ORIGINAL RESEARCH

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Predictive value of cystic fibrosis transmembrane conductance regulator (CFTR) in the diagnosis of gastric cancer

Abstract

Purpose: Gastric cancer is associated with poor prognosis. The high mortality rate of gastric cancer is mainly attributed to late detection, so diagnosis and treatment are crucial to decreasing mortality. The purpose of this study was to examine the predictive accuracy and discriminative ability of cystic fibrosis transmembrane conductance regulator (CFTR) in gastric cancer patients, in addition to the classical cancer tumor biomarkers carbohydrate antigen 199 (CA199) and carcinoembryonic antigen (CEA).

Methods: The study was performed on 78 serum samples from gastric cancer patients and 88 serum samples from healthy adults. Serum levels of CFTR, CA199, CEA and CHN were determined by enzyme-linked immunosorbent assay (ELISA)

Results: Spearman's coefficient analysis showed that, in some cases, CFTR was strongly correlated with CA199 and that CFTR levels increased with age. Kruskal-Wallis testing indicated concentrations of CFTR and CA199 had statistically significant association with stage. Logistic regression showed that CFTR and CA199 independently predicted gastric cancer. Receiver operating characteristics (ROC) showed that combinations of CFTR, CA199, and CEA yielded the best ROC curve, with an AUC of 0.875.

Conclusions: The results of this study indicate that the serum CFTR has a broad application prospects for detection of GC.

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Despite decreasing incidence and mortality, gastric cancer (GC) is the fourth most common cancer and the second most common cause of cancer-related death worldwide [1,2]. In China, the incidence and mortality rates of GC were 35.02 and 26.08 per 100,000 people in 2006, placing it second to lung cancer [3]. The initial diagnosis of GC is often delayed because up to 80% of patients are asymptomatic during the early stages of the disease [4]. For this reason, early diagnosis and treatment are crucial to reducing mortality in patients with GC. Since the last century, numerous tumor biomarkers have been discovered for the detection of malignancy. Carbohydrate antigen 199 (CA199) and carcinoembryonic antigen (CEA) are currently the most commonly used biomarkers for diagnosis of GC and it has been recommended that the serum levels of CA199 and CEA be monitored for detection and evaluation of GC [5, 6]. Other studies have shown that these biomarkers are not effective in the detection of GC due to their relatively poor diagnostic sensitivity and specificity [7, 8]. For this reason, novel tumor biomarkers are required for the screening, diagnosis and determining the prognosis of GC.

In our previously proteomics study, cystic fibrosis transmembrane conductance regulator (CFTR), a glycoprotein with 1480 amino acids, was found to be highly expressed in leukocyte and to have a strong association with innate immunity [9]. CFTR is a cAMP-activated ATP-gated anion channel that transports chloride and thiocyanate ions across epithelial cell membranes. Any defect in CFTR expression and function may lead to various pathological conditions, especially chronic inflammatory diseases [10–14]. Some studies have confirmed the role of CFTR in chronic lung infections with mucoid *Pseudomonas aeruginosa* [11, 12], while other studies have demonstrated that variations of CFTR gene may increase or decrease the risk of a variety of inflammation-associated cancers, including lung cancer and pancreatic cancer [13–14]. Since both CFTR and cancers are closely-associated with chronic inflammation, it would be interesting to determine whether there is a functional interaction between CFTR and cancers. We hypothesized that CFTR may prove useful for detection of cancers.

The aim of the present study was to investigate whether CFTR in serum was helpful for detection of GC, either alone or in combination with the classical GC biomarkers (CA199 and CEA). The association between serum concentration of CFTR and clinicopathological parameters was also explored.

Materials and Methods

Patients and specimens

Ethical statement

The study was performed in the Gastrointestinal Department of the First Affiliated Hospital of Anhui Medical University. Study protocols were approved by the medical ethics committee of the First Affiliated Hospital of Anhui Medical University. All participants were asked to donate blood samples for the current study and to provide their written informed consent to participate in this study. The complete protection of their personal data was agreed in a written form.

Sample collection

Cases consisted of patients in whom gastric carcinoma was present. Of the 78 patients who were diagnosed with gastric carcinoma, seven patients were in Stage I, 26 in Stage II, 31 in Stage III and 14 in Stage IV. Controls were normal, healthy people (n=88). Any participant who had a history of serious or chronic disease, such as sepsis, diabetes mellitus, hypertension or immunologic disease, was excluded from the study.

The serum samples were obtained on the morning of the day of surgery, before any treatment. Peripheral blood samples were drawn by venipuncture after overnight fasting between 8:00 p.m. and 8:30 a.m. Serum samples were centrifuged at 1,500 g at ambient temperature for 45 min and then stored at -80°C.

CFTR, CA199, and CEA measurement

Serum levels of CFTR (Uscn, CHN), CA199 and CEA (Abcam, UK) were determined by enzyme-linked immunosorbent assay (ELISA) in accordance with the kit manufacturer's instructions. For CFTR, the assay has a detection limit of 0.15 ng/mL and a dynamic range of up to 10 ng/mL. The upper limit of normal reference for CA199 is 600 U/mL and 250 ng/mL for CEA.

Data analysis and statistics

Spearman's rank correlation coefficient was used to investigate correlations among biomarkers and between age and biomarkers. The Kruskal-Wallis test, a nonparametric method, was used to examine the relationship among biomarkers and tumor characteristics. Logistic regression was performed to calculate the odds ratio (OR) between biomarkers and case status. Receiver operating characteristic curve (ROC) analysis was performed to evaluate the diagnostic value of the markers in GC.

TABLE 1. Correlations among biomarkers from cases

	CFTR	CEA	CA199
CFTR	1	$P=0.2$	$P<0.001$
CEA		1	$P=0.093$
CA199			1

CFTR was strongly correlated with CA199 ($P<0.001$, Spearman's rank correlation coefficient).

TABLE 2. Association of biomarkers with age in cases and controls

	Age	
	Cancer (P)	Normal (P)
CFTR	0.005	0.95
CEA	0.115	0.865
CA199	0.073	0.97

CFTR levels were found to increase along with age in cases ($P=0.005$, Spearman's rank correlation coefficient).

For each ROC curve, the area under the curve (AUC) was calculated. To determine the whether a given marker panel could lead to improved performance, ROC analysis was first conducted on individual markers and then on combinations of markers. All analyses were performed using SPSS software (version 16.0; SPSS inc., Chicago, IL, U.S.), and $P<0.05$ was considered statistically significant.

Results

During the study period, a total of 78 serum samples from GC patients and 88 serum samples from healthy adults were evaluated. Of the GC patients, 56 (71.8%) were male and the mean age was 62.6 ± 10.2 years. Of the control subjects, 55 (62.5%) were male and the mean age was 59.8 ± 9.3 years. There were no differences with regards to gender ($P=0.206$) or age ($P=0.079$) between the two groups.

Correlations among biomarkers

Spearman's rank correlation coefficients were used to assess the correlations among biomarkers, and results are listed in Table 1. In cases, CFTR was found to be strongly correlated with CA199 ($P<0.001$), but in controls CFTR was only weakly correlated with CEA ($P=0.04$) and CA199 ($P=0.003$).

Association of biomarkers with age

Because age is an important factor in tumor genesis, an experiment was performed to determine whether CA199, CEA and CFTR levels varied with age. No change in CA199 or CEA

TABLE 3. Association of biomarkers with tumor characteristics among cases

	Number	CFTR	CEA	CA199
		median	median	median
Age (years)				
≤ 50	9	6.91	27.97	266.45
51-69	52	5.28	9.38	64.35
≥ 70	17	4.24	6.95	58.37
P		0.015	0.359	0.087
Stage				
I	7	2.18	4.32	10.78
II	26	4.51	11.6	36.89
III	31	5.88	15.36	95.86
IV	14	6.73	3.57	195.03
P		$P<0.001$	0.629	$P<0.001$
BMI				
< 18.5	12	6.01	6.44	114.64
18.5-24	49	5.36	11.61	91.62
> 24	17	4.36	12.45	51.27
P		0.12	0.643	0.123

CFTR was correlation with age ($P=0.015$, Kruskal-Wallis test).

CFTR ($P<0.001$, Kruskal-Wallis test) and CA199 ($P<0.001$, Kruskal-Wallis test) were significantly associated with stage.

concentrations with age was observed for either patients or controls; however, CFTR levels were found to increase along with age in patients but not in controls (Table 2).

Relationship between biomarkers and tumor characteristics in cases

Kruskal-Wallis testing was used to examine the relationship among biomarkers (CFTR, CA199 and CEA) and tumor characteristics such as age, tumor stage (diameter, grade, histology, ratio of positive lymph nodes to total number of lymph nodes) and body mass index (BMI). The results are shown in Table 3. CFTR showed strong positive correlation with age ($P=0.015$). Both CFTR and CA199 were found to be significantly associated with stage, but CEA showed only limited association with stage. No statistically significant relationship was observed between biomarkers and BMI.

FIGURE 1. Distribution of the three biomarkers in patients with gastric cancer and in healthy controls. (a) CFTR, (b) CA199, and (c) CEA in the two groups (gastric cancer patients and healthy controls) examined using an immunoassay specific to each molecule. The solid horizontal line indicates the median value for both groups.

TABLE 4. Results from logistic regression models

Marker	OR	95%CI	P
CFTR	1.709	(1.354, 2.158)	<0.001
CEA	1.05	(0.985, 1.119)	0.133
CA199	1.02	(1.007, 1.033)	0.002

CFTR ($P<0.001$, logistic regression) and CA199 ($P=0.002$, logistic regression) were found to be independent predictive factors of GC.

Connection of biomarkers with GC

Figure 1 shows the distribution of three biomarkers in this study. The nonparametric Mann-Whitney test showed the median value of CFTR in healthy volunteers to be markedly lower than that in patients with GC (2.37 *vs.* 5.25 ng/mL; $P<0.0001$). A similar tendency was also observed in CA199 (16.67 *vs.* 86.36 U/mL; $p<0.0001$) and CEA (2.88 *vs.* 11.0 ng/mL; $P<0.0001$).

A logistic regression model was then used to investigate the correlation between biomarkers and GC. Through this univariate linear analysis, all three biomarkers, CFTR, CA199 and CEA, were found to be predictive of GC. Another logistic regression analysis was performed, this time using a stepwise method, and only CFTR and CA199 were found to be independent predictive factors of GC. The results of the logistic regression analysis are shown in Table 4.

Diagnostic values of three biomarkers

The diagnostic value of the three biomarkers was evaluated with ROC curve analysis (Fig. 2). Calculations showed that CEA had the lowest score (AUC: 0.715), CA199 had a moderate score (AUC: 0.824) and CFTR had the best performance (AUC: 0.851). Combining CFTR, CA199 and CEA yielded the highest ROC curve, with an AUC of 0.875. Combining any two of the three biomarkers did not result in any improvement in ROC curves relative to combining CFTR, CA199, and CEA (Table 5).

Discussion

In the present study, the serum level of CFTR in GC patients was markedly higher than that in healthy controls. There are two possible reasons for this. First, GC cells, which are antigens within the body, activate the inherent immune system. Afterward, the expression of CFTR in the neutrophils increases significantly to kill tumor cells by releasing considerable numbers of chloride ions. Second, CFTR may act as a pattern recognition molecule and inhibit the process of tumor proliferation.

FIGURE 2.

- (a) ROC curves for the three biomarkers respectively (— CFTR, AUC=0.851; --- CA199, AUC=0.824; CEA, AUC=0.715)
 (b) ROC curves for the combined three biomarkers (— CFTR + CA199 + CEA, AUC=0.875)

Previous studies have shown that CFTR exists on surfaces of epithelial cells in the airway, gut and exocrine glands and can also be found in leukocytes [15, 16]. If CFTR dysfunction occurs in the lung, the chemical composition of airway secretions may also be disrupted. This abnormal airway secretions may disable antimicrobial peptides, preventing them from responding to any pathogenic invasion of the surface of the airway, which further increases the susceptibility of the host to *P. aeruginosa* and other infections [17-19]. In a study on the mechanism of *P. aeruginosa* infection in lungs of cystic fibrosis patients, Pier *et al.* proposed that CFTR may be an epithelial cell receptor for *P. aeruginosa* and that CFTR peptides 108-

TABLE 5. ROC analysis of biomarkers

	CFTR	CEA	CA199
CFTR	0.851	0.858	0.869
CEA		0.715	0.846
CA199			0.824
Combined			0.875

Combining CFTR, CA199 and CEA yielded the highest ROC curve (AUC : 0.875, receiver operating characteristic curve).

117 may bind to the conserved outer-core oligosaccharide of the bacterium [20]. In this sense, CFTR serves as a pattern recognition molecule, although with a more limited recognition of bacterial ligands than other pattern recognition molecules [21]. In the dextran sodium sulfate-induced colitis murine model, CFTR deficiency decreased the innate immunity of the biliary epithelium and reduced its tolerance to endotoxin, resulting in a Src-dependent inflammatory response through TLR4 and NF- κ B [22].

There have been a few studies on CFTR expression in GC. In the field of lung cancer research, Naren *et al.* showed that CFTR is located at the apical surface of plasma membrane in polarized lung epithelial cells. Functional coupling has been shown between CFTR and MRP2 (multidrug resistance protein-2), which improves the function of CFTR chloride channels in cultured lung epithelial cells [23]. Furthermore, changes in cystic fibrosis have been shown to be associated with increased risk of developing pancreatic cancer. The two commonest mutations of CFTR were found to be the Δ F508 mutation and the 5T polymorphism. F508 mutation gives rise to the deletion of one amino acid from the CFTR protein, and the 5T polymorphism is located in intron 9 and produces lower levels of an otherwise normal CFTR transcript [24]. Patients with cystic fibrosis are at increased lifetime risk of developing digestive tract cancers and pancreatic cancer [25-27].

It is generally recognized that no single cancer biomarker can provide sufficient information for optimal cancer diagnosis. The current trend is to focus on the identification of multiple biomarkers that can be used in combination. The present data provide evidence that serum CFTR should be considered as a novel biomarker for GC diagnosis. This biomarker displays higher diagnostic sensitivity to GC than commonly-used markers, such as CA199 and CEA (Table 4). Moreover, among the 33 cancer patients who had normal levels of CA199 (< 37 U/mL), 18 of them (54%) had elevated levels of CFTR (values of 4.5 ng/mL or greater; the cutoff for 95% specificity). For this reason, CA199 measurement in combination with CFTR was found to facilitate improvement in the diagnostic

sensitivity of GC over either measurement alone. In addition, a strong correlation between elevated CFTR levels and increasing age was observed in GC patients (Table 2); however, no similar correlation was observed in healthy volunteers. This indicates that the difference in age between cancer cases and controls was not a confounding factor in the present study.

In conclusion, our results indicate that the serum CFTR has a broad application prospects for detection of GC. The combination of CFTR with CA199 and CEA would further improve the diagnostic sensitivity. The limitation of the present study should also be acknowledged: the number of GC patients was too small to generalize the outcomes and the results need to be confirmed in larger numbers of patients.

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References

- Parkin DM, Bray F, Ferlay J, Pisani P. (2005) Global cancer statistics, 2002. *CA Cancer J Clin*;55:74-108. PubMed: 15761078.
- Crew KD, Neugut AI. (2006) Epidemiology of gastric cancer. *World J Gastroenterol*;12:354-62. PubMed: 16489633.
- Zhang SW, Lei ZL, Li GL, Zou XN, Zhao P et al. (2010) A report of cancer incidence and mortality from 34 cancer registries in China, 2006. *China Cancer (Chin)*;19:356-365.
- Koh TJ, Wang TC. (2002) Tumors of the stomach. In: Feldman M, Friedman LS, Sleisenger MH. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease*. 7th ed. Philadelphia, PA.: Saunders:829-44.
- Takahashi Y, Takeuchi T, Sakamoto J, Touge T, Mai M et al. (2003) The usefulness of CEA and/or CA19-9 in monitoring for recurrence in gastric cancer patients: a prospective clinical study. *Gastric Cancer*;6:142-5. doi: 10.1007/s10120-003-0240-9. PubMed: 14520526.
- Lai IR, Lee WJ, Huang MT, Lin HH. (2002) Comparison of serum CA72-4, CEA, TPA, CA19-9 and CA125 levels in gastric cancer patients and correlation with recurrence. *Hepatogastroenterology*;49:1157-60. PubMed: 12143226.
- Camlica H, Duranyildiz D, Oguz H, Oral EN, Yasasever V. (2008) The diagnostic value of macrophage migration inhibitory factor (MIF) in gastric cancer. *Pathol Oncol Res*;14:79-83. doi: 10.1007/s12253-008-9002-7. PubMed: 18347935
- Vitzthum F, Behrens F, Anderson NL, Shaw JH. (2005) Proteomics: from basic research to diagnostic application. A review of requirements & needs. *J Proteome Res*; 4:1086-1097. doi: 10.1021/pr050080b. PubMed: 16083257
- Zhou Z, Ren J, Liu H, Gu G, Li J. (2011) Serum proteomic analysis from bacteremic and leucopenic rabbits. *J Surg Res*;171:749-54. doi: 10.1016/j.jss.2010.04.056. PubMed: 20691998
- Chan HC, Ruan YC, He Q, Chen MH, Chen H et al. (2009) The cystic fibrosis transmembrane conductance regulator in reproductive health and disease. *J Physiol*; 587:2187-95. doi: 10.1113/jphysiol.2008.164970. PubMed: 19015188
- Buchanan PJ, Ernst RK, Elborn JS, Schock B. (2009) Role of CFTR, *Pseudomonas aeruginosa* and Toll-like receptors in cystic fibrosis lung inflammation. *Biochem Soc Trans*;37:863-7. doi: 10.1042/BST0370863. PubMed: 19614608
- Swiatecka-Urban A, Moreau-Marquis S, Maceachran DP, Connelly JP, Stanton CR et al. (2006) *Pseudomonas aeruginosa* inhibits endocytic recycling of CFTR in polarized human airway epithelial cells. *Am J Physiol Cell Physiol*; 290:C862-72. doi: 10.1152/ajpcell.00108.2005. PubMed: 16236828
- Son JW, Kim YJ, Cho HM, Young LS, Man LS, et al. (2011) Promoter hypermethylation of the CFTR gene and clinical/pathological features associated with nonsmall cell lung cancer. *Respirology*;16:1203-9. doi: .1111/j.1440-1843.2011.01994.x. PubMed: 21585618
- Neglia JP, FitzSimmons SC, Maisonneuve P, Schoni MH, Schoni-Affolter F, et al. (1995) The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. *N Engl J Med*;332:494-9. doi: 10.1056/NEJM199502233320803. PubMed: 7830730
- Li C, Naren AP. (2011) Analysis of CFTR interactome in the macro molecular complexes. *Methods Mol Biol*;741:255-70. doi: 10.1007/978-1-61779-117-8_17. PubMed: 21594790
- Döring G, Gulbins E. (2009) Cystic fibrosis and innate immunity: how chloride channel mutations provoke lung disease. *Cell Microbiol*;11:208-16. doi: 10.1111/j.1462-5822.2008.01271.x. PubMed: 19068098
- Van Heeckeren AM, Schluchter MD, Drumm ML, Davis PB. (2004) Role of Cfr genotype in the response to chronic *Pseudomonas aeruginosa* lung infection in mice. *Am J Physiol Lung Cell Mol Physiol*;287:L944-52. doi: 10.1152/ajplung.00387.2003. PubMed: 15246977
- Grasmé H, Becker KA, Zhang Y, Gulbins E. (2010) CFTR-dependent susceptibility of the cystic fibrosis-host to *Pseudomonas aeruginosa*. *Int J Med Microbiol*;300:578-83. doi: 10.1016/j.ijmm.2010.08.011. PubMed: 20951085
- Goldberg JB, Pier GB. (2000) The role of the CFTR in susceptibility to *Pseudomonas aeruginosa* infections in cystic fibrosis. *Trends Microbiol*; 8:514-520. PubMed: 11121762
- Pier GB. (2000) Role of the cystic fibrosis transmembrane conductance regulator in innate immunity to *Pseudomonas aeruginosa* infections. *Proc Natl Acad Sci U S A*. 2000;97:8822-8. PubMed: 10922041

21. Medzhitov R, Janeway CJ Jr. (2000) Innate immunity. *New Engl J Med*;343:338-344. doi: 10.1056/NEJM200008033430506 . PubMed: 10922424
22. Fiorotto R, Scirpo R, Trauner M, Fabris L, Hoque R et al. (2011) Loss of CFTR affects biliary epithelium innate immunity and causes TLR4-NF- κ B-mediated inflammatory response in mice. *Gastroenterology*;141:1498-1508. doi: 10.1053/j.gastro.2011.06.052. PubMed: 21712022
23. Li C, Schuetz JD, Naren AP. (2010) Tobacco carcinogen NNK transporter MRP2 regulates CFTR function in lung epithelia: implications for lung cancer. *Cancer Lett*;292:246-53. doi: 10.1016/j.canlet.2009.12.009. PubMed: 20089353
24. Liu X, Jiang Q, Mansfield SG, Puttaraju M, Zhang Y et al. (2002) Partial correction of endogenous Δ F508 CFTR in human cystic fibrosis airway epithelia by spliceosome-mediated RNA trans-splicing. *Nat Biotechnol*; 20:47-52 doi: 10.1038/nbt0102-47. PubMed: 11753361
25. Neglia JP, FitzSimmons SC, Maisonneuve P, Schöni MH, Schöni-Affolter F et al. (1995) The risk of cancer among patients with cystic fibrosis. Cystic Fibrosis and Cancer Study Group. *N Engl J Med*; 332:494-9. doi: 10.1056/NEJM199502233320803. PubMed: 7830730
26. Schöni MH, Maisonneuve P, Schöni-Affolter F, Lowenfels AB. (1996) Cancer risk in patients with cystic fibrosis: The European data. CF/CSG Group. *J R Soc Med*; 89:38-43. PubMed: 8778449.
27. Sheldon CD, Hodson ME, Carpenter LM, Swerdlow AJ. (1993) A cohort study of cystic fibrosis and malignancy. *Br J Cancer*; 68:1025-8. PubMed: 8217592