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Detection of eight antibodies in cancer patients' sera against proteins derived from the adenocarcinoma A549 cell line using proteomics-based analysis☆

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Abstract

To screen cancer for specific autoantibodies, we applied the approach established by Brichory et al., who reported annexins I and II as specific antigens. Solubilized proteins from a cancer cell line (A549) were separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), followed by Western blotting (WB) analysis, in which the sera of individual patients were tested for primary antibodies. We found 11 positive spots on PVDF membrane using a WB/enhanced chemiluminescence detection Kit, and identified eight proteins, such as α-enolase, inosine-5′-monophosphate dehydrogenase, aldehyde dehydrogenase, 3-phosphoglycerate dehydrogenase, 3-oxoacid CoA transferase, chaperonin, peroxiredoxin 6 and triosephosphate isomerase, that reacted with these antibodies in patients' sera using MALDI-TOF/TOF. All eight antibodies were not detected in the sera derived from lung tuberculosis and healthy controls.

Keywords: Lung adenocarcinoma-specific antibody; Two-dimensional polyacrylamide gel electrophoresis; Lung adenocarcinoma; Matrix-assisted laser desorption ionization/time-of-flight mass spectrometry

1. Introduction

Lung cancer is the leading cause of cancer death and its prevention is a major worldwide challenge. Therefore, it is important to identify biomarkers for the early detection of lung cancer. Tumor antigens associated with cancer cells can induce autoantibodies, as has been demonstrated for a number of different intracellular and surface antigens in patients with various tumor types [1–5]. Remarkably, tumor regression has been demonstrated in some patients with small cell lung carcinoma and autoantibodies to onconeural antigens [5]. These autoantibodies are detectable in the sera and may serve to monitor tumor progression, and such antigens may also have utility in immunotherapy against tumors [4,6]. It is still unclear why and when autoantibodies are found in human serum, although immunogenicity may depend on the levels of expression, post-translational modification, unusual processing of a protein or the extent of variety among cancers of a similar type. Brichory et al. [7,8] have implemented a proteomics-based approach to identify tumor markers based on their occurrence as tumor antigens that elicit a humoral response during tumorigenesis. They subjected aliquots of solubilized proteins from a lung adenocarcinoma cell line (A549) to two-dimensional (2D) PAGE, followed by Western blot (WB) analysis, in which individual sera were tested for primary antibodies. They used a matrix-assisted laser desorption ionization-time-of-flight mass spectrometer (MALDI-TOFMS) to identify proteins that reacted with patients' autoantibodies. They identified glycosylated annexins I and/or II as circulating tumor antigens and their related autoantibodies in lung cancer. These antigens and antibodies are expected to provide a means for early cancer diagnosis, as well as leads for therapy. We followed the approach by Brichory et al. [7,8] to examine whether

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the same antigens would be detected in our patient group, and to find other antigens as well. We used the same cell line, and studied the sera or plasma from various diseases, including lung cancer.

We examined the sera from patients with lung tuberculosis to differentiate the antigens caused by inflammation from those caused by tumorigenesis. We found various positive spots specific for the eight antigens, such as α-enolase, corresponding to these WB spots. Some of the 5 patients with lung adenocarcinoma and 10 patients with esophageal cancer showed positive WB spots for these antibodies. All 10 patients with lung tuberculosis and all of the 10 healthy individuals were negative for these antibodies. All eight antibodies that we identified in the present study were specific to lung adenocarcinoma, and they were not detected in any sera derived from patients with lung tuberculosis or healthy individuals.

2. Materials and methods

2.1. Chemicals and materials

The human adenocarcinoma A549 cell line (JCRB0076) was purchased from the Health Science Research Resources Bank (Tokyo, Japan). The cells were harvested in 300 μL of solubilization buffer (9 M urea/2% Nonidet P-40/2% 2-mercaptoethanol/10 mM phenylmethylsulfonyl fluoride (PMSF)) using a cell scraper and were stored at −80 °C until use. Sera from 5 patients with lung adenocarcinoma, 10 with esophageal cancer, 10 with lung tuberculosis and 10 healthy subjects were analyzed.

Urea, glycine, sodium dodecyl sulphate (SDS), iodoacetamide, CHAPS, acrylamide, *N*,*N*-methylene bisacrylamide, tris (hydroxymethyl)-aminomethane (Tris), ammonium bicarbonate, glycerol, 2-mercaptoethanol, dithiothreitol (DTT), Tween 20, ammonium persulfate, PMSF, Nonidet P-40, silver nitrate and ethylenediamine tetraacetic acid were purchased from Nakalai tesque (Kyoto, Japan). Methanol, ethanol, acetonitrile, acetone, acetic acid, formic acid and distilled water were from Merck (Darmstadt, Germany). Immobilized pH 3–10 non-linear gradient strips (17 and 7 cm long) were purchased from Amersham Pharmacia Biosciences (Arlington Hights, IL). 10–20% gradient slub-gel (20 and 9 cm long) was obtained from Daiichi Chemical Co. Ltd. (Tokyo, Japan). Polyclonal anti-α-enolase antibody (sc-7455) and a monoclonal anti-γ-enolase antibody (sc-21738) were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA).

2.2. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and Western blotting (WB)

To detect autoantibody against tumor antigens in cancer patients' sera, we followed the approach established by Brichory et al. [7,8]. Proteins solubilized from the cultured cells were applied to isoelectric focusing (IEF) gel electrophoresis (first dimensional electrophoresis) and subjected to SDS-PAGE (second dimensional electrophoresis). Samples of 50 g protein were applied to an IEF gel. IEF was conducted with the immobilized pH 3–10 non-linear gradient strips at 500 V for 1 h, followed by 3500 V for an additional 3 h using a Multiphor II Electrophoresis Unit (Amersham Biosciences, CA). After IEF gel electrophoresis, the IEF gel strip was equilibrated with 50 mM Tris (pH 6.8) containing 10% glycerol, 2% SDS, 1% DTT and bromphenol blue and then the treated gel strip was loaded on a second-dimensional (2D) gel. Separated proteins were transferred to a membrane, or visualized using silver staining. Proteins separated by 2D-PAGE were transferred onto polyvinylidene fluoride (PVDF) membrane (Hybond P, Amersham Biosciences) for 1 h at a constant of 20 V, using a Mini Trans-Blot system (Bio-Rad, Hercules, CA). After transfer, PVDF membranes were incubated with a blocking buffer consisting of Tris-buffered saline (TBS) and 3% nonfat dry milk overnight at 4 °C, then washed with washing buffer (TBS/0.05% Tween 20) and incubated with diluted test sera at a 1:500 dilution for 1 h at room temperature. After four washings, the membranes were reacted with horseradish peroxidase-conjugated mouse antihuman IgG (Amersham-Pharmacia Biosciences), at 1:2000 dilution for 1 h at room temperature, washed, and the blots were developed with an enhanced chemiluminescence system (ECL plus, Amersham Biosciences). The 2D gel was stained with silver nitrite and the pieces of gel corresponding to WB positive spots were excised. Each piece of the gel spots was reduced, alkylated and then digested with TPCK-trypsin in Eppendorf tubes at 37° for 16 h. Protein identification was performed as previously reported [9]. The gel pieces were alternately washed with 50 mM ammonium bicarbonate pH 8.5 and acetonitrile and finally dehydrated with acetonitrile. These pieces were completely dehydrated in a Speedvac device at 30 °C and then covered with 25 μL of TPSK modified Trypsin (0.02 mg/mL: Promega Co. Ltd., Madison, WI) in NH_4HCO_3 buffer (40 mM, pH 8.5) and left at 37 °C overnight. After enzymatic digestion, the resultant peptides were extracted in 100 μL of 0.5% (v/v) formic acid and then in 100 μL of acetonitrile/H_2O 1% (v/v) formic acid (50/50). The extraction was conducted in an ultrasonic bath for 15 min each time. The extracts were concentrated and were desalted in ZipTip C18 micro-columns (Millipore Co. Ltd., Bedford, MT).

2.3. Protein identification by mass spectrometry

The extracted peptides were loaded onto the MALDI target plate by mixing 1 μL of each solution with the same volume of a matrix solution that was prepared fresh every day by dissolving 0.3 g/mL of α-cyano-4-hydroxycinnamic acid (Wako Purified Reagent Co. Ltd., Kyoto) in acetone–ethanol (1:1, v/v) solvent. Measurements were performed using an Ultraflex MALDI-TOF/TOF mass spectrometer (Brucker Daltonics, Germany) with an accelerating voltage of 20 kV. The laser wavelength was 337 nm and the laser pulse frequency was 25 Hz. Calibration was accomplished by using external peptide standards (Bruker Daltonics) or using the trypsin autodigestion peptide signal as an internal standard. The final mass spectra were produced by averaging 50–200 laser shots. The peptide mass fingerprint was used for protein identification from the tryptic fragment size using the Mascot Search engine based on the entire NCBIn and SwissProt protein databases, using the

assumption that peptides are monoisotopic, oxidized at methionine residues and carbamidomethylated at the cystine residues. Up to one missed trypsin cleavage was allowed and a tolerance of 100 ppm was the window of error allowed for matching the peptide mass values. Probability-based MOWSE scores were estimated by comparison of the search results against estimated random match population and were reported as $-10\log_{10}(p)$, where p is the absolute probability. All protein identifications were in the expected size range based on the position in the gel.

3. Results

Fig. 1 shows a silver stain profile of the 2D-PAGE of proteins isolated from A549 cells. More than 1000 spots are visible. Fig. 2 shows a comparison of WB profiles among the sera from a lung adenocarcinoma patient (a), from a lung tuberculosis patient (b), from a healthy individual (c) and with polyclonal anti-α-enolase antibody (d). Several positive spots are seen in the WB profile from the lung adenocarcinoma patient, but only a few spots were seen in the profiles from the lung tuberculosis patient (b) and the healthy individual (c). Most sera from cancer patients showed several WB positive spots; in contrast, all control sera showed no or only a few spots. As shown in Fig. 2a, we found four continuously connected spots (Nos. 1–4) in lung adenocarcinoma patients. We confirmed that these spots were reacted with a commercially obtained polyclonal α-enolase antibody (Fig. 2d), but these spots were not reacted with a commercially obtained polyclonal γ-enolase antibody (data not shown). The spot (No. 4) of the highest pI was the same pI and molecular weight of a native α-enolase. WB spots found to be positive in cancer patients and negative in controls were numbered in the profile (Fig. 2a).

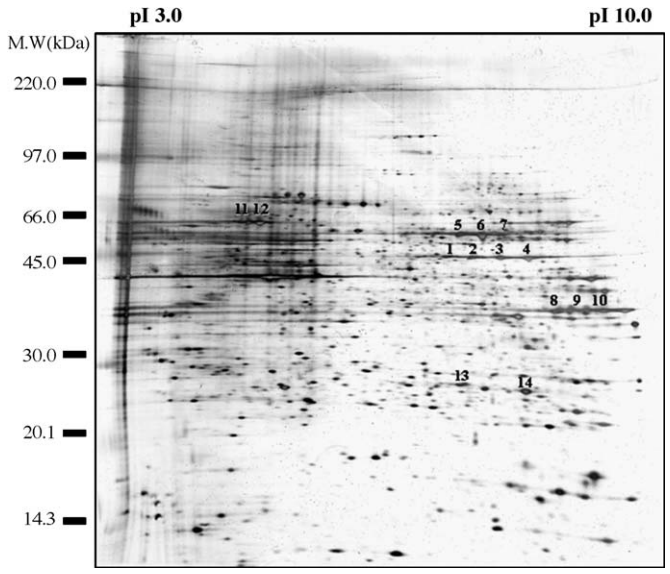

Fig. 1. Silver staining of A549 lung adenocarcinoma cell proteins separated by 2D-PAGE.

The spots in the silver stained gels corresponding to these WB positive spots in lung adenocarcinoma were analyzed with the proteomics-based technique using MALDI-TOF/TOFMS. Fig. 3a shows that the MS spectra (mass fingerprint) obtained from the protein in spot #1 and those of the other three spots

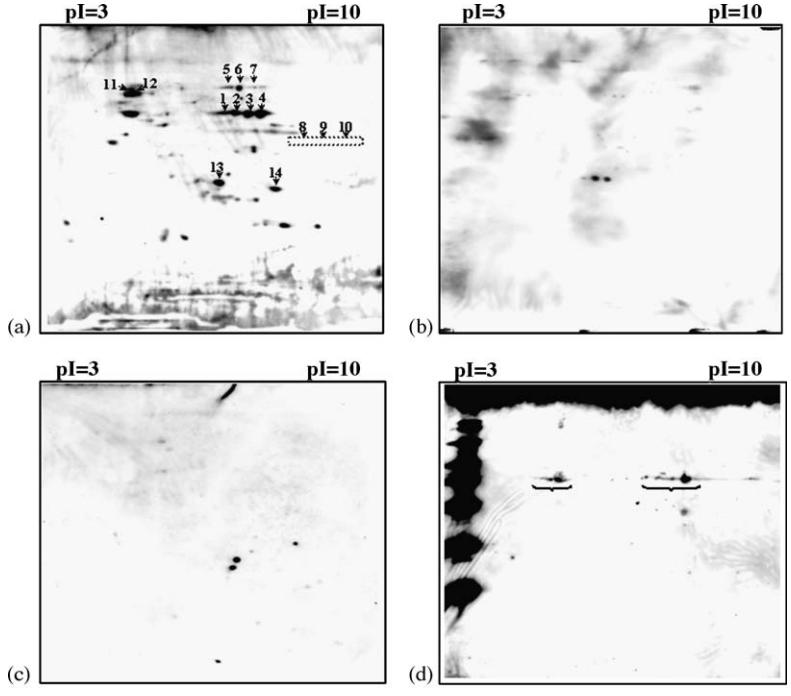

Fig. 2. Western blot analyses of adenocarcinoma and tuberculosis patients', control sera and polyclonal anti-α-enolase antibody. WB of 2D-PAGE slabs of Fig. 1 with serum from a lung adenocarcinoma patient (a), with serum from lung tuberculosis (b), with normal serum (c) and with polyclonal human anti-α-enolase antibody (d). The spots numbered were identified by MALDI/TOFMS with tryptic digests of extracted protein, and the data are shown in Table 1. Spots #1–7, 11, 12 were WB positive. Spots #8–10 were WB negative.

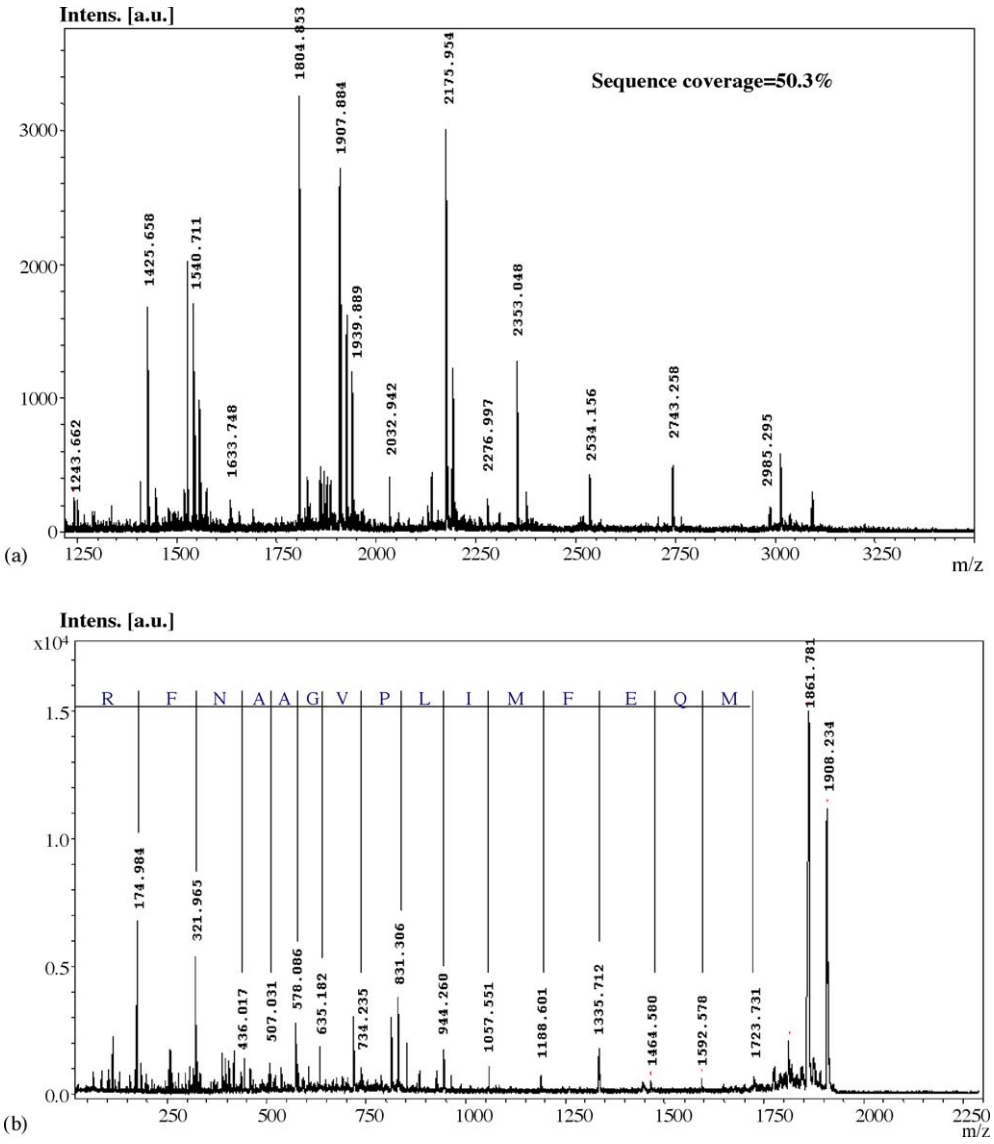

Fig. 3. (a) Matrix-assisted laser desorption ionization mass spectrum obtained from the protein in spot #1 after trypsin digestion. Marked numbers indicate the *m*/*z* of tryptic digests of α-enolase. (b) MALDI-TOF/TOF mass spectra obtained from a peak, $MH^+ = 1908.234$, which revealed the sequence 163–179 of α-enolase.

(spots #2–4 in Fig. 2a) are almost the same (data not shown). Peaks numbered in the figure are identified as the *m*/*z* of tryptic peptides from α-enolase by their high score. As shown in Fig. 3b, the MALDI-TOF/TOF mass spectrum of the peak, $MH^+ = 1907.884$ in Fig. 3a reveals a sequence of α-enolase from 163 to 179 amino acid residues. The MS/MS spectrum of the peak, $MH^+ = 2176.18$ in Fig. 3a revealed a sequence of α-enolase from 234 to 253 residues (spectra not shown). The WB spots of #1–4 were linked together and showed a rosary shape. Mass fingerprints and MS/MS spectra of peptides from #2, 3 and 4 also unambiguously showed α-enolase. The proteins of these spots were identified by their mass fingerprints and MS/MS by their high score (more than 90). The numbers of cases for all positive these eight antibodies are listed in Table 1. Three of the five patients with lung adenocarcinoma, and 3 of the 10 patients with esophageal cancer showed positive WB spots for anti-α-enolase, inosine-5′-monophosphate dehydrogenase, aldehyde dehydrogenase, 3-phosophogycerate dehydrogenase, 3-oxoacid CoA transferase and chaperonin. Two of the five patients with lung adenocarcinoma were positive for peroxiredoxin 6 and triosephosphate isomerase antibodies. The three antibodies against chaperonin, peroxiredoxin 6 and triosephosphate isomerase were not detected in patients with esophageal cancer. In addition, 10 patients with lung tuberculosis and 10 healthy individuals were negative for all eight antibodies.

Spots #8–10 were analyzed, and were silver stain positive and WB negative, and were marked with a dotted rectangle in Fig. 2a. These regions were studied despite being WB negative, as Brichory et al. found autoantibody against annexin II in this region. The MS spectra of the mass fingerprint and the MS/MS of spots from this part identified annexin II. In the present study, we did not detect antibodies against annexin I or II, which was reported by Brichory et al., although we identified annexin I protein in the silver-staining gel.

Table 1
Positive cases of all eight antibodies against identified proteins

Spot	Proteins	Lung adenocarcinoma	Esophageal cancer	Lung tuberculosis	Normal control
1	α-Enolase	3/5	3/10	0/10	0/10
2	α-Enolase	3/5	3/10	0/10	0/10
3	α-Enolase	3/5	3/10	0/10	0/10
4	α-Enolase	3/5	3/10	0/10	0/10
5	Inosine-5′-monophosphate dehydrogenase	3/5	3/10	0/10	0/10
	Aldehyde dehydrogenase	3/5	3/10	0/10	0/10
6	3-Phosphoglycerate dehydrogenase	3/5	3/10	0/10	0/10
	Aldehyde dehydrogenase	3/5	3/10	0/10	0/10
7	3-oxoacid CoA transferase	3/5	3/10	0/10	0/10
8*	Glyceraldehyde-3-phosphate dehydrogenase	0/5	0/10	0/10	0/10
	Annexin II	0/5	0/10	0/10	0/10
9*	Glyceraldehyde-3-phosphate dehydrogenase	0/5	0/10	0/10	0/10
	Annexin II	0/5	0/10	0/10	0/10
10*	Glyceraldehyde-3-phosphate dehydrogenase	3/5	0/10	0/10	0/10
11	Chaperonin	3/5	0/10	0/10	0/10
12	Chaperonin	3/5	0/10	0/10	0/10
13	Peroxiredoxin 6	2/5	0/10	0/10	0/10
14	Triosephosphate isomerase	2/5	0/10	0/10	0/10

* The spots of WB were negative but those of silver stained gel were positive, follow-up with Brichory et al. [7].

4. Discussion

In the present study, we found eight antibodies in the sera derived from lung adenocarcinoma patients according to the method introduced by Brichory et al. [7,8], but nor all antibodies were detected in the sera from patients with lung tuberculosis and healthy controls. Although we detected annexin I proteins of the cell line, the antibody against annexin I was not found in any of our experimental sera. We identified eight proteins that reacted with antibodies in the sera derived from lung adenocarcinoma patients by MALDI-TOF/TOFMS. Those were, α-enolase, inosine-5′-monophosphate dehydrogenase, aldehyde dehydrogenase, 3-phosphoglycerate dehydrogenase, 3-oxoacid CoA transferase, chaperonin, peroxiredoxin 6, and triosephosphate isomerase.

An α-enolase autoantibody has been detected in the sera from various autoimmune diseases, i.e., systemic lupus erythematosus with renal disease, rheumatoid arthritis and Hashimoto's encephalopathy [10–12]. In the present study, we found a specific WB profile of the α-enolase antibody in patients' sera derived from lung adenocarcinoma. The profile of the spots of α-enolase antibody in the sera from patients showed four continuously connected spots at the pI range from 6.0 to 7.5 in the WB profile, as shown in Fig. 2a. Ochi et al. [11] reported that the profile of α-enolase antibody in patients' sera derived from Hashimoto's encephalopathy and the profile of these spots were predominantly existed at the highest pI = 7.5 (No. 4), but the others were only present in trace amounts. The pI and molecular weight of the spot were the same as those of a native α-enolase. The difference of the profile may be caused by a post-translational modification, such as phosphorylation or sulfonation, and this WB profile may be specific to lung adenocarcinoma.

Adams et al. [13] were the first to report that the presence of serum antibodies against retinal enolase in patients with cancer and cancer associated retinopathy, but the mechanisms of the immunogenicities were still unclear. We identified α-enolase in the spots of WB #1–4 in Fig. 2 and the linked shape was differed from that of WB in the reaction of patient serum against 2D-PAGE with purified enolase shown by Adams et al. [13]. Weleber et al. [14] reported that the acquisition of autoantibody was directly associated with retinal degradation in paraneoplastic and autoimmune retinopathies. The retina was not examined in patients in the present research; however, the patients had no evident visual complaints. Future detailed investigation is required in this area. Enolase is a glycolytic enzyme. Recent findings have shown that enolase is a multifunctional protein, and its ability to serve as a plasminogen receptor on the surface of variety of hematopoetic, epithelial and endothelial cells suggests that it may play an important role in the intravascular and pericellular fibrinolytic system [12]. In addition, it was recognized as a heat-shock protein, which may play a crucial role in the transcription of a variety of pathophysiological processes [12]. Jankowska et al. [15] first reported that autoantibodies against α-enolase, recoverin and retinal antigens were found in lung cancer and sarcoidosis. Autoantibodies to retinal antigens, including α-enolase, occur more frequently and in higher titers in lung cancer, especially small-cell lung cancer, than in sarcoidosis or normal controls. Moreover, Dot et al. [16] clarified changes of the response against α-enolase after tumor resection and that the phenomena were due to the acquisition of a disease-causing epitope of α-enolase.

Chaperonin is a 60 kDa heat-shock protein, and anti-chaperonin was reported to be associated with subsequent cardiovascular events [17], colorectal adenocarcinoma [18] and prostate cancer [19]. It was reported that patients with Crohn's disease and ulcerative colitis had elevated antibody against peroxiredoxin [20]. Autoantibody against triosephosphate isomerase has been reported in the sera from various diseases, i.e., hepatitis A virus [21], malaria [22], infectious mononu-

cleosis [23] and osteoarthritis [24]. Peroxiredoxin 6 was one of the peroxiredoxin families where both antioxidants and regulators of peroxide mediated signaling in eukaryotic cells. Chang et al. reported that an antibody against peroxiredoxin-I existed in non-small cell lung cancer [25]. They indicated that the expression of the peroxiredoxin increased in non-small cell lung cancer and the over-expressed peroxiredoxin could induce humoral immune response. In the present study, we first identified an antibody not against peroxiredoxin 1, but peroxiredoxin 6, in lung adenocarcinoma. Autoantibodies against inosine monophosphate dehydrogenase, 3-oxoacid CoA transferase and 3-phosphoglycerate dehydrogenase have not been reported in any literature. It is not clear why autoantibodies are found in human serum, although immunogenicity may depend on the levels of expression, post-translational modification, abnormal processing and varieties among similar types of tumors.

In our study, we did not detect antibodies against annexin I and II, which were reported by Brichory et al. [7,8], in any of the patients' or control sera, although we identified annexin I proteins derived from a lung adenocarcinoma A549 cell line. The antigens identified varied, possibly depending on the combinations of the source of antigens, immunoglobulin class (IgG or IgM), target diseases, stage of the diseases and ethnicity. The present research is our starting work to screen candidate antigens specific to cancer patients. In addition, we believe further research is required for the identification of more antigens from WB positive spots, confirmation of the antigens using purified antigens and analyses of more cases with various cancers and control individuals. It will require more data from a larger group of patients to determine whether autoantibodies to all eight proteins, as well as α-enolase, are a chance observation or occur more frequently in patients with lung adenocarcinoma.

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References

[1] R. Sahin, O.E. Tureci, H. Schmitt, B. Cochlovius, T. Johannes, R. Schmits, et al., Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 11810.
[2] L.J. Old, Y.T. Chen, J. Exp. Med. 187 (1998) 1163.
[3] M. Cheever, M.L. Disis, H. Bernhard, J.R. Gralow, S.L. Hand, E.S. Huseby, et al., Immunol. Rev. 145 (1995) 33.
[4] K. Angelopoulou, E. Diamandis, D.J.A. Sutherland, J.A. Kellen, P.S. Bunting, Int. J. Cancer 58 (1994) 480.
[5] R.B. Darnell, L.M. DeAngelis, Lancet 341 (1993) 21.
[6] T. Soussi, Cancer Res. 60 (2000) 1777.
[7] F.M. Brichory, D.E. Misek, A.M. Yim, M.C. Krause, T.J. Giordano, D.G. Beer, S.M. Hanash, Proc Natl. Acad. Sci. U.S.A. 98 (2001) 9824.
[8] F.M. Brichory, D. Beer, F. LeNaur, T. Giordano, S.M. Hanash, Cancer Res. 61 (2001) 7908.
[9] T. Nakanishi, R. Koyama, T. Ikeda, A. Shimizu, J. Chromatogr. B 776 (2002) 89.
[10] K.H. Lee, H.S. Chung, H.S. Kim, S.H. Oh, M.K. Ha, J.H. Baik, S. Lee, D. Bang, Arthritis Rheum. 7 (2003) 2025.
[11] H. Ochi, I. Horiuchi, N. Araki, T. Toda, T. Araki, K. Sato, H. Murai, M. Osoegawa, T. Yamada, K. Okamura, T. Ogino, K. Mizumoto, H. Yamashita, H. Saya, J. Kira, FEBS Lett. 528 (2002) 197.
[12] V. Pancholi, Cell Mol. Life Sci. 58 (2001) 902.
[13] G. Adams, N. Aptsiauri, J. Guy, J. Heckenlively, J. Flannery, P.A. Hargrave, Clin. Immunol. Immunopathol. 78 (1996) 120.
[14] R.G. Weleber, R.C. Watzke, W.T. Shults, K.M. Trzupek, J.R. Heckenlively, R.A. Egan, G. Adamus, Am. J. Ophthalmol. 139 (2005) 780.
[15] R. Jankowska, D. Witkowska, I. Porebska, M. Kuropatwa, E. Kurowska, W.A. Gorczyca, Pathobiology 71 (2004) 323.
[16] C. Dot, J. Guigay, G. Adams, Am J. Ophthalmol. 139 (2005) 746.
[17] A. Veres, G. Füst, M. Smieja, M. McQueen, et al., Circulation 106 (2002) 2775.
[18] M. Otaka, S. Otani, H. Itoh, T. Kuwabara, A. Zeniya, S. Fujimori, Y. Tashima, O. Masamune, J. Clin. Gastroenterol. 21 (1995) 244.
[19] P.A. Cornford, A.R. Dodson, K.F. Parsons, A.D. Desmond, A. Woolfenden, M. Fordham, J.P. Neoptolemos, Y. Ke, C.S. Foster, Cancer Res. 60 (2000) 7099.
[20] C.A. Jonsson, L. Svensson, H. Carlsten, Clin. Exp. Immunol. 116 (1999) 534.
[21] K. Ritter, A. Uy, S. Ritter, R. Thomssen, Scand J. Infect. Dis. 26 (1994) 379.
[22] F. Geurs, K. Ritter, A. Mast, V. Van Maele, Acta Haematol. 88 (1992) 142.
[23] K. Ritter, A. Kuhlencord, R. Thomssen, W. Bommer, Lancet 342 (1993) 1333.
[24] Y. Xiang, T. Sekine, H. Nakamura, S. Imajoh-Ohmi, H. Fukuda, K. Nishioka, T. Kato, Arthritis Rheum. 50 (2004) 1511.
[25] J.W. Chang, S.H. Lee, J.Y. Jeong, H.Z. Chae, Y.C. Kim, Z.Y. Park, Y.J. Yoo, FEBS Lett. 579 (2005) 2873.