UICC
global cancer control

IJC
International Journal of Cancer

Detection of gastric carcinoma-associated MG7-Ag by serum immuno-PCR assay in a high-risk Chinese population, with implication for screening

Lian Zhang[1], Jun Ren[1], Kaifeng Pan[1], Junling Ma[1], Jiyou Li[1], Lin Shen[1], Xiaodong Zhang[1], Jie Li[1], Daiming Fan[2], Mitchell Gail[3] and Weicheng You[1]

[1] Department of Cancer Epidemiology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University School of Oncology, Beijing Cancer Hospital & Institute, Beijing 100142, China
[2] Department of Digestive Diseases, Fourth Military Medical University, Xijing Hospital, Xian 710032, China
[3] Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892

To evaluate gastric carcinoma-associated antigen, MG7-Ag, for detection of gastric cancer in a high-risk population, a population-based screening of gastric cancer was conducted in Linqu County, Shandong Province, China. In 2002 and 2003, a total of 2,710 participants aged 35–65 years received an endoscopic examination with 5 biopsies taken from standard sites with pathological diagnosis, and serum samples were collected to detect MG7-Ag by serum-based Immunopolymerase chain reaction (PCR) assay. The sensitivity and specificity of MG7-Ag Immuno-PCR assay in detecting of gastric cancer were assessed. Of 2,710 participants, 148 (5.46%) were determined to be MG7-Ag positive. The sensitivity of MG7-Ag Immuno-PCR assay for the detection of gastric cancer was 77.5% (31 of 40 gastric cancer cases), the specificity was 95.62% (2,553 of 2,670 nongastric cancer subjects) and the accuracy was 73.12%. A total of 24 gastric cancer cases were in Stage I or II, of which 17 (70.8%) were MG7-Ag positive. However, the proportion of MG7-Ag positivity in subjects with superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, indefinite dysplasia or dysplasia was ranged from 3.00% to 5.61% in comparison with 77.5% in those with gastric cancer. Our findings suggest that MG7-Ag was a sensitive and specific serum biomarker and may have a potential for gastric cancer screening in the high-risk population.

Gastric cancer (GC) is the second most common cancer in the world and in China.[1,2] Nearly a million of new GC cases occur annually worldwide, and 40% of them are in China.[3] The prognosis of GC varies remarkably by the stage of cancer, and the 5-year relative survival rate can reach to 90% in Stage I but less than 5% in Stage IV.[4] Therefore, much effort has been made to detect GC in the early stages in many countries, including China.

In Japan, a program of mass screening has been implemented using conventional barium X-ray examination as an initial screening method after by an endoscopic examination. As a result, more than 50% of GC cases were diagnosed in the early stages.[5,6] From 1989 to 1990, we conducted a mass screening among 3,400 adults aged 35–64 years in a high-risk population in Linqu County, Shandong Province, where the age-adjusted mortality rate of GC was 70/100,000 for men and 25/100,000 for women, accounting 42% of total cancer deaths in this region.[7] A total of 13 GCs (detection prevalence rate: 0.38%) were detected in this screening, of which 64% of GCs were in Stage I or II.[8]

Because the cost of endoscopic screening is high, we have been interested in finding less expensive preliminary screening tests that could make endoscopic screening cost-effective by identifying subjects at highest risk and most in need of endoscopy. In early 1990, we evaluated the pepsinogen (PG) I:II ratio, which is low in patients with GC, for prescreening[9] but found that it was not sensitive and specific enough because the ratio could also be decreased in subjects with various benign gastric lesions.

To identify more specific and sensitive biomarkers for GC detection, members of our group (Fan and Ren) developed a human gastric carcinoma-associated antigen (MG7-Ag)-specific monoclonal antibody[10,11] and found by immunohistochemistry that expression of MG7-Ag was dramatically elevated in GC tissues compared with normal mucosa and benign lesions.[12] This work suggested that MG7-Ag might be

Key words: gastric cancer, screening, MG7-Ag, Immuno-PCR assay
Grant sponsor: National Basic Research Program of China (973 program); **Grant numbers:** 2004CB518702 and 2010CB529303; **Grant sponsor:** National 10.5 program; **Grant number:** 2004BA703B04
The first two authors contributed equally to this work
DOI: 10.1002/ijc.24739
History: Received 19 Apr 2009; Accepted 23 Jun 2009; Online 8 Jul 2009
Correspondence to: Weicheng You, Department of Cancer Epidemiology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University School of Oncology, Beijing Cancer Hospital & Institute, 52 Fu-cheng Road, Hai-dian District, Beijing 100142, People's Republic of China, Fax: +86-10-88122437, E-mail: weichengyou@yahoo.com

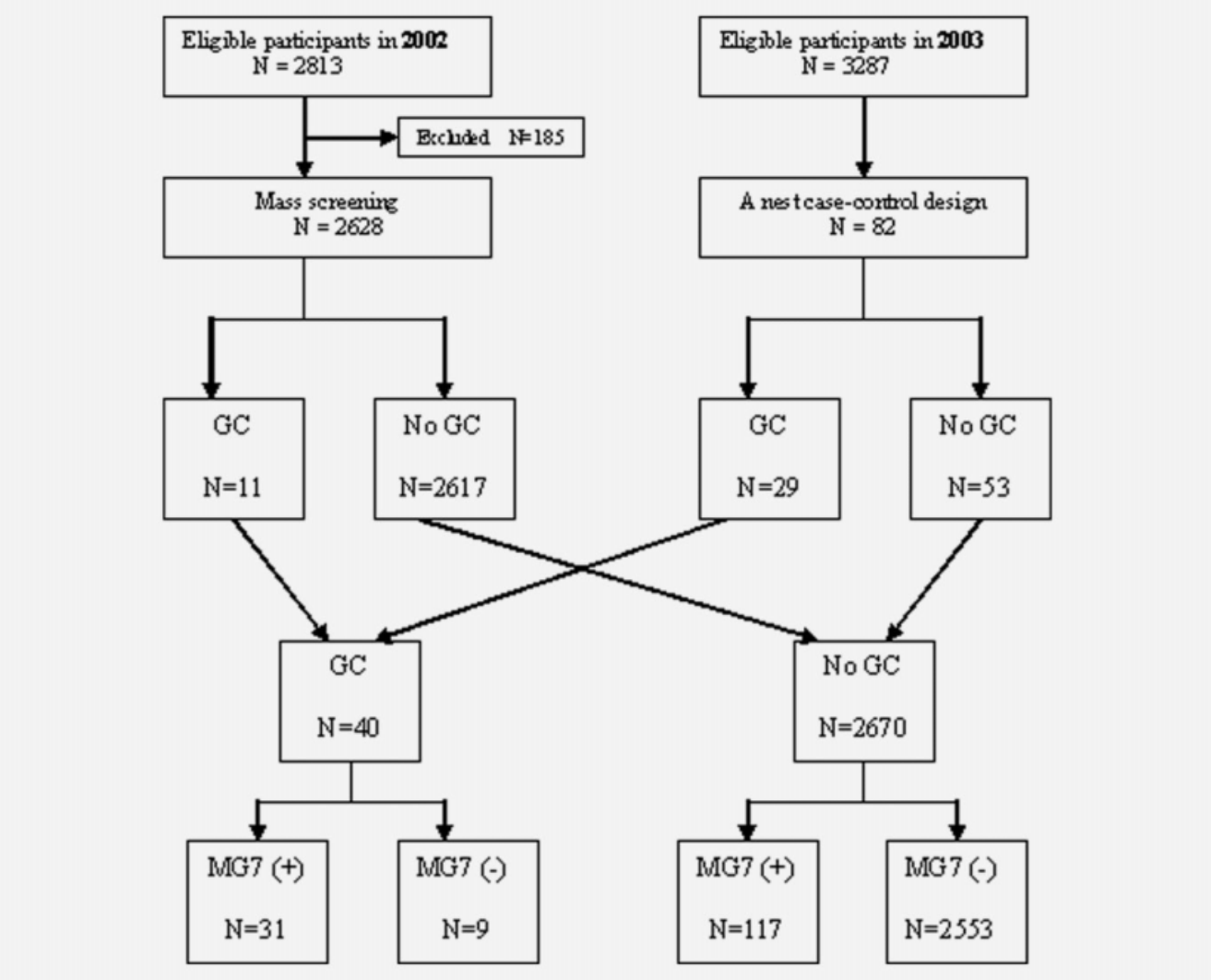

Figure 1. A mass screening with MG7 among the high-risk population of gastric cancer in 2002–2003.

used as a biomarker for GC screening. However, detection of tissue MG7-Ag expression requires gastric biopsies obtained by endoscopy. Therefore, we sought a simple method to measure MG7-Ag in serum, and a serum-based MG7-Ag immunopolymerase chain reaction (PCR) assay was developed.[11,13,14] We tested this method in a hospital-based study and found 82.8% of 198 GCs were serum MG7-Ag positive.[11]

In this study, we evaluated the potential usefulness of this serum-based MG7-Ag immuno-PCR assay for screening high-risk population in Linqu county.

Material and Methods

Study participants

The subjects were recruited in 2 separate gastroscopic screening surveys of subjects aged 35–64 years. In both surveys, subjects with a previous diagnosis of GC, subjects who did not give informed consent and subjects with severe illness (*e.g.*, cardiac, respiratory, high blood pressure and hepatic insufficiency) were excluded. In 2002, a total of 2,813 residents, representing 89% of eligible residents aged 35–64 years in 12 villages in 4 townships in Linqu County were invited to participate in a mass screening for early detection of GC.[15,16] After exclusion of 185 ineligible subjects, 2,628 residents were screened. All participants were given a brief physical examination, and their medical histories were recorded. A similar endoscopic survey was conducted in 13 separate villages in 2003, and 2,927 eligible subjects received endoscopic screening.[17]

A total of 2,710 subjects, including 2,628 subjects in 2002 and 82 subjects in 2003 were enrolled for this study as illustrated in Figure 1. All 40 subjects with prevalent GC and 2,670 subjects without GC were assessed by the MG7-Ag immuno-PCR assay to estimate sensitivity and specificity, respectively.

The project was approved by the Institutional Review Board of Peking University School of Oncology, and an informed written consent was obtained from each participant.

Endoscope and pathology

An endoscopic examination was performed by 4 experienced gastroenterologists using fiberoptic and video endoscopes (Olympus). The gastric mucosa was examined, and 5 biopsies were taken from standard sites of stomach according to Updated Sydney System[18]: lesser curvature of antrum, greater curvature of antrum, angulus, lesser curvature of body and greater curvature of body. The biopsy specimens were immediately fixed in 10% neutral formalin solution in individually labeled vials. Subsequently, the specimens were embedded and stained with hematoxylin and eosin. Each slide was reviewed by a panel of 3 senior pathologists and interpreted according to the Updated Sydney System and Padova International Classification.[18,19] The pathological diagnosis was performed in the pathology laboratory of Beijing Cancer Hospital. Each biopsy was given a diagnosis based on the most severe histology found in the biopsy, and each participant was assigned a global diagnosis based upon the most severe diagnosis among any of the biopsies.

Serologic analysis

A 5-ml blood sample was collected from each subject during the physical examination and allowed to clot in the dark at room temperature for 30 min and then centrifuged at 1,000*g* at room temperature for 15 min. The serum sample was stored at −20°C in the field immediately and then transferred into a freezer at −80°C in the Beijing Cancer Hospital within 2–3 days after collection.

The MG7-Ag immuno-PCR assay was preformed as previously reported.[11,14] Briefly, 100 μL of diluted serum were coated with 0.05 M carbonate buffer (pH 9.6) at 4°C overnight or 37°C for 2 hr. After washing 3 times, 100 μL of biotinylated MG7 (10 μL/mL) was added and incubated at 37°C for 2 hr, then the biotinylated pXJ19 was added to the complex and incubated at room temperature for 30 min. Approximately 50 μL of PCR mixture was added to the wells, and predenaturation for 3 min at 96°C was performed to allow the bound biotinylated plasmid pXJ19 to detach.

PCR mixture contained 10 mM of tris-HCl (pH 8.3), 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.8 mM of dNTP, 2 M of each primer (5′-TCCCAGTCACGACGTTG-3′ and 5′-AACAGCTATGAC CATG-3′) and 1.25 units of Taq DNA polymerase. PCR was accomplished by an initial denaturing temperature of 96°C for 3 min and subsequent 35 cycles of denaturing (94°C, 1 min), annealing (40°C, 1 min), extension (72°C, 1 min), with the last circle followed by a 5-min extension. These primers were expected to generate a 420 bp fragment, and the PCR product resolved on 2% agarose gels containing ethidium bromide.

Rigorous quality control procedures were applied throughout the MG7-Ag immuno-PCR assay. The specificity of the recombinant marker DNA pXJ19 was verified without the homology form the GeneBank to exclude the possibility of cross linkage from the serum taken. The negativity of immuno-PCR represents that nonamplified product is observed by 2 independent technicians. For such blinded screening, the serological testing was conducted in the laboratory of Xijing Hospital, the Fourth Military Medical University, Xian, China, whereas the coding and analysis of serum MG7-Ag samples were double blinded with histopathology information of each subject at Beijing Cancer Hospital.

Table 1. The characteristics of the study participants

	MG7-Ag positive	MG7-Ag negative	Total
Subjects, n (%)	148 (5.46)	2,562 (94.54)	2,710
Male	92 (7.07)	1,209 (92.93)	1,301
Female	56 (3.97)	1,353 (96.03)	1,409
Mean age ± SD	51.3 ± 8.89	49.4 ± 6.79	49.6 ±6.94
Male	51.4 ± 9.55	49.3 ± 6.91	49.5 ± 7.16
Female	51.1 ± 7.77	49.6 ± 6.68	49.6 ± 6.73

Statistical analysis

We calculated sensitivity and specificity of the MG7-Ag immuno-PCR assay by estimating the proportion positive among GC cases and the proportion negative among those without GC, respectively. The differences of proportion of MG7-Ag positivity in the subjects with each gastric lesions and GC stages were calculated by chi square test. All p values were 2 sided, and $p < 0.05$ was considered to indicate statistical significance. The statistical analyses were performed using the SAS (releases 8.2, SAS Institute Inc. Cary, NC).

Results

A total of 2,710 subjects were conducted a pathological diagnosis and MG7-Ag immuno-PCR assay. Among those, 1,301 were men and 1,409 were women. The age ranged from 35 to 64 years, and the mean age was 49.6 ± 6.94 years.

Table 1 showed the characteristics of the study population according to the serum MG7-Ag status by sex. The mean age of the subjects with MG7-Ag positive was 51.3 ± 8.89 years, significantly higher than those with MG7-Ag negative (49.4 ± 6.79 years, $p < 0.05$). Among 2,710 subjects, 148 (5.46%) were MG7-Ag positive, and 2,562 (94.5%) were negative. The proportion of MG7-Ag positive in men was 7.07%, significantly higher than those in women (3.97%, $p < 0.05$).

A total of 11 and 29 GCs were detected by gastroscopy and pathological diagnosis in 2002 and 2003, respectively. Among 40 GCs, 31 were MG7-Ag positive, and 9 were negative (Table 2). However, among 2,670 subjects without GC, 2,553 were negative and 117 were positive. Therefore, the sensitivity of serum MG7-Ag immuno-PCR to detect GC was 77.5% (31 of 40), and the specificity for subjects without GC was 95.62% (2,553 of 2,670). The accuracy of MG7-Ag to identify GC was 73.12%.

To further evaluate the relationship between MG7-Ag status and the stages of GC, we stratified the GCs according to

Table 2. The prevalence of serum MG7-Ag positive in the study population

	GC (*n*)	Non-GC (*n*)	Total
MG7-Ag			
Positive	31	117	148
Negative	9	2,553	2,562
Total	40	2,670	2,710

The sensitivity = $31/40 \times 100 = 77.5\%$.
The specificity = $2{,}553/2{,}670 \times 100 = 95.62\%$.
The accuracy = $(0.775 + 0.9562 - 1) \times 100 = 73.12\%$.

Table 3. Correlation between MG7- Ag and TNM classification of GC

Stages	MG7-Ag positive, *n* (%)	MG7-Ag negative, *n* (%)	Total (*n*)
I + II	17 (70.83)	7 (29.17)	24
Others	14 (87.50)	2 (12.50)	16
Total	31 (77.50)	9 (22.50)	40

TNM classification. As shown in Table 3, among 40 GCs, 31 (77.5%) were MG7-Ag positive. A total of 24 GCs were in Stage I or II, among those, 17 (70.8%) were MG7-Ag positive.

We also assessed the relationship between serum MG7-Ag and precancerous lesions in the study population (Table 4). The proportion of serum MG7-Ag positive was from 3.00% to 5.61% among subjects with superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, indefinite dysplasia or dysplasia, significantly lower than those with GC (77.50% *vs.* 3.00%–5.61%, $p < 0.001$).

Table 4. Correlation between MG7-Ag and gastric lesions

Histological status	MG7-Ag positive, *n* (%)	MG7-Ag negative, *n* (%)	Total
Superficial gastritis	16 (3.84)	401 (96.16)	417
Chronic atrophic gastritis	36 (4.03)	857 (95.97)	893
Intestinal metaplasia	13 (3.00)	420 (97.00)	433
Indefinite dysplasia/ dysplasia	52 (5.61)	875 (94.39)	927
Gastric cancer	31 (77.50)	9 (22.50)	40
Total	148 (5.46)	2,562 (94.54)	2,710

Discussion

This population-based study demonstrated that a serum-based MG7-Ag immuno-PCR assay can be useful in screening a population with a high prevalence of GC in rural China by identifying a small set of subjects who require endoscopy for a definitive diagnosis. Such a screen can identify about 77% of the subjects with prevalent GC, of which 55% were in Stage I or II. Such a 2-phase screening program may have a potential for early detection of GC and greatly reduce the costs associated with endoscopy, making it feasible in areas with limited resources for endoscopy. The study used coded specimens to assure that assays were performed without knowledge of histopathology.

The high prevalence of GC in parts of China and high mortality rates make it desirable to detect GC in its early stages, for which the 5-year relative survival rate can reach 90%.[4] A screening tool is ideally measurable in easily accessible biospecimens, such as serum, and has sufficient sensitivity and specificity that most GC will be detected without having too many false-positive results. This latter condition implies that the positive predictive value should be reasonably high, a goal that is hard to achieve when the disease prevalence is small. For this reason, many previously investigated analytes have not proved useful for screening a general population, including carcinoembryonic antigen (CEA).[20] The prevalence of GC is enriched in populations with low pepsinogen and low pepsinogen I/II ratios,[21] and prescreening with pepsinogen has been advocated as a means of finding high-risk subjects. However, the positive predictive values of 0.77%–1.25% from a meta-analysis of pepsinogen data[22] are much lower than we found for the MG7-Ag immuno-PCR assay.

MG7-Ag is a gastric carcinoma-associated antigen discovered in the GC cell line KATO III and later found to predict GC in immunohistochemical analyses.[10] To measure the analyte in serum, a very sensitive immuno-PCR method was developed to detect as few as 3.8×10^{-14} MG7-Ag molecules.[14,23] Immuno-PCR combines the amplification power of PCR with a sensitive immunoassay. By attaching a reporter DNA, which can be amplified by PCR, to the antibody to MG7-Ag, instead of attaching an enzyme to the antibody, the test sensitivity can be greatly enhanced. In a blinded study of patients in a hospital, the MG7-Ag immuno-PCR assay was positive in 82.8% of patients with GC, it was also positive in 44.4% of patients with colon cancer but in no patients with primary liver or ovarian cancers.[11] The outcome of the MG7-Ag immuno-PCR assay is dichotomous, so the user does not need to specify a cutoff value to determine positivity. Moreover, the prevalence of serum MG7-Ag positivity was consistently low in various precancerous gastric lesions (from 3.0% to 5.6%) in this study, supporting that MG7-Ag may have a potential as a specific biomarker for early detection of GC.

Although the MG7-Ag immuno-PCR assay performed very well, there remains room for improvement. The assay was negative in about 23% of those with GC, and the reasons for this lack of sensitivity are not clear. MG7-Ag-specific monoclonal antibody has a high sensitivity and specificity in detecting GC, but the MG7 antigen has not been fully characterized. Recently, a heterogeneous nuclear ribonucleoprotein A2/B1 was identified as a target antigen for MG7,[24] but its role in gastric carcinogenesis and potential use in screening remain to be determined.

In conclusion, our study showed that a serum-based MG7-Ag Immuno-PCR assay may be useful as a preliminary screen and early detection for GC in general populations with high GC mortality, such as Linqu County, Shandong Province, China. Subjects with positive results would need to have endoscopy to obtain a definitive diagnosis, but the amount of endoscopic screening would be greatly reduced by the preliminary screening with the MG7-Ag Immuno-PCR assay.

References

1. Parkin DM, Muir CS, Whelam SL, Gao YT, Ferlay J, Powell J. Cancer incidence in five continents (VI), IARC Scientific Publication No102. Lyon: IARCPress, 1992.
2. Ministry of Publish Health, China (Cancer Control Office). Data on cancer mortality in China. Beijing: Cancer Control Office on the Ministry of Health, 1980.
3. World Health Organization. The World health report. Geneva: WHO, 1997.
4. You WC. Gastric cancer screening and early detection. In: Wei-cheng Y, ed. Gastric cancer. Beijing, China: Chinese Medicine Publishing House, 2006. 52–7.
5. Kampschoer GH, Fujii A, Masuda Y. Gastric cancer detected by mass survey. Comparison between mass survey and outpatient detection. *Scand J Gastroenterol* 1989;24:813–7.
6. Hisamichi S. Screening for gastric cancer. *World J Surg* 1989;13: 31–7.
7. Wang TG, You WC, Henderson BE, Blot WJ. A case-control study of stomach cancer in Shandong Province. *Natl Cancer Inst Monogr* 1985;69:9–10.
8. You WC, Blot WJ, Li JY, Chang YS, Jin ML, Kneller RW, Zhang L, Han ZX, Zeng XR, Liu WD, Zhao L, Correa P, et al. Precancerous gastric lesions in a population at high risk of stomach cancer. *Cancer Res* 1993;53:1317–21.
9. You WC, Blot WJ, Zhang L, Kneller RW, Li JY, Jin ML, Chang YS, Zeng XR, Zhao L, Fraumeni JF, Jr, Xu GW, Samloff MI. Serum papsinogens in relation to gastric cancer and precancerous lesions at a high-risk population in China. *Cancer Epidemiol Biomarkers Prev* 1993;2:113–17.
10. Fan DM, Zhang XY, Chen XT, Mu ZX, Hu JL, Qiao TD, Chen BJ. Mouse and human monoclonal antibodies against gastric cancer preparation and clinic application. *Chin Med J* 1988;101:488–9.
11. Ren J, Chen Z, Juan SJ, Yong XY, Pan BR, Fan DM. Detection of circulating gastric carcinoma-associated antigen MG7-Ag in human sera using an established single determinant immuno-polymerase chain reaction technique. *Cancer* 2000;88:280–5.
12. Liu J, Hu JL, Zhang XY, Qiao TD, Chen XT, Wu KC, Ding J, Fan DM. The value of MG7Ag in predicting cancerous change in dysplastic gastric mucosa. *Int J Clin Pract* 2002;56:169–72.
13. Fan DM, Zhang X, Chen X. Bird's-eye view on gastric cancer research of the past 25 years. *J Gastroenterol Hepatol* 2005;20: 360–5.
14. Fan DM, Zhou SJ, Zhang XY, Yang AG, Li MF, Chen Z. Establishment of immuno-PCR technique for the detection of tumor associated antigen MG7-Ag on the gastric cancer cell line. *Chin J Cancer Res* 1995;7: 247–50.
15. Liu F, Pan KF, Zhang XM, Zhang Y, Zhang L, Ma JL, Dong CX, Shen L, Li JY, Deng DJ, Lin DX, You WC. Genetic variants in cyclooxygenase-2, expression and risk of gastric cancer and its precursors in a Chinese population. *Gastroenterology* 2006;130:1975–84.
16. Dong CX, Deng DJ, Pan KF, Zhang L, Zhang Y, Zhou J, You WC. Promoter methylation of p16 associated with helicobacter pylori infection in precancerous gastric lesions: a population-based study. *Int J Cancer* 2009; 124:434–9.
17. You WC, Brown LM, Zhang L, Li JY, Jin ML, Chang YS, Ma JL, Pan KF, Liu WD, Hu Y, Crystal-Mansour S, Pee D, et al. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. *J Natl Cancer Inst* 2006; 98:974–83.
18. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The Updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161–81.
19. Rugge M, Correa P, Dixon MF, Hattori T, Leandro G, Lewin K, Riddell RH, Sipponen P, Watanabe H. Gastric dysplasia: the Padova International Classification. *Am J Surg Pathol* 2000;24:167–76.
20. Mackay IR. Carcinoembryonic antigen: use in screening. In: Herberman RB, Mcintire KR, eds. Immunodiagnosis of cancer, part I. New York: Marcel Dekker, 1979. 255–64.
21. Miki K. Gastric cancer screening using the serum pepsinogen test method. *Gastric Cancer* 2006;9:245–53.
22. Miki K, Urita Y. Using serum pepsinogens wisely in a clinical practice. *J Dig Dis* 2007; 8:8–14.
23. Xu L, Jin BQ, Fan DM. Selection and identification of mimic epitopes for gastric cancer-associated antigen MG7 Ag. *Mol Cancer Ther* 2003;2:301–6.
24. Lee CH, Lum JH, Cheung BP, Wong MS, Butt YK, Tam MF, Chan WY, Chow C, Hui PK, Kwok FS, Lo SC, Fan DM. Identification of the heterogeneous nuclear ribonucleoprotein A2/B1 as the antigen for the gastrointestinal cancer specific monoclonal antibody MG7. *Proteomics* 2005;5:1160–6.