Detection of Gastric Cancer and Premalignant Lesions by Novel Marker Glycoprotein 87 Using Monoclonal Antibody Adnab-9

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Abstract

Gastric adenocarcinoma (GC), the second most frequent cancer in the world, is highly prevalent in Asia. A screening test for early-stage GC would represent a major advance in the management of this disease. Associated conditions such as chronic atrophic gastritis (CAG) with intestinal metaplasia are manifested by specialized intestinalized tissue that often includes Paneth cells. Adenoma-associated antigen glycoprotein 87 (GP87), defined by the Adnab-9 monoclonal antibody and shed into the gut, is associated with epithelial cells, some of which resemble Paneth cells. We evaluated the diagnostic value of GP87 for cancer and gastric premalignant lesions. One hundred and seven sections of normal, benign, and premalignant gastric mucosa and 79 sections of pericancerous tissue were evaluated for expression of GP87 by immunohistochemistry. Eighty-two patients with GC, 34 patients with benign chronic disease, 35 patients with premalignant conditions, and 80 normal controls were evaluated by ELISA for GP87 in feces and gastric juice. Adnab-9 immunostaining for GP87 was significantly positive in CAG and intestinal metaplasia (>77.8%), compared with 0% in normal controls (P < 0.05). Fecal GP87 was positive in 79.3% of patients with gastric cancer, including early-stage lesions, and in 84% of patients with CAG versus 10% of controls (P < 0.05). Positive proportions for each pathological group in tissue correlated with that in feces (r = 0.99; P < 0.02). GP87 is differentially expressed in premalignant gastric lesions that appear to be the origin for fecal GP87, which may be useful for early detection of GC.

Introduction

The incidence and death rate of GC in China and other parts of Asia is very high. In some areas of China, it is the first or second leading cause of cancer-related deaths in males, and overall, it is the third most common form of cancer-related death in the general population in Asia (1). The early stage of GC is often asymptomatic, which makes diagnosis complicated and extremely difficult outside of a screening program where the proportion of early-stage lesions may approach 50% (2). The development of ancillary methods for this diagnosis incorporates the desire for efficient, noninvasive diagnostic procedures such as the identification of serum markers specifically associated with a high percentage of patients diagnosed with GC. Among the serum markers currently available for diagnosis of gastric cancer are CEA and CA19-9, but their positive rates are not high and lack specificity (3). Only 20% of GC patients had positive CEA levels, and 26–72% had positive CA19-9 (4, 5). These data suggest that it is necessary to evaluate other markers for their potential role in the management of GC, especially early-stage cancer, because early-stage cancers have a high complete resectability rate and a better prognosis (2). IM is considered a strong concomitant of GC and frequently contains fully differentiated or immature Paneth cells that may be important for proliferation in gastric mucosa (6, 7).

A premalignant antigen, GP87, has been described previously (8, 9), was originally identified and partially characterized using MAb Adnab-9, and is thought to identify a subpopulation of neoplasia-associated Paneth-like cells at the base of intestinal crypts in colon, ileum (10), and upper small bowel (11), but not normal Paneth cells. Because Adnab-9 is shed into the bowel lumen (10), and preliminary data show that it is detectable in stool (12), we studied tissue, gastric juice, and fecal GP87 detection to evaluate the clinical efficacy of this new digestive tract preneoplastic marker for the diagnosis of GC and premalignant lesions.

Materials and Methods

Patient Specimens. GC and nonmalignant gastric tissue specimens were obtained from the Department of Pathology of the General Hospital of the People’s Liberation Army, fixed in 10% formalin, embedded in paraffin, and stained immunohistochemically by the streptavidin–biotin complex method described previously (13). A pathologist reviewed all specimen histopathology. Feces for measurement of Adnab-9 were obtained from patients with different gastric disease states. Gastric
juice was obtained by aspiration during endoscopy. These patients were all from the General Hospital of the People’s Liberation Army. The diagnosis was based on clinical findings, laboratory tests, endoscopic examination, and histopathology of endoscopic biopsy or the surgically removed specimen. For stool studies, normal cases were defined as patients who had negative gastroscopy (n = 33) and/or those who had no symptomatic or other risk factors for GC. GC patient details are summarized in Table 1.

MAb Adnab-9. To raise this antibody, we immunized BALB/C mice with membrane-associated tissue constituents derived from human colonic adenomas. Adnab-9 hybridomas were produced in BALB/c mice for use in the assays below and are also available from DakoCytomation Inc. (Carpinteria, CA). The antigen recognized is a glycoprotein with a molecular weight of 87,000 (9, 14).

Adnab-9 IHC. IHC was performed on 4-μm sections of paraffin-embedded tissue blocks by the method of Hsu et al. (15), as reported previously (9, 11). The staining degree was scored by area staining in a section of one slide: −, <5%; +, 5–25%; ++, 25–50%; and ++++, >50% (11).

Detection of GP87 in Feces by ELISA. We determined stool reactivity with Adnab-9 by ELISA. Briefly, a fecal sample (150 mg) was obtained and, within 4 h of collection, suspended in 1 ml of 0.08N NaCl and centrifuged at 10,000 rpm for 10 min. The supernatant was stored at −20°C until the assay was performed. Stored feces were diluted in PBS at a dilution ratio of 1:4, and each sample was plated in quadruplicate and incubated overnight at 50 μl/well in a 96-well microtiter plate at 4°C. The wells were then blocked with 5% BSA for 2 h at 37°C and washed three times with PBS, and 50 μl of Adnab-9 were added to two wells of test specimen, and PBS was added to the remaining two wells. The plates were then incubated for 2 h and washed, and 50 μl of biotinylated rabbit antimouse antibody (BRAM; 1:5,000) were added to all wells. The plates were further incubated at 37°C for 30 min and washed, and 50 μl of peroxidase-streptavidin (provided by Zymed Laboratories, South San Francisco, CA) at a dilution of 1:5,000 were added to all wells that were incubated at room temperature for 30 min. After a final washing step, substrate (orthophenylenediamine) and H2O2 were added, and after 10 min, the absorbance value at 490 nm was obtained using an ELISA reader (Bio-Rad, Hercules, CA). The final result for each sample was derived by subtracting the average reading of the two PBS-treated wells from the average reading of the two Adnab-9-treated wells. To ensure reproducibility, we assayed representative stool samples from five normal and five cancer patients for GP87 using a similar ELISA in a blinded fashion.

GP87 in Gastric Juice. To demonstrate that the likely source of fecal GP87 is the stomach, gastric juice was aspirated during endoscopy and collected in a specimen trap. GP87 binding to Adnab-9 MAb was determined as described above by an ELISA after homogenization, clarification of the specimen by a 15-min, 10,000 rpm centrifugation, and adjustment of the pH to 7.5.

Statistical Evaluation. Data were analyzed using Student’s t test and nonparametric methods of χ2 (16) and linear correlation by method of least squares using a statistical computer software package (Instat; Graphpad Software Inc., San Diego, CA). P < 0.05 was considered significant.

Results

Expression of GP87 in Gastric Tissues. The results are shown in Table 2. GP87 was absent in normal mucosa of stomach but highly expressed in premalignant tissues and pericancerous tissues. There was 100% positivity in tissue section of CAG with IM and 77.8% positivity in sections with CAG alone (P < 0.05 compared with normal tissues). The highest intensity of staining was seen only in premalignant lesions such as CAG with ATP, where the proportion of 3 + staining of all sections was 46.7%, and in CAG with IM (Fig. 1), where 45.5% of sections stained positive (P < 0.01 when compared with normal tissues). When comparing the relative intensity of expression of GP87 in normal mucosa adjacent to GC with other forms of gastric lesions not associated with GC, we found a high intensity of expression (3+) compared with no cases with that level of intensity in non-cancer-associated CSG. A total of 48.7% of 39 cases of CAG and 46% of 37 cases of CAG with ATP had a staining intensity of 3 + (Table 3). In addition, the positive rate of CAG with ATP in adjacent mucosa of GC (81.1%) tended to be higher than that seen in non-cancer-associated CAG without ATP (73.3%).

Fecal GP87. Fecal specimens from 82 patients with primary GC and 149 patients with benign gastric diseases or normal individuals were evaluated for the presence of GP87 using MAb Adnab-9. As shown in Table 4, the average levels in patients with GC and CAG were significantly higher than the levels of normal individuals (79.3% versus 10%, respectively; P < 0.05) and those with gastric ulcers (21.4%) or CSG (20%). We used a cutoff limit of mean A490 nm value from normal individuals plus 2 SDs, that is 0.068. In 33 of 80 normal individuals, confirmed as normal by endoscopy, only one case was higher than 0.068, thus the false positive rate of normal individuals may be as low as 3%.

The relationships between GP87 levels and the clinical staging of the patients with gastric cancer were also evaluated, as shown in Table 5. Positive fecal levels were found at all

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<th>Table 1 Cancer patient characteristics</th>
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a Stage by TMN system.
b Three patients were unclassifiable.

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<th>Table 2 Immunohistochemical expression of GP87 in gastric premalignant tissues</th>
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a P < 0.05.
stages. Of particular note was that three of the four patients with early-stage cancer were positive.

When the positive proportion of Adnab-9 testing in fecal specimens was compared with that obtained in tissue IHC specimens for each diagnostic category (normal, CSG, and CAG), there was a highly positive correlation ($r = 0.99; P = 0.01$). When the code was broken on the stool samples used to test reproducibility, GP87 activity was higher in the gastric cancer samples than in those of the normal patients, and no overlap was seen (data not shown).

GP87 in Gastric Juice. The positive rate of GP87 in gastric juice was 86.3% for GC and 60% for CAG, higher than that seen in the CSG group (26.1%; $P = 0.05$) or normal group (16%; $P < 0.05$). These results were similar to those obtained in feces, strongly suggesting that the source of fecal antigen is the stomach, and positive proportions for each category correlated significantly ($r = 0.97; P < 0.05$; Fig. 2).

### Discussion

GP87 defined by MAb Adnab-9 is detectable in the colonic effluent, and higher binding levels to the Adnab-9 MAb correlate strongly with levels in noncancerous tissue samples from patients with CRC (9, 10). In the present study, the data indicate that GP87 is expressed in premalignant lesions of gastric origin and detectable in stool, correlating with the presence of both GC and premalignant lesions. The tissue expression seen in premalignant lesions is not expressed in stomach tissue from normal individuals. We may therefore consider GP87 as both a diagnostic marker for gastric cancer and a risk marker for the disease. By using this biomarker to noninvasively detect premalignant lesions of the stomach due to its expression at the level of tissue and gastric secretions, shedding antigens that can be conveniently detected in feces. Fecal GP87 levels may be used as a component in the mass screening and diagnosis of patients with GC and associated premalignant lesions. When compared with other tumor markers that are currently available [i.e., CEA, CA19-9, and TAG-72 (4, 5, 17)], a higher percentage of patients with GC had positive GP87 fecal levels (79.3%) than described historically with the above markers. In addition, unlike these other markers (where the level generally increases with increasing stage), GP87 also detects early-stage cancers (18). GP87 can also identify patients with conditions that render them at increased risk of GC because it is also expressed in premalignant lesions, especially in IM of gastric mucosa, which may be the source of fecal GP87 because of the close correlation between the data from the two sets of samples.

Other markers for GC have been described with variable sensitivity and specificity but have not been approved for GC
Diagnosis of Stomach Cancer Study by Antiadenoma Marker

Diagnosing stomach cancer remains a challenge due to its strong association with non-cardia GC (19). Suggested theoretical strategies for using tumor markers are currently directed at screening and treatment of \textit{H. pylori} to prevent GC (20). Serum pepsinogen I/II ratios have been extensively examined epidemiologically and generally correlate with GC, but with some limitations. In one study, the correlation was strong only in men (21), and in another, the sensitivity for GC was only 55% when considering serum pepsinogen I levels <50 ng/ml and I/II ratio <3.0 (22). Recently, using levels of pepsinogen I cutoff of <70 ng/ml and the same ratio of <3, other workers found improved sensitivity of 80% with a specificity of 70% but a modest positive predictive value of 1.5% (23). The relatively low positive predictive value suggests that a single test based on the above criteria is insufficient for GC screening. Markers for detection of sporadic GC have therefore been somewhat disappointing, and efforts have been directed mainly to markers for detection of predisposing lesions such as CAG or IM (24). These markers had good sensitivity for antibodies to \textit{H. pylori}, Cag A, and serum gastrin levels (92%, 83%, and 83%, respectively) but poor specificity (18%, 41%, and 22%, respectively). The converse was shown for serum pepsinogen I <25 μg/liter and pepsinogen ratios of I/II of <2.5 (sensitivity = 6% and 14%, respectively; specificity = 100% and 96%, respectively). The advantage of stool GP87 based on this study data is its high putative sensitivity and specificity in this Chinese population. In addition, other cancers liable to give a positive fecal GP87 test result such as CRC are relatively less common in China. Another advantage is the ability to collect and send feces at ambient temperature through the mail, in that we have shown that when GP87-positive stool samples are left on fecal occult blood stool cards for at least 6 days at room temperature, Adnab-9 binding is undiminished (25).

When comparing the expression of GP87 in tissues with its detection in feces, we found that the level of GP87 in feces of gastric cancer patients was somewhat lower than it is in patients with a premalignant lesion (CAG). It is generally agreed that the sensitivity as well as the specificity of tumor markers for the diagnosis of sporadic carcinoma is limited. No single tumor marker will unambiguously predict the presence of malignant disease or differentiate between benign versus malignant disease. Despite fecal GP87 having a good sensitivity (79.3%) in GC, it also has a similar rate for premalignant lesions of stomach (84.4%), and therefore cannot be used to differentiate between benign and malignant lesions. However, GP87 was conceptually introduced as a risk marker (26), although it may in also have application as a cancer detection marker in GC. We have shown that proportions of fecal GP87 binding for GC-associated lesions correlate well with proportions of binding in gastric juice (Fig. 2). This may be because GP87 in feces is shed from the superficial mucosa and, due to its relative resistance to bacterial and digestive enzyme breakdown, is relatively well preserved and therefore detectable in the stool (25). This is in contrast with the condition of differing tumor antigen cancer content and levels of shed antigen into the serum that have been described for tumor markers such as CEA (27). The relatively equivalent potential for diagnosis of both GC and CAG by fecal GP87 determination (sensitivity, 79.3% versus 84.4%, respectively) suggests that it may be most useful for the mass screening for GC. It may be used to both diagnose GC and help select a subgroup at high risk for GC. This high-risk group, once identified, could be followed endoscopically to enable an early diagnosis of GC.

Historically, attempts to detect tumor using tumor markers have usually used serum samples that may be more specific for gastric cancer. However, we have found gastric juice, analogous to colonic effluent (26) in CRC (28), to be more sensitive than serum (29). In this study, we find that detection of a tumor marker in feces is more convenient and sensitive than we have previously found in gastric juice (18, 29). In a limited number of cases, GP87 has also been identified in pancreatic cancer and biliary tract cancer tissues and can also be detected in the feces of patients with pancreatic cancer (18). Thus, if GP87 in the feces is positive, other diagnostic modalities may be needed to detect the disease state existing in the positive test if gastroscopy is negative. However, this would probably not be an insurmountable problem in other luminal tumors, which are less common in Chinese patients, and which are accessible to endoscopy, and GP87 could be combined with other markers such as mutated stool k-ras in the case of pancreatic cancer (30). Prospective clinical trials of fecal GP87 should be conducted to investigate the efficacy of this test in reducing the considerable worldwide mortality from GC.
Acknowledgments

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