



Comparative autoantibody profiling before and after appearance of malignance: Identification of anti-cathepsin D autoantibody as a promising diagnostic marker for lung cancer

Xue Luo^{a,b}, Feng Lu^c, Hong-Li Wang^a, Na Wang^a, Wei-Hua Li^a, Ning Guo^a, Hong-Xia Wang^a, Qing Xia^{a,*}

^a National Center of Biomedical Analysis, 27 Taiping Road, Beijing 100850, China

^b Department of Occupational Health, Third Military Medical University, 30 Gao Tan Yan Road, Chong qing 400038, China

^c Department of Molecular Immunology, Institute of Basic Medical Science, 27 Taiping Road, Beijing 100850, China

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ABSTRACT

Cancer patients frequently develop autoantibodies. To test the hypothesis that the appearance of autoantibodies precedes the clinical diagnosis of cancer, we applied an immunoproteomic approach to compare autoantibody profiles before and after appearance of malignances. Proteins from A549 cells, a lung adenocarcinoma cell line, were separated by two dimensional electrophoresis and then immunoblotted with serum samples from 8 individuals who were eventually diagnosed with lung cancer. Compared with autoantibody profiles from 3 years prior to the appearance of malignances, 21 immunoreactive spots newly appeared or presented with stronger staining intensity when clinical diagnoses were made. Among them, 10 matched spots on 2-DE gels were identified by mass spectrometry analysis as 5 proteins. With immunoprecipitation analysis, the antigenicity of protein cathepsin D was confirmed, and notably, in lung cancer sera, the occurrences of autoantibodies against the specific forms of cathepsin D differed significantly from the control groups ($p < 0.05$). Our findings suggest that harnessing immunity may have utility for early cancer marker discovery, and that comparing autoantibodies to specific forms of cathepsin D may be a promising early marker of lung cancer.

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1. Introduction

Lung cancer remains at epidemic proportions and continues to be the leading cause of cancer death in both males and females. Early tumor markers to detect lung cancers when they are smaller and presumably at earlier and more curable stages are urgently needed to improve its survival rate. Theoretically, there is no detection instrument that rivals the sensitivity and specificity of the immune system. Hence, one promising approach to early detection is to identify and examine the immune response to cancer, as opposed to the cancer itself [1].

In the present study, we evaluated a prospectively assembled collection of frozen serum samples to test the hypothesis that the appearance of autoantibodies precedes the diagnosis of lung cancer. We analyzed the immunoreactivity of serum antibodies to proteins from A549 cells in lung cancer patients by immunoproteomic technique. Specially, we compared the autoantibody profiles of sera sample from 8 lung cancer patients, whose sera were

collected at the time of diagnosis and correspondingly 3 years before the appearance of malignancy. By comparing each pair of immunoblot patterns, 21 immunoreactive spots newly appeared or presented with stronger staining intensity in sera after the diagnosis than observed in the 3 years prior to diagnosis. Among them, 10 matched spots on 2-DE gels were identified by mass spectrometry as 5 proteins. With immunoprecipitation analysis, the antigenicity of protein cathepsin D was further confirmed, and the occurrences of autoantibody to cathepsin D were also demonstrated in lung cancer patients.

2. Materials and methods

2.1. Sera and Cell line

Since 2002, the Department of Laboratory Diagnostics (General Hospital, Chinese PLA), has stored serum samples (at -70°C) which were obtained from annual physical examinations of Chinese Armed Forces. A review of medical records identified 8 individuals who were clinically diagnosed with lung cancer and for whom stored serum samples were obtained before the discovery of a malignancy. Serum samples of these 8 patients with lung cancer at the time of diagnosis and correspondingly 3 years, 2

* Corresponding author. Address: Department of Molecular Immunology, Institute of Basic Medical Science, 27 Taiping Road, Beijing 100850, China. Fax: +86 10 68159436.

E-mail address: xiaqing@proteomics.cn (Q. Xia).

years, and 1 year before diagnosis, were enrolled in this study. For immunoprecipitation analysis, Sera from malignant ($n = 47$) and nonmalignant ($n = 23$) lung disease patients and 20 healthy subjects were analyzed. The malignant samples were comprised of squamous cell carcinoma ($n = 14$), adenocarcinoma ($n = 15$), and small cell carcinoma ($n = 18$; Table 1). Informed consent was obtained from all study participants.

The A549 cell line used in this study was cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 unites/ml streptomycin.

2.2. 2-DE and Western blot

Proteins derived from the extracts of A549 cells were separated by 2-DE as described previously. The 2-DE images were captured using ImageScanner (Amersham Pharmacia Biotech). For hybridization with serum, unstained PVDF membranes were incubated

with serum obtained from patients or with normal control serum at a 1:500 dilution for 1 h at room temperature.

2.3. Protein identification by MS analysis

After the Western blot and film exposure to the PVDF membrane incubated with ECL, we stained the PVDF membranes with CBB R250. The profile of the proteins on the membrane was the same as that of the map of 2-DE gels visualized by CBB R250 staining. We put the film exposed to the PVDF membrane (which was incubated with the ECL reagents) onto the stained PVDF membrane to localize the exposed spot on the PVDF membrane. We matched the spots on the PVDF membrane to the 2-DE map of the same sample. Next, we excised the identified protein spots from the 2-DE gel stained by CBB R250 and processed them for mass spectrometry analysis as described previously [5]. Protein identification was repeated at least twice using spots from different gels. Briefly,

Table 1

The pathological characteristics and the five forms of cathepsin D distribution in patients with lung cancer and controls.

Feature	No. cases examined	Cathepsin D-Abs+ (%)				
		66 kDa	52 kDa	48 kDa	42 kDa	34 kDa
<i>Control</i>						
Health	20	0 (0)	4 (20.0)	3 (15)	2 (10)	2 (10)
Nonmalignant lung disease	23	0 (0)	5 (21.7)	4 (17.4)	2 (8.7)	3 (13.0)
Lung cancer	47	17 (36.2) ^A	42 (89.4) ^A	29 (61.7) ^A	16 (34.0) ^A	28 (59.6) ^A
<i>Sex</i>						
Male	28	14 (50.0) ^B	25 (89.3)	15 (53.6)	11 (39.3)	15 (53.6)
Female	19	3 (15.8)	17 (89.5)	14 (73.7)	5 (26.3)	13 (68.4)
<i>Age at diagnosis (yr)</i>						
≥60	20	12 (60.0)	18 (90.0)	15 (75.0)	9 (45.0)	16 (80.0) ^C
<60	27	15 (55.6)	24 (88.9)	14 (51.9)	7 (25.9)	12 (44.4)
<i>Stage</i>						
Early disease (1–2)	13	4 (30.8)	9 (69.2) ^D	7 (53.8)	2 (15.4)	8 (61.5)
Advanced disease (3–4)	34	13 (38.2)	33 (97.1)	22 (64.7)	14 (41.2)	20 (58.8)
<i>Histologic type</i>						
Adenocarcinoma	15	5 (33.3)	13 (86.7)	7 (46.7)	3 (20.0) ^E	5 (33.3) ^F
Squamous cell carcinoma	14	9 (64.3) ^G	13 (92.3)	9 (64.3)	9 (64.3) ^G	10 (71.4)
Small cell carcinoma	18	3 (16.7)	16 (83.3)	13 (72.2)	4 (22.2)	13 (72.2)

Note: A: lung cancer vs. healthy control and nonmalignant lung disease; B: male vs. female; C: <60 vs. >60; D: early disease vs. advanced disease; E: adenocarcinoma vs. squamous cell carcinoma; F: adenocarcinoma vs. small cell carcinoma; G: squamous cell carcinoma vs. small cell carcinoma; significantly different by χ^2 -square test.

Table 2

Autoantigens Proteins identified by PMF.

Item No.	Spot No.	Acc No.	Protein name	M_{Theor}	M_{obv}	pI_{Theor}	pI_{obv}	Score	Sequence coverage (%)	Number of matched peptides	Number of non-matched peptides	RMS error (ppm)
1.	4	gi 4503571	Enolase 1	47139	47290	7.01	7.37	145	41	13	4	97
2.	5	gi 4503571	Enolase 1	47139	47290	7.01	7.09	143	38	12	2	48
3.	6	gi 4503571	Enolase 1	47139	47290	7.01	6.94	160	57	20	13	30
4.	7	gi 4503571	Enolase 1	47139	47290	7.01	7.40	118	37	13	7	121
5.	11	gi 4504447	Heterogeneous nuclear ribonucleoprotein A2/B1 isoform A2	35984	41120	8.67	7.29	153	55	16	17	62
6.	12	gi 154355000	KH-type splicing regulatory protein (FUSE binding protein 2)	68393	76330	8.52	7.51	245	44	26	9	42
7.	13	gi 154355000	KH-type splicing regulatory protein (FUSE binding protein 2)	68393	76330	8.52	7.38	232	45	24	9	81
8.	16	gi 4505753	Phosphoglycerate mutase 1	28786	27100	6.67	7.37	128	43	10	3	64
9.	17	gi 4504447	Heterogeneous nuclear ribonucleoprotein A2/B1 isoform A2	35984	22820	8.67	8.35	105	43	13	26	54

pI_{Theor} and M_{Theor} , calculated isoelectric point and molecular weight according to the protein sequence; pI_{obv} and M_{obv} , the calculation of experimental pI and Mr based on migration of the protein spot on 2-DE gels.

Peptide mass finger printings (PMF) were acquired using a MALDI-TOF REFLEX III instrument (Bruker Daltonics, Bremen, Germany). Nine spots (Table 2) and protein band (66 kDa) immunoprecipitated from cell lysates were analyzed by NanoLC–HDMS MS/MS on Acquity™ Nano UPLC system (Waters Corp., Milford, USA) and a Synapt high-definition mass spectrometry (HDMS) with nano-spray ion source (Waters). Spot eight was analyzed by NanoLC–FT ICR MS/MS on 9.4T APEX Qe (Bruker Daltonics, Billerica, MA, USA) with an HApollon II nanoESI source in positive ion mode. PMF data was searched on local Mascot v2.1 against NCBI nr 070815 (5371018 sequences; 1856562799 residues). The MS/MS ion database search was conducted using Mascot on the freely accessed internet website (<http://www.matrixscience.com>); the database is the non-redundant protein database NCBI nr 20070926 (5519594 sequences; 1911975371 residues).

2.4. Immunoprecipitation

Immunoprecipitations were performed by incubating whole cell extracts from A549 cells with polyclonal anti-cathepsin D antibody (SC10725, Santa Cruz Biotechnology) and preincubated with protein A/G-Sepharose (Santa Cruz Biotechnology).

2.5. Statistical analysis

The chi-squared (χ^2) test was used to compare the association between the presence of the five forms of cathepsin-D and several clinicopathologic parameters, as well as differences between the lung cancer and normal groups. All comparisons were two-tailed, and $P < 0.05$ was considered statistically significant. All data analysis was performed with SPSS V11.5 (SPSS, Inc., Chicago, IL).

3. Results

3.1. Comparison of autoantibody profiles of serum samples

To observe the changing of humoral response during the process of tumorigenesis, we compared autoantibody profiles of 8 individuals newly diagnosed with lung cancer and correspondingly

3 years before the diagnosis. We first attempted to establish their autoantibody profiles individually. A549 cellular proteins were separated by 2-DE (Fig. 1A) and transferred onto PVDF membranes. These 8 pairs of serum samples were investigated for antibodies to A549 cell protein extracts individually. Each membrane was treated with one 1:500 diluted serum sample as the primary antibody and with 1:10000 diluted sheep antihuman IgG as secondary antibody. Western Blotting with each serum sample was repeated at least twice, and the results were highly replicable. Two of the 8 patients had the same autoantibody patterns before and after clinical diagnosis, while distinct changes of autoantibody profiles were observed in the other six pairs of serum samples. Generally, compared with that of prediagnosis, numbers of immunoreactive spots increased and the intensity was stronger in autoantibody profile at the time of the diagnosis. As representative results of WB (Fig. 1B) three years before appearance of malignancy, no obvious immunoreactive spots could be observed in serum from Patient 1, while seven (spots 1–7) immunoreactive spots appeared when clinical diagnosis was confirmed. All together, compared with 3 years before the diagnosis, 21 immunoreactive spots newly appeared or with stronger staining intensity were observed in sera after the diagnosis was confirmed. Among them, 10 matched spots on 2-DE gels were identified by MS analysis, as indicated in Fig. 1A.

3.2. Identification of immunoreactive protein spots

These 10 matched positive spots were excised from the corresponding CBB R250 stained 2-DE gels, in-gel digested by trypsin. The resulting peptides were analyzed by MALDI-TOF MS and ESI-MS/MS. Spot eight was subjected to NanoLC–FT ICR MS/MS analysis on 9.4T APEX Qe. A total of 5 proteins were identified from 10 spots, since some contiguous spots with similar molecular mass were identified to be isoforms of 1 protein. The contiguous spots 4, 5, 6, and 7 were identified as Enolase 1, spots 12, 13 as KH-type splicing regulatory protein (FUSE binding protein 1, FBP1), and spots 11, 17 as heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP-A2/B1) isoform A2 (Table 2). For spot eight, protein mixture of cathepsin D preproprotein and tyrosine was identified with low confidence by PMF; therefore, NanoLC–Q-FT ICR MS/MS was

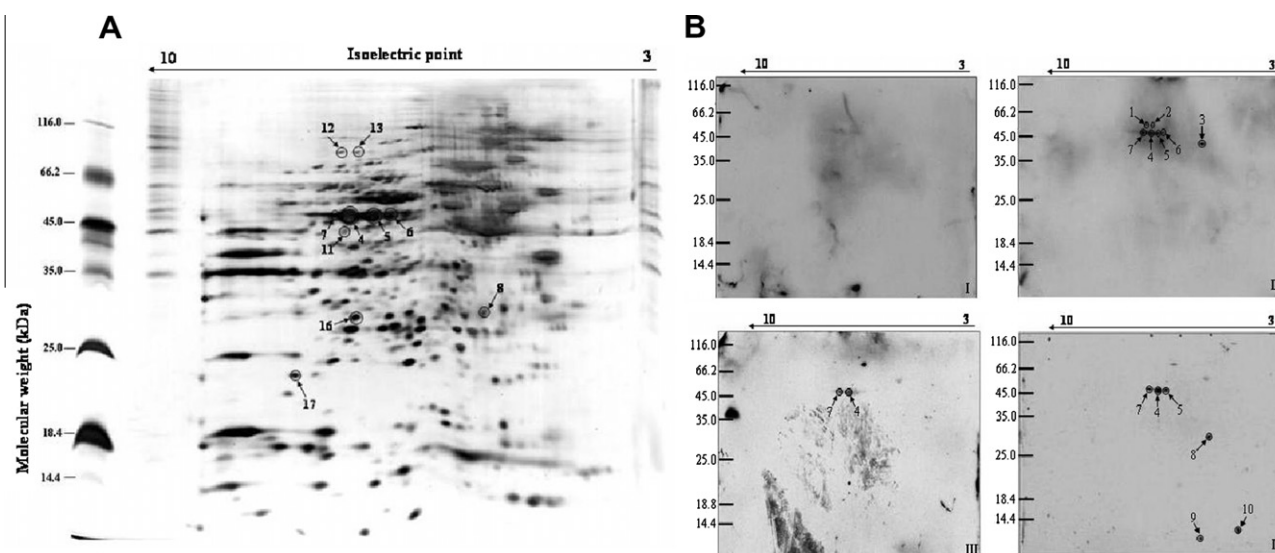


Fig. 1. Comparison of autoantibody profiles of sera from individuals before and after diagnosed with lung cancer. (A) CBB R250 staining of A549 cell-derived proteins separated by two-dimensional PAGE. Ten spots that differentially recognized by serum 3 years before and after clinical diagnosis, and got identification by MS analysis are circled and marked. The original gel size was $8 \times 7 \times 1.0 \text{ mm}^3$. After transferred onto nitrocellulose membranes, proteins were reacted with serum samples diluted at 1/500 from 8 pairs of individuals who eventually developed into lung cancer. (B) Representative 2D-immunoblotting patterns of A549 cell proteins with sera sample from Patient 1 before (i) and after diagnosis (ii), and from Patient 2 before (iii) and after diagnosis (iv).

used for further confirmation (Table 3). For ESI MS/MS measurements, 4 proteins were identified from 8 spots, Enolase 1 from spot 4, 5, 6 and 7; FBP1 from spot 12, 13, and phosphoglycerate mutase

1 from spot 16. Cathepsin D preproprotein from spot 8 was identified by both nanoLC ESI-MS/MS and 9.4T Q-FT. However, spot 11, 17 did not show confident results by ESI MS/MS.

Table 3

Autoantigens proteins identified by MS/MS.

Item No.	Spot No. or band	Acc No.	Protein name	M_{Theor}	M_{obv}	pI_{Theor}	pI_{obv}	Score	Sequence Coverage (%)	Number of matched peptides	RMS error (ppm)	Instrument
1.	4	gi 4503571	Enolase 1	47139	47290	7.01	7.37	1324	50	25	14	HDMS
2.	5	gi 4503571	Enolase 1	47139	47290	7.01	7.09	903	38	18	67	HDMS
3.	6	gi 4503571	Enolase 1	47139	47290	7.01	6.94	444	23	12	103	HDMS
4.	7	gi 4503571	Enolase 1	47139	47290	7.01	7.40	147	12	4	43	HDMS
5.	8	gi 4503143	Cathepsin D preproprotein	44524	28820	6.10	5.76	235	37	11	3	9.4T Q-FT-MS/MS
6.	12	gi 154355000	Tyrosine KH-type splicing regulatory protein (FUSE binding protein 2)	68393	76330	8.52	7.51	207	6	4	21	HDMS
7.	13	gi 154355000	Tyrosine KH-type splicing regulatory protein (FUSE binding protein 2)	68393	76330	8.52	7.38	145	4	3	18	HDMS
8.	16	gi 4505753	Phosphoglycerate mutase 1	28786	27100	6.67	7.37	216	12	4	18	HDMS
9.	66 kDa band	gi 4503143	Cathepsin D preproprotein	44524	28820	6.10	5.76	299	14	6	42	HDMS

pI_{Theor} and M_{Theor} , calculated isoelectric point and molecular weight according to the protein sequence; pI_{obv} and M_{obv} , the calculation of experimental pI and Mr based on migration of the protein spot on 2-DE gels.

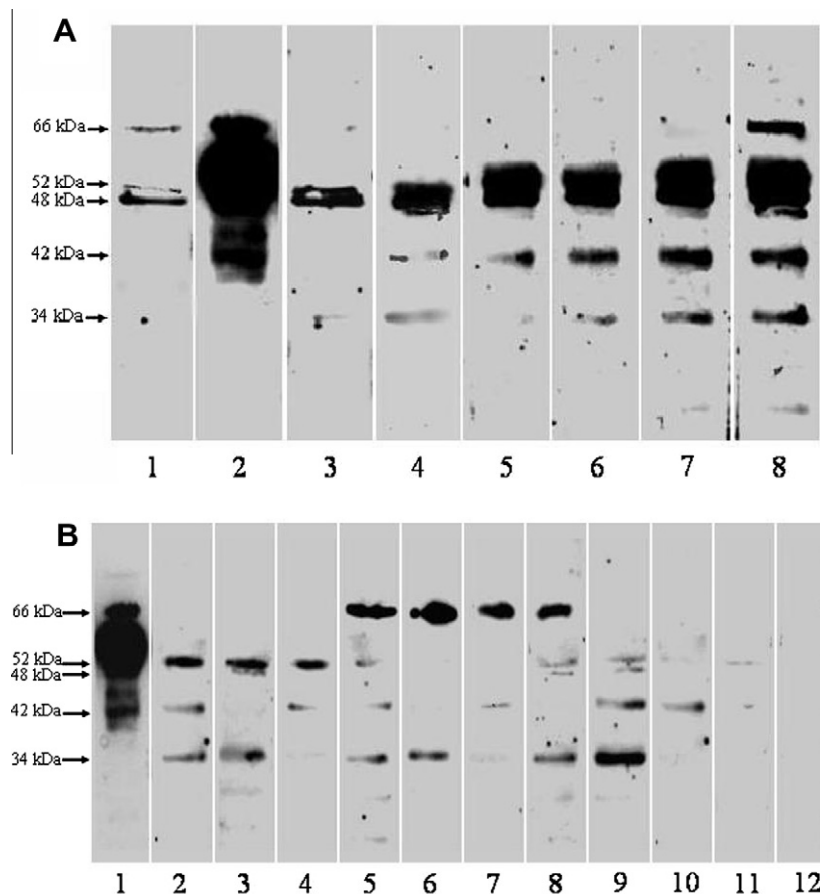


Fig. 2. Validation of the antigenicity of cathepsin D. (A) Western blot analysis of proteins, immunoprecipitated from A549 cells by anti-cathepsin D antibody and separated by 1-D PAGE, was performed with sera from Patient 2 collected when the diagnosis was made and correspondingly 1, 2, 3 years before discovery of malignancy. Whole cell extracts (WCL) were immunoprecipitated with an anti-cathepsin D antibody, and equal amounts of the immunoprecipitated protein were used for immunoblot analysis with anti-cathepsin D antibody (lane 2), sera from Patient 2 (lane 8), and his sera before discovery of malignancy [before 3 years (lane 5), 2 years (lane 6), and 1 year (lane 7)]. Lane 1, input; Lane 3, serum from healthy control; Lane 4, serum from patient with nonmalignant lung disease. (B) Occurrence of cathepsin D autoantibodies in serum from lung cancer patients. In order to find out the occurrence of cathepsin D autoantibody in cancer, Western blot analysis of proteins, immunoprecipitated from A549 cells by anti-cathepsin D antibody and separated by 1-D PAGE, was performed with 47 lung cancer patient sera, 20 normal control sera and 23 nonmalignant lung disease sera. Only typical results are shown. Lane 1, immunoblot analysis with anti-cathepsin D antibody. Lanes 2–10, sera from patients with lung cancer. Lane 11, serum from patient with nonmalignant lung disease. Lane 12, sera from healthy volunteer.

3.3. Confirmation of the antigenicity of cathepsin D in lung cancer patients

Spot 8, which was negative in sera from Patient 2 three years before the appearance of cancer and showed strong staining intensity when the diagnosis confirmed (Fig. 1B III, IV), was identified to be cathepsin D preproprotein with MS analysis. In order to confirm the antigenicity of cathepsin D, an additional type of experiment was conducted. Western blot analysis of proteins, immunoprecipitated from A549 cells by anti-cathepsin D antibody and separated by 1-D PAGE, was performed with sera from Patient 2 (Fig. 2). Whole cell lysates (WCL) were immunoprecipitated with an anti-cathepsin D antibody, and equal amounts of the immunoprecipitated protein were used for immunoblot analysis with anti-cathepsin D antibody (Fig. 2, lane 2), sera from Patient 2 the year when diagnosis confirmed (lane 8), as well as 3 years (lane 5), 2 years (lane 6), and 1 year (lane 7) before discovery of malignancy respectively. All of the four serum samples showed positive reactivity against pro-forms of cathepsin D (48, 52 kDa), while the intensity of immunoreactivity to mature molecular weight form (34 kDa) increased by degree. Interestingly enough, besides recognizing the three well-known forms (34, 48, 52 kDa), a novel form of cathepsin D (66 kDa) was recognized by sera at the year of diagnosis, while negative or weakly recognized in the three prediagnosis periods, normal control (lane 3) and nonmalignant lung disease (lane 4).

3.4. Occurrence of cathepsin D autoantibodies in sera from lung cancer patients

To examine the occurrence of cathepsin D autoantibody in lung cancer, the immunoprecipitation analysis was performed with lung cancer patient as well as control sera. Sera from 43 of 47 lung

cancer, 4 of 20 healthy control, and 6 of 23 nonmalignant lung disease subjects showed positive reactivity against cathepsin D. Notably, two other forms with molecular weight as 66 and 42 kDa were observed immunoreactive in lung cancer (Fig. 2B), except for three well-known forms (52, 48, 34 kDa) of cathepsin D. Statistic analysis showed that positive reactivity against five forms of cathepsin D was significantly higher in the lung cancer group when compared to control groups (Table 1).

3.5. Identification of 66 kDa protein the band by NanoLC-HDMS MS/MS

In the present study, 66 kDa, which showed a sensitivity of 36.2% and a specificity of 100% to lung cancer patients, was an area of interest. We confirmed that it was a novel form of cathepsin D by immunoprecipitation and Synapt HDMS DDA (the latest Q-TOF premiere version) analysis. A549 cells were lysed and immunoprecipitations were performed by incubating whole cell extracts with polyclonal anti-cathepsin D antibody. Immunoprecipitates were separated by SDS-PAGE and stained with CBB R250. Interest band (66 kDa) was excised and subjected to in-gel tryptic digestion. The resulting peptides were analyzed by ESI-MS/MS (Fig. 3, Table 3) and showed that 66 kDa form cathepsin D might be another type modification isoform of cathepsin D in lung cancer patients.

4. Discussion

Since tumor-reactive antibodies are elicited and detectable prior to the development of a palpable tumor or circulating antigens, the assessment of the tumor-reactive immune response against autoantigens should represent an early diagnostic and

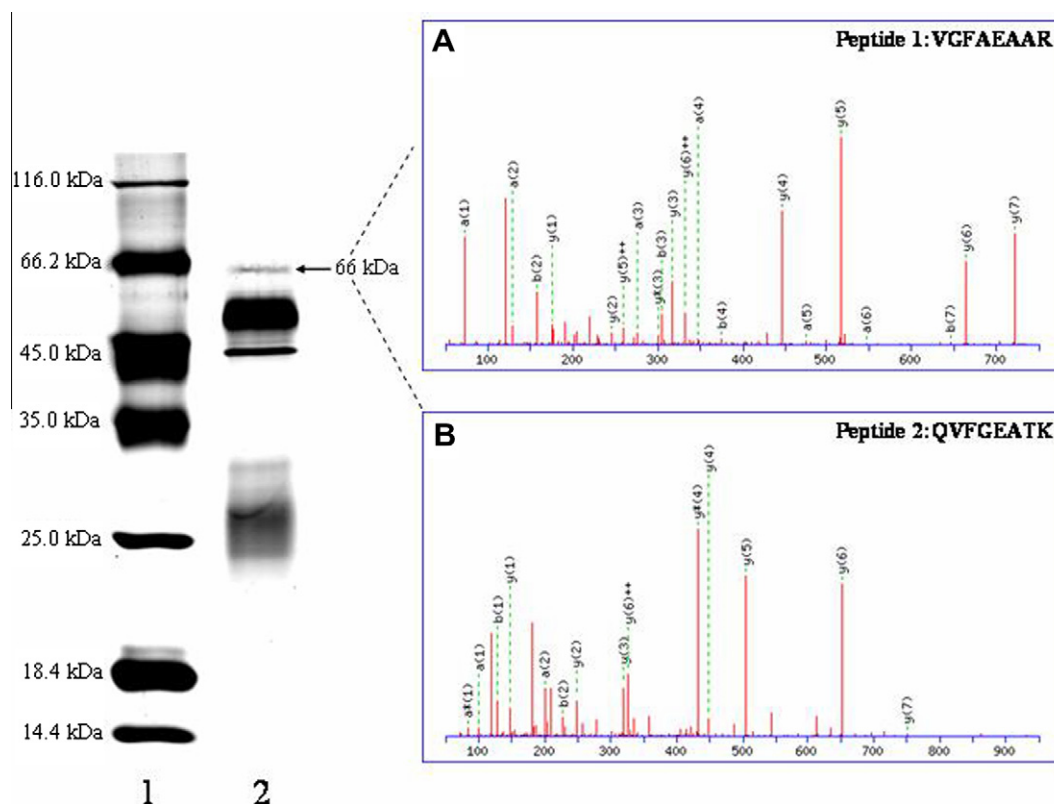


Fig. 3. Identification of protein (66 kDa band) with immunoprecipitation followed by MS/MS analysis. Proteins from cell lysates were immunoprecipitated with antibodies to cathepsin D and then processed for NanoLC-HDMS MS/MS analysis on 9.4T APEX Qe. Lane 1, standard protein molecular marker; Lane 2, proteins immunoprecipitated from A549 cells by anti-cathepsin D antibody and then separated by 1-D SDS-PAGE. (A) MS/MS spectra for inferred peptide1 VGFFAEAR and (B) inferred peptide 2 QVFGEATK.

prognostic marker [2]. Although there are many reports of autoantibodies in patients with malignancies, including lung cancer [3], to our knowledge, this is the first report on the comparative analysis of global autoantibody profiles of sera from lung cancer patients before and after clinical diagnosis. In the present study, we found 21 immunoreactive spots newly appeared or with stronger staining intensity in sera after the diagnosis compared with that of correspondingly 3 years before the diagnosis, and with MS analysis, 10 matched spots on 2-DE gels were identified as 5 proteins. Lung cancer was preceded by or coincided with seroconversion from antibody negative to positive status, suggesting that the novel immune responses are driven by proteins involved in the transformation process, and the five identified autoantigens might be candidates for such tumor markers in lung cancer.

Enolase 1 autoantibody has been detected in the sera from lung cancer patients [4] and also in more than 50% of healthy individuals with low titers [5]. In the present study, we found changes of titer and/or pattern of anti-enolase 1 autoantibody in lung cancer patients' sera. The profile of the spots of enolase 1 antibody in the sera from Patient 1 (after diagnosis) showed three continuously connected spots at the pI range from 6.94 to 7.37 in the 2D blot profile, while only weak intensity could be observed in the sera from the same individual 3 years before appearance of malignancy (Fig. 1BI–II). FBP1 is one of the three far-upstream element binding protein (FBP) family members. Recent findings have shown that FBP is essential for c-myc expression, and reductions in FBP levels reduce c-myc expression and correlate with slower cell growth [6]. To our knowledge this is the first report on FBP1 recognized as an autoantigen by sera from patients with lung cancer, and it will require more data from a larger group of patients to determine whether autoantibodies to FBP1 are a chance observation or occur more frequently in patients with lung cancer.

Cathepsin D is a lysosomal acid proteinase, which is translated as procathepsin D in rough endoplasmic reticulum. In normal cells, the mature cathepsin D (34 kDa) participates in intracellular protein catabolism, hormone and antigen processing, as well as in the apoptotic pathway, and its preform, procathepsin D (pCD52, 48 kDa) is believed to cause enhanced proliferation and neoplastic transformation [7,8]. Bosschers et al. [2] demonstrated that circulating pro-forms of cathepsin D (52, 48 kDa) were detected in 31 of 40 endometrial cancer patients and none of the control volunteers. Circulating IgG reactive with cathepsin D could be demonstrated in 29/31 patients with circulating procathepsin D, while none of the normal controls. In this study, anti-cathepsin D was identified as a newly appeared autoantibody in a patient who was diagnosed with lung cancer, whereas the corresponding specific signal was undetectable in serum correspondingly 3 years before the appearance of malignancy (spot eight in Fig. 1B IV). In the following immunoprecipitation analysis, anti-cathepsin D autoantibodies were observed in 91.5% lung cancer patients, while only 20% of normal controls and 26.1% patients with nonmalignant lung disease did. In contrast to current assays for circulating cathepsin D, the presence of antibodies reactive with specific epitopes of cathepsin D is associated with not only the over expression of cathepsin D, but also the presence of the aberrant processing and modification of the pro-enzyme. Since immune responses generally precede detectable circulating antigen, the assessment of antibodies against these specific epitopes may be a more useful diagnostic or prognostic marker than current assays measuring circulating antigen. In this sense, these results strongly suggest that serum anti-cathepsin D autoantibodies would become a possible novel early tumor marker in lung cancer.

Notably, besides the occurrence, the pattern of anti-cathepsin D autoantibodies was different between lung cancer patients and

control groups. Specifically, the patterns of anti-cathepsin D autoantibodies were more diverse in that each patient had one main intense reaction. Of the 43 patients producing antibodies reactive with cathepsin D, 7 (16%) had most intense reaction against 66 kDa band, 17 (39.5%) against 52 kDa, 5 (11.6%) against 48 kDa, and 14 (32.6%) against the 34 kDa band. While in the four normal controls and six patients with nonmalignant lung disease-producing antibodies, the most intense reaction appeared to be directed at the procathepsin D bands (52 kDa). Sreenavasa et al. [9] isolated cathepsin D from human ovarian tumor cell line UL-1 and was further analyzed by immunoblotting with three sera samples from ovarian cancer patients. They found that the patient-derived antibodies reacted with three forms (52, 48, 32 kDa) and the most intense reaction was directed at the procathepsin D bands (48, 52 kDa), while only weak reactivity with mature 34 kDa cathepsin D. Whether the immune response to the novel forms of cathepsin D (66 kDa) arise from altered glycosylation or other mechanisms still needs further studies. However, these results suggest that novel immune responses to cathepsin D are driven by the process of malignant transformation, and the detection of autoantibodies against specific forms of cathepsin D might have utility for the diagnosis of lung cancer. It still requires more data from a larger group of patients to determine whether the diverse pattern of autoantibodies to cathepsin D is associated with the histology, progression and response to therapy in lung cancer.

Although the data gathered in this paper are somewhat preliminary, it is of interest to perform further studies on the antigens detected in this study, which might be candidates of diagnostic marker and therapy targets in lung cancer. It is necessary to analyze a larger number of samples to identify the relative specific lung cancer-associated autoantibodies and the specific antigens recognized by these autoantibodies.

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