Differential Proteomic Analysis of Human Lung Adenocarcinoma Cell Line A549 and Normal Embryo Cell Line HELF

Zunling Li[1] Xueyan Yu[2] Guoqiang Hu[3] Shuhong Shao[4] Keli Tian[2] Shilian Liu[5] Xiaoyan Hu[2]

1. Department of Biochemistry and Molecular Bioloy, Binzhou Medical College, Binzhou, 256603,
2. Institute of Biochemistry and Molecular Biology, School of Medicine, Shandong University, Jinan 250012,
3. Department of Thoracic Surgery , Qilu Hospital of Shandong University, Jinan 250012,
4. Department of psychology, Binzhou Medical College, Binzhou, 256603,
5. Experimental Centre of Biochemistry and Molecular Biology, School of Medicine, Shandong University, Jinan 250012, Shandong, China)

Abstract **Objective** To find the novel tumor markers and explore the proteins related tumorigenicity, we studied the differential proteomic expressions between human lung adenocarcinoma cell line A549 and normal embryo cell line HELF. **Methods** The differential proteomic expressions between A549 and HELF were seperated by two–dimensional electrophoresis, and the spots of interest were digested and identified by matrix–assisted laser desorption/ionization peptide mass fingerprinting. **Result** The average deviations of spot position were1.87±0.12 mm in isoelectrofocusing direction and 1.02±0.21 mm in sulfate–polyacrylamide gel electrophoresis direction. For the A549 cells, a total of 1154±86 spots were detected, 893 spots were matched and 329 spots was not matched. For the HELF cells, a total of 1338±79 spots were detected and 445 spots were not matched. Part spots were characterized. Some proteins were the products of oncogenes and others were involved in the regulation of cell cycle and inflammation. **Conclusion** There is significant difference between A549 and HELF, and some proteins can be considered as a novel tumor markers.

Key words 2–DE; MALDI–TOF–MS; Proteome; Lung cancer

Lung cancer is the first reason resulting death in the western world and the 5–year survival rate of the disease has not been improved, so to find the novel tumor markers is essential for early diagnosis of the lung cancer. There is a growing interest in applying proteomics to gain insight into disease processes, develop new markers for diagnosis and early detection of diseases and accelerate drug development. Proteomics has already been utilized for the study of lung cellular lineages, bronchoalveolar lavage fluid, and for the intrinsic comparison among different tumors. Some tumor–related markers have been identifed and are applied in clinic diagnosis due to the proteomic approach. For example, AOE372 (an antioxidant enzyme), ATP5D(an ATP synthase subunit) and HSP27 are significantly over–expressed in lung adenocarcinoma[1,2].

Two–dimension electrophoresis (2–DE) is the principal step of proteomics and widely used in comparative studies of protein expression levels between healthy and diseased states. Hence, in this study, the total proteins from A549 and control HELF were separated and identified with 2–DE and matrix–assisted laser desorption/ionization peptide mass fingerprinting (MALDI–TOF MS). The results presented here will no doubt provide clues to further study of the carcinogenic mechanisms, diagnosis, and the therapy of lung cancer[3].

MATERIALS AND METHODS

Cell lines and cell culture

The human non–small cell lung cancer cell line A549 was servere by Biochemistry Lab of Medical school, Shandong Universty. The normal embryo cell

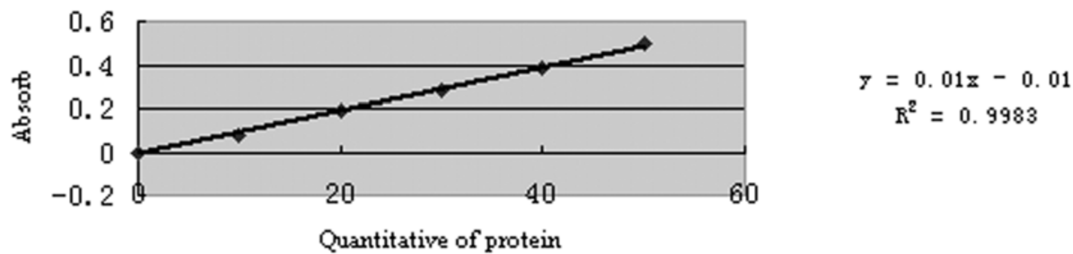

Fig.1 The standard curve by Bradford

line HELF was gained as a precious gift from experimental center of Qilu Hospital. Cells were maintained in RPME1640 containing 10% fetal bovine serum and 1% penicillin/streptomycin.

2-DE

The A549 and HELF cells was lysed with lysis buffer(8 M urea, 2 M thiourea, 2% NP-40, 1% TritonX-100, 4% CHAPS, 40mM Tris-HCl, 65mM DTT, 2% IPGphore buffer)respectively. The lysates were vortexed followed by incubution at 37℃ for 1h. The lysates were centrifuged at 15,000 rpm for 30 min at 4℃[4].The concentration of the total proteins was determined with the BCA Protein Assay Kit. Isoelectrofocusing (IEF) was performed successively using gradient voltage 30V 6h; 60V 6h; 200V 1h; 500V 1h; 1000V 1h; 8000V 2h; 8000V 6h. The second-dimension sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)was conducted at 580V in running buffer (25mM Tris-HCl, pH 8.8, 198mM glycine, and 1% SDS). After electrophoresis, the protein spots were visualized by silver-based staining technique with the protein silver kit. Differential protein expression was determined using PDQust software[3].

MALDI-TOF MS analysis

Twenty differential spots of interest were excised from preparative gels using biopsy punches and transferred to a 1.5 ml siliconized Eppendorf tube. The dried gel pieces were then rehydrated with 50 mM ammonium bicarbonate containing modified trypsin, and digested overnight at 37℃. Peptide were eluted using matrix solution. Peptide masses were determined by MALDI-TOF mass spectrometer. Proteins were identified with peptide mass fingerprinting data by searching software Peptident.

Statistical analysis

Using SPSS11.0 software, the spots of A549 and HELF were treated by a two-sided Students *t* test. $p<$ 0.05 was considered statistically significant.

RESULTS

Quantitative analysis of protein by Bradford

We performed quantitative analysis of protein by Bradford and gained the Regression equation, $y = 0.01x-0.01$ ($R^2=0.998$). The concentration of protein of HELF was 1.2μg/μl and that of A549 was 2.7μg/μl.

The sliver containing maps of A549 and HELF

Taking into account the reproducibility, 2-DE was repeated three times for a same sample. 30 spots were chosen randomly to calculate the deviation of the spot position. The spot positional deviation was 1.87±0.12mm in IEFdirection and 1.02±0.21mm in SDS-PAGE direction. For the A549 cells, a total of 1154±86 spots were detected ,893 spots were matched and 329 spots was not matched. For the HELF cells, a total of 1338±79spots were detected and 445 spots were not matched(Fig. 2).

Analysis of the differential protein by MALDI PMF

Taking into account the accuracy and complexity, only the most prominent proteins overexpressed in

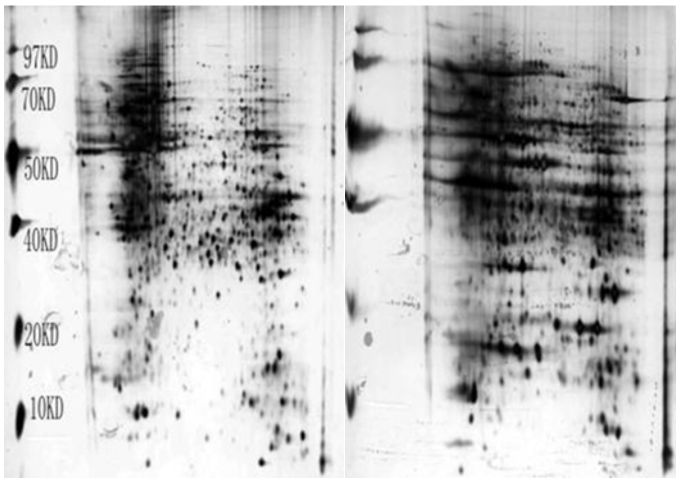

Fig. 2 The sliving maps of A549 and HELF

Table 1 Differential protein expression in A549 and HELF cells

Spot number	Protein	pI value	Biologica category
1	ATP synthase Dchain(ATPQ)	5.20	energy metabolism
2	Gastrointestinal tumor–associated	7.56	products of oncogenes
3	Ras–related protein Rab–39	6.90	products of oncogenes
4	G1/S–specific cyclin D2	5.06	the regulation of cell cycle
5	CapthepsinD	5.31	degradation
6	Glutathione–S–transferaseP	5.45	metabolism associated
7	Cytokeratin18	5.00	cytoskeleton
8	IgG dependent histamine releasing factor	4.46	inflammation
9	β–Tubulin	4.85	cytoskeleton
10	Protein phosphatase2	5.89	Metabolism associated

A549 with respect to the corresponding spots in HELF were selected for further mass spectrometric analysis. The intensity of these proteins was decreased at least 2–fold in HELF cells. A total number of 20 protein spots were selected for tryptic digestion and mass spectrometric analysis. Protein species were classified into five main groups according to their biological function: the products of oncogenes, cytoskeleton, inflammation, chaperones, energy metabolism and so on (Table 1).

DISCUSSION

It is a new pathway of treating cancer to find out the differential proteins between healthy and cancerous cell by proteomic technology. The goals of cancer proteomics are to dicover more sensitive biomarkers for early diagnosis, treatment and prediction. In our present study, we found out the differential proteins and identified the characters of these proteins. Some differential proteins were significant to early diagnosis and prognosis in lung cancer. But the results still lack powerful persuasion beacause of two reasons. One was to use cell line not primary tissues from patient, the other was to use human embryo cell line. It is all known that embryo proteins differ from adult proteins due to the temporal specificity of gene expression. Though there were shortcoming, some identified proteins were still considered as biomarkers. We will conquer above reasons to further study.

It is important to establish the reproducible 2–DE, which guaranteed the experimental consistency and the accurate results. The reproducibility of 2–DE profiles was very important to the key point. The protocols that may affect protein modifications should be avoid-

ed, as these changes may result in specific loss gain or shift of protein species. In our study, 2–DE was repeated three times for a same sample. 30 spots were chosen randomly to calculate the deviation of the spot position; which was 1.87±0.12mm in IEF direction and 1.02±0.21mm in SDS–PAGE direction, which indicated that we established the reproducible 2–DE and insured the uniformity and reliabilty of results.

A549 cell and HELF cell originated from epithelium. The protein spots which were not matched indicated there were differential proteins, but we did not eliminate the influnence of embryo. Identifying the character of part proteins by MALDI–TOF MS, we found some proteins were associated with embryo and others were mainly the products of oncogenes, cytoskeleton, inflammation, chaperones and energy metabolism. For example: ATP synthase Dchain (ATPQ), IgG dependent histamine releasing factor , Cytokeratin18 and so on. Cathepsin D is currently used as markers of breast and lung cancer, respectively. We found the expression of cathepsin D was up–regulated in A549 cells, but the mechanism was still not known. The presence of cathepsin D in lung tumors should be further studied in our future study, since this protein could be of clinical relevance to the diagnosis and other cancers[5]. G1/S–specific cyclin D2 is considered as the regulator of cell cycle. Research indicated Cyclin D was related with carcinogenesis of lung cancer and a potential prognosis marker for non–small cell lung cancer [6]. Gastrointestinal tumor–associated and Ras–related protein Rab–39 were the products of the proto–oncogene. The mechanism of up–regulatin in tumor cells needs further study. Other proteins are associated with metabolism , inflammation and cytoskeleton, which are general proteins and only indicate the metabolism of tumor blooms. So far no tumor markers are related to these proteins.

The comparative proteomics is an effective platform to study cancer[7]. In conclusion, in our study we establish the reproducible 2–DE and find Cathepsin D as a tumor marker, which is important for early diagnosis of lung cancer.

REFERENCE

1. G.A Chen, T.G Gaharib, C.C Huang, et al. Proteomic analysis of lung adenocarcinoma: identification of a highly expressed set of proteins in tumors. Clin Cancer Res, 2002,8: 2298–2305.

2. Patricia Alfonso, Myriam Catala, Maria Luisa Rico–Morales, et al. Proteomic analysis of lung biopsies: Differential protein expression profile between peritumoral and tumoral tissue. Proteomics, 2004,4:442–447.

3. Li Cui, Chen Zhuchu, Xiao Zhiqiang, et al. Comparative proteomics analysis of human lung squamous carcinoma. Biochemical and Biophysical Research Communications. 2003, 309:253–260.

4. N. Araki, T. Morimasa, T. Sakai, et al. Comparative analysis of brain proteins from P53–deficient mice by two–dimensinal electrophoresis. Electrophoresis. 2000, 21:1880 1889.

5. Patricia Alfonso, Myriam Catala, Maria Luisa Rico–Morales, et al. Proteomic analysis of lung biopsies: Differential protein expression profile between peritumoral and tumoral tissue. Proteomics, 2004,4: 442–447.

6. J. Niklinski, W.Niklinska, J.Laudanski, et al. Prognostic molecular markers in non–small cell lung cancer. Lung cancer, 2001,34(suppl.2):53–58.

7. W.Wu, J.J.Kavanagh. Proteomics in cancer research. Int J Gynecol Cancer, 2002, 12: 409–423.