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ORIGINAL ARTICLE – CANCER RESEARCH

Autoantibody against 14-3-3 zeta: a serological marker in detection of gastric cancer

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Abstract

Purpose Autoantibody to 14-3-3 zeta was identified in gastric cancer (GC) by serological proteome analysis (SERPA) in our previous study. We comprehensively evaluated its ability to detect GC, determined its association with clinical characteristics, and explored its temporal change in GC patients before and after gastrectomy resection in this study.

Methods Anti-14-3-3 zeta antibody was examined by immunoassay in sera from 465 GC patients and 465 normal individuals, and also in 69 serial sera from 26 GC patients before and after resection.

Results The frequency of anti-14-3-3 zeta were significantly higher in GC group than in control group, with AUC of 0.627. The appearance of anti-14-3-3 zeta showed no difference in different tumor stage, tumor size, tumor differentiation, and lymphatic metastasis, but was higher in GC patients with family tumor history than without family tumor history. When anti-14-3-3 zeta was combined with clinical markers (CEA, CA199 and CA724), the sensitivity increased to 52.7%. In the follow-up analysis, the titer of anti-14-3-3 zeta was higher in post-resection sera than pre-resection sera, and no difference was observed in CEA, CA199 and CA724. Anti-14-3-3 zeta showed an increase from negative status to positive status in six patients after resection, while other three clinical markers presented different change in GC patients after resection.

Conclusions Autoantibody against 14-3-3 zeta could be a potential diagnostic biomarker and improve the sensitivity of CEA, CA199 and CA724 in diagnosis of GC. Further largescale studies will be needed to validate its performance in GC patients after resection.

Keywords Gastric cancer · 14-3-3 zeta · Autoantibody · Biomarker

Introduction

Gastric cancer (GC) is an important health problem and serious burden, the second leading cause of cancer death not only in China, but also in many other countries (Chen et al. 2016; Van Cutsem et al. 2016). 5-year survival of GC is low because less than 20% of patients are diagnosed at an early stage. In addition, patients with advanced stage GC have an extremely poor survival rates (Hartgrink et al. 2009). At present, endoscopy and histopathological examination are common techniques, but have certain drawbacks in diagnosis of GC. Therefore, it is essential to identify novel, reliable, and non-invasive blood test to improve detection of early stage gastric cancer (Kim et al. 2018). Serum biomarkers have been used for GC diagnosis and prognosis in clinics, e.g., carcinoembryonic antigen (CEA), carbohydrate antibody 199 (CA199), and carbohydrate antibody 724 (CA724). However, generally, these serum biomarkers have limited sensitivity and specificity for GC screening (Carpelan-Holmstrom et al. 2002; Fernandez-Fernandez et al. 1996).

Many studies have demonstrated autoantibodies to tumor-associated antigens (TAAs) can be presented before clinical symptoms in cancer (Dai et al. 2016b; Zhang et al. 2003). Therefore, tumor-associated antigen and autoantibody

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systems were reported as potential biomarkers for early detection of cancer (Dai et al. 2013; Hoshino et al. 2017; Zhong et al. 2008). Our team identified a potential gastric cancer-associated autoantibody (autoantibody to 14-3-3 zeta) by proteomics-based analysis (Liu et al. 2017).

14-3-3 proteins are a family of conserved regulatory molecules that are expressed in all eukaryotic cells. There are seven distinct 14-3-3 proteins in mammals, including 14-3-3 beta, 14-3-3 epsilon, 14-3-3 gamma, 14-3-3 eta, 14-3-3 tau, 14-3-3 zeta and 14-3-3 sigma. 14-3-3 zeta is a ubiquitously expressed 14-3-3 family member that has been implicated to have oncogenic potential through its interaction with target proteins involved in cancer initiation as well as cancer progression (Oh et al. 2013). 14-3-3 zeta protein was reported overexpressed in multiple cancer cell lines, such as colon cancer (Lu et al. 2007), and pancreatic cancer (Shen et al. 2004). Nishimura et al. explored the oncogenetic role of YWHAZ (14-3-3 zeta) in GC, and the results suggested that the overexpression of 14-3-3 zeta protein had a pivotal role in tumor cell proliferation, and was a prognostic factor and potential therapeutic target in GC (Nishimura et al. 2013). Some studies indicated that 14-3-3 zeta protein was overexpressed in early stage of cancers, such as breast cancer (Wulfkuhle et al. 2002), oral and esophageal cancer (Bajpai et al. 2008; Matta et al. 2007). The importance of 14-3-3 proteins in cancer has become apparent, while its exact role in cancer progression as well as the mechanisms by which 14-3-3 zeta protein mediated cancer cell function remains unknown (Goc et al. 2012). Elevated expression of 14-3-3 zeta has been observed in a variety of tumor types (Hatzipetros et al. 2013; He et al. 2010). However, autoantibody against 14-3-3 zeta was only simply evaluated in limited sample size (85 GC patients and 85 normal individuals) in our previous study (Liu et al. 2017). This study also did not explore association of autoantibody against 14-3-3 zeta with tumor stage, metastasis, differentiation and other clinical characteristics.

In the present study, we comprehensively evaluated the ability of autoantibody against 14-3-3 zeta to detect GC and determined its association with clinical characteristics based on the previous study. In addition, the performance of combination of anti-14-3-3 zeta antibody and clinical biomarkers (CEA, CA199, and CA724) was evaluated in diagnosis of GC.

Materials and methods

Serum samples

A total of 465 patients with gastric cancer were included in this study from the First Affiliated Hospital of Zhengzhou University (January 2012 to June 2017) and 465 normal health individuals as control groups were matched to gastric cancer patients by age and gender. All cancer patients were confirmed by pathological examination and did not receive operation and any radiotherapy and chemotherapy treatments before sera were obtained. Sera were obtained from all subjects and stored at − 80 °C.

In addition, 26 gastric cancer patients from the First Affiliated Hospital of Zhengzhou University were followed up for 10 months before and after gastrectomy treatment, without other treatment. Finally, 69 serial serum samples collected at different time points. This study was performed in accordance with the rules of the Declaration of Helsinki of 1975, revised in 2013 and was approved by the Institutional Review Board of Zhengzhou University. Informed consent forms were obtained from all subjects before they participated in the study.

Expression and purification of 14-3-3 zeta recombinant protein

Prokaryotic expression system of *E.coli* was used to express 14-3-3 zeta. The recombinant expression plasmids of 14-3-3 zeta were constructed and stored in our lab. The target genes were inserted into expression vector pET-28a which produced a fusion protein with N-terminal 6 × histidine. Recombinant 14-3-3 zeta protein was further expressed in *E.coli* BL21 cells and purified using nickel column chromatography. The protocol for expression and purification of 6 × His-tagged proteins were performed as described (QIAGEN Inc., Valencia, CA, USA). Elution buffer of 8M urea, 0.1M NaH2PO4, 0.01M Tris, pH4.5, was used to elute the recombinant protein. The purified recombinant proteins were identified by western blotting using monoclonal anti-14-3-3 zeta antibody. Monoclonal anti-14-3-3 zeta antibody were purchased from Invitrogen (RRID:AB_779118).

Detection of 14-3-3 zeta, CA199, CA724 and CEA in sera

The autoantibodies against 14-3-3 zeta were detected in serum samples by enzyme-linked immunosorbent assay ELISA, which was described in detail in our previous study (Wang et al. 2018).

CA199, CA724 and CEA were detected using the electrochemiluminescence immunoassay (ECLIA) kit followed the manufacturer's instruction (Roche, Switzerland).

Statistical analysis

All statistical analysis was performed using SPSS (version 20.0) and Prism software (version 7.0, GraphPad) and receiver operating characteristic (ROC) curves were generated by Medcalc software (versions 11.4.2.0). The ROCs

analysis of anti-14-3-3 zeta for the distinguishing of gastric cancer from controls, leaded to estimate area under the curve (AUC) with 95% confidence interval (CI). Shapiro–Wilk tests were used to detect normal distribution of data. Due to the autoantibody against 14-3-3 zeta was not normally distributed (Shapiro Wilk's test), Mann–Whitney *U* tests were used to compare differences of antibody levels between two groups. χ^2 tests were used to compare the differences of frequency between two groups. As this study was case-matched study with a loose matching, a standard unconditional multivariable logistic regression model was used to calculate odds ratios (ORs) for age-, family tumor history- and sex-adjusted cases associated with GC according to serum anti-14-3-3 zeta levels. Spearman's test was used to evaluate the correlation between anti-14-3-3 zeta autoantibody level and levels of CEA, CA199, and CA724. The optimal cutoff thresholds for designating positive reaction were determined at the point on the ROC curve at which specificity was 90%. Differences were considered statistically significant when $P < 0.05$.

Results

The general information of all subjects

Table 1 shows the detailed clinical information of all subjects. There was no significant difference of age and gender between GC group and normal group in the case–control study ($P > 0.05$). In addition, the frequency of family cancer history (84/454, 18.1%) was significantly higher in case group compared with that of control group (44/406, 10.8%) ($P < 0.05$). Clinical information for all patients, including tumor stage, differentiation, lymphatic metastasis and tumor size was obtained.

Detection of anti-14-3-3 zeta autoantibody in sera from GC patients and normal individuals

To validate the potential of autoantibody against 14-3-3 zeta as biomarker in GC detection, the recombinant 14-3-3 zeta protein was used as antigen in ELISA to detect anti-14-3-3 zeta in sera from 465 GC patients and 465 normal individuals in case and control study. The level of anti-14-3-3 zeta autoantibody was significantly higher in sera from GC patients (median ± IQR 0.17 ± 0.08 ng/ml) than that in normal individuals (median ± IQR 0.14 ± 0.06) ($P < 0.001$, Fig. 1a). ROC analysis showed that anti-14-3-3 zeta can identify GC patients from normal individuals with AUC (95% CI) of 0.627 (0.595–0.658), sensitivity of 22.58%, specificity of 92.26%, positive likelihood ratio (+LR) of 2.92 and negative likelihood ratio (−LR) of 0.84 (Fig. 1b). The autoantibody levels of all subgroups including differentiation (Fig. 1c), metastasis (Fig. 1d), stages (Fig. 1e), tumor size (Fig. 1f), were observed to be higher compared to normal control group ($P < 0.05$). However, there was no difference among subgroups ($P > 0.05$).

The purified recombinant 14-3-3 zeta proteins were stained by coomassie blue on 10% running SDS polypropylene gel. Figure 2a shows that the purified protein presented high purity and concentration. Western blot analysis of recombinant 14-3-3 zeta protein is shown in Fig. 2b, indicating that the protein is able to conjugate anti-14-3-3 zeta antibody. 5 GC sera and 5 normal sera were randomly selected from GC patients and normal individuals for western blot analysis to confirm the results of ELISA. The results of ELISA and western blot are similar in Fig. 2c, indicating that the sensitivity of 14-3-3 zeta protein is higher in GC patients than normal individuals.

Performance of anti-14-3-3 zeta autoantibody in different clinical characteristics of GC

All GC patients were layered by different clinical characteristic, including (lymphatic metastasis, tumor size, differentiation, TNM stage, family tumor history, gender, and age). Every subgroup was compared to normal group to generate ROC curves and the results are shown in Fig. 3. Anti-14-3-3 zeta was observed to significantly distinguish GC patients from normal individuals in every subgroup (Fig. 3a–n) ($P < 0.05$). The AUCs of subgroups ranged from 0.603 to 0.679 ($P < 0.05$). The highest AUC of 0.679 were found in patients without family tumor history (Fig. 3j), and the lowest was observed in patients with lymphatic metastasis (Fig. 3b). The cutoff value was chosen while the sensitivity was the largest, and the specificity was more than 90% to ensure the high specificity and AUC in GC. The frequencies of anti-14-3-3 zeta autoantibody were not observed to be significantly different in comparison group (tumor size: ≤ 5 cm vs > 5 cm, lymphatic metastasis: yes vs no differentiation: poor vs moderate and high, stage: I + II vs III + IV, age: ≤ 60 years vs > 60 years) ($P > 0.05$). However, it was obviously different in the comparison group (family tumor history: yes vs no) ($P < 0.05$) and showed boundary significance in gender (male vs female) ($P = 0.054$) (Fig. 3o). In addition, smoke and alcohol will increase the incidence of gastric cancer and are highly related to GC, so we also explored the correlation of anti-14-3-3 zeta in serum with smoke or alcohol. The results showed that the expression of anti-14-3-3 zeta are not significantly different in non-smoking ($n = 312$) and smoking ($n = 145$) GC patients ($P = 0.789$), as well as non-drinking ($n = 359$) and drinking ($n = 98$) GC patients ($P = 0.648$).

In addition, multivariable logistic regression analyses revealed that anti-14-3-3 zeta autoantibody could be used as potential diagnostic biomarker for the identification of

Table 1 Characteristics of all subjects in this study

Characteristics	Gastric cancer ($n=465$)	Normal individuals ($n=465$)	P	GC patients for serial sera ($n=26$)
Age				
Mean ± SD	58.9 ± 12.0	58.8 ± 11.8	0.943*	55.96 ± 10.494
Range	23–94	23–89		34–77
≤ 60	238 (51.2%)	240 (51.6%)	0.896#	18 (69.2%)
> 60	227 (48.8%)	225 (48.4%)		8 (30.8%)
Gender				
Female	119 (25.6%)	119 (25.6%)	1.000#	9 (34.6%)
Male	346 (74.4%)	346 (74.4%)		17 (65.4%)
Family cancer history				
Yes	84 (18.1%)	44 (9.5%)	0.002#	5 (19.2%)
No	370 (79.6%)	362 (77.8%)		21 (80.8%)
Unknown	11 (2.4%)	59 (12.7%)		0
TNM stage, *n* (%)				
Stage I	76 (16.3%)	–		2 (7.7%)
Stage II	90 (19.4%)	–		6 (23.1%)
Stage III	147 (31.6%)	–		15 (57.7%)
Stage IV	49 (10.5%)	–		2 (7.7%)
Unknown	103 (22.2%)	–		1 (3.8%)
Differentiated degree				
Poor	166 (35.7%)	–		19 (73.1%)
Moderate	173 (37.2%)	–		5 (19.2%)
High	6 (1.3%)	–		0
Unknown	120 (25.8%)	–		2 (7.7%)
Tumor size				
< 2 cm	54 (11.6%)	–		5 (19.2%)
2–5 cm	108 (23.2%)	–		16 (61.5%)
> 5 cm	87 (18.7%)	–		4 (15.4%)
Unknown	216 (46.5%)	–		1 (3.8%)
Lymph node metastasis				
No	123 (26.5%)	–		4 (15.4%)
1–3	75 (16.1%)	–		10 (38.5%)
4–9	64 (13.8%)	–		6 (23.1%)
> 10	45 (9.7%)	–		5 (19.2%)
Unknown	158 (34%)	–		1 (3.8%)

*Independent samples Mann–Whitney U test

#Chi's-square tests

patients and normal after adjusting age, gender and family tumor history ($P<0.05$) (Table 2).

Combination of anti-14-3-3 zeta antibody and clinical biomarkers (CEA, CA199, and CA724) can improve sensitivity in diagnosis of GC

CEA, CA199, and CA724 were commonly used in diagnosis of gastric cancer in China and has been evaluated in many studies (Carpelan-Holmstrom et al. 2002; Gartner et al. 1998). The results showed that the CEA, CA199 be useful in diagnosing GC. Serum levels of CEA, CA19-9, and CA72-4 were assayed with electrochemiluminescence (ECL) method (Roche, Switzerland) in the present study. The threshold values were 5.0 ng/ml, 37.0 U/ml, 6.7 U/ml for CEA, CA19-9, and CA72-4, as recommended by the manufacturer. The threshold value for anti-14-3-3 zeta was chosen while the sensitivity was the largest, and the specificity was more than 90% to ensure the high specificity and AUC in 465 GC sera. CEA was tested in 244 patients (Median ± IQR 2.17 ± 3.24 ng/ml), and CA199 was tested in 242 patients (Median ± IQR 9.92 ± 13.96 U/

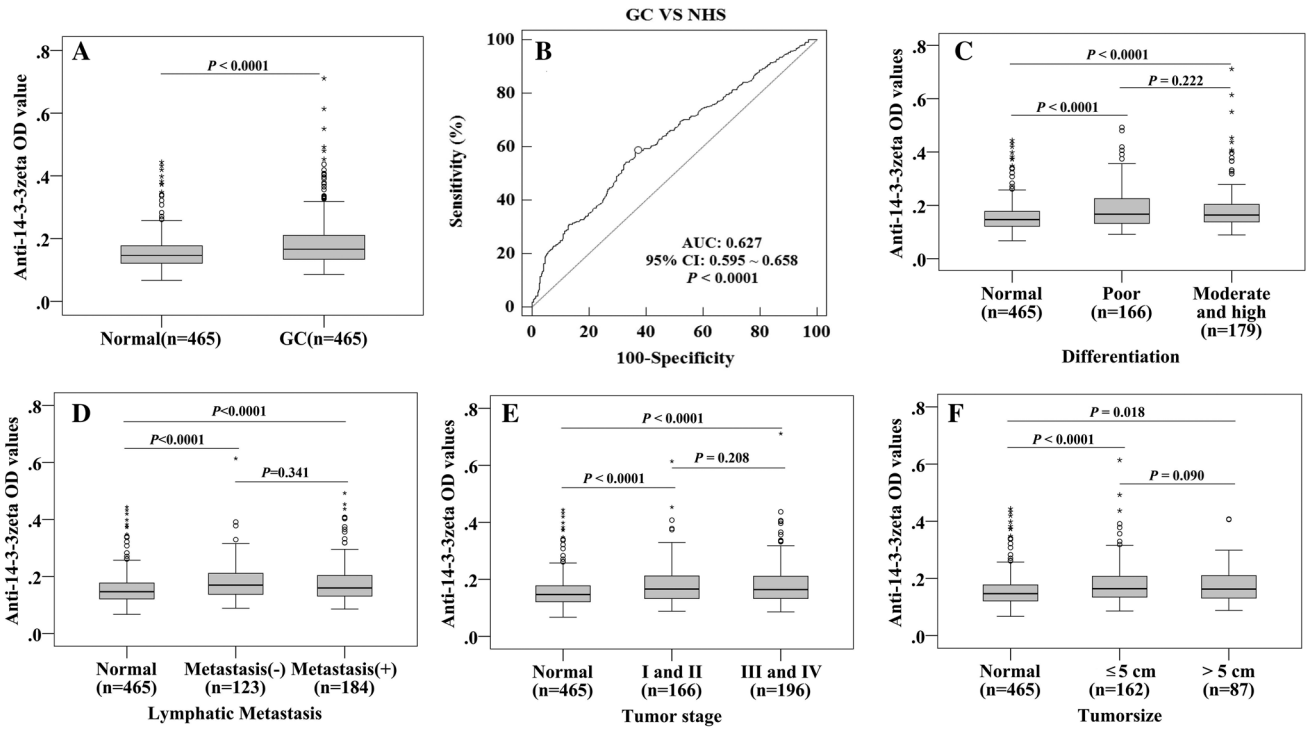

Fig. 1 The distribution of anti-14-3-3 zeta autoantibody in GC patients and NHS. *GC* gastric cancer, *NHS* normal health samples; **a** titers of anti-14-3-3 zeta autoantibody in GC patients and normal individuals, P: Mann–Whitney *U* Test. **b** ROC curve of autoantibody to 14-3-3 zeta. (**c**–**f**) titers of anti-14-3-3 zeta autoantibody in normal individuals and subgroups; **c** differentiation, **d** lymphatic metastasis, **e** tumor stage, **f** tumor size, P: One-way ANOVA: Post hoc multiple comparisons (LSD). Box and Whisker plots showed serum level of anti-14-3-3 zeta in patients and normal individuals. The line within the box marks the median, and the 25th and 75th percentiles are presented by the edges of the area, which is known as interquartile range (IQR). The bars indicate 1.5 times of the IQR from upper or lower percentiles

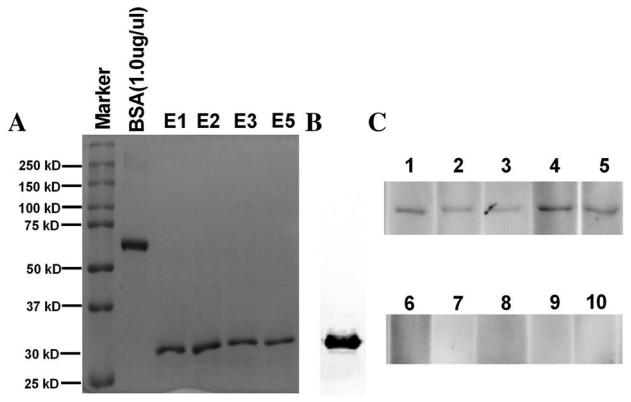

Fig. 2 Western blotting analysis of 14-3-3 zeta. **a** The coomassie blue staining for the purified 14-3-3 zeta protein on 10% SDS polypropylene gel. The lane E1, E2, E3 and E5 are 14-3-3 zeta protein eluted in buffer E; loading quantity of protein sample for each lane is 10 µl. **b** The western blotting result of 14-3-3 zeta protein conjugating with anti-14-3-3 zeta antibody. **c** The western blotting result of 14-3-3 zeta in 5 GC patients and 5 normal individuals. Lane 1–5 are GC sera selected randomly from GC patients; Lane 6–10 normal sera selected randomly from normal individuals

ml), and CA 724 was tested in 214 patients (Median ± IQR 2 0.56 ± 6.61 U/ml) in this study. We further analyzed the correlation of anti-14-3-3 zeta level with CEA ($R = -0.0117$, $P = 0.068$), CA199 ($R = 0.049$, $P = 0.466$), or CA724 ($R = 0.090$, $P = 0.188$) (Fig. 4a–c), indicating that anti-14-3-3 zeta and CEA, CA199, CA724 are independent biomarkers in sera from GC patients. In addition, we also explored the correlation among the expression of CEA, CA199, and CA724 through spearman correlation analysis. The results showed that CEA is correlated with CA199 and CA724, and the correlation coefficient is 0.219 ($P = 0.001$) and 0.239 ($P < 0.0001$), respectively. But there is no correlation between CA199 and CA724. The positive rates of anti-14-3-3 zeta, CEA, CA724, and CA199 were 23.0%, 23.0%, 28.0% and 17.8%, respectively (Fig. 4d) in GC patients. When CEA, CA724 and CA199 were combined, the frequency was up to 42.8%. When combination of anti-14-3-3 zeta, CEA, CA724, CA199 were used to detect GC patients, the sensitivity increased to 57.2% (Fig. 4d).

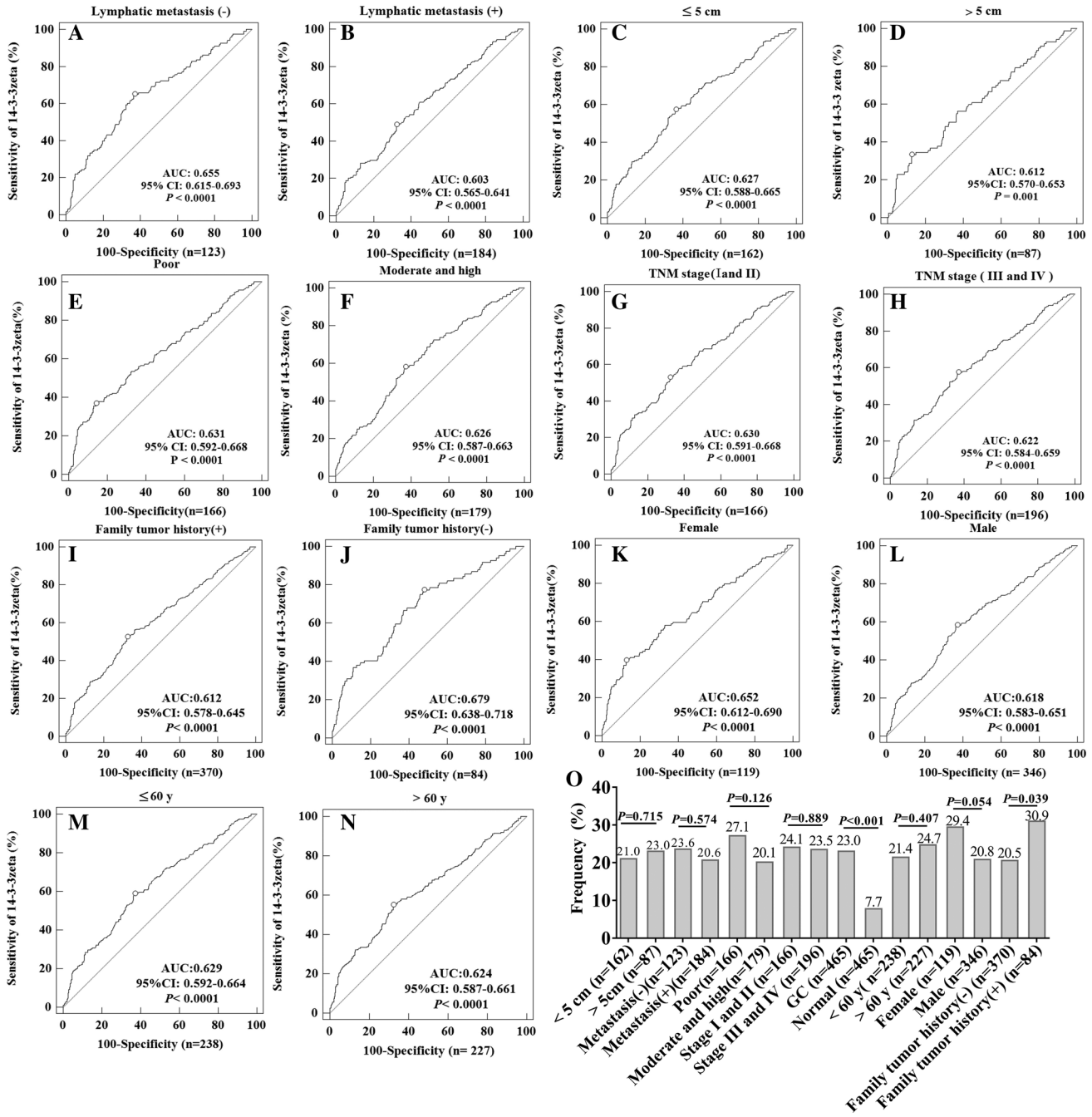

Fig. 3 The performance of 14-3-3 zeta in different characteristics of GC patients

Table 2 Logistic regression analyses of anti-14-3-3 zeta in GC patients compared to normal individuals

Variables	OR (95% CI)	*P*
Gender (female vs male)	0.805 (0.590–1.098)	0.170
Age (≥60 years vs < 60 years)	1.139 (0.864–1.502)	0.357
Family tumor history (yes vs no)	1.749 (1.172–2.612)	0.006
14-3-3 zeta (positive vs negative)	2.969 (1.962–4.492)	< 0.001

The levels of CA199, CA724, CEA and anti-14-3-3 zeta in serial sera before and after tumor resection

CA199, CA724, CEA and anti-14-3-3 zeta were detected in 69 serial sera from 26 GC patients before and after tumor resection to investigate the association with tumor resection. At first, the levels of these four markers were compared in sera before and after resection. If single GC patient had more than one serum before and after

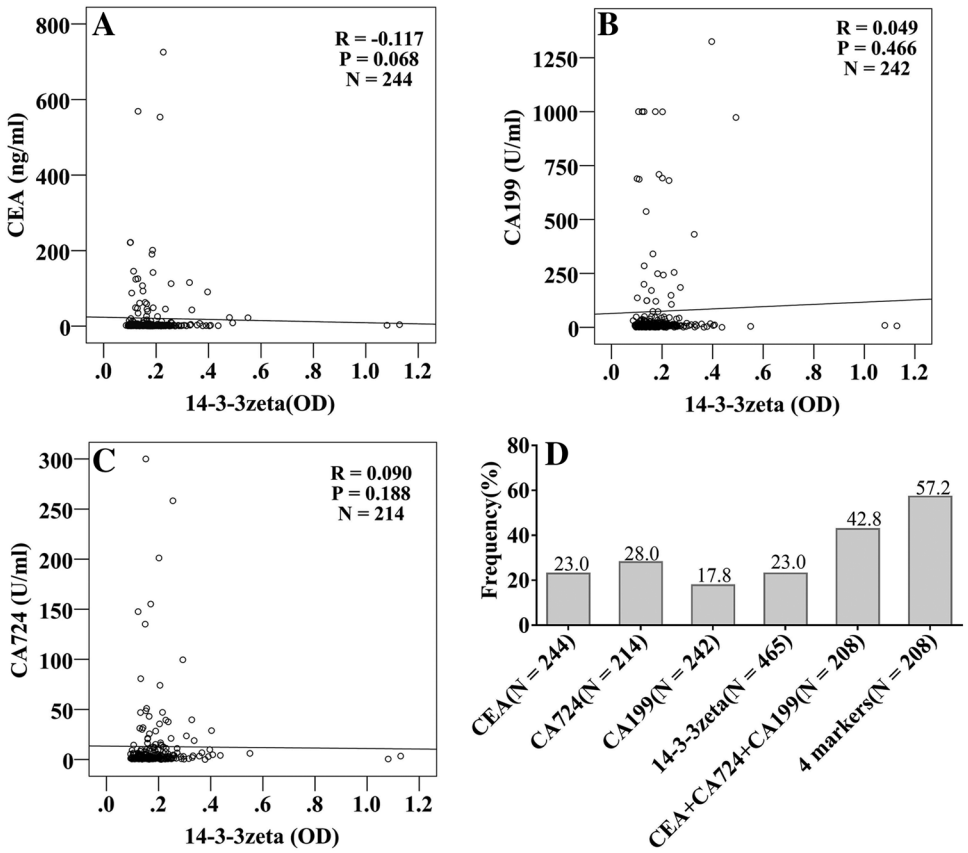

Fig. 4 Correlation analysis of anti-14-3-3 zeta with CEA, CA199 and CA174 in GC sera

resection, the means of OD values from the sera before resection and after resection was calculated, respectively. The results showed that levels of CA199, CA724 and CEA were same in sera before and after resection (Fig. 5a, b) ($P > 0.05$), and the level of anti-14-3-3 zeta were higher in sera after resection than before (Fig. 5c) ($P < 0.05$). Figure 5d–g shows the temporal changes for these four markers in serial sera. Interestingly, all four markers showed significant change in several patients after resection. For example, in patient 12 that donated four sera, 3 months, 2 months, 1 month before resection and 2 months after resection, CA199, CA724, and CEA levels showed a decrease after resection from positive values to negative values Fig. 5d–f, while anti-14-3-3 zeta antibody showed an opposite result from negative value to positive value (Fig. 5g). In patient 15, CA199, CA724, and CEA status changed from negative to positive after resection while the status of anti-14-3-3 zeta did not change. In addition, for CA199 level, only patient 12 showed a decrease and patient 15 showed an increase after resection (Fig. 5d). For CA724 level, patient 1, 2, 12 showed decreases and patient 15, 19 showed increases after resection (Fig. 5e). For CEA level, patient 5, 12 showed decreases and patient 15 showed increase after resection (Fig. 5f). For anti-14-3-3 zeta levels, six patients showed an increase after resection (Fig. 5g).

Discussion

Anti-14-3-3 zeta was identified as potential biomarker in GC sera by serological proteome analysis (SERPA) in the previous study (Liu et al. 2017). Even though SERPA has some drawbacks due to limits of 2-DE, it remains a very robust to evaluate humoral responses to cancer (Hamrita et al. 2008; Suzuki et al. 2010). Our previous study showed that autoantibody to 14-3-3 zeta showed high performance in diagnosis of lung cancer and hepatocellular carcinoma (Dai et al. 2016b; Liu et al. 2014). To determine the performance of anti14-3-3 zeta in GC detection, anti-14-3-3 zeta antibody was examined in sera from 465 GC patients and 465 normal individuals. The results showed that anti-14-3-3 zeta were significantly overexpressed in GC patients, which is line with the previous study (Liu et al. 2017; Wang et al. 2018). In addition, no study was found to report the association of anti-14-3-3 zeta antibody with general clinical information in GC, so we further explored the performance of anti-14-3-3 zeta in difference tumor stage, tumor size,

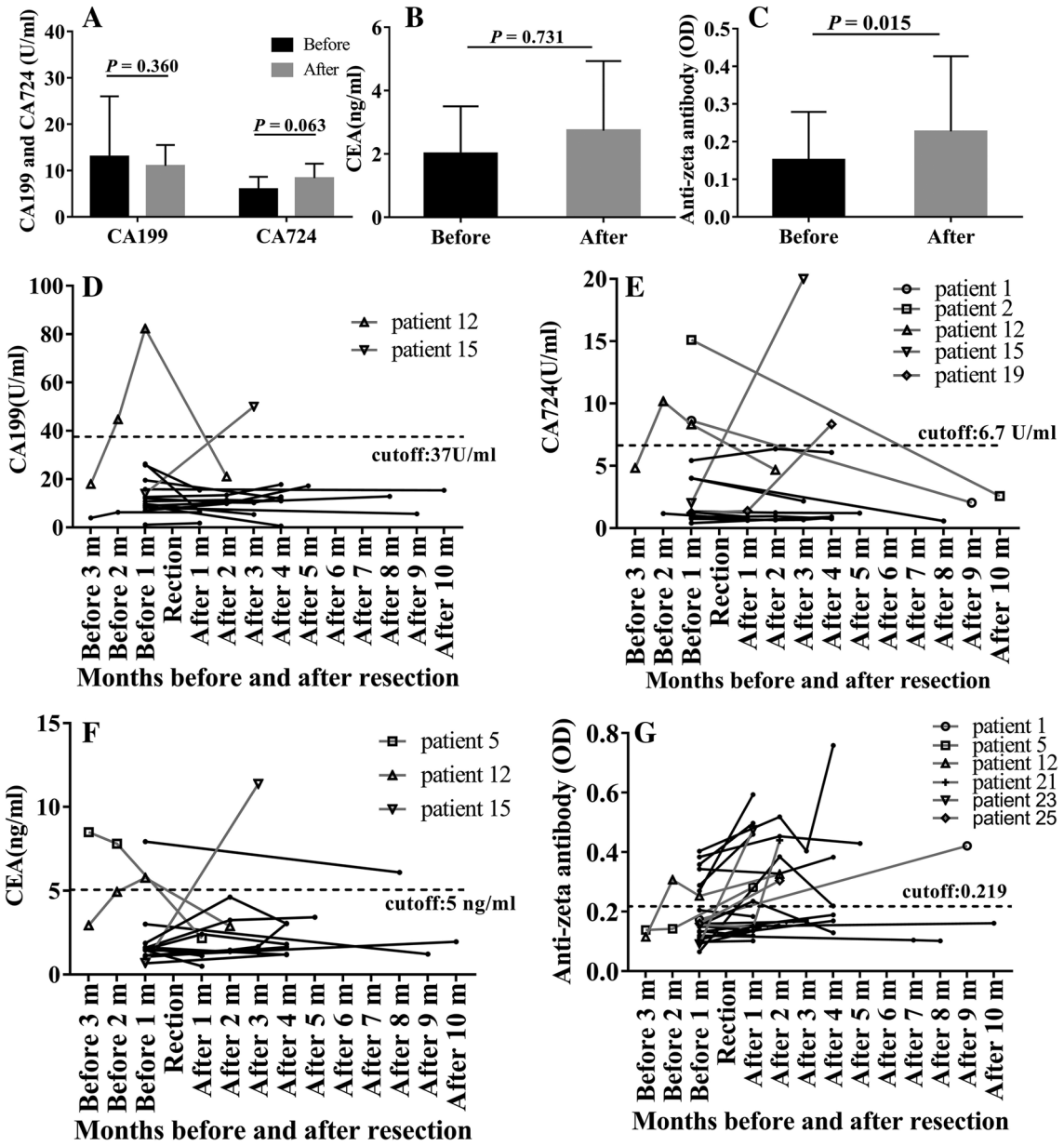

Fig. 5 The levels of CA199, CA724, CEA and anti-14-3-3 zeta in GC sera before and after tumor resection

differentiation or lymphatic metastasis status of GC. But no significantly difference was observed, and the same results were observed in lung cancer and oral cancer (Dai et al. 2016b; Matta et al. 2007). Some studies also reported similar results for other markers in GC. Werner et al. reported that 5 autoantibodies to MAGEA4, CTAG1, TP53, ERBB2_C and SDCCAG8 showed no difference between early stage and late stage (Werner et al. 2016). Meistere et al. reported that six antigens of CTAG1B/CTAG2, DDX53, IGF2BP2, TP53, and MAGEA3, were predominantly reacting with sera from patients with intestinal-type gastric cancer, but not with stage (Meistere et al. 2017). These results indicated that the appearance of anti-14-3-3 zeta may have association with the development of GC, but have no association with the progression of GC.

To date, many serum markers have been identified and applied in detection of GC, and the most used tumor markers are: CA 72-4, CEA and CA 19-9 (Cainap et al. 2015; Ning et al. 2018; Xu et al. 2018). Even though the combined testing for these markers is useful for clinical diagnosis of GC, they have not been generally considered as a tool for the early detection of GC due to their low sensitivity and specificity (Cainap et al. 2015). In this study, we combined detection of autoantibodies to 14-3-3 zeta, CEA, CA199 and CA724, and

found this panel could enhance sensitivity for the diagnosis of GC, indicating that autoantibody to 14-3-3 zeta could potentially act as a complementary clinical biomarker, such as CEA, CA199 and CA724, for serological detection of GC. Although this panel was simple and inexpensive, it was not ideal screening method due to low sensitivity of 57.2% in this study. The ideal tumor marker should have a specificity of at least 70% and a sensitivity of 90% (Cooner 1993).

CEA, CA199 and CA724 also has been used in monitoring the effectiveness of treatments (Waddell et al. 2014). In this study, we focused on the temporal change of anti-14-3-3 zeta, CA-72-4, CA 19-9, and CEA in 69 sera from 26 GC patients before and after resection. The level of anti-14-3-3 zeta antibody were higher in sera after resection than before, which is line with our previous study (Dai et al. 2016a; Wang et al. 2018), while the levels of CA-72-4, CA 19-9, and CEA showed no difference. Danna et al. reported that surgical removal of primary tumor reversed tumor-induced immunosuppression (Danna et al. 2004), which may result in increasing of antibody level in sera after surgery. In addition, only certain patients were sensitive to tumor resection and their biomarkers' levels significantly changed after resection, such as patient 12 and patient 15. Interestingly, not all markers' levels significantly changed in certain patient after resection. For example, CA199, CA724 and CEA increased from negative status to positive status in patient 15, while anti-14-3-3 zeta was observed not different after resection. Therefore, it is necessary to expand sample size and extend follow-up time to identify these special markers and the characteristics of these post-resection patients who are sensitive to certain markers.

In summary, autoantibody against 14-3-3 zeta could be a potential diagnostic biomarker and improve the sensitivity of CEA, CA199 and CA724 in diagnosis of GC. Further largescale studies will be needed to validate its performance of the sensitivity, specificity and AUC value in real-world screening scenarios. Furthermore, antibody to 14-3-3 zeta may be potential prognostic biomarkers after resection treatment.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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