RESEARCH ARTICLE

# Mini-Array of Multiple Tumor-associated Antigens (TAAs) in the Immunodiagnosis of Esophageal Cancer

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## Abstract

**Sera of cancer patients may contain antibodies that react with a unique group of autologous cellular antigens called tumor-associated antigens (TAAs). The present study aimed to determine whether a mini-array of multiple TAAs would enhance antibody detection and be a useful approach in esophageal cancer detection and diagnosis. Our mini-array of multiple TAAs consisted of eleven antigens, p53, pl6, Impl, CyclinB1, C-myc, RalA, p62, Survivin, Koc, CyclinD1 and CyclinE full-length recombinant proteins. Enzyme-linked immunosorbent assays (ELISA) were used to detect autoantibodies against eleven selected TAAs in 174 sera from patients with esophageal cancer, as well as 242 sera from normal individuals. In addition, positive results of ELISA were confirmed by Western blotting. In a parallel screening trial, with the successive addition of antigen to a final total of eleven TAAs, there was a stepwise increase in positive antibody reactions. The eleven TAAs were the best parallel combination, and the sensitivity and specificity in diagnosing esophageal cancer was 75.3% and 81.0%, respectively. The positive and negative predictive values were 74.0% and 82.0%, respectively, indicating that the parallel assay of eleven TAAs raised the diagnostic precision significantly. In addition, the levels of antibodies to seven antigens, comprising p53, Impl, C-myc, RalA, p62, Survivin, and CyclinD1, were significantly different in various stages of esophageal cancer, which showed that autoantibodies may be involved in the pathogenesis and progression of esophageal cancer. All in all, this study further supports our previous hypothesis that a combination of antibodies might acquire higher sensitivity for the diagnosis of certain types of cancer. A customized mini-array of multiple carefully-selected TAAs is able to enhance autoantibody detection in the immunodiagnosis of esophageal cancer and autoantibodies to TAAs might be reference indicators of clinical stage.**

**Keywords:** Esophageal cancer - tumor-associated antigen - autoantibody - diagnosis

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## Introduction

Estimated 482,300 new esophageal cancer cases and 406,800 deaths occurred in 2008 worldwide, and the highest rates were found in Southern and Eastern Africa and Eastern Asia (Jemal et al., 2011). Esophageal cancer is one of the most common malignancies of the digestive tract and the fourth most frequent causes of cancer deaths in China (He et al., 2011). Esophageal cancer shows a poor prognosis largely due to the lack of early screening strategy and is often presented in an advanced stage at the first time of diagnosis (Shang et al., 2010). Despite of considerable diagnostic and therapeutic advances in the treatment of esophageal cancer in recent years, new diagnostic and therapeutic tools are essential for the optimal management of esophageal cancer. Therefore, extensive studies have been conducted to identify and validate new biomarkers to increase the sensitivity and specificity of esophageal cancer detection. Chen et al reported a novel and cost-effective quartz crystal microbalance (QCM) immunosensor to detect early lung cancer on animal model (Chen et al., 2011). Despite the high sensitivity of 80%, the diagnostic value of this method for esophageal cancer of human is still in question.

Numerous studies have demonstrated that cancer sera contain antibodies that react with a unique group of autologous cellular antigens called tumor-associated antigens (TAAs) (Tan et al., 2008; Zhang et al., 2010). In addition, the available data show that a number of the target antigens are cellular proteins, such as p53 whose aberrant regulation or overexpression is capable of leading to tumorigenesis, as well as CyclinB1 (Crawford et al., 1982; Winter et al., 1992; Soussi, 2000; Himoto et al., 2005; Ersvaer et al., 2007). It has been also demonstrated that the immune surveillance system in certain cancer patients has the capability of recognizing these antigenic

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changes in cancer cells and produce autoantibodies, which can amplify the response signal to the TAAs (Tan, 2001; Tan et al., 2008). Thus, cancer-associated autoantibodies might be considered as reporters identifying aberrant or disregulated cellular mechanisms in tumorigenesis (Tan et al., 2008; Zhang et al., 2010).

However, because of the low sensitivity, no single marker has been used for early diagnosis. Many studies have shown that the sensitivity can be enhanced by using a mini-array of multiple TAAs as target antigens (Zhang, 2007a; 2007b; Li et al., 2008; Liu et al., 2009; Ye et al., 2013). For example, a previous study showed that the frequency of antibodies against any individual antigen rarely exceeded 15-20%, but with the successive addition of antigens to the panel, there was a stepwise increase in the percentage of positive reactors between 44% and 68% against a combination of seven antigens (Zhang et al., 2003). In addition, lung, breast, and prostate cancers showed separate and distinctive profiles of antibody responses, indicating that customized TAA panels could be developed for different types of cancer (Zhang et al., 2003). Mini-arrays of TAAs might provide another approach to tumor detection and diagnosis. The current study evaluated whether a mini- array of multiple TAAs would enhance autoantibody detection and be an effective tool in the immunodiagnosis of esophageal cancer.

## Materials and Methods

*Serum samples*

Sera from 174 patients with esophageal cancer were obtained from the first Affiliated Hospital of Zhengzhou University (Henan Province, China) from January 1, 2009 to January 27, 2010. All cancer sera were collected at the first time of cancer diagnosis before patients being treated with chemotherapy or radiation therapy. Of 174 esophageal cancer patients, 115 (66.1%) were male and 59 (33.9%) were female. Mean age was 61.85±9.11 years (range, 34-84 years). As control, sera were obtained from 242 individuals without obvious evidence of malignancy and autoimmune diseases during annual health examination in the Affiliated Hospitals of Zhengzhou University from March to April in 2011. The distribution of gender and age in case and control groups were not different significantly. The names and identification numbers of study populations were blinded to all investigators due to the regulations concerning studies of human subjects. In addition, informed consent has been obtained from all the participants involved. All persons gave their informed consent prior to their inclusion in the study.

*Recombinant proteins and antibody*

All TAAs used in the present study, including p53, p16, Impl, CyclinB1, C-myc, RalA, p62, Survivin, Koc, CyclinD1 and CyclinE, were constructed and purified. Horseradish peroxidase (HRP)-conjugated goat anti-human IgG was purchased (Stratagene company, USA).

*Enzyme-linked immunosorbent assay (ELISA)*

All purified recombinant proteins were individually diluted in Phosphate buffered saline (PBS) to a final concentration of 0.5μg/ml, and 100μl were added into each well to coat onto microtitre plates (Gibico, USA) overnight at 4°C. The human serum samples diluted at 1:100, were incubated with the antigen-coated wells at 37°C for 60 min followed by washing with PBS containing 0.05% Tween 20 (PBST), and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Stratagene company, USA) as a secondary antibody diluted at 1:4000 for 60 min followed by washing with PBST. The solution of 3,3',5,5'-TMB (3,3',5,5'-tetramethyl benzidine, TMB)–$H_2O_2$-urea was used as the detecting reagent. Finally, the average optical density (OD) of each well was read at 450 nm. Each sample was tested at least twice. The cut-off value for determining a positive reaction was designated as the mean absorbance of the 242 normal human sera plus 2 standard deviations (mean±2SD) (Fang et al., 2004; Liu et al., 2009; Ye et al., 2013).The detailed protocol of ELISA has been described previously (Zhang et al., 1999; Zhang et al., 2003).

*Western blotting*

Western blot analysis was used to confirm the positive sera samples detected by ELISA. In brief, the purified proteins (p53, p16, Impl, CyclinB1, C-myc, RalA, p62, Survivin, Koc, CyclinD1 and CyclinE) were electrophoresed on 12% SDS-PAGE and subsequently transferred to a PVDF membrane. After blocking in TBST with 5% non-fat milk for 2h at room temperature, the PVDF membrane was incubated for 90 min with patient's serum diluted at 1:300. Then, the PVDF membrane was incubated with HRP-conjugated goat anti-human IgG diluted at 1:2000 for 1h followed by washing with TBST solution. Finally, the ECL-kit was used to detect immunoreactive bands according to the manufacturer's instructions (Kangwei biological technology company, Beijing, China).

*Statistical analysis*

To determine whether the frequency of autoantibodies to the selected TAAs in the esophagus cancer sera was significantly higher than that in sera from normal cohort, the data were analyzed using the $\chi^2$ tests and $\chi^2$ tests with Yates' correction. The level of statistical significance (0.05) was used and $p<0.05$ was considered to show statistically significant differences. The comprehensive evaluations of testing results for every anti-TAA antibody, including the methods for calculating the sensitivity, specificity, positive and negative likelihood ratio, Youden's index (YI), positive (PPV) and negative predictive value (NPV), agreement rate and $\kappa$-value, were based on the methodology provided in the Epidemiology textbook (Chen et al., 2004).

## Results

*Prevalence of antibodies against a mini-array of multiple TAAs in esophageal cancer*

In the current study, a mini-array of eleven TAAs was used as coating antigens in ELISA, and sera from 174 patients with esophageal cancer and sera from 242 normal

individuals were examined for the presence of antibodies to the individual TAA and cumulatively to the entire panel of eleven TAAs. The frequency of antibodies against eleven TAAs in sera from 174 patients with esophageal cancer and 242 normal human sera is shown in Table 1. A positive test for antibodies was taken as an absorbance reading above the mean+2SD of the 242 normal human sera. The combined antibody frequency was 75.3% (131/174), significantly higher than the frequency (19.0%) in sera from normal individuals (46/242). Antibody frequency to any individual TAA in esophageal cancer varied from 10.3% to 21.8%. The ELISA results were also confirmed by Western blotting analysis. The higher frequency of antibodies to an individual TAA in esophageal cancer was against p53 (21.8%), p16 (18.4%), IMP1 (16.1%), CyclinB1 (16.1%), C-myc (15.5%) RalA (15.5%), p62 (12.1%), Survivin(12.1%), Koc (11.5%), CyclinD1 (10.3%), and followed by CyclinE (10.3%). The reactivity of normal human sera was low, ranging from 0.8 to 3.7% to any individual TAA, with a combined frequency of reactivity of 19.0% against the complete panel of eleven TAAs.

In addition, clinical stages of all the patients with esophageal cancer were determined according to TNM standards. The distribution of OD was non-normal in various stages of esophageal cancer. We found that the levels of antibodies to seven TAAs, including p53, Imp1, p62, C-myc, RalA, Survivin, and CyclinD1, were significantly different in different stages of esophageal cancer, using Kruskal-Wallis H test. Spearman rank correlation analysis was used to assess the correlation between levels of antibodies and stages of esophageal cancer. The result revealed the positive correlation of antibodies to seven TAAs and stages of esophageal cancer, including p53, Imp1, C-myc, RalA, Survivin, CyclinD1, and CyclinE, as well as the negative correlation of antibody to p62 and the stages. The detailed information is shown in Table 2.

*Evaluation of diagnostic values of a mini-array of eleven TAAs in immunodiagnosis of esophageal cancer*

The validity of a test method is defined as its ability to distinguish between individuals with a disease and those without the disease. In order to assess the value of the approach of antibody detection to a mini-array of multiple TAAs in separating individuals with and without cancer, a group of parameters, such as the YI , sensitivity/specificity, and PPV/NPV, were calculated and are shown in Tables 3 and 4. Table 3 shows the comprehensive evaluation of antibodies to a panel of eleven TAAs. With the successive addition of TAAs to a total of eleven antigens, there was a stepwise increase of positive antibody reactions up to 75.3% and there was also a slight decrease of specificity from 96.3% with one TAA to 81.4% with a panel of eleven TAAs. It is consistent with the results of other two parameters (PPV/NPV). The PPV / NPV were also

**Table 1. Frequency of Antibodies to Eleven TAAs in Esophageal Cancer and Evaluation of Diagnostic Value of Antibodies in Esophageal Cancer Detection**

| TAAs | No.(%) of antibodies in: ESCC | NHS | Sensitivity (%) | Specificity (%) | YI | PPV (%) | NPV (%) | AUC |
|---|---|---|---|---|---|---|---|---|
| p53 | 38[a](21.8) | 9(3.7) | 21.8 | 96.3 | 0.2 | 80.9 | 63.1 | 0.6 |
| p16 | 32[a](18.4) | 3(1.2) | 18.4 | 98.8 | 0.2 | 91.4 | 62.7 | 0.6 |
| Imp1 | 28[a](16.1) | 4(1.7) | 16.1 | 98.3 | 0.1 | 87.5 | 62.0 | 0.6 |
| CyclinB1 | 28[a](16.1) | 5(2.1) | 16.1 | 97.9 | 0.1 | 84.8 | 61.9 | 0.6 |
| C-myc | 27[a](15.5) | 3(1.2) | 15.5 | 98.8 | 0.1 | 90.0 | 61.9 | 0.6 |
| RalA | 27[a](15.5) | 8(3.3) | 15.5 | 96.7 | 0.1 | 77.1 | 61.4 | 0.6 |
| p62 | 21[a](12.1) | 10(4.1) | 12.1 | 95.9 | 0.1 | 67.7 | 60.3 | 0.5 |
| Survivin | 21[a](12.1) | 1(0.4) | 12.1 | 99.6 | 0.1 | 95.5 | 61.2 | 0.6 |
| Koc | 20[a](11.5) | 5(2.1) | 11.5 | 97.9 | 0.1 | 80.0 | 60.6 | 0.5 |
| CyclinD1 | 18[a](10.3) | 9(3.7) | 10.3 | 96.3 | 0.1 | 66.7 | 59.9 | 0.5 |
| CyclinE | 18[a](10.3) | 2(0.8) | 10.3 | 99.2 | 0.1 | 90.0 | 60.6 | 0.5 |

*$P$-values relative to NHS: [a]$P<0.05$. TAA, tumor-associated antigen; ESCC, esophageal cancer; NHS, normal human sera; YI, Youden's index; PPV, positive predictive value; NPV, negative predictive value; AUC: area under the receiver operating characteristic (ROC) curve

**Table 2. Positive Rate of Antibodies to eleven TAAs in Different Clinical Stages of Esophageal Cancer**

| TAAs | Levels of antibodies M (Q) | | | | | $p$ value | r |
|---|---|---|---|---|---|---|---|
| | Stage0 (n=3) | Stage1 (n=8) | Stage2 (n=79) | Stage3 (n=52) | Stage4 (n=18) | | |
| p53 | 0.37(0.14) | 0.41(0.11) | 0.52(0.13) | 0.59(0.23) | 0.67(0.07) | 0.000[#] | 0.481* |
| p16 | 0.42(0.18) | 0.56(0.09) | 0.47(0.09) | 0.50(0.10) | 0.51(0.06) | 0.184 | 0.07 |
| Imp1 | 0.45(0.16) | 0.43(0.16) | 0.47(0.12) | 0.51(0.24) | 0.59(0.16) | 0.010[#] | 0.269* |
| CyclinB1 | 0.52(0.08) | 0.34(0.16) | 0.39(0.13) | 0.37(0.08) | 0.37(0.08) | 0.087 | -0.143 |
| C-myc | 0.35(0.24) | 0.36(0.25) | 0.36(0.15) | 0.46(0.29) | 0.65(0.09) | 0.000[#] | 0.453* |
| RalA | 0.34(0.16) | 0.38(0.14) | 0.42(0.09) | 0.45(0.13) | 0.46(0.13) | 0.025[#] | 0.261* |
| p62 | 0.55(0.30) | 0.58(0.13) | 0.48(0.14) | 0.43(0.12) | 0.50(0.14) | 0.017[#] | -0.162 |
| Survivin | 0.34(0.27) | 0.37(0.21) | 0.41(0.14) | 0.45(0.18) | 0.60(0.16) | 0.000[#] | 0.411* |
| Koc | 0.27(0.15) | 0.29(0.18) | 0.35(0.13) | 0.32(0.15) | 0.31(0.15) | 0.187 | -0.053 |
| CyclinD1 | 0.24(0.27) | 0.35(0.16) | 0.35(0.13) | 0.40(0.28) | 0.65(0.21) | 0.000[#] | 0.375* |
| CyclinE | 0.38(0.31) | 0.39(0.15) | 0.38(0.16) | 0.49(0.23) | 0.50(0.17) | 0.055 | 0.225* |

*$p<0.05$; [#]$p<0.05$; M, median; $Q=P_{75}-P_{25}$, quartile range; r, rank correlation coefficient

**Table 3. Evaluation of Diagnostic value of Antibodies to Different Combination of TAAs in Esophageal Cancer Detection**

| Panel of TAAs | Positive No.(%) ESCC n=174 | NHS n=242 | Sensitivity (%) | Specificity (%) | YI (%) | PPV | NPV | AUC |
|---|---|---|---|---|---|---|---|---|
| p53 | 38[a] (21.8) | 9 (3.70) | 21.80 | 96.30 | 0.18 | 80.90 | 63.10 | 0.59 |
| p53, p16 | 63[a] (36.2) | 12 (4.96) | 36.20 | 95.00 | 0.31 | 84.00 | 67.40 | 0.66 |
| p53, p16, Imp1 | 73[a] (42.0) | 16 (6.61) | 42.00 | 93.40 | 0.35 | 82.00 | 69.10 | 0.68 |
| p53, p16, Imp1, CyclinB1 | 85[a] (48.9) | 21 (8.68) | 48.90 | 91.30 | 0.40 | 80.20 | 71.30 | 0.70 |
| p53, p16, Imp1, CyclinB1, C-myc | 93[a] (53.4) | 23 (9.50) | 53.40 | 90.50 | 0.44 | 80.20 | 73.00 | 0.72 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA | 101[a] (58.0) | 30 (12.4) | 58.00 | 87.60 | 0.46 | 77.10 | 74.40 | 0.73 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA, p62 | 108[a] (62.1) | 38 (15.7) | 62.10 | 84.30 | 0.46 | 74.00 | 75.60 | 0.73 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA, p62, Survivin | 116[a] (66.7) | 38 (15.7) | 66.70 | 84.30 | 0.51 | 75.30 | 77.90 | 0.76 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA, p62, Survivin, Koc | 125[a] (71.8) | 43 (17.8) | 71.80 | 82.20 | 0.54 | 74.40 | 80.20 | 0.77 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA, p62, Survivin, Koc, CyclinD1 | 128[a] (73.6) | 45 (18.6) | 73.60 | 81.40 | 0.55 | 74.00 | 81.10 | 0.78 |
| p53, p16, Imp1, CyclinB1, C-myc, RalA, p62, Survivin, Koc, CyclinD1, CyclinE | 131[a] (75.3) | 46 (19.0) | 75.30 | 81.00 | 0.56 | 74.00 | 82.00 | 0.78 |

*$p$ values relative to NHS: [a]$P < 0.05$; TAA, tumor-associated antigen; EC, esophagus cancer; NHS, normal human serum; YI, Youden's index; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the receiver operating characteristic (ROC) curve

**Table 4. Summary of the Diagnostic value of Antibodies for a Panel of Eleven TAAs in EC**

| Mini-array | EC | NHS | Total |
|---|---|---|---|
| Any TAA positive | 131(75.3) (A) | 46 (19.0) (B) | 177 (C1) |
| All TAA negative | 43 (24.7) (C) | 196 (81.0) (D) | 239 (C2) |
| Total | 174 (R1) | 242 (R2) | 416 (N) |

*Fourfold table $\chi^2$ tests: $\chi^2$=131.154, P<0.01. Sensitivity (%)=A/(A+C)=131/174=75.3%. Specificity (%)=D/ (B+D)=196/242=81.0%. Youden's index=Sensitivity+Specificity -1=0.753+0.810-1=0.56. Positive (+) likelihood ratio=Sensitivity/(1-Specificity) =0.753/(1-0.810)=3.96. Negative (-) likelihood ratio=(1- Sensitivity)/Specificity=(1-0.753)/0.810=0.305. Agreement rate=(A+D)/(A+B+C+D) ×100=(131+196)/(131+46+43 +196) ×100=78.6%. ϰ=[N (A+D)-(R1C1+R2C2)]/ [N2-(R1C1+R2C2)] =0.56. NHS, normal human sera

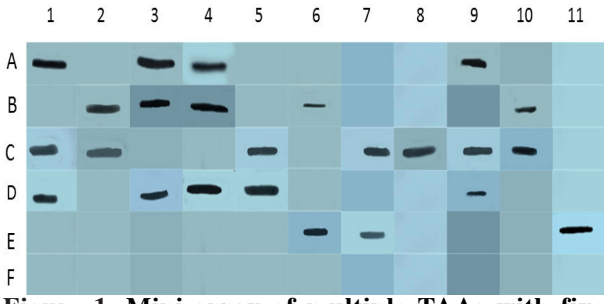


**Figure 1. Mini-array of multiple TAAs with five representative esophageal cancer sera using western blot analysis.** Lane 1, p53; Lane 2, p16; Lane 3, Imp1; Lane 4 CyclinB1; Lane 5, C-myc; Lane 6, RalA; Lane 7, Survivin; Lane 8, p62; Lane 9, Koc; Lane 10, Cyclin D1; Lane 11, CyclinE.(A-E) Five representative esophageal cancer sera showed different antibody profiles with the eleven TAAs. TAA, tumor-associated antigen. (F) Normal human serum showed no reactivity to any of the eleven TAAs

variable in different combinations of TAAs. In the panel of eleven TAAs, the PPV was 74.0%, and the PPV was 82.0%. The Youden's index also increased from 0.18 with one TAA to 0.56 with eleven TAAs, which indicated that the clinical diagnostic value of parallel assay of eleven TAAs was high. It also suggests that the parallel assay of eleven TAAs can raise the diagnostic precision greatly. The positive and negative likelihood ratio was 3.96 and 0.305 respectively. The concordance rate and Kappa value were 78.6% and 0.56, respectively, indicating that the observed value of this parallel assay had a middle range coincidence with the actual value. Taken together, these data showed the utility of the mini-array of eleven TAAs in increasing the clinical diagnostic quality and value in esophageal cancer. Positive results of ELISA were also confirmed by western blot. Western Blot analysis of five representative esophageal cancer sera is shown in Figure 1.

## Discussion

As demonstrated in many studies, cancer has long been recognized as a multi-step and multi-factor biological process in which proteins are the final executants of various life functions. This biological nature of cancer was well demonstrated by the frequent observation that many cancer patients produce autoantibodies to several TAAs (Zhang et al., 2001). In addition, the autoantibodies are generally absent, or present in extremely low titers, in normal individuals and in non-cancer conditions (with the exception of autoimmune conditions) (Tan, 2001; Tan et al., 2008). The stability and persistence of autoantibodies to TAAs in sera from patients with cancer is an advantage over other potential markers, including the TAAs themselves, which are released by tumors but are rapidly degraded or cleared after circulating in sera for a limited time (Anderson et al., 2005). Moreover, the widespread availability of methods and reagents to detect serum autoantibodies promotes their characterization in cancer patients and assay development. Thus, anti-TAA antibodies have been extensively investigated and evaluated as serological markers for cancer diagnosis (Paradis et al., 2007).

It is disappointed that no single autoantibody can completely identify the cancer group from the healthy controls due to the low frequency and sensitivity. However, the combination of multiple autoantibodies may provide a promising method for early detection of cancer. Our previous studies showed that combinations of multiple antigen-antibody systems might acquire higher sensitivity for diagnosis of cancer (Liu et al., 2006). Wang and his coworkers (Wang et al., 2005) used a phage display library derived from prostate cancer tissue and developed a phage

protein micro-array for the analysis of autoantibodies in sera from prostate cancer patients and individuals without history of prostate cancer. In the study, a 22-phage-peptide detector was constructed for prostate cancer screening, with 81.6% sensitivity and 88.2% specificity. Koziol et al. (2003) used five anti-TAA antibodies to acquire 79% sensitivity and 86% specificity, which are equivalent to those of Wang. In addition, different types of cancer showed distinct profiles of autoantibody in which specific TAAs were preferentially targeted (Zhang et al., 2003; Koziol et al., 2003; Zhang et al., 2007).These studies support the hypothesis that ''customized" TAA arrays enhance autoantibody detection in cancer and constitute promising and powerful tools for the immunoserological diagnosis of certain types of cancer, such as breast, liver and prostate cancer.

Our further aim is to increase the sensitivity and specificity of anti-TAA antibodies as diagnostic markers of cancer by expanding the TAA array, including TAAs which may be more selectively associated with one specific type of cancer such as esophageal cancer and not with others (Zhang et al., 2011). We expect that our mini-array of multiple TAAs may be used as a novel non-invasive approach to distinguish cancer in the common population and high-risk individuals. In fact, the TAAs, such as p53, p16 and C-myc, which were used in the current mini-array, are associated with several types of cancer, including lung, liver, colon, ovarian and prostate cancer (Zhang, 2007a; 2007b; Li et al., 2008; Liu et al., 2009; Ye et al., 2013). Hence, the approach may not be suitable for distinguishing one type of cancer from another. In addition, OD value varies between experiments. Wu et al used a Combination of p53- and phage-ELISA to solve this issue (Wu et al., 2011). We believe it is more reliable if we calculate the absolute concentration of autoantibody by using standard curve instead of OD number. In the future studies, we propose that carefully selected antibody-antigen systems might be unique to one type of cancer and others may not. A comprehensive analysis and evaluation of various combinations of carefully selected antibody-antigen systems may be useful for the development of autoantibody profiles involving various arrays of TAAs and the diagnosis of certain other types of cancer. In the present study, a mini-array of multiple TAAs was used as coating antigens in an ELISA to detect autoantibodies to these antigens in sera from 174 patients with esophageal cancer and sera from 242 normal individuals. Antibody frequency to the individual TAAs in esophageal cancer was variable and ranged from 10.3% to 21.8%, which does not meet the requirements of clinical early diagnosis of esophageal cancer. With the successive addition of TAAs to a total of eleven antigens, there was a stepwise increase in positive antibody reactions, reaching a sensitivity of 75.3% and a specificity of 81.0% in esophageal cancer. The positive and negative likelihood ratios were 3.96 and 0.305, respectively, indicating that the clinical diagnostic value of a parallel assay of eleven TAAs was high. The concordance rate of 78.6% revealed that the observed value of this assay had a middle range coincidence with the actual value . The PPV of 74.0% and NPV of 82.0% indicated that the parallel assay raised the diagnostic precision significantly. In addition, the levels of antibodies to seven antigens, including p53, Impl, C-myc, RalA, p62, Survivin, and CyclinD1, were significantly different in different stages of esophageal cancer, suggesting antibodies may involve in the development of esophageal cancer.

In conclusion, our data further supports our previous hypothesis that a customized mini-array of multiple carefully-selected TAAs is able to enhance autoantibody detection in the immunodiagnosis of esophageal cancer. In addition, additional esophageal cancer-specific TAAs are likely to be necessary to maximize the sensitivity and specificity by defining systematically the optimal combination of TAAs and autoantibodies against TAAs might be reference indicators of clinical stage of cancer.

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