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Original contribution

Different expression pattern and significance of p14ARF-Mdm2-p53 pathway and Bmi-1 exist between gastric cardia and distal gastric adenocarcinoma ☆

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Summary Recent studies have suggested that adenocarcinoma of gastric cardia (GCA) is distinct from distal stomach, with different risk factors, tumor characteristics, and biological behavior. The aim of this study is to evaluate the possible difference in the expressions of p14ARF, Mdm2, p53, and Bmi-1 by immunohistochemical staining on paraffin-embedded tissues of gastric cardia adenocarcinoma (GCA; n = 74) and distal gastric adenocarcinoma (DGA; n = 41). The results showed that the percentage of p14ARF-negative expression, Mdm2 overexpression, p53-positive expression, and p53 pathway abnormality ($p14ARF^{-}/Mdm2^{+}/p53^{+}$) were all significantly higher in GCA than those in DGA ($P < .05$). Further analysis showed that in GCA, the negative expression of p14ARF was significantly associated with poor differentiation, Mdm2 overexpression with tumor stage and lymph node metastasis, and positive p53 expression with tumor stage ($P < .05$), whereas in DGA, only Mdm2 overexpression was related with well/moderate differentiation ($P < .05$). Abnormality of the p53 pathway was significantly correlated with poorer differentiation only in GCA ($P < .05$). The positive expression of Bmi-1 in all cases of GCA and DGA was significantly higher than normal gastric mucosa epithelium, but no difference was found between GCA and DGA ($P > .05$). Thus, the results in this study confirmed that different expression pattern and clinicopathologic significance of the p14ARF-Mdm2-p53 pathway did exist between GCA and DGA. The results further support the hypothesis that different mechanisms may be involved in the development and progression of adenocarcinoma from cardia and distal portion of stomach.

1. Introduction

Gastric adenocarcinoma is the second leading cause of cancer-related mortality in the world [1]. Recent decades have witnessed a dramatic shift in the subsite of gastric cancers. In contrast to the decrease in distal gastric adenocarcinoma (DGA), the adenocarcinoma incidence from the gastric cardia (GCA) increased dramatically among Western and American populations [2]. Similar

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changes in the incidence of GCA and DGA in southern and central rural areas in Hebei province of China were also found in the past 2 decades [3].

Recent studies have suggested that GCA is distinct from distal stomach carcinoma, with different risk factors, tumor characteristics, and biological behavior [4-6]. GCA usually shows a greater tendency toward deeper wall penetration, more lymph node metastasis, and poorer prognosis than gastric cancer at other sites [7,8]. Our preliminary study showed that differences in patterns of immunophenotypic markers (MUC2, CK14, and CK20) and expressions of signaling molecules (p38 and MNK1) did exist between gastric cardiac and distal adenocarcinomas in Chinese patients [9]. Some other studies have also shown differences in the genetic aberrations between GCA and DGA. The frequency and spectrum of *TP53* gene mutations in GCA displayed a similar prevalence with adenocarcinoma of the esophagus than that in DGA [10-12].

The p14ARF-Mdm2-p53 pathway, commonly referred to as the *p53 pathway*, is disrupted in the development of cancer. The p53 pathway is usually inactivated by a *TP53* mutation, *p14ARF* deletion, or amplification of *MDM2* in many human cancers [13,14]. Currently, there are several investigations on the differences of alterations in the members of the p53 pathway between cardia adenocarcinoma and DGA; however, the results are conflicting rather than conclusive. Some studies suggested that p53 alterations were more frequently found in adenocarcinoma of the cardia than that of the antrum [15]. Significant differences in the prevalence of promoter hypermethylation in *p14ARF* among esophageal, cardia, and gastric adenocarcinoma were also found [16]. However, at protein level, Jovanović et al [17] found no significant differences in p53 expression among Barrett adenocarcinoma, adenocarcinoma of the cardia, and adenocarcinoma of the antrum. Therefore, further comparative study on the expression pattern of the p14ARF-Mdm2-p53 pathway and its significance between GCA and DGA is imperative.

Bmi-1 (B-cell-specific Moloney murine leukemia virus integration site 1) was originally isolated as an oncogene that cooperates with c-myc in the generation of mouse pre–B-cells lymphomas [18]. It has been reported that increased expression of Bmi-1 in primary human tumor cells leads to down-regulation of the *INK4a-ARF* locus and thereby impact the p14ARF-Mdm2-p53 pathway [19], suggesting that Bmi-1 is a potent repressor of the p53 pathway. The possible role and putative relationship between Bmi-1 and the p53 pathway in adenocarcinomas of gastric cardia and distal stomach are still unclear.

In this study, we comparatively examined the expressions of p14ARF, Mdm2, p53, and Bmi-1 at protein level in GCA and DGA of Chinese patients using immunohistochemical methods. The aims of this study are to evaluate the expression pattern and clinical pathological significance of the p53 pathway in gastric adenocarcinoma arising from the 2 different subsites of stomach and to explore the possible differences in the carcinogenesis between GCA and DGA.

2. Materials and methods

2.1. Tissue samples

One hundred fifteen formalin-fixed and paraffin-embedded resection tissue samples of gastric adenocarcinoma were obtained from the archives of the Department of Pathology, the Second Hospital of Hebei Medical University, in March 2007 and December 2008. The average age of the patients was 50 years. Based on the location of primary tumor, the type II tumors according to Siewert classification were defined as *cardia adenocarcinomas* (GCA) [20,21], whereas the cases with a primary tumor center located in antrum and angle of stomach were defined as *DGA* in this series. Among the cases, 74 were GCAs and 41 were DGAs. None of the patients had received chemotherapy or radiotherapy before resection of the tumor. Twenty normal gastric mucosa tissues free from inflammation, metaplasia, dysplasia, or carcinoma were used for comparison.

The morphologic classification of the adenocarcinomas was conducted according to the fourth edition of World Health Organization criteria [22], whereas the staging was based on the seventh edition of TNM system [23]. The cases were subdivided according to 3 grades of malignancy: well differentiated, moderately differentiated, and poorly differentiated. For statistical purposes, well- and moderately differentiated adenocarcinomas were taken together to compare with those of poorly differentiated cases.

No significant difference was found in age, sex, tumor size, tumor stage, and lymph node metastasis between GCA and DGA cases in this series (Table 1).

Table 1 Clinicopathologic parameters of adenocarcinomas of gastric cardia and distal stomach

Clinicopathologic parameters	GCA (n = 74), n (%)	DGA (n = 41), n (%)
Age (y)		
≤50	9 (12.2)	9 (22.0)
>50	65 (87.8)	32 (78.0)
Sex (male/female)		
Male	56 (75.7)	29 (70.7)
Female	18 (24.3)	12 (29.3)
Differentiation		
Well/moderate	32 (43.2)	17 (41.5)
Poor	42 (56.8)	24 (58.5)
Tumor size (cm)		
<5	51 (68.9)	28 (68.3)
≥5	23 (31.1)	13 (31.7)
Tumor stage		
T1/T2	20 (27.0)	9 (22.0)
T3/T4	54 (73.0)	32 (78.0)
Lymph node metastasis		
Negative	31 (41.9)	12 (29.3)
Positive	43 (58.1)	29 (70.3)