

Evaluation of Tumour-Associated Antigen (TAA) Miniarray in Immunodiagnosis of Colon Cancer

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Abstract

Previous studies demonstrated that cancer sera contain antibodies, which react with a unique group of autologous cellular antigens called tumour-associated antigens (TAAs). This study determines whether a mini-array of multiple TAAs would enhance antibody detection and be a useful approach in colon cancer detection and diagnosis. The mini-array of multiple TAAs was composed of five TAAs including Imp1, p62, Koc, p53 and c-myc full-length recombinant proteins. Enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies against these five TAAs in 46 sera from patients with colon cancer and also 58 sera from normal individuals. Antibody frequency to any individual TAA in colon cancer was variable and ranged from 15.2% to 23.9%. With the successive addition of TAAs to a final total of five antigens, there was a stepwise increase of positive antibody reactions reaching a sensitivity of 60.9% and a specificity of 89.7% in colon cancer. Positive and negative likelihood ratio was 5.91 and 0.43 respectively, which showed that the clinical diagnostic value of parallel assay of five TAAs was high. Positive and negative predictive values were respectively 82.4% and 74.3% indicating that parallel assay of five TAAs raised the diagnostic precision greatly. Agreement rate and Kappa value were 76.9% and 0.52 respectively, which indicated that the observed value of this assay had middle range coincidence with actual value. The data from this study further support our previous hypothesis that detection of autoantibodies for diagnosis of certain type of cancer can be enhanced by using a mini-array of several TAAs as target antigens. In 19 colon cancer sera with carcinoembryonic antigen (CEA) negative, 11 (57.9%) were found to have anti-TAA antibodies. When CEA and anti-TAAs were used together as markers in colon cancer detection, the diagnostic sensitivity could be raised from 60.9% to 82.6%. A customized antigen mini-array using a panel of appropriately selected TAAs can enhance autoantibody detection in immunodiagnosis of colon cancer. Anti-TAA and CEA were independent markers and the simultaneous use of these two markers significantly raised the sensitivity of colon cancer detection.

Introduction

The highly specific autoantibody response in systemic autoimmune disease generally predicts the biological phenotype of the disease making autoantibodies clinically valuable and diagnostically useful. Whether a similar mechanism is operating in the humoral immune response in cancer remains to be established, but appears to be a possibility. Many studies have demonstrated that cancer sera contain antibodies, which react with a unique group of autologous cellular antigens generally known as

tumour-associated antigens (TAAs) [1–3]. These autoantibodies can be used as reporters identifying aberrant cellular mechanisms in tumorigenesis and also serve as immunodiagnostic markers for cancer detection. Although the mechanism underlying the production of such autoantibodies in cancer patients are not completely understood, emerging evidence indicates that many of the target antigens are cellular proteins whose dysregulation could have tumourigenic potential including mRNA binding proteins p62 [4] and cell-cycle control proteins cyclinB1 [5–7], or are proteins whose aberrant regulation

or overexpression could lead to tumourigenesis, such as p53 [8, 9], HER-2/neu [10]. The immune system in certain cancer patients appears to have the capability of sensing these abnormalities and we have proposed that autoantibodies might be regarded as reporters or sentinels identifying aberrant cellular mechanisms in tumourigenesis [1].

Many investigators have been interested in the use of autoantibodies as serological markers for cancer diagnosis, especially because of the general absence of these autoantibodies in normal individuals and non-cancer conditions. Enthusiasm for this approach has been tempered by low sensitivity. We have observed that this drawback can be overcome by using a panel of carefully selected TAAs and that different types of cancer may require different panels of TAAs to achieve the sensitivity and specificity required to make immunodiagnosis a feasible adjunct to tumour diagnosis. This feature is one of the innovative notions we have proposed in our study. One of the previous studies showed that detection of autoantibodies in cancer can be enhanced by using mini-array of seven TAAs as target antigens [11].

Colon cancer is one of the most common tumour types in the world. In the population of the United States, colon cancer is the second leading cause of cancer death among men and third in women [12]. In China, the incidence rate was initially low, but in recent years, because of the changes of life style and nutritional habits, the rate is increasing [13]. Risk factors for colon cancer include a personal or family history of colon cancer or polyps and inflammatory bowel disease. Other risk factors include a sedentary life-style (not enough exercise), high fat/low fibre diet and inadequate ingestion of fruits and vegetables. As with all cancer, early detection is one of the most significant predictors of survival. Although several approaches, such as digital rectal exams, faecal occult blood loss and sigmoidoscopy are currently available for colon cancer screening, the high mortality rate of colon cancer can be still attributed to lack of diagnostic methods that allow early detection. Carcinoembryonic antigen (CEA) is an important tumour marker to diagnose colon cancer and now being used in clinical laboratory, but its sensitivity and specificity is not satisfactory [14–16]. In the present study, we determine whether a mini-array of multiple TAAs would enhance autoantibody detection and be a useful approach in colon cancer diagnosis and also evaluate whether anti-TAAs and CEA could be used simultaneously in colon cancer detection.

Materials and methods

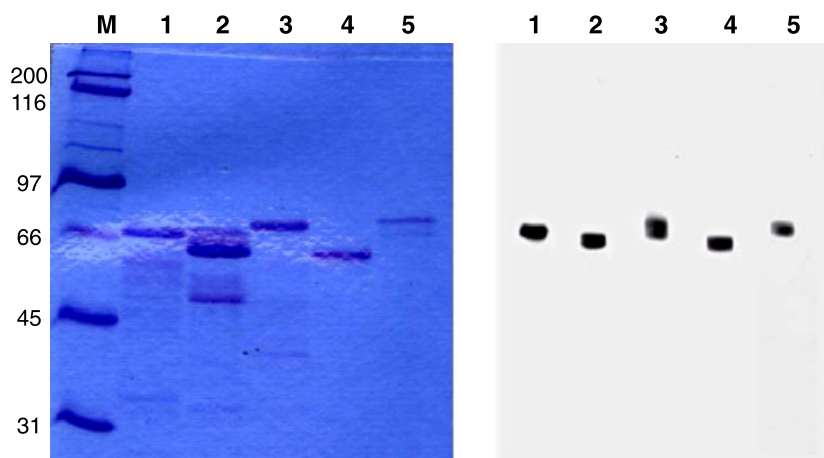
Serum samples and antibodies. In this study, sera from 46 patients with colon cancer and 58 normal human sera (NHS) were obtained from the Department of Clinical Laboratory Technology of Dalian Municipal Central

Hospital (Liaoning Province, China). All cancer sera were collected at the time of cancer diagnosis when the patients had not yet received treatment with any chemotherapy or radio-therapy, 58 normal human sera were collected from adults during annual health examination in people who had no obvious evidence of malignancy. Of 46 colon cancer patients, 35(76.1%) were male and 11(23.9%) were female. Mean age was 56.8 ± 13.2 year (range, 25–76 years). Because of the regulations concerning studies of human subjects, the patient's name and identification number were blinded to investigators. This study was approved by the Institutional Review Boards of Dalian Municipal Central Hospital and collaborating academic institutions.

Expression and purification of recombinant TAAs. Five antigens, Imp1, p62, Koc, p53 and c-myc, were selected for expression of recombinant proteins. All these five antigens were used for detection of different types of cancer in our previous study [11] and the sensitivity and specificity of antibodies against these antigens in cancer sera have been confirmed [11]. In brief, recombinant p62 has been expressed from a clone isolated from cDNA expression library by immunoscreening with antibody from a patient with HCC [4]. p62 cDNA was subcloned into pET28a vector producing a fusion protein with NH-terminal 6 $\times$ histidine and T7 epitope tags. The recombinant protein expressed in *Escherichia coli* BL21 (DE3) was purified using nickel column chromatography. Koc cDNA cloned in the pcDNA3 vector [17] was similarly subcloned to pET28a vector and recombinant protein expressed as above. Imp1 construct pCMV5-Imp1 was provided by F.C. Nielsen [18] and p53 clone (p53SN3) by Yuxin Yin of Columbia University, New York, NY and subcloned into pET28a for protein expression. cDNA from c-myc was amplified by polymerase chain reaction from human foetal liver tissue and survivin cDNA from human survivin EST clone (BG258433) before subcloning in pET28a vector. As shown in Fig. 1, expression of adequate amounts of recombinant protein was examined in SDS-PAGE and Coomassie blue staining was used to determine that expression products of expected molecular sizes were produced. In addition, Western blot analysis was used to confirm that the bands seen in SDS-PAGE were reactive with reference antibodies. The antibodies used were rabbit polyclonal anti-Imp1 and kindly supplied by F.C. Nielsen [18], anti-Koc/IMP3 from F. Müller-Pillasch [17], which were raised against specific COOH-terminal peptides of the respective proteins, and anti-p62 raised against the full-length protein [4]. Reactivities of p53 and c-myc were determined with monoclonal antibodies obtained from Oncogene Research Products, Boston, MA.

Enzyme-linked immunosorbent assay (ELISA). Purified recombinant proteins (Imp1, p62, Koc, p53 and c-myc) were individually diluted in PBS to a final concentration

Figure 1 Expression of five recombinant proteins. (A) Five recombinant proteins Imp1 (lane 1), p62 (lane 2), Koc (lane 3), p53 (lane 4) and c-myc (lane 5) expressed as a 6 × histidine tag protein in *Escherichia coli* were purified using nickel column chromatography. Five proteins were detected in SDS-PAGE with Coomassie blue staining. (B) Western blotting analysis showing immuno-reactivity of each protein with corresponding antibody. Lane 1, Imp1; lane 2, p62; lane 3, Koc; lane 4, p53; lane 5, c-myc.



of 0.5 µg/ml, and 200 µl were added into each well to coat Immunolon2 microtitre plates (Fisher Scientific, Houston, TX, USA) overnight at 4 °C. The human serum samples were diluted at 1:200, incubated with the antigen-coated wells at 37 °C for 90 min followed by washing with PBS containing 0.05% Tween 20 (PBST), then incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Caltag Laboratories, Burlingame, CA, USA) as a secondary antibody diluted 1:4000 for 90 min followed by washing with PBST. The solution of 3,3',5,5'-TMB (3,3',5,5'-tetramethyl benzidine, TMB)-H₂O₂-Urea was used as the detecting agent. The OD of each well was read at 450 nm. Each sample was tested in duplicate. The cut-off value for determining a positive reaction was designated as the mean absorbance of the 58 normal human sera plus 2 standard deviations (mean + 2SD). As several hundred test sera were analyzed at different time periods, each run of ELISA included four NHS samples and two positive control samples. The four NHS samples representing a range of 2SD above and below the mean of the 58 normal human sera were always used in each experiment and the average value of the four NHS samples was used in each run to normalize all absorbance values to the standard mean of the entire 58 normal samples. In addition, all positive sera were confirmed with repeating test, as were some negative sera. The detailed protocol of ELISA has been described by Rubin [19].

Western blotting and slot blot. Western blot analysis was used to confirm that the bands seen in SDS-PAGE were reactive with reference antibodies. In brief, the purified recombinant proteins (Imp1, p62, Koc, p53 and c-myc) were electrophoresed by SDS-PAGE and subsequently transferred to a nitrocellulose membrane. The individual strips were preblocked in PBST with 5% non-fat milk for 30 min at room temperature, then incubated for 90 min with patients' sera diluted 1:100 and finally incubated with HRP-conjugated goat anti-human IgG diluted 1:3000 for 90 min followed by washing with

PBST solution. Positive signals were captured by autoradiography. Slot blot analysis was used to confirm the positive sera samples detected by ELISA. The method for Slot blot was identical to Western blotting with the exception that the purified recombinant protein (100 ng/well) was applied directly to the nitrocellulose membrane using a vacuum source. Membranes were not cut into strips and therefore, the detection of autoantibodies in an individual patient's serum to all five TAAs could be performed in one blot simultaneously.

Electro chemiluminescence immunoassay (ECLIA). ECLIA was used to measure the serum carcinoembryonic antigen (CEA). Experiment was performed according to the instruction of CEA kit (Roche, Mannheim, Germany). The reference ranges of CEA were 0–4.7 ng/ml. If the concentration of CEA is >4.7 ng/ml, it can be considered as positive reaction.

Statistical analysis. To determine whether the frequency of autoantibodies to five TAAs in each cohort of patients' sera was significantly higher than that in sera from normal cohort, the data were analyzed using the χ^2 tests with Yates' correction. Two statistical significant levels (0.05 and 0.01) were used. The comprehensive evaluation of testing results for each anti-TAA antibody including the methods for calculating the sensitivity, specificity, Youden's index, positive and negative likelihood ratio, positive and negative predictive value, agreement rate and Kappa value was based on the methodology provided in Epidemiology textbook [20].

Results

Prevalence of antibodies to a mini-array of five TAAs in colon cancer

In the present study, a mini-array of five TAAs was used as coating antigens in ELISA, and sera from 46 patients with colon cancer and sera from 58 normal individuals were examined for the presence of antibodies to the

individual TAA and cumulatively to the entire panel of five TAAs. The mini-array of TAAs used in this study includes Imp1, p62, Koc, p53 and c-myc full-length recombinant proteins rather than peptide fragments of these proteins. p53 is tumour suppressor gene product [9] and c-myc is oncogene product [21]. p62 [4], Koc [17] and Imp1 [18] are three proteins containing similar RNA-binding motifs, and their mRNAs are either over-expressed in cancer [22, 23] or antibodies have been identified to the protein products [4, 24]. Table 1 shows the frequency of antibodies to a mini-array of five TAAs in sera from 46 patients with colon cancer and 58 normal human sera, which were obtained from the same region as controls. A positive test for antibodies was taken as an absorbance reading above the mean + 2SD of the 58 normal human sera. Antibody frequency to any individual TAA in colon cancer was variable from 15.2% to 23.9%. Out of 46 sera from patients with colon cancer analyzed, 60.9% (28/46) were shown to have antibodies cumulatively to any of these five TAAs, which was significantly higher than the frequency in sera from normal individuals (10.3%). The ELISA results were also confirmed by immunoblotting analysis. The higher frequency of antibodies to an individual TAA in colon cancer was against p53 (23.9%) and p62 (21.7%), IMP1 (21.7%), c-myc (21.7%) and followed by Koc (15.2%). The reactivity of normal human sera was low, ranging from 0 to 3.4% to any individual TAA, with a combined frequency of reactivity of 10.3% against the complete panel of five TAAs.

Evaluation of diagnostic values of a mini-array of five TAAs in immunodiagnosis of colon cancer

The validity of a test is defined as its ability to distinguish between who has a disease and who does not. To address the question of how valuable is the approach of antibody detection to a mini-array of multiple TAAs in separating people with and without cancer in question, a group of parameters, such as the sensitivity/specificity, Youden's index, the positive predictive value/negative

predictive value (+PV/−PV) and so on were calculated and summarized in Tables 2 and 3. Table 2 shows the comprehensive evaluation of antibodies to a panel of five TAAs. With the successive addition of TAAs to a total of five antigens, there was a stepwise increase of positive antibody reactions up to 60.9% and there was also a slight decrease of specificity from 100% with one TAA to 89.7% with a panel of five TAAs. It is consistent with the results of other two parameters (+PV/−PV). The +PV/−PV were also variable in different combination of TAAs. In the panel of total of five TAAs, the +PV was 82.4%, and the −PV was 74.3%. The Youden's index was also increased from 0.152 with one TAA to 0.506 with five TAAs. The positive and negative likelihood ratio was 5.91 and 0.43 respectively, which indicated that the clinical diagnostic value of parallel assay of five TAAs was high. It also suggests that parallel assay of five TAAs can raise the diagnostic precision greatly. Agreement rate and Kappa value were 76.9% and 0.52 respectively, which indicated that the observed value of

Table 2 Evaluation of diagnostic value of antibodies to different combination of TAAs in colon cancer detection.

TAAs	Colon Cancer (%)	NHS (%)	Sen. (%)	Spe. (%)	YI	+PV (%)	−PV (%)
Koc	7 (15.2)	0	15.2	100	0.152	100	59.8
Koc and p62	15 (32.6)	1 (1.7)	32.6	98.3	0.309	93.8	64.8
Koc, p62 and Imp1	20 (43.5)	1 (1.7)	43.5	98.3	0.418	95.2	68.7
Koc, p62, Imp1 and c-myc	24 (52.2)	4 (6.9)	52.2	93.1	0.453	85.7	71.1
Koc, p62, Imp1, c-myc and p53	28 (60.9)	6 (10.3)	60.9	89.7	0.506	82.4	74.3

Sen, sensitivity; Spe, specificity; YI, Youden's index; +PV, positive predictive value; −PV, negative predictive value.

Table 3 Summary of diagnostic value of antibodies to a panel of five TAAs in colon cancer.

	Any TAA positive	All TAA negative	Total
Colon cancer	28 (A)	18 (C)	46 (R1)
NHS	6 (B)	52 (D)	58 (R2)
Total	34 (C1)	70 (C2)	104 (N)

Fourfold table χ^2 tests: $\chi^2 = 37.45$, $P < 0.001$.

Sensitivity (%) = $A/(A + C) = 28/46 = 60.9\%$.

Specificity (%) = $D/(B + D) = 52/58 = 89.7\%$.

Youden's index = Sensitivity + Specificity − 1 = $0.609 + 0.897 - 1 = 0.506$.

Positive (+) Likelihood: Ratio = Sensitivity/(1 − Specificity) = $0.609/(1 - 0.897) = 5.91$.

Negative (−) Likelihood Ratio = (1 − Sensitivity)/Specificity = $(1 - 0.609)/0.897 = 0.43$.

Percent agreement = $(A + D)/(A + B + C + D) \times 100\% = 113/156 = 76.9\%$.

Kappa = $[N(A + D) - (R_1C_1 + R_2C_2)]/[N^2 - (R_1C_1 + R_2C_2)] = 0.52$.

Table 1 Sensitivity and specificity of anti-TAA antibodies and CEA in colon cancer detection.

TAAs	Frequency (%)		Sensitivity (%)	Specificity (%)	Youden's index
	Colon Cancer (n = 46)	NHS (n = 58)			
Koc	7 (15.2) ^a	0 (0.0)	15.2	100.0	0.15
p62	10 (21.7) ^a	1 (1.7)	21.7	98.3	0.20
Imp1	10 (21.7) ^a	0 (0.0)	21.7	100	0.22
c-myc	10 (21.7) ^b	3 (5.2)	21.7	94.8	0.17
p53	11 (23.9) ^a	2 (3.4)	23.9	96.6	0.21
CEA	27 (58.7) ^a	1 (1.7)	58.7	98.3	0.57

Relative to NHS: ^a $P < 0.01$, ^b $P < 0.05$.

this assay had middle range coincidence with actual value. Taken together, these data show the utility of the multiple antigen array in increasing the clinical diagnostic quality and value in cancer. Positive results of ELISA were also confirmed by slot blot. Slot blot analysis of four representative colon cancer sera is shown in Fig. 2.

Simultaneous use of both CEA and anti-TAAs as markers in colon detection

Carcinoembryonic antigen (CEA) is a complex glycoprotein produced by 90% of colorectal cancers and contributes to the malignant characteristic of a tumour. It can be measured in serum quantitatively and its level in blood can be used as a marker of disease. Because of its lack of sensitivity in the early stages of colorectal cancer, CEA measurement is an unsuitable marker for colon cancer screening in population. The sensitivity of CEA in detection of colon cancer is variable depending on a different cutoff value which was used [16]. As shown in Table 4, 27 of 46 (58.7%) colon cancer sera were tested as CEA positive, which was significant higher than that in normal human sera (1/58, 1.7%). Although 58.7% of patients with colon cancer can be detected by CEA, there are still 41.3% (19/46) of patients, which can not be detected by this marker. However, the sensitivity of simultaneous use of both CEA and anti-TAAs as markers in colon cancer detection is demonstrated in Table 4. Of interest was that 11 of 19 (57.9%) colon cancer sera with

Table 4 Sensitivity of simultaneous use of both CEA and anti-TAAs as markers in colorectal cancer detection.

	Anti-TAAs (+)	Anti-TAAs (-)	Total
CEA (+)	17 (A)	10 (C)	27
CEA (-)	11 (B)	8 (D)	19
Total	28	18	46

Sensitivity (%) = (A+B+C)/(A+B+C+D) = 38/46 = 82.6%.

CEA negative were anti-TAA positive. If both anti-TAA and CEA were simultaneously used as diagnostic markers, 38 (17 + 11 + 10) of 46 (82.6%) colon cancer patients could be correctly identified. Elevated CEA and anti-TAA appear to be independent, but complementary serological markers for the diagnosis of colon cancer.

Discussion

Many studies indicated that no single marker can completely distinguish the cancer group from the healthy controls. However, the combination of multiple markers may provide a promising way for early detection of cancer. In the other hand, the multi-factorial and multi-step nature in the molecular pathogenesis of human cancers can be also considered in both the design and interpretation of studies to identify biomarkers, which will be useful for early detection of cancer. One of our previous studies indicated that combinations of multiple antigen-antibody systems might acquire higher sensitivity for diagnosis of cancer [11, 25]. Another study by Wang *et al.* [26] was to use a phage display library derived from prostate cancer tissue and develop a phage protein microarray for the analysis of autoantibodies in serum samples from 119 patients with prostate cancer and 138 individuals with no history of prostate cancer. In Wang's study, a 22-phage-peptide detector was constructed for prostate-cancer screening, with 81.6% sensitivity and 88.2% specificity. Compared to one of our studies, a decision tree was constructed for classifying prostate cancer, using five TAAs, with 79% sensitivity and 86% specificity [27]. With a smaller panel, these rates are equivalent to those of Wang. Approaches from Wang *et al.* as well as our panel of TAAs do not as yet justify adoption in a screening programme and could be improved by the inclusion of additional tumour antigens.

Our efforts will be aimed at increasing both the sensitivity and specificity of autoantibodies as markers in cancer by expanding TAA array to include antigens, which might be more selectively associated with one specific cancer such as colon cancer and not with others. We expect that our mini-array of multiple TAAs can be used as a novel non-invasive approach to identify cancer in normal population and also in high-risk individuals. Our

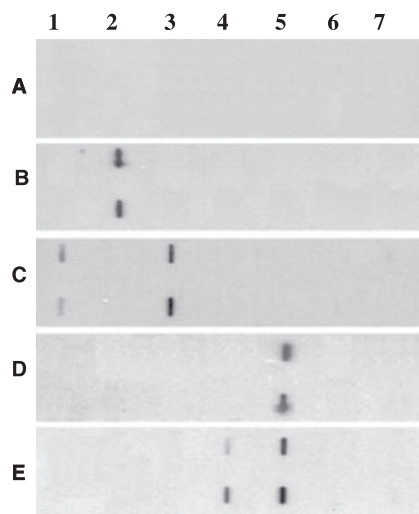


Figure 2 Slot blot analysis of four representative colon cancer sera. Lane 1, Imp1; lane 2, p62; lane 3, Koc; lane 4, p53; lane 5, c-myc; lane 6 and lane 7, PBS. (A) Normal human serum shows no reactivity to any of the five TAAs. (B) One colon cancer serum shows reactivity to only p62. (C) Another colon cancer shows a strong reactivity to Koc, and also a weak reactivity to Imp 1. (D) A third colon cancer serum shows reactivity against c-myc. (E) A fourth colon cancer serum shows reactivity against p53 and c-myc.

concern is that the approach may be not suitable to distinguish one type of cancer with another. The reason is that certain TAAs, which we have used in our mini-array approach (for example p62, p53 and so on), are associated with several types of cancer such as breast, gastric, lung and prostate cancer. In the future study, we propose that some of the selected antibody-antigen systems may be unique to one type of cancer and some may not. A comprehensive analysis and evaluation of various combinations of selected antibody-antigen systems will be useful for the development of autoantibody profiles involving different panels or arrays of TAAs in the future and the results could be useful for diagnosis of certain types of other cancer. Important additional studies would be to determine whether mini-arrays of antibody markers might be useful in identifying in early stage of certain types of cancer, such as colon, lung, breast, gastric and liver cancer in high-risk individuals. In searching the literature, we found that the notion of using panels of TAAs for detecting cancer autoantibodies is rarely discussed and is fairly a new approach. We stress the notion that panels of 'customized' TAAs should be used for different types of tumour and that these customized panels should be rigorously tested for sensitivity and specificity not only against other tumours but also against other disease conditions. For instance, in the case of lung cancer, the nature of precondition would be heavy cigarette smoking. It has been well known that cigarette smoking is the major cause of lung cancer and is currently estimated to cause 85% of all lung cancer deaths. In the case of HCC, the natural conditions would be chronic hepatitis and liver cirrhosis. Tumour-associate antigen panels might conceivably be used for early detection of tumours in high-risk individuals. Anti-p53 antibodies were detected in two heavy smokers before clinical detection of lung cancer and in one patient, early treatment resulted in good response, which correlated with total disappearance of p53 antibodies [28, 29].

Autoantibodies against several cancer-related antigens have been demonstrated in colon cancer [30–32]. For example, the presence of p53 autoantibodies has been detected in 18% of patients with colon cancer [9]. In the present study, five TAAs were used as coating antigens in ELISA to detect autoantibodies against these antigens in 46 sera from patients with colon cancer and 58 sera from normal individuals. Antibody frequency to any individual TAA in colon cancer was variable and ranged from 15.2% to 23.9%. It cannot meet the needs of clinical early diagnosis to colon cancer. With the successive addition of TAAs to a final total of five antigens, there was a stepwise increase of positive antibody reactions reaching a sensitivity of 60.9% and a specificity of 89.7% in colon cancer. Positive and negative likelihood ratio was 5.91 and 0.43 respectively, which showed that the clinical diagnostic value of parallel assay of five TAAs was high. Positive and

negative predictive values were 82.4% and 74.3% respectively indicating that parallel assay of five TAAs raised the diagnostic precision greatly. Agreement rate and Kappa value were 76.9% and 0.52 respectively, which indicated that the observed value of this assay had middle range coincidence with actual value.

CEA is an important tumour marker in detection of colon cancer, and now being used in clinical laboratory, but the concern is that there are about 40% patients of colon cancer with CEA negative. The interesting notion was that at the present study, 58.7% colon cancer sera with CEA negative were anti-TAAs positive. If both anti-TAA and CEA were simultaneously used as diagnostic markers, 82.6% colon cancer patients could be correctly identified suggesting that CEA and anti-TAAs seem to be independent, but complementary serological markers in detection of colon cancer. Because of the limited sample size of the current study, additional studies in colon cancer patients with different clinical stages should be performed to further evaluate whether both CEA and anti-TAAs could be used together to enhance colon cancer detection and might be a clinically useful non-invasive approach in colon cancer diagnosis.

In conclusion, our data suggest that TAA mini-arrays may provide a promising and powerful tool for enhancing cancer detection, but their utility in a clinical setting is currently in its infancy. Before the approach of TAA arrays are widely implemented in screening programmes for cancer diagnosis or as tools for monitoring cancer progression and guiding therapeutic interventions, it is important to maximize their sensitivity and specificity by defining systematically the optimal combination of TAAs.

Acknowledgments

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