

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



From Biomarker Discovery to Clinical Evaluation for Early Diagnosis of Lung Surgery-Induced Injury

Mei-Ling Tsai, Shu-Hui Chen,
Chih-Ching Chang and Ming-Ho Wu
*National Cheng Kung University,
Taiwan, Republic of China*

1. Introduction

Lung cancer is one of the most common cancers in the world (Chiang et al., 2010; Landis et al., 1998). Surgical removal of the tumor mass offers the best chance for a cure in patients with non-small-cell lung cancer. A tumor in stages I (confined to the lung without nodal or distant metastasis), II (involvement of only lymph nodes within the lung), and IIIA (involvement of nodes on the same side as the tumor) is considered potentially resectable for cure (Martini et al., 1995).

Based on tumor size and location, lung surgery is mainly divided into three types: wedge resection (removal of a small area in one lobe of either right or left lung), lobectomy (removal of one lobe from a right or left lung), and pneumonectomy (removal of an entire right or left lung). The mortality rate is much higher after pneumonectomy (61%) than lobectomy (35%) (Gunluoglu et al., 2011). Among the post-surgical factors, aberrant local inflammation and abnormal fluid drainage are the most common for inducing pulmonary edema. Excessive accumulation of fluid in the alveoli causes lung injury and hinders functional recovery. A severe form of acute lung injury results in acute respiratory distress syndrome. Sudden and life-threatening lung failure is the most detrimental factor in post-surgical mortality (Jordan et al., 2000).

With the progression from lung injury to acute respiratory distress syndrome, proinflammatory cytokines are increased, such as interleukin-1 β (Donnelly et al., 1996; Geiser et al., 2001) and tumor necrosis factor- α (Tremblay et al., 2002). However, the production of vascular endothelial growth factor is reduced in the early stage but not altered in the late stage (Medfor & Millar, 2006). Interleukin-1 β and tumor necrosis factor- α are further elevated in the sustained phase (Bhatia & Moochhala, 2004). The increases in proinflammatory cytokines are not correlated with injury-induced mortality (Donnelly et al., 1996). Corticosteroid which alters host inflammatory responses do not show beneficial effects in the early stage of acute respiratory distress syndrome (Kollef et al., 1995). Current reviews suggest that activation of inflammation-independent pathways in the early stage and inflammation-dependent pathways in the late stage contribute to the development of acute respiratory distress syndrome (Spragg et al., 2010; Bhatia & Moochhala, 2004).

To effectively reduce post-surgical mortality, early detection of acute respiratory distress syndrome may provide in-depth information for the design of management plans, including non-pharmacological therapies (Villar et al., 2011).

2. Proteomic analysis of bronchoalveolar lavage fluid in biomarker studies

To effectively identify the biomarkers of various lung diseases, bronchoalveolar lavage fluid from the lower airways and alveoli is collected for genomic or cytological analysis of cellular components (Meyer, 2007). This lung-specific fluid can be used for protein analysis. Identification of the non-cellular components in bronchoalveolar lavage fluid may provide valuable data for the early detection of acute lung injury.

2.1 Current advances in proteomic analysis of bronchoalveolar lavage fluid

In the past decades, over 100 human proteins or protein isoforms have been identified in bronchoalveolar lavage fluid from patients with various lung diseases (Wattiez et al., 1999; Lenz et al., 1993; Sadaghdar et al., 1992; Sabounchi-Schütt et al., 2001; Vesterberg et al., 2001). The major challenge today is to identify the lead proteins and validate the potential biomarkers (Turtoi et al., 2011a). To overcome this difficulty, integration of clinical studies with proteomic analysis of bronchoalveolar fluid is a potential solution (Turtoi et al., 2011b).

2.2 Sampling concern in proteomic analysis of bronchoalveolar lavage fluid

In clinical proteomics, the most difficult challenge before sample analysis is patient selection and sample collection (Apweiler et al., 2009). In the case of lung cancer patients, the major concern is to collect bronchoalveolar lavage fluid from those who may develop post-surgical lung edema. Although both bronchoalveolar lavage fluid and bronchial washings are collected using similar procedures, the former is collected from terminal alveoli after instilling more than 140 ml of sterile saline and the latter is collected from major airways after instilling less than 140 ml of saline. Because of the concern that excessive fluid accumulation may cause the complication of lung edema, bronchial washing is a better choice for conducting clinical proteomics.

2.3 Technical limitations in proteomic analysis of bronchoalveolar lavage fluid

In conventional proteomic analysis, two-dimensional gel electrophoresis provides good protein separation. However, it restricts the discovery of proteins with extreme biochemical properties such as size, isoelectric point, and solubility (Rabilloud, 2002). In comparison, one-dimensional gel electrophoresis provides easy comparison of banding patterns in protein profiling but is less efficient in protein separation. Moreover, the high salt concentration in the bronchoalveolar lavage fluid interferes with the resolution of protein separation to a lesser extent in one-dimensional gel electrophoresis (Plymoth, 2003).

2.4 Application of 1D gel with liquid chromatography and MS/MS in biomarker discovery

The rapid development of LC/MS/MS offers a better solution to one-dimensional gel electrophoresis (Schirle et al., 2003). A similar approach has been used to discover proteins

with molecular weight greater than 100 kDa (such as α 2-macroglobulin). The discovery of hundreds of proteins in bronchoalveolar lavage fluid demonstrates its feasibility in biomarker identification (Wu et al., 2005; Chang et al., 2007).

2.5 Sensitivity and specificity of the lead proteins after proteomic analysis

To accelerate the translation of biomarker discovery from bench to bedside, the development of techniques has been divided into 5 stages (Pepe et al., 2001). In Phase 1, potential biomarkers are discovered by various approaches, such as proteomic analysis. After the leads are identified by biochemical studies, measurable classifiers or outcomes are developed in Phase 2. Based on the analysis of their specificity and sensitivity, the cutoff point of the measurable outcome is determined and used in Phase 3. Based on patient history and clinical data, the number and nature of clinical cases is well defined in Phase 3. Suitable criteria for a clinical trial are determined in Phase 4. Phase 5 is a randomized trial to compare the specificity and sensitivity of the leads with those of current biomarkers in the market.

Today, the importance of sensitivity and specificity in biomarker selection has shifted proteomic studies from large-scale analysis to clinically-relevant validation. In addition to large-scale analysis in protein or metabolite identification (Mou et al., 2011; Huang et al., 2011), the leads are selected based on their sensitivity and specificity.

3. Translational study from protein identification to clinical application

The purpose of this study was to discover potential biomarkers for the early detection of acute respiratory distress syndrome. To avoid sampling-induced complications, bronchial washings from lung cancer patients before and after surgical therapy (lobectomy) were collected. To reduce population heterogeneity, cancer stage, hormonal variation, and tumor location were well-defined. Only patients older than 60 years, had right lung cancer at stages IA and IB, and agreed to receive right lung lobectomy were recruited.

Those patients who met the inclusion and exclusion criteria were selected as controls. The inclusion criteria were: defined cancer in any lobe of the right lung, non-smoker, age ≥ 60 years, elective operation, operation period < 210 min, forced expiratory volume in 1 s (FEV1) $> 80\%$, and no prior major lung resection or thoracic irradiation. Exclusion criteria were: age < 60 years, operation period > 210 min, FEV1 $< 60\%$, emergency or urgent operation, and prior major lung resection or distant thoracic irradiation. Based on our criteria, 7 patients (5 females and 2 males with ages ranging between 61 and 77 years) were included as controls. A review was conducted from the medical records and prospective database. The study protocol was approved by the Human Medical Studies Committee at National Cheng Kung University Medical College Hospital. Informed consent was given by all participants or their legal guardians.

Prior to a large-scale analysis of proteins in bronchial washings, the protein profiles of washings from different compartments of the lungs before and after lobectomy were compared. In the right lung where tumor tissues were identified, no clear bands at molecular weights > 75 kDa were found in washings collected before or after lobectomy. In the left lung, no clear bands at molecular weights > 75 kDa were found in the washings collected before lobectomy. After lobectomy, more bands at molecular weights > 75 kDa were found. The intensity of each band was much greater (Fig. 1). Similar patterns were found in all samples studied.

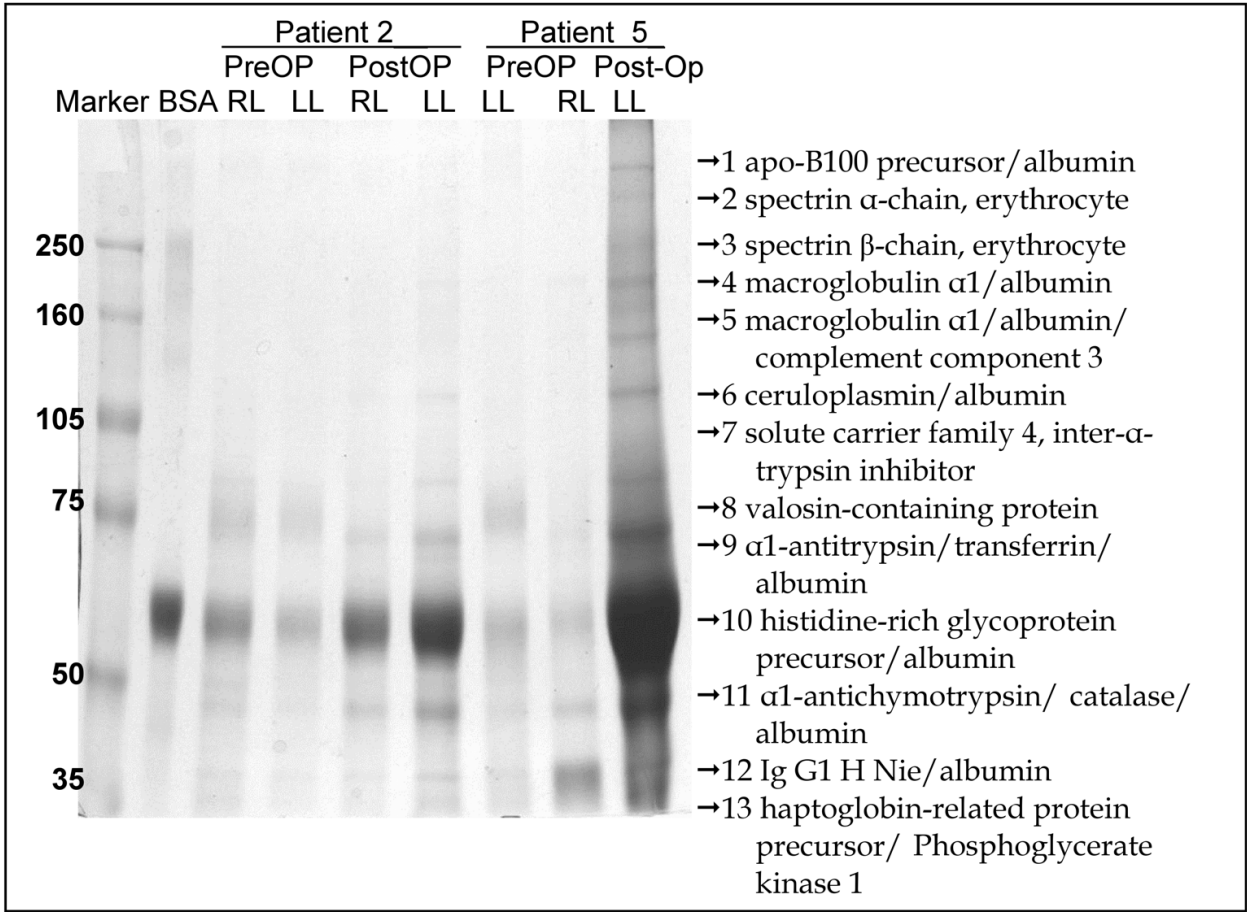


Fig. 1. Protein profiling of bronchial washes from right (RL) and left lungs (LL) from patients before (Pre-Op) and after (Post-Op) right lung lobectomy. Bovine serum albumin (BSA) was used a positive control because albumin was identified in various bands.

The banding pattern allowed us to hypothesize that the proteins at molecular weights >75 kDa are exuded into alveoli after surgery. One-dimensional gel electrophoresis coupled with LC/MS/MS allowed us to identify the proteins in 13 major bands. As listed in Table 1, 8 proteins had molecular weights >100 kDa, including α 2-macroglobulin.

To test our hypothesis that protein exudation is surgery-dependent, the relative abundance of α 1-antitrypsin (47 kDa) and α 2-macroglobulin (162 kDa) in bronchial washings was measured by Western blot analysis. α 1-antitrypsin was found in washings collected before and after lobectomy (data not shown) but α 2-macroglobulin was only found after lobectomy (Fig. 2).

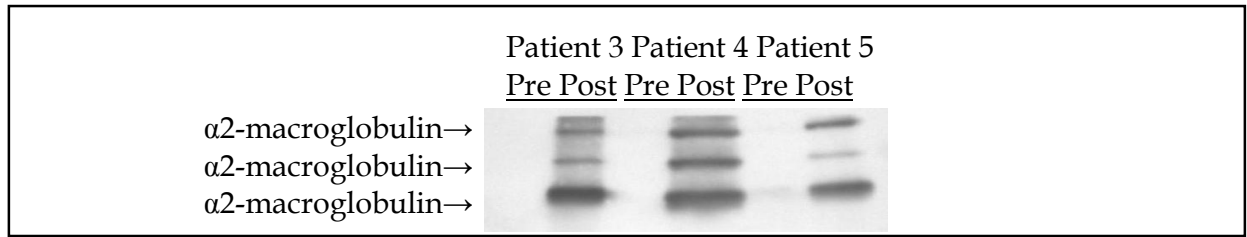


Fig. 2. Relative abundance of α 2-macroglobulin in bronchial washings before (Pre) and after (Post) lobectomy.

No	GI number	Protein name	MW (kDa)	No. of matched peptides	Sequence coverage	Score
1	28780	apo-B100 precursor	516.384	6	2%	285
2	1174412	spectrin α chain, erythrocyte	280.904	5	3%	257
3	134798	spectrin β chain, erythrocyte	247.040	7	4%	291
4	179674	complement component C4A	194.365	5	4%	216
5	4557385	complement component 3 precursor	188.612	8	7%	451
6	224053	α 2-macroglobulin	162.096	2	1%	86
7	4557485	ceruloplasmin (ferroxidase)	122.998	2	2%	91
8	1483187	inter- α -trypsin inhibitor family heavy chain-related protein (IHRP)	103.553	2	2%	82
9	4507021	solute carrier family 4, anion exchanger, member 1	102.017	3	4%	213
10	6005942	valosin-containing protein	89.962	4	9%	130
11	28592	serum albumin	71.351	12	19%	615
12	3287489	hsp89- α - δ -N	63.850	2	5%	85
13	4504489	histidine-rich glycoprotein precursor	60.527	2	4%	69
14	553788	transferrin	55.233	4	10%	177
15	69990	α 1-glycoprotein	52.488	2	6%	84
16	386789	hemopexin precursor	52.266	2	2%	81
17	38408	immunoglobulin M heavy chain	50.135	3	9%	112
18	229601	Ig G1 H Nie	49.812	3	12%	127
19	177827	α 1-antitrypsin	46.790	2	7%	67
20	10334547	immunoglobulin heavy chain	42.319	2	8%	57
21	123510	haptoglobin-related protein precursor	39.505	2	6%	114
22	121039	Ig gamma-1 chain C region	36.605	3	14%	118
23	121043	Ig gamma-2 chain C region	36.500	2	11%	55
24	183817	β -globin	19.209	4	33%	221
25	442753	Chain D, Hemoglobin Ypsilanti	16.021	5	50%	206

Table 1. Proteins identified in bronchial washings from the left lung of a patient receiving right lung lobectomy.

3.1 Vascular endothelial growth factor and lobectomy-induced inflammation

Since vascular endothelial growth factor is a potent inducer of vascular permeability (Lee, 2005) and its expression is positively correlated with inflammation-induced protein exudation and leukocyte infiltration (Chang et al., 2005), it is plausible to suggest that an increase in vascular endothelial growth factor is associated with surgery-induced protein exudation and leukocyte infiltration.

As shown in Table 2, the vascular endothelial growth factor level was positively correlated with total protein concentration ($y = 0.0025x + 1.0755$, $R^2 = 0.7359$, $P < 0.05$) and cell count ($y = 0.0696x - 0.6441$, $R^2 = 0.8463$, $P < 0.05$) but not with operation duration or $\text{PaO}_2/\text{FiO}_2$. The correlation analysis supported the hypothesis that the induction of vascular endothelial growth factor after surgery contributes to leukocyte infiltration and protein exudation.

Patient No	Operation duration (min)	$\text{PaO}_2/\text{FiO}_2$	Cell count (10^4 cells/ml)	Protein conc. ($\mu\text{g/ml}$)	VEGF (pg/ml)
1	152	270.167	2.5	0.641	162.80
2	191	202.500	67.0	3.412	613.68
3	234	435.000	100.0	4.826	1517.40
4	160	220.800	22.5	1.737	89.95
5	196	336.000	3.5	2.462	99.79
6	140	338.333	0.5	0.660	109.64
7	221	505.600	5.0	1.302	359.69

PaO_2 : arterial partial pressure of oxygen; FiO_2 : inspired oxygen fraction; $\text{PaO}_2/\text{FiO}_2$: oxygenation index
Table 2. Clinical data of 7 patients who received right lung lobectomy without complications

3.2 $\alpha 2$ -macroglobulin and $\alpha 1$ - antitrypsin in lobectomy-induced inflammation
The relative expression of $\alpha 2$ -macroglobulin at bands 2, 4, and 5 from bronchial washings was correlated with protein concentration, leukocyte number, and the level of vascular endothelial growth factor (Fig. 3).

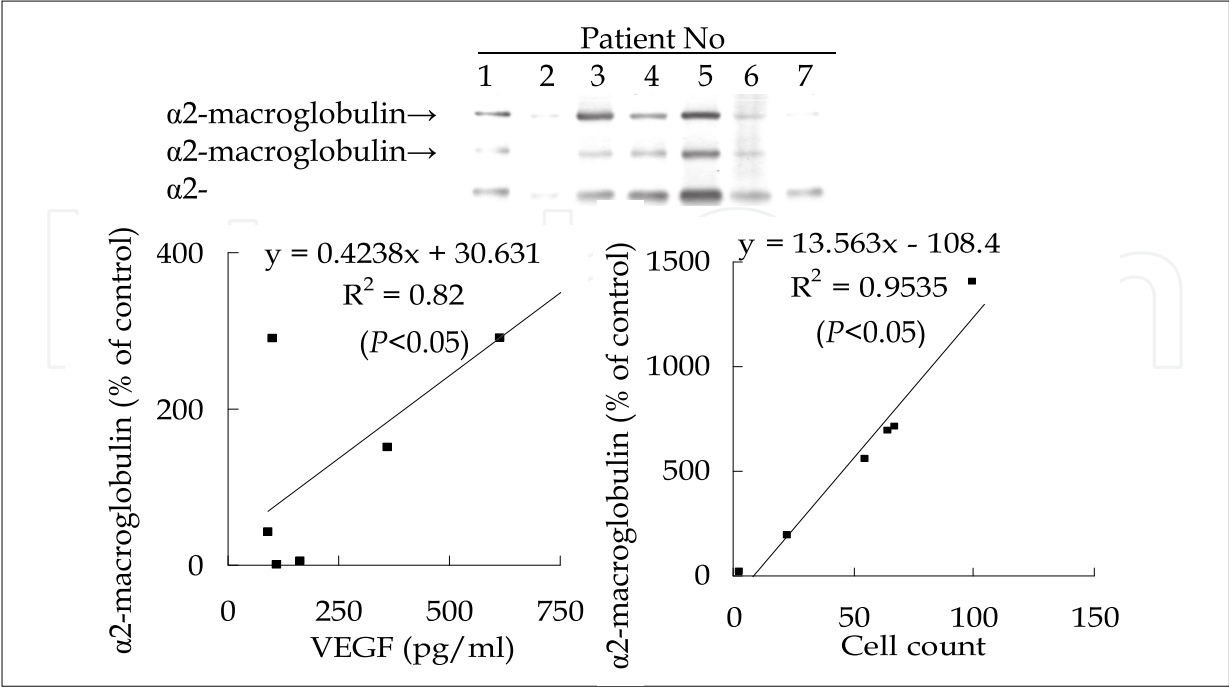


Fig. 3. Correlation analysis of $\alpha 2$ -macroglobulin and VEGF/total cells in bronchial washings.

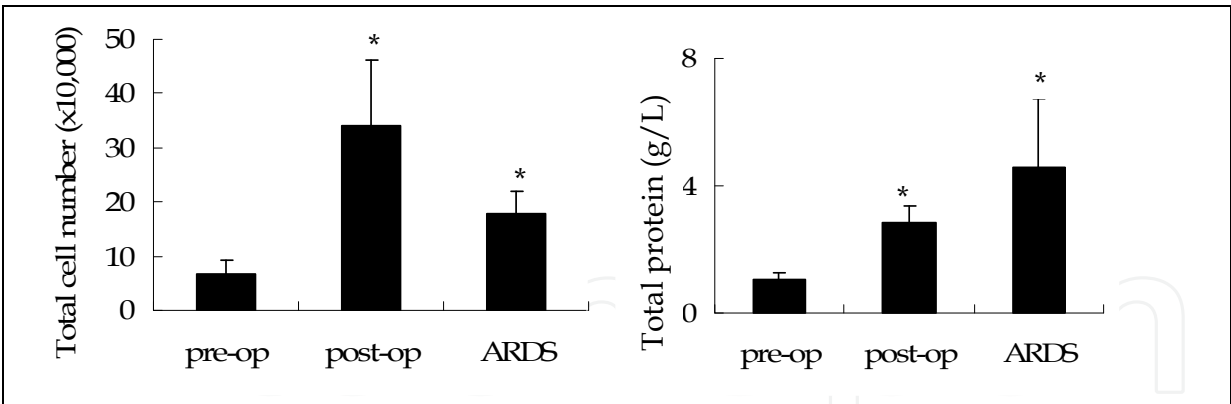
Likewise, the relative expression of α 1-antitrypsin at bands 5, 7, and 8 from bronchial washing was positively correlated with protein concentration, leukocyte number, and the level of vascular endothelial growth factor (data not shown). These data supported our hypothesis that the increase of vascular endothelial growth factor after surgery facilitates leukocyte infiltration and the exudation of acute-phase proteins (such as α 1-antitrypsin and α 2-macroglobulin) into alveoli.

3.3 Characterization of α 2-macroglobulin and α 1-antitrypsin in lobectomized patients with acute respiratory distress syndrome

Based on the report of the joint American-European Consensus Conference, the acute respiratory distress syndrome is well defined as follows: bilateral infiltrates on frontal chest radiography, the absence of left atrial hypertension (pulmonary capillary wedge pressure <18 mmHg or no clinical signs of left ventricular failure), and severe hypoxemia with a PaO₂/FiO₂ ratio <200 mmHg (Bernard et al., 1994). Five patients who received lung surgery and met these criteria were studied.

3.3.1 Characterization of patients with acute respiratory distress syndrome

The group with lobectomy free of complications had levels of total protein and total leukocyte numbers in their bronchial washings similar to those who developed acute respiratory distress syndrome ($P > 0.05$, Fig. 4). These data indicate that lung surgery induces inflammation (leukocyte infiltration and protein exudation) in the groups with and without the complication of acute respiratory distress syndrome. So, factors other than inflammation contribute to the development of this syndrome.



*Significant difference from pre-op.

Fig. 4. Total leukocyte number and protein concentration in patients before (pre-op) and after lobectomy (post-op) with no complication and those with acute respiratory distress syndrome (ARDS).

In lung cancer patients, an increase of vascular endothelial growth factor is positively associated with poor prognosis ($P = 0.018$; Han et al., 2001) but not with a worse postoperative year-survival rate ($P = 0.0643$; Liao et al., 2001). These reports are also consistent with our finding that the increase of vascular endothelial growth factor after lung surgery does not contribute to surgery-induced acute respiratory distress syndrome.

3.3.2 Protein profiling of bronchial washings from lobectomized patients with acute respiratory distress syndrome

Unlike patients with no complications, those with acute respiratory distress syndrome showed white or gray patches on the chest X-ray. In one-dimensional gel electrophoresis, the protein profiling of bronchial washings from patients without complications showed a much clearer banding pattern than those from patients with acute respiratory distress syndrome (Fig. 5). Eight bands from each gel were cut and subjected to LC/MS/MS for protein identification. No protein was identified in Lane 1. The most significant difference was that albumin appeared in almost every band of the samples from patients without complications but not in those with acute respiratory distress syndrome. In contrast, α 1-antitrypsin was identified only in bands 6 and 7 from the group without complications but was found in bands 2, 3, 4, 5, 6, and 7 in the group with the complication (Fig. 5).

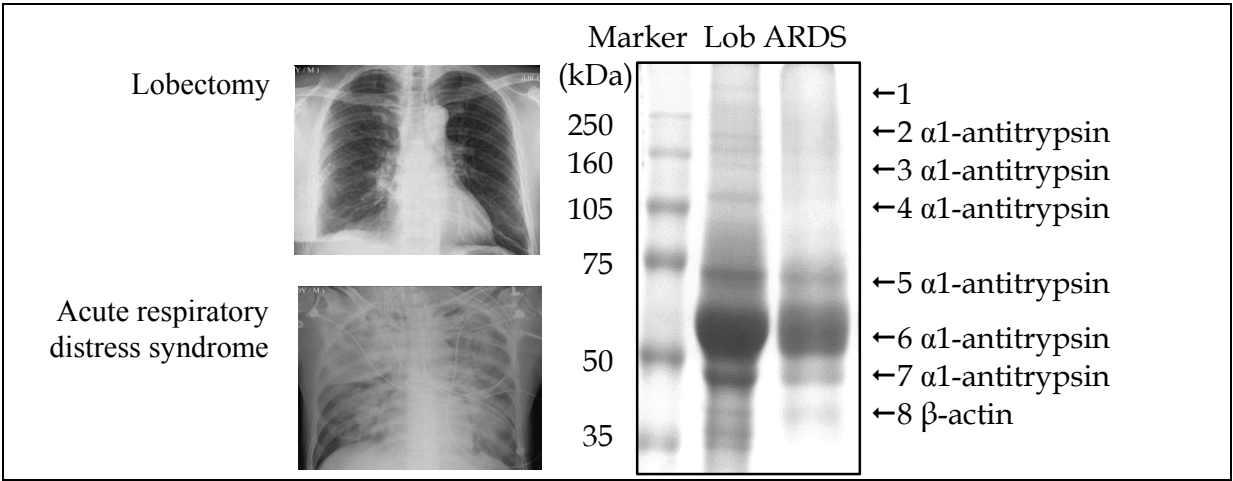


Fig. 5. Comparison of chest X-rays and protein profiling of bronchial washings in lobectomized patients with no complications (lobectomy, Lob) and those with acute respiratory distress syndrome (ARDS).

3.3.3 α 2-macroglobulin and α 1-antitrypsin in bronchial washings from lobectomized patients with acute respiratory distress syndrome

As shown in Fig. 6, both α 2-macroglobulin and α 1-antitrypsin were detected in bronchial washings after surgery. After quantification, the total amounts of α 2-macroglobulin at bands 2, 4, and 5 and α 1-antitrypsin at bands 5, 7, and 8 did not show any statistical difference between the groups with and without complications. The most important finding was lower levels of α 1-antitrypsin at bands 7 and 8 in the group without complications than the acute respiratory distress syndrome group (Fig. 6). It is likely that α 1-antitrypsin variants at bands 5, 7, and 8 can be used as biomarkers for the early detection of acute respiratory distress syndrome. In bronchial washings collected from the patients with acute respiratory distress syndrome, leukocyte number was not correlated with the total amounts of α 2-macroglobulin or α 1-antitrypsin. Our analyses again supported the notion that surgery-induced inflammation is not an important indicator in the early phase of acute respiratory distress syndrome. It has been reported that α 1-antitrypsin can be produced by lung epithelial cells (Venember et al., 1994) but α 2-macroglobulin cannot. Our preliminary data confirmed the expression of

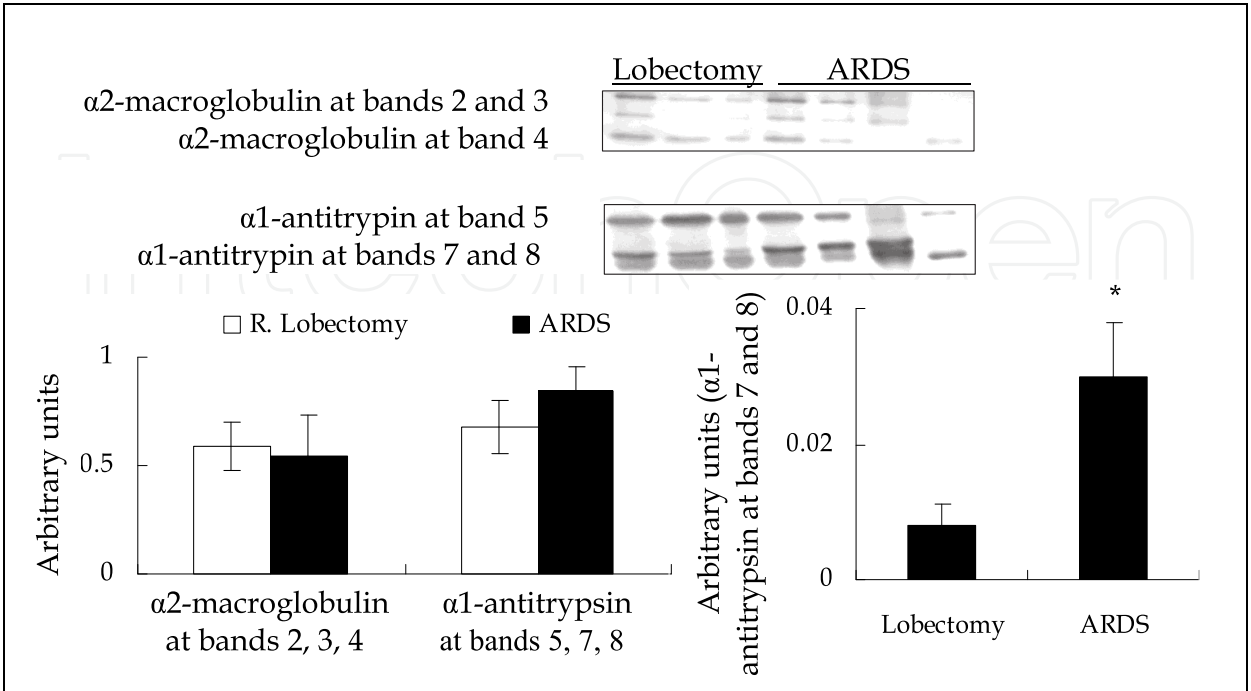


Fig. 6. Relative expression of $\alpha 1$ -antitrypsin and $\alpha 2$ -macroglobulin (macroglobulin) in the lobectomized group without complications (lobectomy) and in the group with acute respiratory distress syndrome (ARDS).

$\alpha 1$ -antitrypsin in A549, a lung epithelial cell line. The changes in $\alpha 1$ -antitrypsin variants could be due to functional changes in lung epithelial cells.

3.4 Specificity and sensitivity of $\alpha 1$ -antitrypsin variants as potential biomarkers for acute respiratory distress syndrome

It is of importance to turn the relative expression of $\alpha 1$ -antitrypsin in bronchial washings into a measurable outcome because only the measurable outcome is used to determine the cutoff value. Based on the cutoff value, sensitivity (the proportion of subjects who test positive among those with the condition) and specificity (the proportion of subjects who test negative among those without the condition) can be calculated.

As shown in Fig. 6, $\alpha 1$ -antitrypsin variants at bands 7 (47 kDa) and 8 (40 kDa) had a lower abundance in the group without complications than the group with acute respiratory syndrome. To avoid variations in sample loading and the intensity in each calculation, the ratio of the expression of $\alpha 1$ -antitrypsin at band 5 (70 kDa) to that at bands 7 and 8 was used as the measurable outcome. Based on this calculation, the cutoff value was 0.5. A ratio <0.5 was considered an indication of acute respiratory distress syndrome.

Table 3 shows the ratio for each patient from the complication-free group. Four out of 7 patients had a ratio <0.5 . The specificity of $\alpha 1$ -antitrypsin for true negative patients was 0.43 (3/7).

Table 4 shows the ratio for each patient from the complication group. Three out of 5 patients had a ratio <0.5 . The sensitivity of $\alpha 1$ -antitrypsin for true positive patients was 0.6 (3/5).

Patient No	Ratio of expression of α 1-antitrypsin at band 5 to that at bands 7 and 8	Cutoff value = 0.5
1	0.000: 0.027	<0.5
2	0.043: 0.099	<0.5
3	0.019: 0.024	>0.5
4	0.017: 0.023	>0.5
5	0.018: 0.087	<0.5
6	0.000: 0.006	<0.5
7	0.042: 0.053	>0.5

Table 3. Ratio of the expression of α 1-antitrypsin at band 5 to that at bands 7 and 8 in the lobectomized patients without acute respiratory distress syndrome.

Patient No	Ratio of expression of α 1-antitrypsin at band 5 to that at bands 7 and 8	Cutoff value = 0.5
A	0.081: 0.177	<0.5
B	0.043: 0.199	<0.5
C	0.081: 0.086	>0.5
D	0.015: 0.040	<0.5
E	0.025: 0.048	>0.5

Table 4. Ratio of the expression of α 1-antitrypsin at band 5 to that at bands 7 and 8 in lobectomized patients with acute respiratory distress syndrome.

3.5 Further improvement of specificity and sensitivity for detecting acute respiratory distress syndrome using dual biomarkers

As shown in Tables 3 and 4, the sensitivity of α 1-antitrypsin variants for detecting acute respiratory distress syndrome (0.6) was better than the specificity (0.43). The major concern is how to optimize the cutoff value and improve the specificity. In table 3, patients 1 and 6 with ratios <0.5 showed the lowest values in cell counts and protein concentration. Meanwhile, the expression of α 2-macroglobulin was almost undetectable, which indicates minor inflammation in the patients. The lower ratio of relative expression of α 1-antitrypsin at band 5 to that at bands 7 and 8 was false-positive. α 1-antitrypsin was found in the lungs before and after surgery; α 2-macroglobulin only occurred in the lungs after surgery. To avoid the lower levels of α 1-antitrypsin variants which may create a false-positive result, α 2-macroglobulin can be recruited as a second biomarker. The ratio of α 1-antitrypsin variants was considered as a true result only when

the sample expressed detectable $\alpha 2$ -macroglobulin in bronchial washings. Accordingly, the specificity for true negative patients changed to 0.71 (5/7). The prediction for true negatives was improved.

4. From identification of leads to further validation using $\alpha 2$ -macroglobulin and $\alpha 1$ -antitrypsin variants as an example

After the discovery of potential biomarkers by proteomic analysis in this study, the first challenge was to identify the leads from the proteins discovered after developing a quick screening test. After Phase 1, the second challenge was to provide clear justification to optimize the cutoff values.

4.1 Contribution of this study to the discovery of biomarkers for detecting acute respiratory distress syndrome

Ideally, quantitative proteomic analysis should be used to reveal lobectomy-induced changes of all proteins in bronchial washings. However, the unique compartment of the lung allowed us to analyze exudate components which may not exist before surgery, such as $\alpha 2$ -macroglobulin. Based on the important mechanism of surgery-induced inflammation in the early phase of lung injury, one-dimensional gel electrophoresis in this study was an easy and suitable tool to identify $\alpha 2$ -macroglobulin as an indicator of vascular endothelial growth factor-mediated permeability.

The second contribution of this study was to take advantage of one-dimensional gel electrophoresis with pattern analysis to reveal the pattern changes of $\alpha 1$ -antitrypsin between the groups with and without post-surgical complications. The difference found allowed us to identify $\alpha 1$ -antitrypsin variants as biomarkers for the early detection of acute respiratory distress syndrome.

4.2 Limitations of this study

In this study, $\alpha 1$ -antitrypsin variants were considered as biomarkers for acute respiratory distress. No mechanistic data are provided to explain why and how the formation of $\alpha 1$ -antitrypsin variants are related to the progression from surgery-induced inflammation to acute respiratory distress syndrome.

The association between $\alpha 1$ -antitrypsin variants and infection was first reported in 2010 (Zhang et al., 2010). The decrease of the $\alpha 1$ -antitrypsin variant at 130 kDa and the increase of the variant at 40 kDa is associated with human immunodeficiency virus-induced infection. Glycoproteomic analysis shows that changes in $\alpha 1$ -antitrypsin variants may be due to a shift of glycosylation. In future, glycoproteomic analysis of $\alpha 1$ -antitrypsin variants should be further explored.

Although the analysis of their specificity and sensitivity, the cutoff point of the measurable outcome, and criteria for patient selection are clearly and easily determined, the small number of clinical cases in this study limits the generalization of $\alpha 2$ -macroglobulin and $\alpha 1$ -antitrypsin as markers for acute respiratory distress syndrome. To use them as measurable biomarkers in Phase 3, it is necessary to increase the number and the complexity of clinical cases for further validation on whether the cutoff points determined are suitable for early diagnosis of acute respiratory distress syndrome.

One-dimensional gel electrophoresis does not offer a good way for protein separation. Comparative proteomic analysis only compares the intensity of each spot. These two

approaches may our discovery of new proteins. The technology of stable isotope dimethyl labeling coupled with LC/MS/MS permits further quantification of specific peptides of each protein and provides a better quantification tool after one-dimensional electrophoresis (Huang et al., 2006). This approach then compensates for the limitation of one-dimensional gel electrophoresis.

5. Conclusion

Both inflammation -dependent and -independent mechanisms contribute to the progression from lung injury to acute respiratory distress syndrome. Stage-dependent changes in biomarkers allow us to monitor the progression of the diseases and develop new treatments in a stage-dependent manner.

In this study, α 2-macroglobulin and α 1-antitrypsin were positively correlated with vascular endothelial growth factor, clearly showing lobectomy-induced inflammation. The total amount of α 1-macroglobulin can be used as a biomarker of increased vascular permeability in the lung. The severity of lobectomy-induced inflammation is similar to that of inflammation in acute respiratory distress syndrome but respiratory function becomes much worse in patients with the syndrome. Concomitantly, the patients with acute respiratory distress syndrome had lower levels of α 1-antitrypsin at higher molecular weights and higher levels of α 1-antitrypsin at lower molecular weights. Similarly, human immunodeficiency virus-induced infection is associated with the decreased abundance of α 1-antitrypsin at higher molecular weights and the increased abundance of α 1-antitrypsin at lower molecular weights (Zhang et al., 2010). Because α 1-antitrypsin exists in lung epithelial cells (Venember et al., 1994), the changes of α 1-antitrypsin variants in the patients with acute respiratory distress may reflect lung epithelial damage.

6. Acknowledgment

The authors appreciate the technical support of Shih-Hsin Ho, Hong-Da Wang, and Yan-Jie Chen, clinical sample collections by Drs. Jia-Ming Chang and Chang-Wen Chen, and grant support from the National Science Council, Taiwan (NSC-95-2314-B-006-125-MY2 and NSC-95-2323-B-006-004).

7. References

- Apweiler, R., Aslanidis, C., Deufel, T., Gerstner, A., Hansen, J., Hochstrasser, D., Kellner, R., Kubicek, M., Lottspeich, F., Maser, E., Mewes, HW., Meyer, HE., Müllner, S., Mutter, W., Neumaier, M., Nollau, P., Nothwang, HG., Ponten, F., Radbruch, A., Reinert, K., Rothe, G., Stockinger, H., Tárnok, A., Taussig, MJ., Thiel, A., Thiery, J., Ueffing, M., Valet, G., Vandekerckhove, J., Wagener, C., Wagner, O., & Schmitz, G. (2009). Approaching clinical proteomics: current state and future fields of application in cellular proteomics. *Cytometry A*, Vol.75, No.10, (October 2009), pp. 816-32, ISSN 1552-4930
- Bernard, GR., Artigas, A., Brigham, KL., Carlet, J., Falke, K., Hudson, L., Lamy, M., Legall, JR., Morris, A., & Spragg, R. (1994). The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial

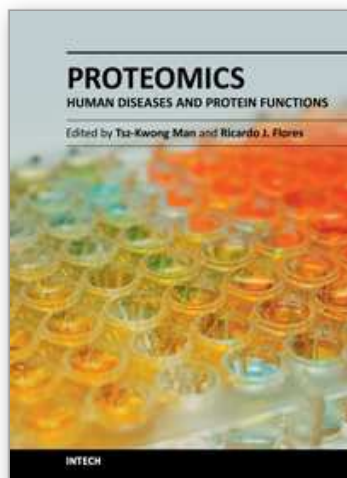
- coordination. *American Journal of Respiratory and Critical Care Medicine*, Vol.149, No. 3 Pt 1, (March 1994), pp. 818-24, ISSN 1073-449X
- Bhatia, M., & Moochhala, S. (2004). Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *Journal of Pathology*, Vol.202, No.2, (February 2004), pp. 145-56, ISSN 1096-9896
- Chang, CC., Chiu, HF., Wu, YS., Li, YC., Tsai, ML., Shen, CK., & Yang, CY. (2005). The induction of vascular endothelial growth factor by ultrafine carbon black contributes to the increase of alveolar-capillary permeability. *Environmental Health Perspectives*, Vol.113, No.4, (April 2005), pp.454-60, ISSN 0091-6765
- Chang, CC., Chen, SH., Ho, SH., Yang, CY., Wang, HD., & Tsai, ML. (2007). Proteomic analysis of proteins from bronchoalveolar lavage fluid reveals the action mechanism of ultrafine carbon black-induced lung injury in mice. *Proteomics*, Vol.7, No.23, (December 2007), pp.4388-97, ISSN 1615-9861
- Chiang, CJ., Chen, YC., Chen, CJ., You, SL., & Lai, MS. (2010). Cancer trends in Taiwan. Taiwan Cancer Registry Task Force. *Japanese Journal of Clinical Oncology*, Vol.40, No.10, (October 2010), pp.897-904, ISSN 0368-2811
- Donnelly, SC., Strieter, RM., Reid, PT., Kunkel, SL., Burdick, MD., Armstrong, I., Mackenzie, A., & Haslett, C. (1996). The association between mortality rates and decreased concentrations of interleukin-10 and interleukin-1 receptor antagonist in the lung fluids of patients with the adult respiratory distress syndrome. *Annals of Internal Medicine*, Vol.125, No.3, (August 1996), pp.191-6, ISSN 1539-3704
- Geiser, T., Atabai, K., Jarreau, PH., Ware, LB., Pugin, J., & Matthay, MA. (2001). Pulmonary edema fluid from patients with acute lung injury augments in vitro alveolar epithelial repair by an IL-1 β -dependent mechanism. *American Journal of Respiratory and Critical Care Medicine*, Vol.163, No.6, (May 2001), pp.1384-8, ISSN 1073-449X
- Gunluoglu, MZ., Demir, A., Turna, A., Sansar, D., Melek, H., Dincer, SI., & Gurses, A. (2011). Extent of lung resection in non-small lung cancer with interlobar lymph node involvement. *Annals of Thoracic and Cardiovascular Surgery*, Vol.17, No.3, (June 2011), pp.229-35, ISSN 1341-1098
- Han, H., Silverman, JF., Santucci, TS., Macherey, RS., d'Amato, TA., Tung, MY., Weyant, RJ., & Landreneau, RJ. (2001). Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. *Annals of Surgical Oncology*, Vol.8, No.1, (January-February 2001), pp.72-9, ISSN 1068-9265
- Huang, SY., Tsai, ML., Tsai, CJ., Wu, JL., Hsu, JL., Ho, SH., & Chen SH. (2006). Quantitation of protein phosphorylation in pregnant rat uteri using stable isotope dimethyl labeling coupled with IMAC. *Proteomics*, Vol.6, No.6, (March 2006), pp.1-12, ISSN 1615-9861
- Huang, Z., Lin, L., Gao, Y., Chen, Y., Yan, X., Xing, J., & Hang, W. (2011). Bladder cancer determination via two urinary metabolites: a biomarker pattern approach. (in press). *Molecular & Cellular Proteomics*, ISSN 1535-9484

- Jordan, S., Mitchell, J.A., Quinlan, GJ., Goldstraw, P., & Evans, TW. (2000). The pathogenesis of lung injury following pulmonary resection. *European Respiratory Journal*, Vol.15, No.4, (April 2000), pp.790-9, ISSN 1399-3003
- Kollef, MH., & Schuster, DP. (1998). The acute respiratory distress syndrome. *The New England Journal of Medicine*, Vol.332, No.1, (January 1995), pp.27-37, ISSN 1533-4406
- Landis, SH., Murray, T., Bolden, S., & Wingo, PA. (1998). Cancer statistics, CA-a Cancer Journal for Clinicians. Vol.48, No.1, (January-February 1998), pp.6-29, ISSN 1542-4863
- Lee, YC. (2005). The involvement of VEGF in endothelial permeability: a target for anti-inflammatory therapy. *Current Opinion in Investigational Drugs*, Vol.6, No.11, (November 2005), pp.1124-30, ISSN 1472-4472
- Lenz, AG, Meyer, B., Costabel, U., & Maier, K. (1993). Bronchoalveolar lavage fluid proteins in human lung disease: analysis by two-dimensional electrophoresis. *Electrophoresis*, Vol.14, No. 3, (March 1993), pp. 242-4, ISSN 1522-2683
- Liao, M., Wang, H., Lin, Z., Feng, J., & Zhu, D. (2001). Vascular endothelial growth factor and other biological predictors related to the postoperative survival rate on non-small cell lung cancer. *Lung Cancer*, Vol.33, No.2-3, (August-September 2001), pp.125-32, ISSN 0169-5002
- Martini, N., Bains, MS., Burt, ME., Zakowski, MF., McCormack, P., Rusch, VW., & Ginsberg, RJ. (1995). Incidence of local recurrence and second primary tumors in resected stage I lung cancer. *The Journal of Thoracic and Cardiovascular Surgery*, Vol.109, No.1, (January 1995), pp.120-9, ISSN 0022-5223
- Medford, AR., & Millar, AB. (2006). Vascular endothelial growth factor (VEGF) in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS): paradox or paradigm? *Thorax*, Vol.61, No.7, (July 2006), pp.621-6, ISSN 1468-3296
- Meyer, KC. (2007). Bronchoalveolar lavage as a diagnostic tool. *Seminars in Respiratory and Critical Care Medicine*, Vol.28, No.5, (October 2007), pp.546-60, ISSN 1069-3424
- Mou, Y., Xing, R., & Liu, C. (2011). Diagnosis of Gallbladder Cancer Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Profiling. (in press). *The American Journal of the Medical Sciences*, ISSN 1538-2990
- Pepe, MS., Etzioni, R., Feng, Z., Potter, JD., Thompson, M., Thornquist, M., Winget, M., & Yasui, Y. (2001). Phases of biomarker development for early detection of cancer. *Journal of the National Cancer Institute*. Vol.93, No.14, (July 2001), pp.1054-61. ISSN 1460-2105
- Plymoth, A., Löfdahl, CG., Ekberg-Jansson, A., Dahlbäck, M., Lindberg, H., Fehniger, TE., & Marko-Varga, G. (2003). Human bronchoalveolar lavage: biofluid analysis with special emphasis on sample preparation. *Proteomics*, Vol.3, No.6, (June 2003), pp.962-72 ISSN 1615-9861
- Rabilloud, T. (2002). Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics*, Vol.2, No.1, (January 2002), pp.3-10, ISSN 1615-9861
- Sabounchi-Schütt, F., Aström, J., Eklund, A., Grunewald, J., & Bjellqvist, B. (2001). Detection and identification of human bronchoalveolar lavage proteins using narrow-range immobilized pH gradient DryStrip and the paper bridge sample

- application method. *Electrophoresis*, Vol.22, No.9, (May 2001), pp.1851–60, ISSN 1522-2683
- Sadaghdar, H., Huang, ZB., & Eden, E. (1992). Correlation of bronchoalveolar lavage findings to severity of *Pneumocystis carinii* pneumonia in AIDS. Evidence for the development of high-permeability pulmonary edema. *Chest* Vol.102, No.1, (July 1992), pp.63-9. ISSN 1931-3543
- Schirle, M., Heurtier, MA., & Kuster, B. (2003). Profiling core proteomes of human cell lines by one-dimensional PAGE and liquid chromatography-tandem mass spectrometry. *Molecular & Cellular Proteomics*, Vol.2, No.12, (December 2003), pp.1297-305, ISSN 1535-9484
- Spragg, RG., Bernard, GR., Checkley, W., Curtis, JR., Gajic, O., Guyatt, G., Hall, J., Israel, E., Jain, M., Needham, DM., Randolph, AG., Rubenfeld, GD., Schoenfeld, D., Thompson, BT., Ware, LB., Young, D., & Harabin, AL. (2010). Beyond mortality: future clinical research in acute lung injury. *American Journal of Respiratory and Critical Care Medicine*, Vol.181, No.10, (May 2010), pp.1121-7, ISSN 1073-449X
- Tremblay, LN., Miatto, D., Hamid, Q., Govindarajan, A., & Slutsky, AS. (2002). Injurious ventilation induces widespread pulmonary epithelial expression of tumor necrosis factor-alpha and interleukin-6 messenger RNA. *Critical Care Medicine*, Vol.30, No.8, (August 2002), pp.1693–1700, ISSN 1530-0293
- Turtoi, A., De Pauw, E., & Castronovo, V. (2011a). Innovative proteomics for the discovery of systemically accessible cancer biomarkers suitable for imaging and targeted therapies. *American Journal of Pathology*, Vol.178, No.1, (January 2011), pp.12-8 ISSN 0002-9440
- Turtoi, A., Dumont, B., Greffe, Y., Blomme, A., Mazzucchelli, G., Delvenne, P., Mutijima, EN., Lifrange, E., De Pauw, E., & Castronovo, V. (2011b). Novel Comprehensive Approach for Accessible Biomarker Identification and Absolute Quantification from Precious Human Tissues. *Journal of Proteome Research*, Vol.10, No.7, (July 2011), pp.3160-82, ISSN 1535-3907
- Venembre, P., Boutten, A., Seta, N., Dehoux, MS., Crestani, B., Aubier, M., & Durand, G. Secretion of alpha 1-antitrypsin by alveolar epithelial cells. *FEBS Letters*, Vol.346, No.2-3, (June 1994), pp.171-4. ISSN 0014-5793
- Vesterberg, O., Palmberg, L., & Larsson, K. (2001). Albumin, transferrin and alpha2-macroglobulin in bronchoalveolar lavage fluid following exposure to organic dust in healthy subjects. *International Archives of Occupational and Environmental Health*, Vol.74, No.4, (May 2001), pp.249-54, ISSN 1432-1246
- Villar, J., Blanco, J., Zhang, H., & Slutsky, AS. (2011). Ventilator-induced lung injury and sepsis: two sides of the same coin? *Minerva Anestesiologica*, Vol.77, No.6, (June 2011), pp.647-53, ISSN 1827-1596
- Wattiez, R., Hermans, C., Bernard, A., Lesur, O., & Falmagne, P. (1999). Human bronchoalveolar lavage fluid: two-dimensional gel electrophoresis, amino acid microsequencing and identification of major proteins. *Electrophoresis*, Vol.20, No.7, (June 1999), pp.1634-45, ISSN 1522-2683
- Wu, J., Kobayashi, M., Sousa, EA., Liu, W., Cai, J., Goldman, SJ., Dorner, AJ., Projan, SJ., Kavuru, MS., Qiu, Y., & Thomassen, MJ. (2005). Differential proteomic analysis of

bronchoalveolar lavage fluid in asthmatics following segmental antigen challenge. *Molecular & Cellular Proteomics*, Vol.4, No.9, (September 2005), pp.1251-64, ISSN 1535-9484

Zhang, L., Jia, X., Zhang, X., Cao, J., Yang, P., Qiu, C., Shen, Y., Ma, F., Liu, L., Sun, J., Shen, F., Yin, L., Liu, L., Yao, Y., & Lu, H. (2010). Alpha-1 antitrypsin variants in plasma from HIV-infected patients revealed by proteomic and glycoproteomic analysis. *Electrophoresis*, Vol.31, No.20, (October 2010), pp.3437-45, ISSN 1522-2683



Proteomics - Human Diseases and Protein Functions

Edited by Prof. Tsz Kwong Man

ISBN 978-953-307-832-8

Hard cover, 438 pages

Publisher InTech

Published online 10, February, 2012

Published in print edition February, 2012

Biomedical research has entered a new era of characterizing a disease or a protein on a global scale. In the post-genomic era, Proteomics now plays an increasingly important role in dissecting molecular functions of proteins and discovering biomarkers in human diseases. Mass spectrometry, two-dimensional gel electrophoresis, and high-density antibody and protein arrays are some of the most commonly used methods in the Proteomics field. This book covers four important and diverse areas of current proteomic research: Proteomic Discovery of Disease Biomarkers, Proteomic Analysis of Protein Functions, Proteomic Approaches to Dissecting Disease Processes, and Organelles and Secretome Proteomics. We believe that clinicians, students and laboratory researchers who are interested in Proteomics and its applications in the biomedical field will find this book useful and enlightening. The use of proteomic methods in studying proteins in various human diseases has become an essential part of biomedical research.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mei-Ling Tsai, Shu-Hui Chen, Chih-Ching Chang and Ming-Ho Wu (2012). From Biomarker Discovery to Clinical Evaluation for Early Diagnosis of Lung Surgery-Induced Injury, *Proteomics - Human Diseases and Protein Functions*, Prof. Tsz Kwong Man (Ed.), ISBN: 978-953-307-832-8, InTech, Available from: <http://www.intechopen.com/books/proteomics-human-diseases-and-protein-functions/from-biomarker-discovery-to-clinical-evaluation-for-early-diagnosis-of-lung-surgery-induced-injury>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen