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Original contribution

Loss of heterozygosity at chromosomes 1p35-pter, 4q, and 18q and protein expression differences between adenocarcinomas of the distal stomach and gastric cardia ☆

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Summary Loss of heterozygosity of 1p35-pter, 4q, and 18q is frequent in gastric carcinoma, suggesting that these regions harbor tumor suppressor genes. However, the differences in these genetic alterations between adenocarcinoma of the gastric cardia and adenocarcinoma of the distal stomach remain unclear. In this study, loss of heterozygosity at chromosomes 1p35-pter, 4q, and 18q were analyzed in adenocarcinoma of the gastric cardia and adenocarcinoma of the distal stomach samples acquired by laser capture microdissection. The expression of several tumor suppressor gene proteins, runt-related transcription factor 3 (1p36), annexin A10 (4q33), SMAD family member 4 (18q21.1), and deleted in colorectal carcinoma (18q21.3), was evaluated immunohistochemically. The adenocarcinoma of the distal stomach and adenocarcinoma of the gastric cardia lesions had a similar trend in total deletion frequency for chromosomes 1p35-pter (36.5% for adenocarcinoma of the distal stomach and 32.5% for adenocarcinoma of the gastric cardia), 4q (42.3% for adenocarcinoma of the distal stomach and 47.5% for adenocarcinoma of the gastric cardia), and 18q (38.5% for adenocarcinoma of the distal stomach and 45% for adenocarcinoma of the gastric cardia). However, loss of heterozygosity patterns were clearly different in the 2 adenocarcinomas. Deletion mapping indicated that 4q32.2-4q34.3, 18q21.2-21.31, 18q22.3-23, and 1p35.2-1p36.13 were involved in adenocarcinoma of the distal stomach, whereas 4q13.3-4q22.3, 4q31.21-4q32.2, 18q21.31-18q22.1, and 1p35.2-1p36.13 were involved in adenocarcinoma of the gastric cardia. Expression of ANXA10 ($P = .038$), SMAD family member 4 ($P = .028$), and deleted in colorectal carcinoma ($P = .004$) was less common in adenocarcinoma of the distal stomach than in adenocarcinoma of the gastric cardia. Expression of runt-related transcription factor 3 ($P = .795$) showed no significant difference in the 2 tumors. The tumors differed in the profile of genetic alterations and protein expression of these well-known tumor suppressor genes. The deleted regions defined in this study may harbor tumor suppressor genes relevant to adenocarcinoma of the gastric cardia.

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1. Introduction

Gastric carcinoma is one of the most common malignancies in much of the world [1]. However, carcinomas arising from different regions of the stomach have undergone significant changes in prevalence; in most developed countries, gastric adenocarcinoma in the antrum or distal stomach has decreased, whereas adenocarcinoma of the gastric cardia (AGC) has increased [2,3]. In the past several years, the incidence of AGC exhibited an increasing trend in the high-incidence areas of gastric adenocarcinoma in China [4,5]. Recent studies indicate that AGC and adenocarcinoma of the distal stomach (ADS) differ in their clinicopathologic and epidemiological characteristics [6,7]. However, the etiology of AGC and its relation to ADS remain unsettled.

Loss of heterozygosity (LOH) is a prominent feature of cancer. Previous studies have identified high frequencies of deletion of chromosomes 1p, 4q, 8p, 11p, and 18q in AGC [8,9]. The critical regions for these alterations need to be delineated. Interestingly, these chromosomes are frequently altered in gastric carcinomas [10,11]. The differences in genetic alterations and the molecular mechanism underlying the development of AGC and ADS remain unknown. It, therefore, seems essential to explore the biologic features of AGC.

It is well accepted that frequent LOH in cancer cells could make a greater contribution to the expression of tumor suppressor genes (TSGs). Some TSGs associated with gastric carcinoma have been identified in the regions altered in gastric cancer, such as *runt-related transcription factor 3* (*RUNX3*) (located at 1p36) [12], *annexin A10* (*ANXA10*) (located at 4q33) [13], *SMAD family member 4* (*SMAD4*) (located at 18q21.1) [14], and *deleted in colorectal carcinoma* (*DCC*) (located at 18q21.3) [15]. However, whether AGC also shows abnormal expression of these TSGs is unclear.

Therefore, in this study, we evaluated the difference in LOH in AGC and ADS by detecting the patterns of allelic loss covering chromosomes 4q, 18q, and 1p35-pter using 28 microsatellite markers. Furthermore, we examined the expression profiles of the gastric carcinoma–associated TSGs in these regions to compare the genetics and expression of TSGs in AGC and ADS. Approval for the study was received from the Ethics Committee of China Medical University.

2. Materials and methods

2.1. Samples

Matched control and tumor tissue samples from 106 patients who underwent operations for gastric carcinoma were obtained from the Department of Surgical Oncology, The First Hospital of China Medical University, from January 2003 to October 2005. Tumors were classified according to endoscopic, intraoperative, and pathologic data. An *AGC* (n = 46) was defined as a tumor center within 1 cm above and 2 cm below the anatomic esophagogastric junction, according to the classification of Siewert and Stein [16]. An *ADS* (n = 60) was defined as a tumor located in the middle or the lower third of the stomach. The age, sex, tumor location, tumor size, gross type according to the Bormann classification, grade of differentiation, lymph node metastasis, and pTNM stage were obtained from the operative records and pathology reports and assigned according to the standard criteria of the Seventh TNM staging system [17] (Table 1). No patient received preoperative chemotherapy or radiotherapy.

2.2. Laser capture microdissection and DNA extraction

LOH analysis was performed on 40 cases of AGC and 52 cases of ADS. Hematoxylin and eosin–stained sections were microdissected using a PixCell II laser capture microdissection (LCM) system (Arcturus Engineering Inc,

Table 1 Comparison of clinicopathologic characteristics of patients with ADS and AGC

	AGC (n = 46) (%)	ADS (n = 60) (%)	*P*
Age (y)			.582
≤60	19 (41)	28 (47)	
>60	27 (59)	32 (53)	
Sex			.025
Male	40 (87)	41 (68)	
Female	6 (13)	19 (32)	
Tumor size (cm)			.664
≤4	31 (67)	38 (63)	
>4	15 (33)	22 (37)	
Lauren type			.092
Intestinal	16 (35)	10 (17)	
Diffuse	24 (52)	38 (63)	
Mixed	6 (13)	12 (20)	
Depth of invasion (T stage)			.101
1	4 (9)	15 (25)	
2	14 (30)	19 (32)	
3	25 (54)	21 (35)	
4	3 (6.5)	5 (8)	
Lymph node metastasis			.039
Negative	6 (13)	18 (30)	
Positive	40 (87)	42 (70)	
pTNM stage			.070
1	4 (9)	15 (25)	
2	29 (63)	24 (40)	
3	11 (24)	17 (28)	
4	2 (4)	4 (7)	