskern, D., Rusch, D.B., Salzberg, S., Shao, W., Shue, B., Sun, J., Wang, Z., Wang, A., Wang, X., Wang, J., Wei, M., Wides, R., Xiao, C., et al. (2001) The sequence of the human genome. *Science* **291**, 1304-1351.
43. Strauss, G.M. and Skarin, A.T. (1994) Use of tumor markers in lung cancer. *Hematol. Oncol. Clin. North. Am.* **8**, 507-532.
44. Omenn, G.S. (2006) Strategies for plasma proteomic profiling of cancers. *Proteomics* **6**, 5662-5673.
45. Rifai, N., Gillette, M.A. and Carr, S.A. (2006) Protein biomarker discovery, and validation: the long, and uncertain path to clinical utility. *Nat. Biotechnol.* **24**, 971-983.

46. Kulpa, J., Wojcik, E., Radkowski, A., Kolodziejski, L. and Stasik, Z. (2000) CYFRA 21-1, TPA-M, TPS, SCC-Ag, and CEA in patients with squamous cell lung cancer, and in chemical industry workers as a reference group. *Anticancer Res.* **20**, 5035-5040.
47. Molina, R., Auge, J.M., Filella, X., Vinolas, N., Alicarte, J., Domingo, J.M. and Ballesta, A.M. (2005) Pro-gastrin-releasing peptide (proGRP) in patients with benign, and malignant diseases: comparison with CEA, SCC, CYFRA 21-1, and NSE in patients with lung cancer. *Anticancer Res.* **25**, 1773-1778.
48. Molina, R., Filella, X., Auge, J.M., Fuentes, R., Bover, I., Rifa, J., Moreno, V., Canals, E., Vinolas, N., Marquez, A., Barreiro, E., Borras, J. and Viladiu, P. (2003) Tumor markers (CEA, CA 125, CYFRA 21-1, SCC, and NSE) in patients with non-small cell lung cancer as an aid in histological diagnosis, and prognosis. Comparison with the main clinical, and pathological prognostic factors. *Tumour Biol.* **24**, 209-218.
49. Ardizzoni, A., M.A., Cafferata, M., Tiseo, R., Filiberti, P., Marroni, F. Grossi, and M. Paganuzzi (2006) Decline in serum carcinoembryonic antigen, and cytokeratin 19 fragment during chemotherapy predicts objective response, and survival in patients with advanced nonsmall cell lung cancer. *Cancer* **107**, 2842-2849.
50. Holdenrieder, S., Stieber, P., von Pawel, J., Raith, H., Nagel, D., Feldmann, K. and Seidel, D. (2004) Circulating nucleosomes predict the response to chemotherapy in patients with advanced non-small cell lung cancer. *Clin. Cancer Res.* **10**, 5981-5987.
51. Hampton, R., Walker, M., Marshall, J. and Juhl, H. (2002) Differential expression of carcinoembryonic antigen (CEA) splice variants in whole blood of colon cancer patients, and healthy volunteers: implication for the detection of circulating colon cancer cells. *Oncogene* **21**, 7817-7823.
52. Trauner, M., Grygar, S., Stauber, R.E., Brodatsch-Hausler, E. and Klimpfinger, M. (1994) Carcinoembryonic antigen, cytokeratin expression, and mucin composition in hyperplastic, and neoplastic polyps of the colorectum. *Z. Gastroenterol.* **32**, 626-631.
53. Buccheri, G., Torchio, P. and Ferrigno, D. (2003) Clinical equivalence of two cytokeratin markers in mon-small cell lung cancer: a study of tissue polypeptide antigen, and cytokeratin 19 fragments. *Chest.* **124**, 622-632.
54. Schneider, J., Philipp, M., Velcovsky, H.G., Morr, H. and Katz, N. (2003) Pro-gastrin-releasing peptide (ProGRP), neuron specific enolase (NSE), carcinoembryonic antigen (CEA), and cytokeratin 19-fragments (CYFRA 21-1) in patients with lung cancer in comparison to other lung diseases. *Anticancer Res.* **23**, 885-893.
55. Barak, V., Goike, H., Panaretakis, K.W. and Einarsson, R. (2004) Clinical utility of cytokeratins as tumor markers. *Clin. Biochem.* **37**, 529-540.
56. Lamy, P., Grenier, J., Kramar, A. and Pujol, J.L. (2000) Pro-gastrin-releasing peptide, neuron specific enolase, and chromogranin A as serum markers of small cell lung cancer. *Lung Cancer* **29**, 197-203.
57. Greenberg, A.K. and Lee, M.S. (2007) Biomarkers for lung cancer: clinical uses. *Curr. Opin. Pulm. Med.* **13**, 249-255.
58. Dziadziuszko, R., Witta, S.E., Cappuzzo, F., Park, S., Tanaka, K., Danenberg, P.V., Baron, A.E., Crino, L., Franklin, W.A., Bunn, P.A., Jr., Varella-Garcia, M., Danenberg, K.D. and Hirsch, F.R. (2006) Epidermal growth factor receptor messenger RNA expression, gene dosage, and gefitinib sensitivity in non-small cell lung cancer. *Clin. Cancer Res.* **12**, 3078-3084.
59. Nishio, K., Arao, T., Shimoyama, T., Fujiwara, Y., Tamura, T. and Saijo, N. (2005) Translational studies for target-based drugs. *Cancer Chemother. Pharmacol.* **56 Suppl 1**, 90-93.
60. Barlesi, F., Gimenez, C., Torre, J.P., Doddoli, C., Mancini, J., Greillier, L., Roux, F. and Kleisbauer, J.P. (2004) Prognostic value of combination of Cyfra 21-1, CEA, and NSE in patients with advanced non-small cell lung cancer. *Respir. Med.* **98**, 357-362.
61. Muley, T., Dienemann, H. and Ebert, W. (2003) Increased CYFRA 21-1, and CEA levels are negative predictors of outcome in p-stage I NSCLC. *Anticancer Res.* **23**, 4085-4093.
62. Muley, T., Dienemann, H. and Ebert, W. (2004) CYFRA 21-1, and CEA are independent prognostic factors in 153 operated stage I NSCLC patients. *Anticancer Res.* **24**, 1953-1956.
63. Okada, M., Nishio, W., Sakamoto, T., Uchino, K., Yuki, T., Nakagawa, A. and Tsubota, N. (2004) Prognostic significance of perioperative serum carcinoembryonic antigen in non-small cell lung cancer: analysis of 1,000 consecutive resections for clinical stage I disease. *Ann. Thorac. Surg.* **78**, 216-221.
64. Okamoto, T., Nakamura, T., Ikeda, J., Maruyama, R., Shoji, F., Miyake, T., Wataya, H. and Ichinose, Y. (2005) Serum carcinoembryonic antigen as a predictive marker for sensitivity to gefitinib in advanced non-small cell lung cancer. *Eur. J. Cancer* **41**, 1286-1290.
65. Salgia, R., Harpole, D., Herndon, J.E., 2nd, Pisick, E., Elias, A. and Skarin, A.T. (2001) Role of serum tumor markers CA 125, and CEA in non-small cell lung cancer. *Anticancer Res.* **21**, 1241-1246.
66. Lee, J.H. and Chang, J.H. (2005) Diagnostic utility of serum, and pleural fluid carcinoembryonic antigen, neuron-specific enolase, and cytokeratin 19 fragments in patients with effusions from primary lung cancer. *Chest.* **128**, 2298-2303.
67. Pollan, M., Varela, G., Torres, A., de la Torre, M., Ludena, M.D., Ortega, M.D., Pac, J., Freixenet, J., Gomez, G., Sebastian, F., Diez, M., Arrabal, R., Canalis, E., Garcia-Tirado, J., Arnedillo, A., Rivas, J.J., Minguella, J., Gomez, A., Garcia, M., Aragones, N., Perez-Gomez, B., Lopez-Abente, G., Gonzalez-Sarmiento, R. and Rojas, J.M. (2003) Clinical value of p53, c-erbB-2, CEA, and CA125 regarding relapse, metastasis, and death in resectable non-small cell lung cancer. *Int. J. Cancer* **107**, 781-790.
68. Sakao, Y., Nakazono, T., Sakuragi, T., Natsuaki, M. and Itoh, T. (2004) Predictive factors for survival in surgically resected clinical IA peripheral adenocarcinoma of the lung. *Ann. Thorac. Surg.* **77**, 1157-1161; discussion 1161-1152.
69. Sun, S.S., Hsieh, J.F., Tsai, S.C., Ho, Y.J. and Kao, C.H. (2000) Tissue polypeptide-specific antigen, and carcinoembryonic antigen for early prediction of recurrence in lung adenocarcinoma. *Am. J. Clin. Oncol.* **23**, 605-608.
70. Tomita, M., Matsuzaki, Y., Edagawa, M., Shimizu, T.,

Hara, M. and Onitsuka, T. (2004) Prognostic significance of preoperative serum carcinoembryonic antigen level in lung adenocarcinoma but not squamous cell carcinoma. *Ann. Thorac. Cardiovasc. Surg.* **10**, 76-80.
71. Zhou, B.B. and Bartek, J. (2004) Targeting the checkpoint kinases: chemosensitization versus chemoprotection. *Nat. Rev. Cancer* **4**, 216-225.
72. Kulpa, J., Wojcik, E., Reinfuss, M. and Kolodziejski, L. (2002) Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. *Clin. Chem.* **48**, 1931-1937.
73. Barlesi, F., Tchouhadjian, C., Doddoli, C., Torre, J.P., Astoul, P. and Kleisbauer, J.P. (2005) CYFRA 21-1 level predicts survival in non-small-cell lung cancer patients receiving gefitinib as third-line therapy. *Br. J. Cancer* **92**, 13-14.
74. Hatzakis, K.D., Froudarakis, M.E., Bouros, D., Tzanakis, N., Karkavitsas, N. and Siafakas, N.M. (2002) Prognostic value of serum tumor markers in patients with lung cancer. *Respiration* **69**, 25-29.
75. Kashiwabara, K., Nakamura, H. and Esaki, T. (2000) Prognosis in bronchogenic squamous cell carcinoma groups divided according to serum squamous cell carcinoma-related antigen, and cytokeratin 19 fragment levels. *Clin. Chim. Acta.* **294**, 105-113.
76. Merle, P., Janicot, H., Filaire, M., Roux, D., Bailly, C., Vincent, C., Gachon, F., Tchirkov, A., Kwiatkowski, F., Naame, A., Escande, G., Caillaud, D. and Verrelle, P. (2004) Early CYFRA 21-1 variation predicts tumor response to chemotherapy, and survival in locally advanced non-small cell lung cancer patients. *Int. J. Biol. Markers* **19**, 310-315.
77. Pujol, J.L., Boher, J.M., Grenier, J. and Quantin, X. (2001) Cyfra 21-1, neuron specific enolase, and prognosis of non-small cell lung cancer: prospective study in 621 patients. *Lung Cancer* **31**, 221-231.
78. Pujol, J.L., Molinier, O., Ebert, W., JDaures, .P., Barlesi, F., Buccheri, G., Paesmans, M., Quoix, E., Moro-Sibilot, D., Szturmowicz, M., Brechot, J.M., Muley, T. and Grenier, J. (2004) CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. *Br. J. Cancer* **90**, 2097-2105.
79. Reinmuth, N., Brandt, B., Semik, M., Kunze, W.P., Achatzy, R., Scheld, H.H., Broermann, P., Berdel, W.E., Macha, H.N. and Thomas, M. (2002) Prognostic impact of Cyfra21-1, and other serum markers in completely resected non-small cell lung cancer. *Lung Cancer* **36**, 265-270.
80. Sun, S.S., Hsieh, J.F., Tsai, S.C., Ho, Y.J., Lee, J.K. and Kao, C.H. (2000) Cytokeratin fragment 19, and squamous cell carcinoma antigen for early prediction of recurrence of squamous cell lung carcinoma. *Am. J. Clin. Oncol.* **23**, 241-243.
81. Vollmer, R.T., Govindan, R., Graziano, S.L., Gamble, G., Garst, J., Kelley, M.J. and Christenson, R.H. (2003) Serum CYFRA 21-1 in advanced stage non-small cell lung cancer: an early measure of response. *Clin. Cancer Res.* **9**, 1728-1733.
82. Ando, S., Kimura, H., Iwai, N., Yamamoto, N. and Iida, T. (2003) Positive reactions for both Cyfra21-1, and CA125 indicate worst prognosis in non-small cell lung cancer. *Anticancer Res.* **23**, 2869-2874.
83. Pujol, J.L., Quantin, X., Jacot, W., Boher, J.M., Grenier, J. and Lamy, P.J. (2003) Neuroendocrine, and cytokeratin serum markers as prognostic determinants of small cell lung cancer. *Lung Cancer* **39**, 131-138.
84. Molina, R., Filella, X. and Auge, J.M. (2004) ProGRP: a new biomarker for small cell lung cancer. *Clin. Biochem.* **37**, 505-511.
85. Schneider, J. (2006) Tumor markers in detection of lung cancer. *Adv. Clin. Chem.* **42**, 1-41.
86. Bonner, J.A., Sloan, J.A., Rowland, K.M., Jr., Klee, G.G., Kugler, J.W., Mailliard, J.A., Wiesenfeld, M., Krook, J.E., Maksymiuk, A.W., Shaw, E.G., Marks, R.S. and Perez, E.A. (2000) Significance of neuron-specific enolase levels before, and during therapy for small cell lung cancer. *Clin. Cancer Res.* **6**, 597-601.
87. Satoh, H., Ishikawa, H., Kurishima, K., Yamashita, Y.T., Ohtsuka, M. and Sekizawa, K. (2002) Cut-off levels of NSE to differentiate SCLC from NSCLC. *Oncol. Rep.* **9**, 581-583.
88. Schneider, J., Philipp, M., Salewski, L. and Velcovsky, H.G. (2003) Pro-gastrin-releasing peptide, (ProGRP), and neuron specific enolase, (NSE) in therapy control of patients with small-cell lung cancer. *Clin. Lab.* **49**, 35-42.
89. Shibayama, T., Ueoka, H., Nishii, K., Kiura, K., Tabata, M., Miyatake, K., Kitajima, T. and Harada, M. (2001) Complementary roles of pro-gastrin-releasing peptide, (ProGRP), and neuron specific enolase, (NSE) in diagnosis, and prognosis of small-cell lung cancer, (SCLC). *Lung Cancer* **32**, 61-69.
90. Massacesi, C., Rocchi, M.B., Marcucci, F., Pilone, A., Galeazzi, M. and Bonsignori, M. (2003) Serum tumor markers may precede instrumental response to chemotherapy in patients with metastatic cancer. *Int. J. Biol. Markers* **18**, 295-300.
91. Ferrigno, D., Buccheri, G. and Giordano, C. (2003) Neuron-specific enolase is an effective tumour marker in non-small cell lung cancer, (NSCLC). *Lung Cancer* **41**, 311-320.
92. Ando, S., Suzuki, M., Yamamoto, N., Iida, T. and Kimura, H. (2004) The prognostic value of both neuron-specific enolase, (NSE), and Cyfra21-1 in small cell lung cancer. *Anticancer Res.* **24**, 1941-1946.
93. Bremnes, R.M., Sundstrom, S., Aasebo, U., Kaasa, S., Hatlevoll, R. and Aamdal, S. (2003) The value of prognostic factors in small cell lung cancer: results from a randomised multicenter study with minimum 5 year follow-up. *Lung Cancer* **39**, 303-313.
94. Maeda, T., Ueoka, H., Tabata, M., Kiura, K., Shibayama, T., Gemba, K., Takigawa, N., Hiraki, A., Katayama, H. and Harada, M. (2000) Prognostic factors in advanced non-small cell lung cancer: elevated serum levels of neuron specific enolase indicate poor prognosis. *Jpn. J. Clin. Oncol.* **30**, 534-541.
95. Cho, W.C., Yip, T.T., Yip, C., Yip, V., Thulasiraman, V., Ngan, R.K., Yip, T.T., Lau, W.H., Au, J.S., Law, S.C., Cheng, W.W., Ma, V.W. and Lim, C.K. (2004) Identification of serum amyloid a protein as a potentially useful biomarker to monitor relapse of nasopharyngeal cancer by serum proteomic profiling. *Clin. Cancer Res.* **10**, 43-52.
96. Maciel, C.M., Junqueira, M., Paschoal, M.E., Kawamura, M.T., Duarte, R.L., Carvalho Mda, G. and Domont, G.B. (2005) Differential proteomic serum pattern of low molecular weight proteins expressed by adenocarcinoma lung cancer patients. *J. Exp. Ther. Oncol.* **5**, 31-38.
97. Huang, L.J.,Chen, S.X., Huang, Y., Luo, W.J., Jiang, H.H.,

Hu, Q.H., Zhang, P.F. and Yi, H. (2006) Proteomics-based identification of secreted protein dihydrodiol dehydrogenase as a novel serum markers of non-small cell lung cancer. *Lung Cancer* **54**, 87-94.
98. Heo, S.H., Lee, S.J., Ryoo, H.M., Park, J.Y. and Cho, J.Y. (2007) Identification of putative serum glycoprotein biomarkers for human lung adenocarcinoma by multilectin affinity chromatography, and LC-MS/MS. *Proteomics* **7**, 4292-4302.
99. Liotta, L.A. and Petricoin, E.F. (2006) Serum peptidome for cancer detection: spinning biologic trash into diagnostic gold. *J. Clin. Invest.* **116**, 26-30.
100. Villanueva, J., Shaffer, D.R., Philip, J., Chaparro, C.A., Erdjument-Bromage, H., Olshen, A.B., Fleisher, M., Lilja, H., Brogi, E., Boyd, J., Sanchez-Carbayo, M., Holland, E.C., Cordon-Cardo, C., Scher, H.I. and Tempst, P. (2006) Differential exoprotease activities confer tumor-specific serum peptidome patterns. *J. Clin. Invest.* **116**, 271-284.
101. Biran, H., Friedman, N., Neumann, L., Pras, M. and Shainkin-Kestenbaum, R. (1986) Serum amyloid A, (SAA) variations in patients with cancer: correlation with disease activity, stage, primary site, and prognosis. *J. Clin. Pathol.* **39**, 794-797.
102. Anderson, N.L. and anderson, N.G. (1998) Proteome, and proteomics: new technologies, new concepts, and new words., *Electrophoresis* **19**, 1853-1861.
103. de Hoog, C.L. and Mann, M. (2004) Proteomics. *Annu. Rev. Genomics. Hum. Genet.* **5**, 267-293.
104. Pandey, A. and Mann, M. (2000) Proteomics to study genes, and genomes. *Nature* **405**, 837-846.
105. Conrad, D.H., Goyette, J. and Thomas, P.S. (2008) Proteomics as a method for early detection of cancer: a review of proteomics, exhaled breath condensate, and lung cancer screening. *J. Gen. Intern. Med.* **23 Suppl 1**, 78-84.
106. Alessandro, R., Fontana, S., Kohn, E. and De Leo, G. (2005) Proteomic strategies, and their application in cancer research. *Tumori.* **91**, 447-455.
107. Anderson, N.L. and anderson, N.G. (2002) The human plasma proteome: history, character, and diagnostic prospects. *Mol. Cell Proteomics* **1**, 845-867.
108. Hanash, S.M., Pitteri, S.J. and Faca, V.M. (2008) Mining the plasma proteome for cancer biomarkers. *Nature* **452**, 571-579.
109. Anderson, L. and Hunter, C.L. (2006) Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins. *Mol. Cell Proteomics* **5**, 573-588.
110. Janecki, D.J., Bemis, K.G., Tegeler, T.J., Sanghani, P.C., Zhai, L., Hurley, T.D., Bosron, W.F. and Wang, M. (2007) A multiple reaction monitoring method for absolute quantification of the human liver alcohol dehydrogenase ADH1C1 isoenzyme. *Anal. Biochem.* **369**, 18-26.
111. Anderson, N.L. anderson, N.G. Haines, L.R., Hardie, D.B., Olafson, R.W. and Pearson, T.W. (2004) Mass spectrometric quantitation of peptides, and proteins using Stable Isotope Standards, and Capture by Anti-Peptide Antibodies, (SISCAPA). *J. Proteome. Res.* **3**, 235-244.
112. Keshishian, H., Addona, T., Burgess, M., Kuhn, E. and Carr, S.A. (2007) Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry, and stable isotope dilution. *Mol. Cell Proteomics* **6**, 2212-2229.