Current Proteomic Analysis and Post-translational Modifications of Biomarkers in Human Lung Cancer Materials

Supachok Sinchaikul[1], PhD; Piyorot Hongsachart[1,2], MSc; Supawadee Sriyam[1,2], MSc; Payungsak Tantipaiboonwong[3], PhD; Suree Phutrakul[2], PhD; Shui-Tein Chen[1,4], PhD

Lung cancer is the leading cause of cancer deaths worldwide and is often diagnosed in the advanced or late stages. The discovery of candidate biomarkers in human lung cancer materials holds clinical potential as well as a significant challenge. Due to a large number of proteins in human materials that form the protein complexity and post-translational modifications (PTMs) of proteins, proteomic analysis of lung cancer biomarkers remains a difficult task. Many proteomic approaches have been used to investigate the biomarkers in human lung cancer materials. In this review, we summarize the current proteomic approaches employed for analysis of proteome profiles in human lung cancer materials and some PTMs causing different proteome profiles and expression levels of biomarkers in lung cancer. In addition, bioinformatic tools for PTMs are useful for prediction of glycosylation and phosphorylation sites in proteins, which can help us further understand the correlation of PTMs and tumor development. However, it is necessary that proteome analysis of lung cancer materials be incorporated with new strategies and technologies to improve the efficiency of the analysis and to investigate novel biomarkers that would be very useful for further diagnostic and therapeutic applications. *(Chang Gung Med J 2008;31:417-30)*

Prof. Shui-Tein Chen

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Lung cancer in Taiwan

The increasing number of lung cancer cases worldwide is a serious problem. Nearly 60% of people die within one year of diagnosis, and nearly 75% die within two years. Recently, the American Cancer Society estimated that in the US, lung cancer causes about 31% of all cancer deaths in men and about 26% in women and is responsible for 15% cases of all new cases of cancer, (ranked 2nd).[(1)] In Taiwan, lung cancer has been the most common cause of cancer deaths during past decade. The number of deaths has risen to about 7,479, making it the leading cause of cancer deaths in Taiwan. In 2006, 19.7% of cancer deaths in Taiwan were caused by lung cancer (Fig. 1A).[(2)] The numbers of lung cancer deaths in men and women were 5,149 (21.1% of

From the [1]Institute of Biological Chemistry and Genomics Research Center, Academia Sinica, Taipei, Taiwan; [2]Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [3]School of Cosmetic Science, Mae Fah Luang University, Chiang Rai, Thailand; [4]Institute of Biochemical Sciences, College of Life Science, National Taiwan University, Taipei, Taiwan.

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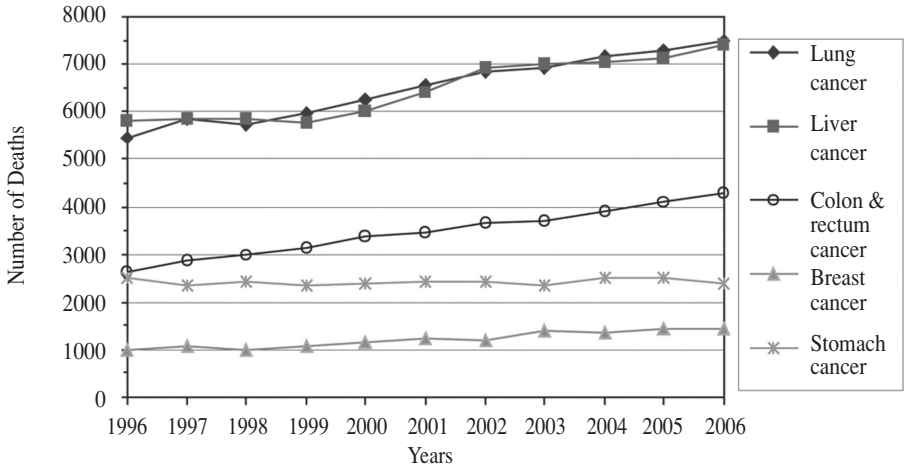
Correspondence to: Prof. Shui-Tein Chen, Institute of Biological Chemistry and Genomics Research Center, Academia Sinica. No. 128 Academia Rd., Sec. II, Nankang, Taipei, 115, Taiwan (R.O.C) Tel.: 886-2-27855696 ext. 7071; Fax: 886-2-27883473; E-mail: bcchen@gate.sinica.edu.tw

deaths, ranked 2nd) and 2,330 (17.2% of deaths, ranked 1st), respectively (Fig. 1B). In addition, the number of adenocarcinoma (AD) deaths was higher than deaths from squamous cell carcinoma (SCC), small cell lung carcinoma (SCLC) and large cell carcinoma (LCC).[3] Most diagnoses of lung cancer are in patients over 55 years old. However, the majority of lung tumors have reached locally advanced stage III or metastatic stage IV by the time of diagnosis. Therefore, early diagnosis of lung cancer is necessary to improve patient survival.

Proteomic analysis of lung cancer materials

Proteomic approaches are important and very useful for studying the human proteome, including differential protein expression, post-translational modifications (PTMs), protein-protein interactions and signaling pathways. Comprehensive analysis of protein expression patterns in human serum, urine and other materials might improve the ability to unravel and understand the proteome complexity of human tumors in tumor development and progression. The current proteome analysis of biomarkers in

A



B

Cancers	No. Deaths	Deaths (%)
Total	24,428	100.0
1. Liver cancer	5,383	22.0
2. Trachea, bronchus, & lung cancer	5,149	21.1
3. Colon & rectum cancer	2,485	10.2
4. Oral cavity cancer	2,044	8.4
5. Stomach cancer	1,571	6.4
6. Oesophagus cancer	1,230	5.0
7. Prostate cancer	957	3.9
8. Pancreas cancer	749	3.1
9. Non-Hodgkin's lamphoma	733	3.0
10. Nasopharynx cancer	611	2.5
11. Others	3,516	14.4

Men

Cancers	No. Deaths	Deaths (%)
Total	13,570	100.0
1. Trachea, bronchus, & lung cancer	2,330	17.2
2. Liver cancer	2,032	15.0
3. Colon & rectum cancer	1,799	13.3
4. Breast cancer	1,439	10.6
5. Stomach cancer	827	6.1
6. Cervix uteri cancer	792	5.8
7. Pancreas cancer	498	3.7
8. Non-Hodgkin's lymphoma	478	3.5
9. Gallbladder cancer	467	3.4
10. Ovary cancer	380	2.8
11. Others	2,528	18.6

Women

Fig. 1 (A) Statistics of the top five cancers causing deaths in Taiwan from 1996-2006. (B) The top ten cancers causing deaths in both genders in Taiwan in 2006.

human lung cancer materials is summarized in Table 1. Human plasma, serum and tissues are mostly used for analysis of biomarkers, whereas urine, cell lines and pleural effusion samples are used less often. The most common proteomic approaches used for analysis of the lung cancer proteome are two-dimensional electrophoresis (2-DE), two-dimensional difference in-gel electrophoresis (2-D DIGE) and mass spectrometry (MS) using matrix assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) as ion sources. The 2-DE and 2-D DIGE methods provide different protein profiles and mass spectrometry identifies differentially expressed proteins corresponding to spots with different intensities between the sample groups. In our study, we used 2-DE to compare the proteome profiles human normal and lung cancer materials, such as serum, urine, tissue and cell lines (Fig. 2). Each paired group of human materials provided different protein profiles and also showed differentially expressed proteins between normal and lung cancer samples. Likewise, 2-DE could classify the differentially expressed proteins or unique proteins in each subtype from non-small cell lung cancer cell lines (Fig. 3). Thereafter, the differentially expressed proteins among paired groups might become useful biomarkers for further diagnostic and therapeutic applications in lung cancer. Although 2-DE is a powerful method and is used to identify the large scale of the proteome, it still has some limitations: (1) it has poor resolution of separation for less abundant proteins; (2) its detection of proteins with extreme properties (small, large, hydrophobic, and strong acidic or basic) is limited; and (3) it is time consuming and expensive.

Recently, many proteomic approaches have been attempted to increase the overall resolution of protein separation; for example, a combination of multi-dimensional liquid chromatography (LC) and

Table 1. Current Proteome Analysis of Biomarkers in Various Human Lung Cancer Materials

Study	Human materials	Proteomic analysis methods	Discovery (Significant proteins)
1. Plasma/Serum			
Fujii *et al*. (2005)[4]	Plasma (AD-2, healthy-3)	1-D and 2-D μLC-MS/MS	Using 2-D and 1-D μLC-MS/MS analyses, approximately 250 and 100 different proteins were detected, respectively, in each HSA- and IgG depleted sample. Twelve specific proteins in both groups of plasma samples were identified.
Maciel *et al*. (2005)[5]	Serum (AD-20, healthy-20)	2-DE, MALDI-TOF MS	Five up-regulated proteins (immunoglobulin lambda chain, transthyretin monomer, haptoglobin-alpha 2 and two isoforms of serum amyloid protein) and one down-regulated protein (fragment of apolipoprotein A-I) were detected in patients.
Yang *et al*. (2005)[6]	Serum (158: SCC-68, AD-53, SCLC-35, bronchioloalve-olar carcinoma-2)	SELDI-TOF MS (WCX2)	Five protein peaks at 11493, 6429, 8245, 5335 and 2538 Da were chosen as biomarker patterns in lung cancer. SELDI marker pattern showed a sensitivity of 91.4% in the detection of NSCLC, which was significantly higher than that in the detection of SCLC, with a sensitivity of 79.1% in the detection of lung cancers in stages I/II.
Okano *et al*. (2006)[7]	Plasma (AD-2, SCC-2, SCLC-1, healthy-5)	Immuno-affinity chromatography, anion-exchange chromatography, 2-D DIGE, LC-MS/MS	The expression level of 364 proteins in lung cancer was more than two-fold higher than in the healthy volunteers. Only 58 proteins, including classic plasma proteins, tissue-leakage proteins, catalase, clusterin, ficolin, gelsolin, lumican, tetranectin, triosephosphate isomerase and vitronectin, were identified.
Liu *et al*. (2007)[8]	Serum (SCC-73, AD-49, SCLC-24, healthy-40)	SELDI-TOF MS, 1-DE, ESI-MS/MS	Three peaks, at 13.78, 13.90 and 14.07 k m/z, were significantly lower in lung cancer sera compared with sera from healthy individuals. The peaks were identified as native transthyretin (TTR), which has two variants and gave 78.5% sensitivity and 77.5% specificity for lung cancer versus normal at the cut-off point of 115 μg/mL.

Table 1. (continued)

Study	Human materials	Proteomic analysis methods	Discovery (Significant proteins)
2. Tissue			
Chen *et al*. (2002)[9]	Tissues (AD-93, nonneoplastic lung tissues-10)	2-DE, MALDI-TOF MS	Antioxidant enzyme AOE372, ATP synthase subunit D (ATP5D), β1,4-galactosyltransferase, cytosolic inorganic pyrophosphatase, glucose-regulated Mr 58,000 protein, glutathione-S-transferase M4, prolyl 4-hydroxylase β subunit, triosephosphate isomerase, and ubiquitin thiolesterase (UCHL1) were identified as significantly overexpressed proteins in lung adenocarcinoma.
Chen *et al*. (2003)[10]	Tissues (AD-90, neoplastic lung tissues-10)	2-DE, MALDI-TOF MS	A total of 682 individual protein spots were quantified in 90 lung adenocarcinomas by using quantitative 2-DE analysis. Thirty-three of 46 survival-associated proteins were identified by using MS, of which 14 are enzymes, 10 represent structural proteins, and 8 include proteins with chaperone, growth factor, potential oncogenic, proteinase inhibitory, or calcium-binding properties.
Li *et al*. (2003)[11]	Tissues (SCC-15, normal bronchial epithelial tissues)	2-DE, MALDI-TOF MS	The total numbers of tumor tissues and normal tissues were 1349 ± 67 spots and 1297 ± 73 spots, respectively. Forty differential proteins were characterized and some were involved in the regulation of the cell cycle and signal transduction.
Li *et al*. (2004)[12]	Tissues (AD-3, SCC-3, LCC-3, Basaloid carcinoma-4, normal tissue)	2-DE, MALDI-TOF MS	Twenty five proteins in 14 cases of lung cancer were differentially expressed compared to matched nontumorous lung tissues. Eleven proteins were down-regulated and fourteen were up-regulated. Alloalbumin venezia, Se-binding protein 1, carbonic anhydrase, heat shock 2-kD-like protein and SM22α protein were down-regulated in all 14 cases of lung cancer, whereas alpha enolase was up-regulated.
Li *et al*. (2006)[13]	Tissues (SCC-20, normal bronchial epithelial tissues)	2-DE, MADLDI-TOF MS	Seventy six differential protein spots with at least a five-fold discrepancy were detected and 68 proteins were identified by PMF. Fourteen proteins in patients' autologous sera were identified and six proteins, alpha enolase, pre-B cell-enhancing factor precursor, triosephosphate isomerase, phosphoglycerate mutase 1, fructose-bisphosphate aldolase A and guanine nucleotide-binding protein beta subunit-like protein, were up-regulated in SCC.
3. Cell line			
Seike *et al*. (2005)[14]	Cell lines (AD-10, SCC-10, SCLC-10)	2-D DIGE, MALDI-TOF MS	Thirty two protein spots were identified and categorized into three histological groups of SCC, AD and a group of carcinomas with other histological types.
Xiao *et al*. (2005)[15]	Cell lines (AD-2, SCC-3, SCLC-1, ASC-2, normal bronchus cells)	1-DE, nano-ESI-MS/MS	Two hundred and ninety nine proteins were identified and shown to be involved in key cellular processes such as cell growth, organogenesis and signal transduction. Eleven proteins were detected in plasma, matrix metalloproteinase 1 levels were elevated and, fascin, ezrin, annexin A4, 14-3-3σ, 14-3-3β, 14-3-3η proteins were detected in 628 blood plasma samples using ELISA.
Tian *et al*. (2007)[16]	Cell lines (AD± metastatic cells; CL1-5 & CL1-0 cells)	2-DE, MALDI-TOF MS & MS/MS	Thirty three differentially expressed proteins were identified unambiguously, among which 16 proteins were significantly up-regulated and 17 proteins were down-regulated in highly metastatic CL1-5 cells.

Table 1. (continued)

Study	Human materials	Proteomic analysis methods	Discovery (Significant proteins)
4. Urine			
Tantipaiboonwong *et al*. (2005)[17]	Urine (AD-5, healthy-10)	2-DE, MALDI-TOF MS & MS/MS	After comparing sample preparation methods, the sequential sample preparation of urinary proteins by gel filtration and ultrafiltration was most suitable and retained most urinary proteins. Seven differentially expressed urinary proteins in lung cancer, such as CD59 glycoprotein, activator of cAMP-responsive element modulator, transthyretin, plasma retinol-binding protein, GM2-activator protein (GM2AP), Ig lambda light chain and Ig kappa chain C region, were identified.
5. Pleural effusion			
Tyan *et al*. (2005)[18]	Pleural effusion (AD-43)	2-DE, nano-HPLC-ESI-MS/MS	Altogether 1415 unique proteins from human pleural effusion were identified. Among these 124 proteins identified with higher confidence levels, some have not been reported in plasma and may represent proteins specifically in pleural effusion samples.

Abbreviations: AD: adenocarcinoma; ASC: adeno-squamous carcinoma; LCC: large cell carcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung carcinoma; HPLC: high performance liquid chromatography; 1-DE: one-dimentional electrophoresis; 2-DE: two-dimentional electrophoresis; 2-D DIGE: two-dimentional difference in-gel electrophoresis; ESI: electrospray ionization; LC: liquid chromatography; MS: mass spectrometry; MALDI-TOF MS: matrix assisted laser desorption/ionization time-of-flight mass spectrometry; SELDI-TOF MS: surface-enhanced laser desorption/ionization time-of-flight mass spectrometry.

2-DE or mass spectrometry (LC-MS) has been applied to plasma proteomics in lung cancer.[4,7] The liquid phase separation of capillary electrophoresis (CE) has also become a new approach in proteome analysis. The improved techniques of capillary isoelectric focusing (CIEF) using a monolithic- immobilized pH gradient (IPG) and the on-line combination of CIEF and capillary non-gel sieving electrophoresis (CNGSE) using a hollow-fiber membrane interface are attributed to high efficiency of the protein separation method and have been used to separate the protein mixtures excreted from lung cancer cells.[19,20] In addition, two-dimensional capillary electrophoresis (2-D CE), in which a sample undergoes separation in the first dimension capillary by sieving electrophoresis and fractions and then is periodically transferred across an interface into a second dimension capillary, has been used to separate the protein mixture of the A548 lung cancer cell line.[21] Although the combination of CE with other approaches plays an important role in proteome analysis, further development is necessary to increase the efficiency of protein separation. On the other hand, protein chip technology coupled with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is also an innovative technique, which is very fast, needs very small amounts of a sample, and can analyze the complex biological mixtures directly. It has been used to analyze the proteome of lung cancer materials.[6,8,22] The immobilizing specific monoclonal antibodies or phage-expressed proteins on the chips, known as protein arrays, allow for quantitation of distinct proteins from different lung cancer samples. For example, it has been used to detect the antibody profiles of human lung cancer materials, such as plasma samples, from 40 non-small cell lung carcinoma (NSCLC) patients (AD, SCC and unknown type), tissue samples from 12 SCC patients, and serum samples from 24 lung cancer patients.[23-25] Array analysis, which can define up- and down-regulated proteins in lung cancer samples with high accuracy, is a promising approach for further diagnosis of lung cancer.

Post-translational modifications (PTMs)

PTMs are covalent processing events that change the properties of a protein by proteolytic cleavage or by addition of a modifying group to one or more amino acids. PTMs of proteins, including glycosylation, phosphorylation, acetylation, ubiquitination, farensylation, methylation, sialylation, oxidation, prolyl isomerization and hydroxylation, may occur at different stages of tumor progression and

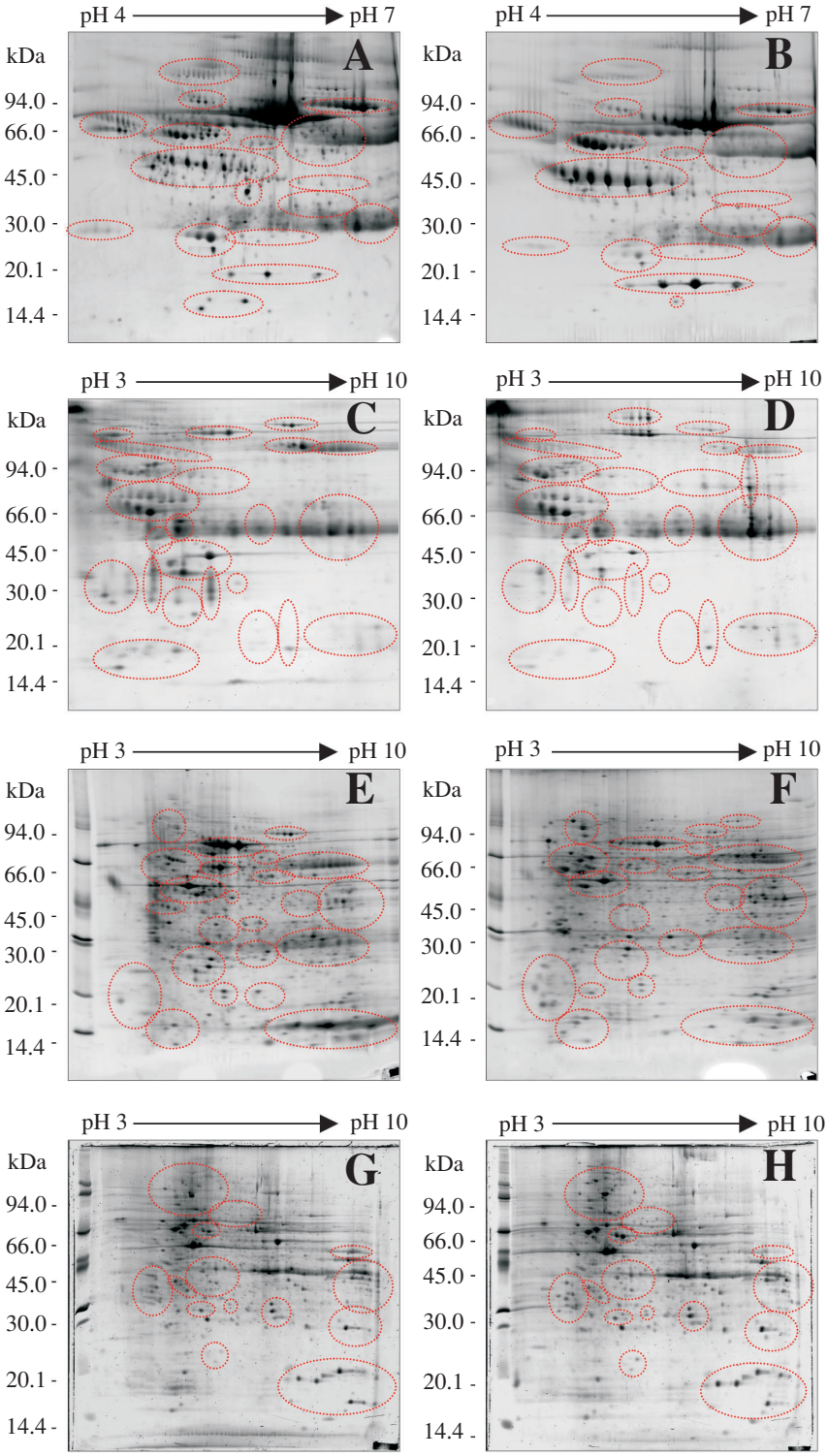


Fig. 2 Differential proteome profiles of human normal (A, C, E) and lung cancer (B, D, F, G, H) materials (serum - A, B; urine - C, D; tissue - E, F; cell lines - G, H). Only the protein profiles of lung cancer cell lines were compared between mock-non-metastatic cells and FuT4-metastatic cells. Circles represent different protein profiles among groups.

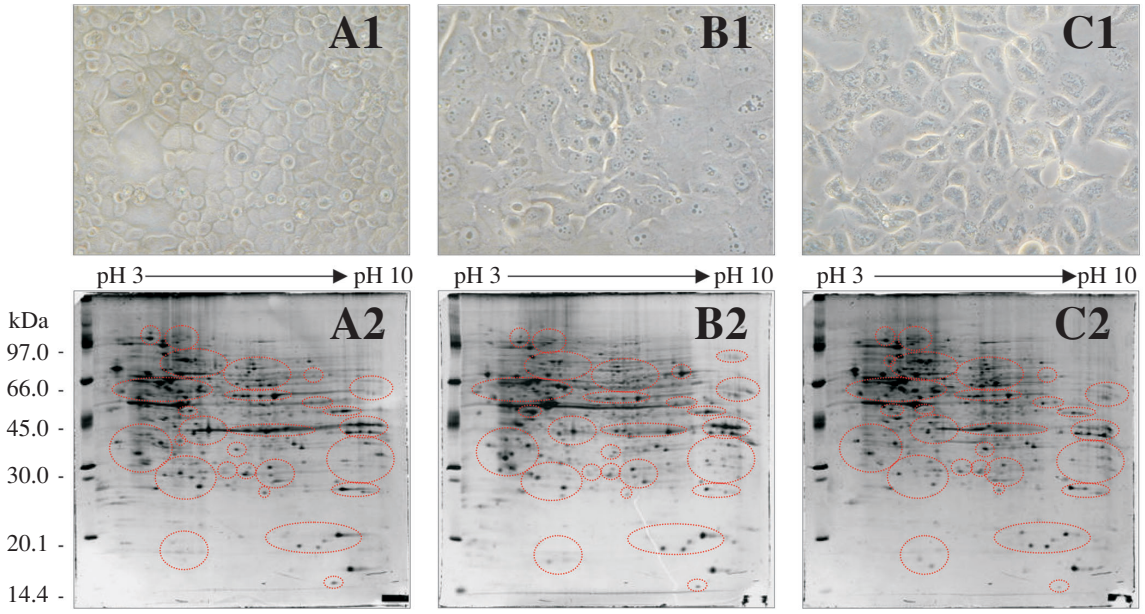


Fig. 3 Cell morphology and proteome profiles of human non-small cell lung cancer cell lines (A, adenocarcinoma - NCI-H838; B, squamous cell carcinoma - SW900; C, large cell carcinoma - NCI-H661). Circles represent different protein profiles among groups.

development of metastases.[26,27] Most PTM studies investigate the modification sites in a protein molecule and define the structure of the modifications at each specific attachment site. Thus, PTM analysis approaches are very important for studying PTMs. In lung cancer, several protein markers were not only identified in human materials, but some specific PTMs were also investigated using proteomic approaches.[7,10,18,28] Current studies of PTMs including detection and identification of biomarkers in lung cancer are summarized in Table 2. In addition, it is well known that glycosylation and phosphorylation are two of the most biologically relevant and ubiquitous PTMs of proteins and have been reported as the key processes in tumor progression in many cancers including lung cancer. Therefore, this review highlights the major aspects of glycosylation and phosphorylation.

Glycosylation, the process of addition of saccharides to proteins or lipids, is one of the most common PTMs in current cancer research. N- and O-linked oligosaccharide variants on glycoproteins (glycoforms) play fundamental roles during molecular and cellular recognition in development, growth and cellular communication, and can lead to alterations in cancer progression and immune responses.[44,45] The study of glycosylation of proteins is defined as glycoproteomics while the identification of glycan molecules is defined as glycomics. Comparative studies of the specific carbohydrate chains of glycoproteins can provide useful information for the diagnosis, prognosis and immunotherapy of tumors.[46] Therefore, the ability to efficiently and comprehensively profile glycoproteins in biological samples of interest is critical to many biological and clinical researchers. In general, glycoproteomic analysis is divided to three major steps, the enrichment of glycoproteins, the separation of glycoproteins and the identification of glycoprotein and glycan structures. The enrichment of glycoproteins from biological samples is useful for isolation of glycoproteins from highly abundant proteins in sample mixtures. Lectins, which are carbohydrate-binding proteins or glycoproteins and highly specific for their sugar moieties, are mostly used in the enrichment of glycoproteins prior to glycoprotein separation. Lectins are not only used to screen carbohydrate specificity by the lectin staining method, but they have also been used to isolate glycoproteins in human materials, depending on the carbohydrate specificity.[47,48] The separation of glycoproteins is usually the same as protein separation methods, such one-dimensional electrophoresis (1-DE), 2-DE and 2-D DIGE sometimes coupled with different glyco-

Table 2. Studies of PTMs in Human Lung Cancer Materials

PTM study	Human materials	Method	Discovery
1. Glycosylation			
Satoh *et al*. (1997)[29]	Serum (AD-198, SCC-140, LCC-22, others-11)	Radioimmunoassay (RIA) kit	In patients with AD or other NSCLC subtypes, there was a correlation between serum sialyl Lewis X-i antigen and stage of disease. Levels of the marker varied significantly depending on the number of metastatic organs in AD and in other NSCLC subtypes. Survival of NSCLC patients with high sialyl Lewis X-i antigen levels was significantly poorer than that of patients with low antigen levels.
Otake *et al*. (2001)[30]	Serum (AD-5, SCC-5, LCC-4, healthy-12)	Pyridylaminated (PA) labeling, size fractionation HPLC and reversed-phase HPLC	Thirty different structures of oligosaccharides were deduced, and these accounted for 84.1% of the total N-linked oligosaccharides present in human sera. The amount of a triantennary trigalactosylated structure with one outer arm fucosylation (A3G3Fo) was found to be markedly increased in NSCLC patients.
Laack *et al*. (2002)[31]	Tissues (AD-39)	Lectin histochemistry	Binding of *Helix pomatia* agglutinin (HPA), *Phaseolus vulgaris* leukoagglutinin and *Ulex europaeus* agglutinin to AD tissues were prognostic indicators for overall and relapse-free survival, whereas *Maackia amurenis* agglutinin and *Sambucus nigra* agglutinin binding had no prognostic value.
Lopez-Ferrer *et al* (2002)[32]	Tissues (AD-21, SCC-21)	Immunohistochemical and Western blot assays	Core and terminal structures were detected more frequently in AD than in SCC, except Lewis y, which was expressed strongly in both types of NSCLC. In AD and SCC, different sets of glycosyltransferases must be expressed and different PTMs of the mucin genes can take place in these 2 tumor types.
Geng *et al*. (2004)[33]	Cell lines (95C-lowly metastases; 95D-highly metastases)	RNAi of α-1,6 FucT	α-1,6 FucT could regulate E-cadherin mediated cell adhesion and thus plays an important role in cancer development and progression. Core fucosylated E-cadherin could serve as a promising prognostic indicator for lung cancer patients.
Gokmen *et al*. (2004)[34]	Serum (AD-38, SCC-64, SCLC-57)	Thiobarbituric acid method	Serum total sialic acid is elevated in lung cancer patients with different histological types with and without metastasis. Serum total sialic acid in lung cancer patients with metastasis is significantly higher than those with no metastasis.
Kossowska *et al*. (2005)[35]	Serum (AD-4, SCC-5, LCC-1, NSCLC unknown-8, SCLC-11)	1-DE and *Aleuria aurantia* lectin staining	Fucosylated oligosaccharides were observed in higher amounts in cancer sera than healthy sera. Glycoproteins of a molecular mass of 29 kDa appear to carry more fucose residues than that the 42-kDa band, comprising α_1-acid glycoprotein and haptoglobin. Glycans of the 26-kDa band were fucosylated to a higher extent in NSCLC vs SCLC.
Ueda *at al*. (2007)[36]	Serum (AD-5, healthy-5)	Sequential purification (immunodepletion of 6 high-abundance proteins and lectin chromatography)	The candidate biomarkers of 34 serum glycoproteins were identified and revealed a significant difference in α1,6-fucosylation level between lung cancer and healthy controls, clearly demonstrating that carbohydrate-focused proteomics could allow for detection of serum components with cancer-specific features

Table 2. (continued)

PTM study	Human materials	Method	Discovery
2. Phosphorylation			
Gharib *et al*. (2003)[37]	Tissue (AD-93)	2-DE, MALDI-TOF MS, ESI MS/MS	Fourteen of 21 isoforms of cytokeratins (CK) 7, 8, 18 and 19 occurred at significantly higher levels in tumors compared to uninvolved adjacent tissues. Specific isoforms of the four types of CK identified correlated with either clinical outcome or individual clinical parameters.
Chen *et al*. (2003)[38]	Tissue (AD-93, uninvolved lung tissues-10)	2-DE, Q-TOF MS & MS/MS	Three phosphorylated forms and one unphosphorylated form of oncoprotein 18 (Op18) were identified and found to be overexpressed in lung AD as compared with normal lung. Both phosphorylated and unphosphorylated Op18 proteins were significantly increased in poorly differentiated tumors.
Liu *et al*. (2004)[39]	Cell lines (NSCLC; H1299, H23, H226)	2-DE, MALDI-TOF MS	The expression of M-type pyruvate kinase with two isoforms (M1 and M2) was altered in NSCLC likely due to changes in translational control and/or differential phosphorylation of the protein.
Flores-Delgado *et al*. (2007)[40]	Cell line (NSCLC; A549)	Protein phosphatase 1 (PP1) antibody array	The antibody array identified 31 potential novel PP1-interacting proteins (PIPs) and 11 of 17 well-known PIPs included as controls. A majority of the interactions between PP1 and putative PIPs were isoform- or cell type-specific. Co-immunoprecipitation with PP1 provided 9 associated proteins of APAF-1, Bax, E-cadherin, HSP-70, Id2, p19Skp1, p53, PCNA and PTEN.
Ma *et al*. (2007)[41]	Cell line (SCLC; NCI-H69)	Phosphoantibody array, siRNA	Strong HGF induction of specific phosphorylation sites in phosphoproteins involved in c-MET/HGF signal transduction was detected. The c-MET serves as an attractive therapeutic target in SCLC, as shown through siRNA and selective prototype c-MET inhibitor.
3. Other PTMs			
Uno *et al*. (2004)[42]	Cell lines (NSCLC; A549, NCI-H1299, -H358, -H226, -H322, -H460)	Immunohistochemical analysis, LCM, SELDI-MS	Loss of expression or a defect of myristoylation of the Fus1 protein was observed in human primary lung cancer and cancer cell lines. Myristoylation is required for Fus1-mediated tumor-suppressing activity.
Beck *et al*. (2006)[43]	Cell line (SCLC; OC-NYH)	LC-MS/MS	A total of 32 acetylations, methylations and ubiquitinations were located in the human histones H2A, H2B, H3 and H4, including seven novel modifications. A relative increase in acetylated peptide from the histone variants H2A, H2B and H4 was accompanied by a relative decrease of dimethylated Lys^{57} from histone H2B.

Abbreviations: AD: adenocarcinoma; LCC: large cell carcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung carcinoma; 1-DE: one-dimentional electrophoresis; 2-DE: two-dimentional electrophoresis; ESI: electrospray ionization; LC: liquid chromatography; LCM: laser capture microdissection; MS: mass spectrometry; HPLC: high performance liquid chromatography; MALDI-TOF MS: matrix assisted laser desorption/ionization time-of-flight mass spectrometry; Q-TOF MS: quadrupole iontrap time-of-flight mass spectrometry; RNAi: RNA interference; SiRNA: small interfering RNA; SELDI-MS: surface-enhanced laser desorption/ionization mass spectrometry; APAF-1: apeptotic protease activating factor 1; HGF: hepatocyte growth factor; PCNA: proliferating cell nuclear antigen; PTEN: phosphatase and tension homolog; c-MET: mesenchymal-epithelial transition factor.

protein staining methods, such as the Pro-Q Emerald 488 glycoprotein stain,[49] lectin fluorescence stain[47] and isotope labeling.[36] Third, the identification of glycoproteins and glycan structures can be processed by various methods, such as chromatographic methods (nano-LC with hydrophilic columns, nano-LC with graphitized carbon packing, anion-exchange chromatography), electromigration approaches (capillary electrophoresis, capillary electrochromatography), capillary liquid chromatography/matrix assisted laser desorption/ionization time-of-flight mass spectrometry (LC/MALDI-TOF/TOF MS) & tandem MS (MS/MS) and chip-based approaches.[50] Although glycoproteomic analysis has been used to discover biomarkers in human lung cancer,[30-36] it is still difficult to analyze the whole glycoproteome of lung cancer materials. There is only one report describing the comparative glycoproteome profiles in human lung cancer adenocarcinoma sera by the sequential steps of (1) immunodepletion of 6 high-abundance proteins, (2) targeted enrichment of glycoproteins by lectin column chromatography, and (3) quantitative proteome analysis using $^{12}C_6$- or $^{13}C_6$-2-nitrobenzensulfenyl (NBS) stable isotope labeling followed by matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF-MS) analysis.[36] Using these approaches, the candidate biomarkers of 34 glycoproteins were identified and revealed significant differences in the α1,6-fucosylation level between lung cancer and healthy controls.

Phosphorylation, the addition of a phosphate group to a protein molecule or a small molecule, is a key regulatory mechanism of cellular signaling processes.[51] The analysis of phosphorylated proteins, or phosphoproteomics, and the characterization of phosphorylation sites under different biological conditions are some of the most challenging tasks in current proteomics research. Using existing technology, it is estimated that there are approximately 100,000 potential phosphorylation sites in the human proteome of which fewer than 2000 are currently known.[52] However, the identification of phosphoproteins remains a difficult task. Currently, several emerging experimental strategies can be employed to explore the phosphoproteome in human materials. The methods to isolate or enrich phosphorylated proteins are immunoaffinity or immunoprecipitation using a specific antibody, chromatofocusing, ion exchange chromatography and affinity chromatography, such as immobilized metal ion affinity chromatography (IMAC).[53] The separation and detection of phosphoproteins generally use 1-DE, 2-DE or 2-D DIGE incorporated with phosphoprotein staining (Pro-Q Diamond phosphoprotein gel stain) or isotope labeling (^{32}P, isotope-coded affinity tag [ICAT], or stable isotope labeling with amino acids in cell culture [SILAC]).[54-56] Analysis and identification methods of phosphoproteins and phosphopeptides are mass spectrometry-based approaches, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS) and MS/MS.[57] Although there are a few reports describing ed the study of phosphorylation in lung cancer materials,[37-41] direct investigation of the lung cancer phosphoproteome using the above approaches has never been reported. There are gaps and some difficulties with this method. However, it would be very interesting to investigate the whole phosphoproteome in each lung cancer material to understand the correlation of phosphorylation and cancer mechanisms.

Bioinformatics of post-translational modifications (PTMs)

Bioinformatics tools are becoming an important factor in proteomic analysis and are useful for prediction of PTMs, which leads to easy investigation of specific modification sites in the protein molecule and understanding the correlation between protein modification and tumor development. The Expert Protein Analysis System (ExPASy) World Wide Web server (http://www.expasy.org), one of the most popular proteomics servers, provides access to a variety of databases and analytical tools dedicated to proteins and proteomics. Useful databases for site prediction of two major PTMs, glycosylation and phosphorylation, are available on this server. For glycosylation, the NetGlyc database, which is available at http://www.cbs.dtu.dk/services/NetGlyc/, predicts N-linked glycosylation sites in human proteins using artificial neural networks that examine the sequence context of Asn-Xaa-Ser/Thr sequences. Meanwhile, the NetOGlyc database, which is available at http://www.cbs.dtu.dk/services/NetOGlyc/, predicts the mucin type N-acetylgalactosamine (GalNAc) O-linked glycosylation sites in mammalian proteins

and has correctly predicted 76% of the glycosylated residues and 93% of the nonglycosylated residues.[58] The YinOYang database, which is available at http://www.cbs.dtu.dk/services/YinOYang/, can also predict O-linked glycosylation, especially the O-β-N-acetylglucosamine (GlcNAc) attachment sites in eukaryotic protein sequences, which have been found in over half of all SwissProt human sequences, 65% of which are nuclear and cytoplasmic.[59] For phosphorylation, the NetPhos database, which is available at http://www.cbs.dtu.dk/services/NetPhos/, predicts potential phosphorylation sites at serine, threonine or tyrosine residues in eukaryotic proteins.[60] The NetPhosK server, which is available at http://www.cbs.dtu.dk/services/NetPhosK, produces neural network predictions of kinase specific eukaryotic protein phosphorylation sites. The Phospho.ELM database, which is available at http://phospho.elm.eu.org/, is an alternative resource containing 1703 phosphorylation site instances for 556 phosphorylated proteins and is used for predicting phosphorylation sites in proteins and searching the substrates of a specific kinase.[61] In addition to the PTM database from ExPASy, the dbPTM database, which is available at http://dbptm.mbc.nctu.edu.tw/, accumulates biological information related to PTM, such as catalytic sites, structural information, solvent accessibility of residues, protein secondary structures, protein domains and protein variations.[62] Version 2.0 of dbPTM integrates the experimentally validated PTM sites with referable studies from Swiss-Prot, Phospho.ELM, O-GLYCBASE, and UbiProt. These can be valuable tools for both molecular biologists working on protein PTMs and bioinformaticians developing computational predictions of the specificity of their reactions.

Conclusion

Although several proteomic approaches have been used to investigate lung cancer biomarkers in various human materials and understand tumor biology, there are few studies of the lung cancer proteome in each type of human material, which could give us different insights into the proteome and a better understanding of the protein complexity in lung cancer. The global identification of PTMs modulating the biological activity of most proteins is still a difficult task that is currently accelerating due to advances in proteomics techniques. The combination of current proteomic approaches, such as multidimensional separations, and new technologies, is becoming a new strategy and/or an alternative method for improving protein separation of complex protein mixtures and analyzing the lung cancer proteome. On the other hand, sample preparation, novel technologies and bioinformatics tools are also needed to incorporate with current approaches for intensive analysis of the lung cancer proteome. If the proteome of lung cancer in each type of human material is elucidated, it will be very useful for further study of tumor biology and applications in diagnostics and therapeutics.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43-66.
2. Hse PY, Statistics on Causes of Death, Health Statistics in Taiwan, 2006. Department of Health, Executive Yuan, Taiwan, R.O.C. 2006;www.doh.gov.tw.
3. Ko YC, Wang JL, Wu CC, Huang WT, Lin MC. Lung cancer at a medical center in southern Taiwan. Chang Gung Med J 2005;28:387-95.
4. Fujii K, Nakano T, Kanazawa M, Akimoto S, Hirano T, Kato H, Nishimura T. Clinical-scale high-throughput human plasma proteome analysis: lung adenocarcinoma. Proteomics 2005;5:1150-9.
5. Maciel CM, Junqueira M, Paschoal ME, Kawamura MT, Duarte RL, Carvalho Mda G, Domont GB. Differential proteomic serum pattern of low molecular weight proteins expressed by adenocarcinoma lung cancer patients. J Exp Ther Oncol 2005;5:31-8.
6. Yang SY, Xiao XY, Zhang WG, Zhang LJ, Zhang W, Zhou B, Chen G, He DC. Application of serum SELDI proteomic patterns in diagnosis of lung. BMC Cancer 2005;5:83.
7. Okano T, Kondo T, Kakisaka T, Fujii K, Yamada M, Kato H, Nishimura T, Gemma A, Kudoh S, Hirohashi S. Plasma proteomics of lung cancer by a linkage of multidimensional liquid chromatography and two-dimensional difference gel electrophoresis. Proteomics 2006;6;3938-48.
8. Liu L, Liu J, Dai S, Wang X, Wu S, Wang J, Huang L, Xiao X, He D. Reduced transthyretin expression in sera of lung cancer. Cancer Sci 2007;98:1617-24.
9. Chen G, Gharib TG, Huang CC, Thomas DG, Shedden KA, Taylor JM, Kardia SL, Misek DE, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, Beer DG. Proteomic analysis of lung adenocarcinoma: identification of a highly expressed set of proteins in tumors. Clin Cancer Res 2002;8:2298-305.

10. Chen G, Gharib TG, Wang H, Huang CC, Kuick R, Thomas DG, Shedden KA, Misek DE, Taylor JM, Giordano TJ, Kardia SL, Iannettoni MD, Yee J, Hogg PJ, Orringer MB, Hanash SM, Beer DG. Protein profiles associated with survival in lung adenocarcinoma. Proc Natl Acad Sci USA 2003;100:13537-42.
11. Li C, Chen Z, Xiao Z, Wu X, Zhan X, Zhang X, Li M, Li J, Feng X, L S, Chen P, Xie JY. Comparative proteomics analysis of human lung squamous carcinoma. Biochem Biophys Res Commun 2003;309:253-60.
12. Li LS, Kin H, Rhee H, Kim SH, Shih DH, Chung KY, Park KS, Paik YK, Chang J, Kim H. Proteomic analysis distinguishes basaloid carcinoma as a distinct subtype of non-small cell lung carcinoma. Proteomics 2004;4:3394-400.
13. Li C, Xiao Z, Chen Z, Zhang X, Li JL, Wu X, Li X, Yi H, Li M, Zhu G, Liang S. Proteome analysis of human lung squamous carcinoma. Proteomics 2006;6:547-58.
14. Seiko M, Kondo T, Fujii K, Okano T, Yamada T, Matsuno Y, Gemma A, Kudoh S, Hirohashi S. Proteomic signatures for histological types of lung cancer. Proteomics 2005;5:2939-48.
15. Xiao T, Ying W, Li L, Hu Z, Ma Y, Jiao L, Ma J, Cai Y, Lin D, Guo S, Han N, Di X, Li M, Zhang D, Su K, Yuan J, Zheng H, Gao M, He J, Shi S, Li W, Xu N, Zhang H, Liu Y, Zhang K, Gao Y, Qian X, Cheng S. An approach to studying lung cancer-related proteins in human blood. Mol Cell Proteomics 2005;4:1480-6.
16. Tian T, Hao J, Xu A, Hao J, Luo C, Liu C, Huang L, Xiao X, He D. Determination of metastasis-associated proteins in non-small cell lung cancer by comparative proteomic analysis. Cancer Sci 2007;98:1265-74.
17. Tantipaiboonwong P, Sinchaikul S, Sriyam S, Phutrakul S, Chen ST. Different techniques for urinary protein analysis of normal and lung cancer patients. Proteomics 2005;5:1140-9.
18. Tyan YC, Wu HY, Su WC, Liao PC. Proteomic profiling of human pleural effusion using two-dimensional nano liquid chromatography tandem mass spectrometry. J Proteome Res 2005;4:1274-86.
19. Liu H, Yang C, Yang Q, Zhang W, Zhang Y. On-line combination of capillary isoelectric focusing and capillary non-gel electrophoresis using a hollow-fiber membrane interface: a novel two-dimensional separation system for proteins. J Chromatogr B Analyt Technol Biomed Life Sci 2005;817:119-26.
20. Zhu G, Yuan H, Zhao P, Zhang L, Liang Z, Zhang W, Zhang Y. Macroporous polyacrylamide-based monolithic column with immobilized pH gradient for protein analysis. Electrophoresis 2006;27:3578-83.
21. Zhu C, He X, Kraly JR, Jones MR, Whitmore CD, Gomez DG, Eggertson M, Quigley W, Boardman A, Dovichi N. Instrumentation for medium-throughput two-dimensional capillary electrophoresis with laser-induced fluorescence detection. Anal Chem 2007;79:765-8.
22. Xiao X, Liu D, Tang Y, Guo F, Xia L, Liu J, He D. Development of proteomic patterns for detecting lung cancer. Dis Markers 2003-2004;19:33-9.
23. Zhong L, Hidalgo GE Stromberg AJ, Khattar NH, Jett JR, Hirschowitz EA. Using protein microarray as a diagnostic assay for non-small cell lung cancer. Am J Respir Crit Care Med 2005;172:1308-14.
24. Bartling B, Hofmann HS, Boettger T, Hansen G, Burdach S, Silber RE, Simm A. Comparative application of antibody and gene array for expression profiling in human squamous cell lung carcinoma. Lung Cancer 2005;49:145-54.
25. Gao WM, Kuick R, Orchekowski RP, Misek DE, Qiu J, Greenberg AK, Rom WN, Brenner DE, Omenn GS, Haab BB, Hanash SM. Distinctive serum protein profiles involving abundant proteins in lung cancer patients based upon antibody microarray analysis. BMC Cancer 2005;5:110.
26. Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nat Biotechnol 2003;21:255-61.
27. Krueger KE, Srivastava S. Posttranslational modifications: current implications for cancer detection, prevention, and therapeutics. Mol Cell Proteomics 2006;5:1799-810.
28. Hanash S, Brichory F, Beer D. A proteomic approach to the identification of lung cancer markers. Dis Markers 2001;17:295-300.
29. Satoh H, Ishikawa H, Kamma H, Yamashita YT, Takahashi H, Ohtsuka M, Hasegawa S. Serum sialyl lewis X-i antigen levels in non-small cell lung cancer: correlation with distant metastasis and survival. Clin Cancer Res 1997;3:495-9.
30. Otake Y, Fujimoto I, Tanaka F, Nakagawa T, Ikeda T, Menon KK, Hase S, Wada H, Ikenaka K. Isolation and characterization of an N-linked oligosaccharide that is significantly increased in sera from patients with non-small cell lung cancer. J Biochem 2001;129:537-42.
31. Laack E, Nikbakht H, Peters A, Kugler C, Jasiewicz Y, Edler L, Hossfeld DK, Schumacher U. Lectin histochemistry of resected adenocarcinoma of the lung: helix pomatia agglutinin binding is an independent prognostic factor. Am J Pathol 2002;160:1001-8.
32. Lopez-Ferrer A, Barranco C, de Bolos C. Differences in the O-glycosylation patterns between lung squamous cell carcinoma and adenocarcinoma. Am J Clin Pathol 2002;118:749-55.
33. Geng F, Shi BZ, Yuan YF, Wu XZ. The expression of core fucosylation E-cadherin in cancer cells and lung cancer patients: prognostic implications. Cell Res 2004;14:423-33.
34. Gokmen SS, Kazezoglu C, Tabakoglu E, Altray G, Gungor O, Ture M. Serum total sialic acid levels in lung cancer patients of different histological types with and no extrapulmonary metastases. Turk J Biochem 2004;29:262-7.
35. Kossowska B, Ferens-Sieczkowska M, Gancarz R, Passowicz-Muszynska E, Jankowska R. Fucosylation of

serum glycoproteins in lung cancer patients. Clin Chem Lab Med 2005;43:361-9.
36. Ueda K, Katagiri T, Shimada T, Irie S, Sato TA, Nakamura Y, Daigo Y. Comparative profiling of serum glycoproteome by sequential purification of glycoproteins and 2-nitrobenzensulfenyl (NBS) stable isotope labeling: a new approach for the novel biomarker discovery for cancer. J Proteome Res 2007;6:3475-83.
37. Gharib TG, Chen G, Wang H, Huang CC, Prescott MS, Shedden K, Misek DE, Thomas DG, Giordano TJ, Taylor JM, Kardia S, Yee J, Orringer MB, Hanash S, Beer DG. Proteomic analysis of cytokeratin isoforms uncovers association with survival in lung adenocarcinoma. Neoplasia 2002;4:440-8.
38. Chen G, Wang H, Gharib TG, Huang CC, Thomas DG, Shedden KA, Kuick R, Taylor JM, Kardia SL, Misek DE, Giordano TJ, Iannettoni MD, Orringer MB, Hanash SM, Beer DG. Overexpression of oncoprotein 18 correlates with poor differentiation in lung adenocarcinomas. Mol Cell Proteomics 2003;2:107-16.
39. Liu Y, Chen Q, Zhang JT. Tumor suppressor gene 14-3-3sigma is down-regulated whereas the proto-oncogene translation elongation factor 1delta is up-regulated in non-small cell lung cancers as identified by proteomic profiling. J Proteome Res 2004;3:728-35.
40. Flores-Delgado G, Liu CW, Sposto R, Berndt N. A limited screen for protein interactions reveals new roles for protein phosphatase 1 in cell cycle control and apoptosis. J Proteome Res 2007;6:1165-75.
41. Ma PC, Tretiakova MS, Nallasura V, Jagadeeswaran R, Husain AN, Salgia R. Downstream signalling and specific inhibition of c-MET/HGF pathway in small cell lung cancer: implications for tumour invasion. Br J Cancer 2007;97:368-77.
42. Uno F, Sasaki J, Nishizaki M, Carboni G, Xu K, Atkinson EN, Kondo M, Minna JD, Roth JA, Ji L. Myristoylation of the fus1 protein is required for tumor suppression in human lung cancer cells. Cancer Res 2004;64:2969-76.
43. Beck HC, Nielsen EC, Matthiesen R, Jensen LH, Sehested M, Finn P, Grauslund M, Hansen AM, Jensen ON. Quantitative proteomic analysis of post-translational modifications of human histones. Mol Cell Proteomics 2006;5:1314-25.
44. Dennis JW, Granovsky M, Warren CE. Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta 1999;1473:21-34.
45. Lowe JB. Glycosylation, immunity, and autoimmunity. Cell 2001;104:2364-9.
46. Kobata A, Amano J. Altered glycosylation of proteins produced by malignant cells, and application for the diagnosis and immunotherapy of tumours. Immunol Cell Biol 2005;83:429-39.
47. Patwa T, Zhao J, Anderson MA, Simeone DM, Lubman DM. Screening of glycosylation patterns in serum using natural glycoprotein microarrays and multi-lectin fluorescence detection. Anal Chem 2006;78:6411-21.
48. Hirabayashi J. Lectin-based structural glycomics: glycoproteomics and glycan profiling. Glycoconj J 2004;21:35-40.
49. Hart C, Schulenberg B, Steinberg TH, Leung WY, Patton WF. Detection of glycoproteins in polyacrylamide gels and on electroblots using Pro-Q Emerald 488 dye, a fluorescent periodate Schiff-base stain. Electrophoresis 2003;24:588-98.
50. Mechref Y, Novotny MV. Miniaturized separation techniques in glycomic investigations. J Chromatogr B Analyt Technol Biomed Life Sci 2006;841:65-78.
51. Mukherji M. Phosphoproteomics in analyzing signaling pathways. Expert Rev Proteomics 2005;2:117-28.
52. Zhang H, Zha X, Tan Y, Hornbeck PV, Mastrangelo AJ, Alessi DR, Polakiewicz RD, Comb MJ. Phosphoprotein analysis using antibodies broadly reactive against phosphorylated motifs. J Biol Chem 2002;277:39379-87.
53. Schmidt SR, Schweikart F, Anderson ME. Current methods for phosphoprotein isolation and enrichment. J Chromatogr B Analyt Technol Biomed Life Sci 2007;849:154-62.
54. Raggiaschi R, Gotta S, Terstappen GC. Phosphoproteome analysis. Biosci Rep 2005;25:33-44.
55. Goshe MB, Veenstra TD, Panisko EA, Conrads TP, Angell NH, Smith RD. Phosphoprotein isotope-coded affinity tags: application to the enrichment and identification of low-abundance phosphoproteins. Anal Chem 2002;74:607-16.
56. Ibarrola N, Kalume DE, Gronborg M, Iwahori A, Pandey A. A proteomic approach for quantitation of phosphorylation using stable isotope labeling in cell culture. Anal Chem 2003;75:6043-9.
57. Carr SA, Annan RS, Huddleston MJ. Mapping posttranslational modifications of proteins by MS-based selective detection: application to phosphoproteomics. Methods Enzymol 2005;405:82-115.
58. Julenius K, Molgaard A, Gupta R, Brunak S. Prediction, conservation analysis, and structural characterization of mammalian mucin-type O-glycosylation sites. Glycobiology 2005;15:153-64.
59. Gupta R, Brunak S. Prediction of glycosylation across the human proteome and the correlation to protein function. Pac Symp Biocomput 2002;310-22.
60. Blom N, Gammeltoft S, Brunak S. Sequence- and structure-based prediction of eukaryotic protein phosphorylation sites. J Mol Biol 1999;249:1351-62.
61. Diella F, Cameron S, Gemund C, Linding R, Via A, Kuster B, Sicheritz-Ponten T, Blom N, Gibson TJ. Phospho.ELM: a database of experimentally verified phosphorylation sites in eukaryotic proteins. BMC Bioinformatics 2004;5:79.
62. Lee TY, Huang HD, Hung JH, Huang HY, Yang YS, Wang TH. dbPTM: an information repository of protein post-translational modification. Nucleic Acids Res 2006;34:D622-7.

現今肺癌檢體的蛋白體學分析與生物標記的轉譯後修飾作用之研究

Supachok Sinchaikul[1] Piyorot Hongsachart[1,2] Supawadee Sriyam[1,2]
Payungsak Tantipaiboonwong[3] Suree Phutrakul[2] 陳水田[1,4]

肺癌是癌症患者中死亡率最高的疾病，肺癌早期症狀不明顯，發現時大都已惡化或爲末期的症狀。從肺癌檢體中發現生物標記，是有臨床上的使用性及具有挑戰性的研究，此挑戰性主因爲生物檢體中蛋白質的多樣性及複雜度，用蛋白體學來尋找肺癌檢體中的生物標記仍是一件艱鉅的工作。許多不同的研究方法，已被使用在肺癌檢體中生物標記的研究。在本回顧論文中，我們收集目前使用蛋白體學來分析肺癌檢體的蛋白體研究，來確認出蛋白質圖譜、蛋白質後修飾形成的蛋白圖譜，以及不同肺癌各別蛋白質表現量的多寡。另外使用生物資訊軟體來分析與預測蛋白質上後修飾作用的氨基酸殘基位置及後修飾在醣化及磷酸化位置。總言之，蛋白體分析研究肺癌檢體所得到的生物標記，可配合新的生技如蛋白晶片等，從特徵分子開發成早期偵檢及醫療上的應用。(長庚醫誌 2008;31:417-30)

關鍵詞：肺癌，蛋白體學，人類檢體，轉譯後修飾作用，生物標記，生物資訊學

[1]中央研究院 生物化學所 基因體研究中心；[2]清邁大學；[3]湄發蠻大學；[4]臺灣大學 生化科學所
受文日期：民國96年9月26日；接受刊載：民國96年12月25日
通訊作者：陳水田教授，中央研究院 生物化學所 基因體研究中心。台北市115南港區研究院路二段128號。
Tel.: (02)27855696轉7071; Fax: (02)27883473; E-mail: bcchen@gate.sinica.edu.tw