Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1762 (2006) 398–403

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

http://www.elsevier.com/locate/bba

Rapid report

Autoantibody biomarker opens a new gateway for cancer diagnosis

M. Nesterova, N. Johnson, C. Cheadle, Y.S. Cho-Chung *

Cellular Biochemistry Section, Basic Research Laboratory, CCR, National Cancer Institute, Building 10, Room 5B05, 9000 Rockville Pike, Bethesda, MD 20892-1750, USA

Received 11 November 2005; received in revised form 21 December 2005; accepted 22 December 2005
Available online 30 January 2006

Abstract

The list of cancer markers of current interest has grown considerably, but none of the markers used in clinical work is a true tumor marker. These cancer biomarkers are based on the determination of tumor antigens. Here, we report a single method of autoantibody enzyme immunoassay (EIA) screens for a spectrum of serum tumor markers. A comparison of the autoantibody-based EIA to conventional antigen EIA kits, using receiver operating characteristic (ROC) plots, showed that the autoantibody EIA can significantly enhance the sensitivity and specificity of tumor markers. The detection of serum autoantibodies for a spectrum of serum tumor markers, as demonstrated here, suggests that most, if not all, serum cancer biomarkers produce autoantibodies. A unique autoantibody biomarker screening method, as presented here, might therefore facilitate achieving the accurate and early diagnosis of cancer.
Published by Elsevier B.V.

Keywords: Extracellular protein kinase A; Autoantibody; Cancer biomarker; Cancer diagnosis; Enzyme immunoassay

An ideal tumor marker would allow a simple blood test to detect cancer, and its levels would correlate with the stage of tumor progression. Due to the lack of sensitivity and specificity, however, no single marker has been recognized as a true cancer marker [1,2]. Currently, available cancer markers measure cancer antigens. For example, the prostate-specific antigen (PSA) is measured for prostate cancer marker [3], the Carcino-Embryonic Antigen (CEA) for colorectal cancer [4], the cancer antigen CA15-3 for breast cancer [5], the cancer antigen CA19-9, for gastrointestinal cancer [6], and the cancer antigen CA125 for the diagnosis of ovarian cancer [7].

In addition to the above-mentioned novel markers, some other proteins, hormones, and enzymes have been used as markers for the past 30 years [2,8]. Notable among these are α-fetoprotein (AFP), a liver cancer marker; human chorionic gonadotropin (hCG), a breast cancer marker; and prostatic acid phosphatase (PAP), a prostate cancer marker. These markers lack specificity, however. Their levels are also increased under benign conditions and during gestation. All these markers are based on the antigen determination method and lack specificity and sensitivity. There is great need to discover novel biomarkers and translate them into routine clinical use. Our present study is directed to this need.

In normal mammalian cells, two types of cAMP-dependent protein kinase (PKA) species are present strictly intracellularly [9]. These protein kinases are designated type I (PKA-I) and type II (PKA-II); they are distinguished by different regulatory subunits (R subunits) RI and RII, but they contain a common catalytic subunit (C subunit) [10]. The ratios of PKA-I to PKA-II change dramatically during cell development, differentiation, and transformation [11]. In cancer cells of various cell types, however, PKA has been shown to be secreted into the conditioned medium [12,13]. This PKA, designated as extracellular PKA (ECPKA), is shown to be the catalytic subunit (C subunit) of PKA [12]. Its activity is specifically inhibited [12,13] by the PKA inhibitor protein, PKI [14], and uses the PKA-specific substrate, kemptide [12,13]. In cancer cells, the ECPKA expression has been modulated by changing the ratios of the intracellular type I PKA (PKA-I) to type II (PKA-II) [12], and down-regulation of ECPKA was shown by a mutant Cα lacking the N-terminal myristyl group [12]. In the serum of cancer patients, ECPKA expression is markedly up-regulated, in contrast to normal serum [12,13], and a surgical removal of melanoma led to a decrease in ECPKA levels in patients [15].

* Corresponding author. Tel.: +1 301 496 4020; fax: +1 301 480 8587.
E-mail address: yc12b@nih.gov (Y.S. Cho-Chung).

0925-4439/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbadis.2005.12.010

We speculated that ECPKA excretion might elicit the induction of serum autoantibodies and that the presence of such autoantibodies could serve as a cancer diagnostic. In order to examine whether ECPKA induces its autoantibody in patients' sera, we developed a novel enzyme immunoassay (EIA) method (Fig. 1) to measure the anti-IgG autoantibody for ECPKA. Fig. 2 shows the data obtained from this ECPKA-autoantibody EIA. It shows the presence of ECPKA autoantibody in the sera of patients with a wide variety of types of cancers. We tested 345 serum samples from cancer patients. The cancers included breast (n=24), cervical (n=13), colon (n=40), lung (n=6), ovarian (n=36), prostate (n=35), pancreatic (n=6), renal cell (n=60), and rectal (n=14) carcinomas and melanomas (n=90), other carcinomas (n=19), including bladder, esophageal, gastric, hepatocellular, and small bowel; and sarcoma, thymoma, liposarcoma, and leiomyosarcoma. As controls, we used normal sera from a blood bank (n=163). The serum samples were aliquoted (5 μl volume) and kept frozen at −80 °C until use. The serum samples were thawed only once before use. Diluted serum samples were never used twice. For statistical analysis, mean, S. D., and confidence intervals (CIs) were used where appropriate. Data on reproducibility of EIA tests were analyzed by CV at relevant concentrations and during appropriate temporal intervals; and measures of intraobserver or interobserver variability. The difference between groups (patients vs. controls) was calculated to have significance at $P<0.05$. The results (antibody titer) were evaluated using receiver operating characteristic (ROC) [16] plots to determine the optimal sensitivity and specificity of EIA (see Fig. 3). The assay was reproducible, with within-run and between-run CVs of <5.0% and <5.8%, respectively.

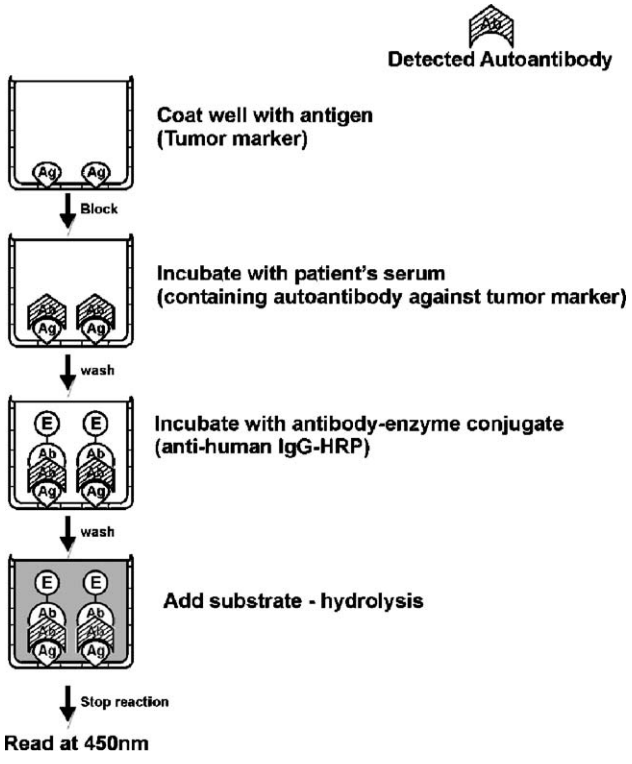


Fig. 1. Schematic illustration of EIA to detect autoantibodies specific to serum tumor markers. Ag=antigen; Ab=antibody; E=enzyme. See detailed information in the legend of Fig. 2.

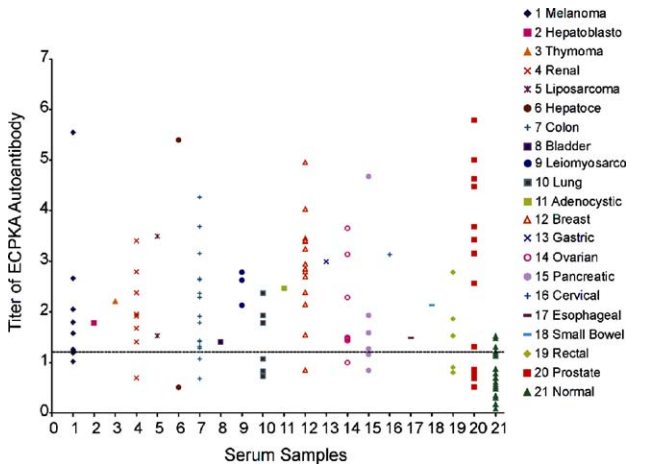
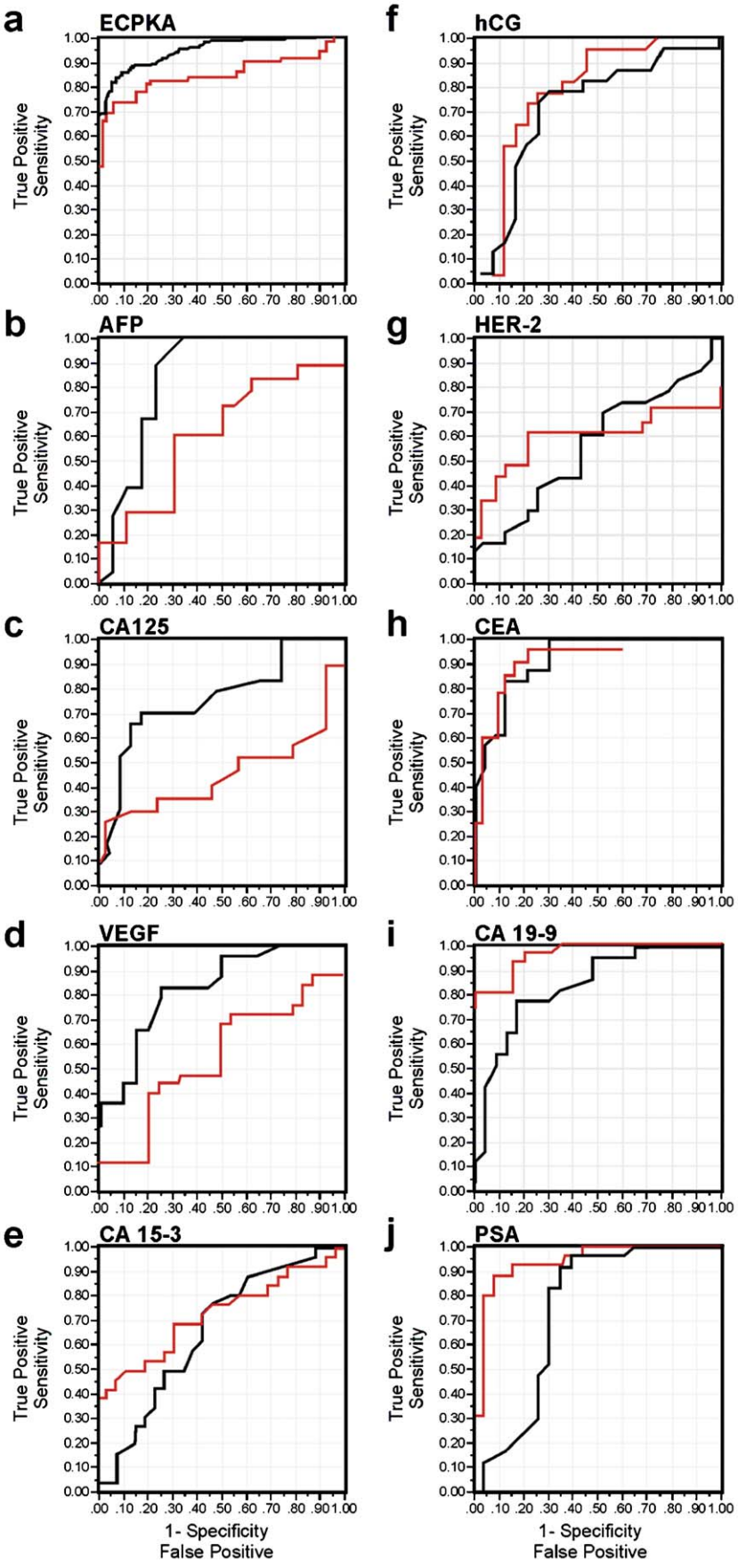


Fig. 2. Titers of ECPKA autoantibodies in sera from cancer patients with various cancer cell types and normal control group. *Enzyme immunoassay* (*EIA*): Anti-Ecpka IgG autoantibodies were measured by solid-phase EIA. The plates were coated with 100 μl of diluted antigen (2 μg per ml PBS) purified recombinant human PKA Cα subunit at room temperature overnight, and washed once with washing buffer (20 mM HEPES, 0.9% NaCl, 30 mM sucrose, 0.1% bovine serum albumin [BSA], pH 7.0), blocked for 2 h at room temperature with 100 μl of Blockace (Serotec, http://www.serotec.com), and washed two times with Na-Citrate washing solution (50 mM Na-Citrate, 0.15 M NaCl, 0.1% Tween® 20, pH 5.0–5.2). We then added 100 μl of 25,000-fold diluted serum samples (dilution buffer: PBS pH 7.4, 0.25% BSA, 0.05% Tween 20), incubated for 1 h at 37 °C. After three washes with the Na-Citrate solution, added 100 μl of 20,000-fold diluted anti-human IgG-HRP antibody-enzyme conjugate (Jackson ImmunoResearch Laboratories, West Grove, PA), in PBS, 0.9% NaCl, 1% BSA, incubated the samples for 1 h at room temperature, washed five times in Na-Citrate solution, and added 100 μl of TMB substrate. The reaction was stopped with 100 μl of 0.45 M H_2SO_4 reagent and the absorbance at 450 nm recorded on an ELISA reader (BioRad [Hercules, CA] microplate reader benchmark). *Purification of PKA Cα*: the recombinant human PKA Cα (1.1 kb) from OT1529-Cα plasmid [12] was infused with pQE31 DNA leading to production of pQE-Cα (Hong, S.H. Seoul National University, Seoul, Korea). pQE-Cα plasmid was expressed in Escherichia coli, and purification of native PKA Cα protein was achieved (Paragon, Baltimore, MD).

The serum-presence of autoantibody directed against ECPKA was highly correlated to cancer. Only representative data from each tumor type and control group are shown in Fig. 2. Antibody titers are arbitrarily expressed as ratios to the mean absorbance of the normal control sera. Values >1.3 (broken line) were considered positive. High anti-ECPKA, autoantibody titers (n=345, frequency=90%, mean titer=3.6) were found in cancer patients, and low or negative titers (n=163, frequency=13%, mean titer=1.0) were found in the control group (Fig. 2). The EIA experiments were performed three or more times with each tumor type consisting of equal numbers of patients and controls with sample sizes varying 24, 46, and 92. Samples of three known titers (low, medium, and high) were run in each test to check for the reproducibility of the assays.

We compared these data with data from a PKA enzymatic assay that measures antigen [12]. A comparison of individual anti-ECPKA autoantibody titers obtained by EIA with those measured by PKA enzymatic assay showed no correlation between the two assays. The sensitivity and specificity of autoantibody EIA and those of the PKA assay were evaluated using


a
ECPKA
b
AFP
c
CA125
d
VEGF
e
CA 15-3
f
hCG
g
HER-2
h
CEA
i
CA 19-9
j
PSA
True Positive
Sensitivity
1- Specificity
False Positive
.00 .10 .20 .30 .40 .50 .60 .70 .80 .90 1.00
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00

ROC plots and are shown in Fig. 3a. The autoantibody EIA had a sensitivity of 90% (95% confidence interval, 0.88 to 0.92), and specificity of 87% (95% confidence interval, 0.80 to 0.95) (AUC: 0.93675), and the enzymatic assay showed a sensitivity of 83%, and specificity of 80% (AUC: 0.86938) (Fig. 3a). These data show that the ECPKA autoantibody-detection method exhibits a higher sensitivity and specificity than the antigen detecting method.

We speculated that the known serum tumor markers might also produce autoantibodies, as was shown for ECPKA, and that the autoantibody-based EIA developed for ECPKA could be extended to assay these serum tumor-marker autoantibodies. For comparison, we also assayed the nine serum tumor markers with their antigen kits, and evaluated the data by ROC plots (Fig. 3b–j). The autoantibody EIAs of tumor markers were performed as described above, except for the use of each specific tumor antigen for coating the EIA plates and appropriate tumor types. Thus, for AFP, we used serum from liver, lung, and pancreatic cancer patients; for CA125, serum from mucinous or nonmucinous ovarian cancer patients; for VEGF, serum from breast and colon and gastric cancer patients; for CA15-3, hCG, and HER-2, serum from breast cancer patients; for CEA and CA19-9, we used colon and gastric cancer patient's serum; and for PSA, we used prostate cancer patients' serum. Each EIA consisted of equal numbers of patients' and control sera. Sample sizes varied, either 24, 46, or 92. Importantly, the same samples assayed by EIA were also assayed by the antigen kit, usually on the same day. The autoantibody EIA and antigen kit assays for each tumor marker were performed with three or more times of repeated experiments. The mean, S.D., and confidence intervals (CIs) were used where appropriate. The difference between groups (patients vs. controls) was calculated to have significance at $P<0.05$. ROC curves were used to calculate the cutoff values for optimal sensitivity and specificity. These results were reproducible, with within-run and between-run CVs of <6.0% and <6.3%, respectively.

This comparison provided a striking difference in the sensitivity and specificity achieved by the autoantibody EIA compared to what the antigen kits of the individual tumor markers produced for some markers. The liver tumor marker α-fetal protein (AFP) [8], measured by autoantibody EIA, showed sensitivity of 89% and specificity of 77% (AUC: 0.84150), compared to measurements by the AFP antigen kit of 60% and 69%, respectively (AUC: 0.60243) (Fig. 3b). Similarly, the nonmucinous ovarian cancer antigen CA125 [7], measured by autoantibody EIA, had sensitivity of 70% and specificity of 83% (AUC: 0.75520), compared to its antigen kit measurements of 45% sensitivity and 70% specificity (AUC: 0.49211) (Fig. 3c). The blood vessel (and thus tumor) growth factor, vascular endothelial growth factor (VEGF) [17], as measured by the autoantibody EIA, showed sensitivity of 66% and specificity of 85% (AUC: 0.84688), compared to 40% sensitivity and 79% specificity (AUC: 0.54333) when measured by the antigen kit (Fig. 3d).

By contrast, in the tumor markers for breast cancer, CA15-3 [5], hCG [18], and Her-2 [19], and for colon and gastrointestinal cancers, CEA [4], and CA19-9 [6], the autoantibody EIA exhibited sensitivity, specificity, and AUC (see the legend of Fig. 3) similar to those obtained with the antigen kits (Fig. 3e–i). These results indicate that a single autoantibody EIA method was applicable for the detection of autoantibodies specific for each of these nine tumor markers. The parallel results (ROC curves almost superimposable) obtained by the autoantibody EIA and antigen kit assay for a given tumor marker, compared to the distinct ROC curves of these assays obtained for different tumor markers, support the autoantibody-EIA method presented here as a universal screen for serum tumor markers.

In the case of PSA [3], however, the autoantibody EIA exhibited a lower sensitivity and specificity than the antigen-determining kit (Fig. 3j). Although use of prostate-specific antigen has been of value in the detection, diagnosis, and clinical management of men with prostate cancer, problems with specificity remain. Serum PSA levels not only reflect changes due to cancer, but also changes due to inflammation, trauma, or benign proliferation [3]. There is also considerable overlap in PSA levels among men with prostate cancer and benign disease despite the discovery of new molecular forms, such as free PSA (fPSA) and complexed derivatives of PSA that offer the potential for improved diagnostic discrimination of prostate cancer from benign conditions [3].

We examined whether PSA autoantibody detection could be valuable to distinguish prostate cancer patients with androgen-sensitive (AS) disease from those with androgen-insensitive (AI) disease. As shown in Fig. 4, the autoantibody EIA clearly distinguished the AS and AI groups of patients. The AS group exhibited a high sensitivity (95%) and specificity (75%) whereas the AI group showed low sensitivity (60%) and specificity (50%) (Fig. 4). With the PSA-antigen kit, however, there was no significant distinction between the AS and AI groups in sensitivity, specificity, or AUC (Fig. 4).

In the present study, we have presented a highly sensitive EIA for measuring IgG autoantibodies against a spectrum of serum tumor markers. Using receiver operating characteristic (ROC) plots, the data obtained with the autoantibody EIA were evaluated in comparison to those obtained with antigen-determination kits. The ROC plots divided the tumor markers into two groups. In the first group, which included tumor

Fig. 3. Receiver operating characteristic (ROC) plots of autoantibody EIA and Antigen EIA kit for serum tumor markers. The sensitivity and specificity of the autoantibody EIA and antigen EIA kit for each tumor marker are presented graphically in an ROC curve. Panel a represents the data of the ECPKA enzymatic assay (red) and those of the anti-ECPKA autoantibody EIA (black). In panels b–j: red = data from antigen-EIA kits; black = data of autoantibody EIA for each designated serum tumor markers. b, AFP; c, CA125; d, VEGF; e, CA15-3; f, hCG; g, HER-2; h, CEA; i, CA19-9; and j, PSA. The tumor antigens, AFP, VEGF, CA125, CA15-3, CA19-9, and hCG were from Biodesign International (Saco, ME); CEA was from Sigma-Aldrich (St. Louis, MO), HER2 was from Invitrogen (Carlsbad, CA); and PSA was from Aspen Bio, Inc. (Castle Rock, CO). The antigen-EIA kits for AFP, PSA, CEA, hCG, CA 125, CA 19-9, and CA 15-3 were from Bio-Quant, Inc. (San Diego, CA); HER-2 was obtained from Sigma-Aldrich (St. Louis, MO); and VEGF were from Assay Designs (Castle Rock, CO).

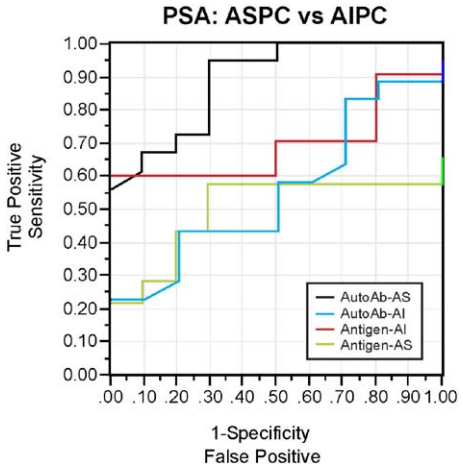


Fig. 4. Receiver operating characteristic (ROC) plots of autoantibody EIA and antigen EIA kit assays in androgen-sensitive (AS) versus androgen-insensitive (AI) prostate cancers. The ROC curves were plotted as described above. Black=the autoantibody EIA of androgen-sensitive (AS) prostate cancer, area under curve (AUC 0.88611); blue=the autoantibody EIA of androgen-insensitive (AI) prostate cancer (AUC 0.62500); green=the antigen kit assay of the AS prostate cancer (AUC 0.49286); red=the antigen-kit assay of the AI prostate cancer (AUC 0.69000). EIA autoantibody experiments were performed as described in the text on 18 serum samples of AS prostate cancer and 14 serum samples of AI prostate cancer, with equal numbers of controls for the EIA and antigen kits.

markers VEGF, CA125, AFP, and ECPKA, the autoantibody EIA showed markedly enhanced sensitivity and specificity compared to results obtained with the antigen kits. By contrast, in the second group of markers, including CA15-3, hCG, Her-2, CEA, CA19-9, and the sensitivity and specificity obtained by the autoantibody EIA and antigen kits were quite similar. The ROC curves from the two assays were, in fact, almost superimposable (Fig. 3). That this spectrum of serum tumor markers exhibited super or equivalent sensitivity and specificity to those obtained with the specific tumor-antigen kits strongly supports that (1) serum tumor markers may all produce autoantibodies and (2) the unique autoantibody EIA presented here may open a new gateway for cancer diagnostics.

The enhancement of sensitivity and specificity of tumor markers elicited by the autoantibody-EIA could have immediate clinical significance. For example, VEGF, the vascular endothelial growth factor, has been shown to be a key mediator of blood vessel growth and thus for tumor growth [17], and its detection gives valuable information to patients for the choice of new treatment [20]. CA125, an ovarian tumor marker, combined with transvaginal ultrasound examination, appears to provide the highest specificity and positive predictive value for the detection of ovarian cancer [7]. Patients with the lymph node-positive and aggressively growing HER-2-positive breast tumors could receive beneficial effects from treatment with herceptin (a monoclonal antibody for HER-2) [21].

Importantly, our PSA analysis data comparing androgen-sensitive (AS) and -insensitive (AI) prostate cancers were the most striking results (Fig. 4). The high PSA autoantibody production in AS and the distinctively low PSA autoantibody production in AI clearly distinguished these two groups, whereas this was not possible using the PSA antigen determination (kit) (Fig. 4). The data are not only valuable for determination of therapeutic choice but are also important for monitoring the progression and prognosis of the disease.

One of the most important criteria for cancer markers is the ability to distinguish cancer from inflammatory and benign diseases such as hepatitis, pancreatitis, and benign disease causing PSA increase. Because known cancer markers all depend on the measurement of cancer antigens, the distinction between inflammation and cancer is difficult. In the present study, we used a single EIA method of autoantibody detection. An increase in cancer biomarkers can occur temporarily with inflammation [3]; but because such an increase in cancer antigens might not increase autoantibodies, our autoantibody-detection method could make it possible to distinguish between the increase in levels of tumor markers caused by cancer and that caused by inflammation.

Monitoring the quantitative relationship of autoantibody titers of cancer antigens, such as ECPKA, with disease progression and prognosis would be of importance, but this is yet to be accomplished. Autoantibody monitoring of cancer antigens would also be critically important in the early detection of relapsing patients. Because ECPKA levels were shown to fall after surgical removal of melanoma [15], if a patient began to show re-increase in ECPKA autoantibody that previously showed a decrease upon surgical removal of a tumor, it could be an early sign of disease recurrence. Such studies have not yet been performed.

The protein products of oncogenes and tumor suppressor genes can be detected in extracellular fluids and serve as potential markers for carcinogenesis in vivo. Antibodies against p53 tumor suppressor protein were found in the sera of patients with breast [23] and lung carcinomas [22] and in children with B-lymphomas [24]. Circulating antibodies against the oncogene product c-*myc* was also found in sera of patients with colorectal cancer [25].

A test based on the demonstration of autoantibodies to tumor antigen in sera of patients, as described here, could be of great importance for early diagnosis because of the prolonged time course of carcinogenesis [26] and the possibility that a very small tumor or a subtle biochemical change in the cell might be able to produce a detectable level of anti-stimulant autoantibody in response to chemical/viral carcinogens, before the emergence of the tumor phenotype [1]. This could happen well before the released tumor antigen reaches a detectable level. Our results thus suggest that the autoantibody detection rather than antigen detection would serve for early diagnosis.

Our results further indicate the utility of the autoantibody-based EIA method presented here as a routine diagnostic procedure to detect cancer of various cell types by measuring various serum tumor biomarkers with a single assay. Thus, the autoantibody biomarker-detecting EIA is superior to antigen-determining kits in time saved for diagnoses, and savings for patients of the costs of the kits. This new assay, autoantibody detection, could remove false positives often associated with conventional testing and provide a novel technology for cancer detection.

Acknowledgements

We thank Drs. Susan Bates and Jeffrey Schlom (NCI, Bethesda, MD), Sridhar Mani (Albert Einstein College of Medicine, Bronx, NY), and Rishab K. Gupta (John Wayne Cancer Institute, Santa Monica, CA) for their generosity in providing us with serum samples. This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute (P.I., Y.S. Cho-Chung), Bethesda, MD.

References

[1] D. Sulitzeanu, Human cancer-associated antigens: present status and implications for immunodiagnosis, in: G. Klein (Ed.), Advances in Cancer Research, vol. 44, Academic Press, Inc., New York, 1985, pp. 1–42.
[2] C.T. Garrett, S. Sell, Cellular Cancer Markers, Humana Press, Totowa, NJ, 1995.
[3] M.B. Gretzer, A.W. Partin, PSA markers in prostate cancer detection, Urol. Clin. North Am. 30 (2003) 677.
[4] N.P. Crawford, D.W. Colliver, S. Galandiuk, Tumor markers and colorectal cancer: utility in management, J. Surg. Oncol. 84 (2003) 239.
[5] K.L. Cheung, F.R. Robertson, Objective measurement of remission and progression in metastatic breast cancer by the use of serum tumour markers, Minerva Chir. 58 (2003) 297.
[6] V. Trompetas, E. Panagopoulos, M. Priovolou-Papaevangelou, G. Ramantanis, Giant benign true cyst of the spleen with high serum level of CA 19-9, Eur. J. Gastroenterol. Hepatol. 14 (2002) 85.
[7] C. Anderiesz, M.A. Quinn, Screening for ovarian cancer, Med. J. Aust. 178 (2003) 655.
[8] S. Sell, Cancer Markers, Humana Press, Clifton, NJ, 1980.
[9] E.G. Krebs, J.A. Beavo, Phosphorylation–dephosphorylation of enzymes, Annu. Rev. Biochem. 48 (1979) 923.
[10] S.J. Beebe, J.D. Corbin, Cyclic nucleotide-dependent protein kinases, in: E.G. Krebs, P.D. Boyer (Eds.), The Enzymes: Control by Phosphorylation, vol. 17, Academic Press, Orlando and London, 1986, pp. 43–111 (Part A).
[11] S.M. Lohmann, U. Walter, Regulation of the cellular and subcellular concentrations and distribution of cyclic nucleotide-dependent protein kinases, in: P. Greengard, G.A. Robinson (Eds.), Advances in Cyclic Nucleotide and Protein Phosphorylation Research, vol. 18, Raven Press, New York, 1984, pp. 63–117.
[12] Y.S. Cho, Y.G. Park, Y.N. Lee, M.K. Kim, S. Bates, L. Tan, Y.S. Cho-Chung, Extracellular protein kinase A as a cancer biomarker: its expression by tumor cells and reversal by a myristate-lacking Calpha and RIIbeta subunit overexpression, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 835.
[13] M.E. Cvijic, T. Kita, W. Shih, R.S. DiPaola, K.V. Chin, Extracellular catalytic subunit activity of the cAMP-dependent protein kinase in prostate cancer, Clin. Cancer Res. 6 (2000) 2309.
[14] S. Whitehouse, D.A. Walsh, Mg X ATP2-dependent interaction of the inhibitor protein of the cAMP-dependent protein kinase with the catalytic subunit, J. Biol. Chem. 258 (1983) 3682.
[15] T. Kita, J. Goydos, E. Reitman, R. Ravatn, Y. Lin, W.C. Shih, Y. Kikuchi, K.V. Chin, Extracellular cAMP-dependent protein kinase (ECPKA) in melanoma, Cancer Lett. 208 (2004) 187.
[16] M.H. Zweig, G. Campbell, Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine, Clin. Chem. 39 (1993) 561.
[17] G. Chodorowska, J. Chodorowski, A. Wysokinski, Vascular endothelial growth factor (VEGF) in physiological and pathological conditions, Ann. Univ. Mariae Curie-Sklodowska [Med.] 59 (2004) 8.
[18] C. Rao, X. Li, S.K. Manna, Z.M. Lei, B.B. Aggarwal, Human chorionic gonadotropin decreases proliferation and invasion of breast cancer MCF-7 cells by inhibiting NF-kappaB and AP-1 activation, J. Biol. Chem. 279 (2004) 25503.
[19] L.N. Klapper, M.H. Kirschbaum, M. Sela, Y. Yarden, Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors, Adv. Cancer Res. 77 (2000) 25.
[20] J.C. Yang, L. Haworth, R.M. Sherry, P. Hwu, D.J. Schwartzentruber, S.L. Topalian, S.M. Steinberg, H.X. Chen, S.A. Rosenberg, A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer, N. Engl. J. Med. 349 (2003) 427.
[21] C.X. Wang, D.C. Koay, A. Edwards, Z. Lu, G. Mor, I.T. Ocal, M.P. Digiovanna, In vitro and in vivo effects of combination of trastuzumab (Herceptin) and tamoxifen in breast cancer, Breast Cancer Res. Treat. 92 (2005) 251.
[22] S.F. Winter, J.D. Minna, B.E. Johnson, T. Takahashi, A.F. Gazdar, D.P. Carbone, Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation, Cancer Res. 52 (1992) 4168.
[23] L.V. Crawford, D.C. Pim, R.D. Bulbrook, Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer, Int. J. Cancer 30 (1982) 403.
[24] R. Lubin, B. Schlichtholz, D. Bengoufa, G. Zalcman, J. Tredaniel, A. Hirsch, C.C. de Fromentel, C. Preudhomme, P. Fenaux, G. Fournier, Analysis of p53 antibodies in patients with various cancers define B-cell epitopes of human p53: distribution on primary structure and exposure on protein surface, Cancer Res. 53 (1993) 5872.
[25] K. Ben Mahrez, I. Sorokine, D. Thierry, T. Kawasumi, S. Ishii, R. Salmon, M. Kohiyama, Circulating antibodies against c-*myc* oncogene product in sera of colorectal cancer patients, Int. J. Cancer 46 (1990) 35.
[26] G.J. Kelloff, C.C. Sigman, K.M. Johnson, C.W. Boone, P. Greenwald, J.A. Crowell, E.T. Hawk, L.A. Doody, Perspectives on surrogate end points in the development of drugs that reduce the risk of cancer, Cancer Epidemiol. Biomark. Prev. 9 (2000) 127.