

Original Article

Relationship between p53 Expression and Gastric Cancers in Cardia and Antrum

Ramin Azarhoush MD*, Abbas Ali Keshtkar MD MPH PhD**, Taghi Amiriani PhD***, Vahideh Kazemi-Nejad MD*

Background: The mutations in p53 gene and accumulation of p53 protein are the most common genetic events in gastric carcinomas. The present study was conducted to compare the frequency of p53 gene overexpression in a consecutive series of adenocarcinomas arising from the cardia and the antrum. This study also evaluates the associations of this gene expression with demographic and clinicopathologic findings (age, sex, histology, and grade of tumor).

Methods: Retrospective analysis was performed on 111 patients with gastric cancer who had undergone upper gastrointestinal endoscopies in 5th Azar Medical Center (northeastern, Iran), during 1998-2005. The series comprised of 25 patients with cardia adenocarcinoma and 86 patients with antral adenocarcinoma. p53 alteration (nuclear p53 overexpression) was detected by immunohistochemistry.

Results: Nuclear p53 overexpression was found in 14 (56%) out of the 25 and 27 (31.3%) out of the 86 patients with cardia and antral adenocarcinomas, respectively. p53 gene overexpression was significantly more frequent in adenocarcinomas of the cardia than the antrum. There were no differences in the clinicopathologic characteristics of the tumors between p53-positive and p53-negative cases in both types of the cancer.

Conclusion: This study shows that p53 alterations correlate well with gastric location, and they are more frequent in adenocarcinoma of the cardia than the antrum. This result reinforces the hypothesis that the cancers of the lower esophagus and upper stomach have distinct epidemiologic, pathogenesis, and molecular characteristics from that observed in cancers of the lower part of the stomach.

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Introduction

Gastric cancer remains the second most common cause of cancer-related deaths world wide.¹⁻³ However, there are large geographic variations in incidence, which may be related to environmental and genetic factors.

In addition to dietary factors such as excessive intake of salt and food contaminations with N-

nitro compounds, cigarette smoking, and *H. pylori* infection have been regarded as presumed environmental factors contributing to gastric carcinogenesis.⁴⁻⁶

Mutations in the tumor suppressor gene p53 are the most frequent genetic alterations in human cancer. Previous studies of their incidence ranged from as low as 4% to as high as 57%.⁷ Recently, one study demonstrated that p53 mutations were frequently found in *H. pylori*-associated gastritis, suggesting a direct role for *H. pylori* infection in p53 mutagenesis.⁸ p53 is a tumor suppressor gene. It is located on the short arm of chromosome 17, producing a 53 kDa nuclear protein. p53 gene, through the production of p53 protein, controls the cell cycle and prevents genetic mutations for carcinogenesis.⁹ The mutations of the p53 gene are

Authors' affiliations: *Department of Pathology, **Department of Preventive and Social Medicine, ***Department of Internal Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Corresponding author and reprints: Abbas Ali Keshtkar MD MPH PhD, Department of Preventive and Social Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Telefax: +98-171-442-1660,
E-mail: abkeshtkar@yahoo.com

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genetic events in the pathogenesis of gastric adenocarcinoma.^{10,11} However, the suggestion awaits further investigation for confirmation.

Rapid increase in p53 levels and activation of p53 as a transcription factor (shown to have major role in carcinogenesis) lead to two major effects: cell-cycle arrest and apoptosis. The wild type gene is capable of arresting growth in transfecting experiments.⁹ In some cases, it sends a cell into a programmed spiral of death.¹⁰ Mutant forms not only deprive beneficial effects of the wild type cells but also can spur abnormal cell growth.¹⁰ p53 mutation is a common event in gastric carcinoma occurring from the early stage of progression with its specific mutation spectrum.¹¹ With homozygous loss of p53, DNA damage goes unrepaired, mutations become fixed in dividing cells, and the cells turn into malignant transformation.^{12,13} Accumulation of p53 protein in the nuclei of carcinomatous cells is known to correlate well with the presence of mutations in the p53 gene.^{14,15}

There are several methods for detecting molecular alterations in cancer including DNA sequencing, polymerase chain reaction (PCR), immunoblot, and immunohistochemistry (IHC). IHC can be applied to relatively small specimens. It is simple and fast and is consistent with other methodologies.^{15,16} Therefore, the IHC method has been widely used for searching for molecular markers of cancer, such as p53.

The aim of our study was to document the frequency and the relationships between immunohistochemical expressions of p53 with demographic and clinicopathologic findings, and with the location of stomach cancer.

Materials and Methods

One hundred and eleven gastric biopsy specimens taken by fiberoptic endoscopy were collected from patients with gastric adenocarcinoma. Twenty-five specimens were gathered from patients with cardia tumor and the remaining 86 specimens were gathered from antral tumor. The cases were identified from pathologic laboratory files. The patients underwent endoscopy in 5th Azar Medical Center in Gorgan (northeastern Iran) during 1998 – 2005. Adenocarcinoma of the cardia was defined as a tumor with a center located within 2 cm of the esophago-gastric junction.¹⁷ No patient had received chemotherapy or radiotherapy before

endoscopic biopsy. Clinicopathologic and demographic data (including age, gender, gastric location of the tumor, and histologic grade) were collected retrospectively in all patients, by reviewing the clinical charts and all histologic slides.

The tissue specimens were received in formaldehyde (10% solution). The samples that were paraffin wax embedded, were sectioned and stained serially with hematoxylin and eosin (H & E) for conventional histopathologic study. For IHC study, dewaxed 5 μ sections were stained with the primary antibody p53 (DAKO, Denmark) using a three step immunoperoxidase technique enhanced by microwave heating. Hematoxylin was used to counterstain the nuclei lightly. Anti-p53 antibody is a monoclonal mouse antiserum against p53 of human origin, which recognizes both wild and mutant type of p53. For negative controls we used the omitted primary antibody and for positive controls, we used a Barrett's adenocarcinoma with a positive immunostaining.

Results were reported positive when at least 20% of cells showed homogeneous and intense nuclear staining. All the slides were examined by two independent observers.

For statistical analysis we used χ^2 test, Fisher's exact test, and Kappa test. $P < 0.05$ was considered significant.

Results

Eighty-six specimens were collected from patients with antral adenocarcinoma and 25 specimens were collected from patients with cardia adenocarcinoma. The patients with cancer of antrum comprised 67 men and 19 women aged 22 – 89 years (median: 59) and those with cancer of the cardia comprised 19 men and 6 women aged 24 – 85 years (median: 65). The tumors of antrum comprised 75 cases of intestinal type, and 11 cases of the diffuse type in Lauren's classification.¹⁷

The same 86 tumors were examined by IHC. Twenty-seven (31.3%) of the adenocarcinomas stained positively with the human p53-specific monoclonal antibody (DAKO, Denmark). In these cases, the staining was exclusively nuclear and stromal cells were unstained. Fifty-nine cases had no p53 immunoreactivity. Omission of the primary antibody resulted in complete abolition of immunostaining. There was no significant difference in the clinicopathologic characteristics

of the antral tumors between p53-positive and p53-negative cases (Table 1).

The tumors of cardia comprised 25 cases, 18 cases of the intestinal and seven cases of the diffuse type in Lauren's classification. The same 25 tumors were examined by IHC. Fourteen (56%) of adenocarcinomas stained positively with the human p53-specific monoclonal antibody (DAKO). There was no significant difference in clinicopathologic characteristics of the cardia tumors between p53-positive and p53-negative cases.

The results did not demonstrate any significant association between p53 expression and age, sex, and histologic type in both locations (cardia and antrum) (Tables 1 and 2). But there was a relationship between p53 expression and location. The frequency of p53 gene expression (p53 protein immunoreactivity) was higher in adenocarcinomas of the cardia (14 of the 25) compared with adenocarcinomas of the antrum (27 of the 86) (Chi-square=5.03, P=0.025).

Discussion

The p53 gene has been indicated to be commonly mutated in various human cancers (including gastric cancer). Mutant p53 can act as a dominant oncogene. The presence of p53 protein overexpression is generally thought to indicate the presence of p53 gene mutation. The frequency and the pattern of p53 mutations might vary with geographic origin in gastric cancer, reflecting different etiologic agents. A number of studies have reported the frequency of p53 alterations in gastric cancer, studying either p53 protein

overexpression or p53 gene mutation.¹⁸⁻²⁰ Of the gastric adenocarcinomas, 18% to 58% have been found to have p53 mutation. P53 protein overexpression has been detected in 26 – 65% of tumors in the stomach.²¹⁻²⁶

We found p53 expression in 41 cases (36.9%) of all gastric cancer tissues. It is compatible with the above mentioned research. We found a relatively high frequency (56%, 14 of 25) of p53 protein overexpression in adenocarcinoma of the cardia. This frequency was higher than that observed in adenocarcinoma of the antrum (31.3%, 27 of 86). The difference was statistically significant.

The frequency of p53 alteration was compared with the location of the tumors in the stomach, in a few studies. Higher frequency of p53 protein expression in adenocarcinoma of the cardia than in the antrum has been reported in various studies.^{19,20,27} Therefore, adenocarcinoma of the cardia appears to show a higher frequency of p53 alteration compared with cancer of the lower part of the stomach. In another study comparing p53 alterations in a consecutive series of esophageal and cardiac adenocarcinoma, a high frequency of gene mutation (70% vs. 63%) and p53 protein production (70% vs. 56%) was observed in both groups of tumors.⁴

Other site specific differences including DNA aneuploidy and microsatellite instability have been reported with respect to the anatomical sites of tumors.²⁸⁻³⁰ Molecular similarities between cardia and esophageal adenocarcinoma are consistent with the hypothesis that these cancers share a common etiology, distinct from that responsible for the development of adenocarcinoma in the

Table 1. Status of p53 expression by gender, age groups, histological types, and gastric tumor location (numbers in the parenthesis are percent).

Factors	P53 expression		Total	χ^2	P value
	Positive	Negative			
Gender				0.79	0.373
	Male	34 (82.9)	53 (75.7)		
	Female	7 (21.1)	17 (24.3)		
Age groups				0.83	0.362
	<50 years	14 (34.2)	22 (31.4)		
	50 – 69 years	18 (43.8)	33 (47.2)		
	≥70 years	9 (22.0)	15 (21.4)		
Histological types				3.2	0.074
	Intestinal	31 (75.6)	62 (88.6)		
	Diffuse	10 (24.4)	8 (11.4)		
Tumor location				5.03	0.025
	Antrum	27 (65.9)	59 (84.3)		
	Cardia	14 (34.1)	11 (15.7)		
Total		41 (100)	70 (100)		
			111 (100)		

Table 2. Location of gastric tumors by gender, age groups, and histological types (numbers in the parenthesis are percent).

Factors	Tumor location			χ^2	P value
	Antrum	Cardia	Total		
Gender				0.04	0.84
	Male	67 (77.9)	19 (76.0)		
	Female	19 (22.1)	6 (24.0)		
Age groups				0.70	0.403
	<50 years	9 (33.7)	7 (28.0)		
	50 – 69 years	40 (46.5)	11 (44.0)		
	≥70 years	17 (19.8)	7 (28.0)		
Histological types				3.3	0.07
	Intestinal	75 (87.2)	18 (72.0)		
	Diffuse	11 (12.8)	7 (28.0)		
Total		86 (100)	25 (100)		

lower part of the stomach. It has been suggested that a comparison of the precise type of p53 mutation might indicate whether similar or distinct mechanisms of carcinogenesis are involved in different tumors.^{3,31}

We found no association between p53 alteration and age, gender, and histological grade. Similar results have been reported in most series of adenocarcinoma of the esophagus and stomach,^{20,27,32} which indicate an early involvement of p53 in the development of these cancers.^{33,34} Some authors reported a higher incidence of p53 alterations in gastric cancer of the intestinal type in comparison with the diffuse type.^{35,36} We were unable to confirm such a feature, maybe because of the limited number of cancers of the antrum showing p53 abnormalities.

In conclusion, our study revealed higher levels of p53 alterations in adenocarcinoma of the cardia compared with the antrum. This result reinforces the hypothesis that the cancers of the lower esophagus and upper stomach have distinct epidemiological, pathogenesis, and molecular characteristics from that observed in cancers of the lower part of the stomach.

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