Immunohistochemical Evidence of p53 Overexpression in Gastric Epithelial Dysplasia[1]

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Abstract

Molecular abnormalities of the *p53* gene in chromosome 17p may be among the most commonly observed in human cancer. Their role in gastric carcinogenesis is suggested by their frequent detection in invasive adenocarcinomas. To investigate the chronology with which these abnormalities appear in the gastric carcinogenesis process, the expression of p53 proteins was investigated in late stages of the process, namely dysplasia, and in superficial carcinomas. A polyclonal antibody, CM-1, against both wild-type and mutant proteins was applied to paraffin-embedded biopsy and gastrectomy specimens previously fixed in buffered formalin. Positive nuclear stain was obtained in 36.4% of 33 cases of gastric epithelial dysplasia, corresponding to 19% of mild, 27.3% of moderate, and 64.3% of severe dysplasias. Eight of 13 (61.5%) invasive carcinomas showed positive stain. The data indicate an increased incidence of *p53* abnormalities in the late stages of gastric carcinogenesis.

Introduction

Deletions and insertions in the short arm of chromosome 17 as well as point mutations of the *p53* gene have been reported frequently in human tumors, especially in the lung, breast, stomach, and colon (1–7). The nuclear proteins encoded by the *p53* gene are suspected to play a regulatory role in the cell cycle. The normal protein has a short half-life, which makes it invisible in normal tissues by the usual immunohistochemical stains. Overexpression of the wild-type protein may occur abnormally in certain tumors (8). The proteins expressed by mutant *p53* genes are more stable and more easily detected by immunochemical methods (9–11). Polyclonal antibody (CM-1) has been developed to detect both wild-type and mutant p53 proteins utilizing pure soluble recombinant human p53. The CM-1 antibody is able to detect protein in archived formalin-fixed and paraffin-embedded human tissues (12). Positivity of p53 protein by immunochemical methods has been reported in invasive gastric carcinomas and has been correlated with prognosis (13).

Our interest has been in the precancerous gastric process, characterized by steps identifiable in tissue sections stained with hematoxylin and eosin. These steps involve atrophy, intestinal metaplasia, and dysplasia of the gastric mucosa (14). Archival material displaying several degrees of dysplasia as well as (predominantly superficial) invasive carcinomas was stained with CM-1 polyclonal antibody to p53 proteins in order to investigate the timing of the abnormal expression of p53 proteins.

Materials and Methods

From a series of Italian patients enrolled in a prospective study of GED,[3] 24 subjects with a histological diagnosis of GED were selected. Each patient underwent multiple biopsies according to a protocol described in a previous study (15). In two of these patients, a gastrectomy was also performed because of the occurrence of GC. In addition, 9 cases of GED detected in stomachs resected for gastric cancer (8 cases) or for severe dysplasia (1 case) were also studied with extensive sampling of dysplastic and carcinomatous areas. All gastric biopsies as well as the surgical specimens were immediately fixed in buffered formalin (12 h for biopsy specimens, 24 h for resected stomachs). Five-μm-thick sections were stained with hematoxylin and eosin. Serial sections were used in the immunohistochemistry study. They were dewaxed in xylene (twice for 5 min each) and rehydrated through serial alcohols (100%, 95%; two changes of 3 min each) to distilled water (twice for 5 min each). Polyclonal antibody against both wild-type and mutant p53 protein (CM-1; Signet Laboratories, Inc., MA) (12) were used at a working concentration of 1:25 at room temperature for 20 min. Hydrogen peroxide, normal goat blocking serum, biotinylated immunoglobulins, avidin-biotin complex, and 3-amino-9-ethylcarbazole substrate solution were used according to the instruction from a commercial kit (Signet ELITE avidin-biotin detection system; Signet Laboratories, Inc.). Sections received a light Mayer's hematoxylin counterstain, were mounted with aqueous mounting medium (Crystal/Mount; Biomeda, CA), and were postmounted with Postmounting Crystal/Mount in Permount (Biomeda). Positive control sections from for-

Received 4/22/92.
[1] Performed under the auspices of the R. Farini Foundation for Gastroenterological Research and NIH Grant P01-CA28842.
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[3] The abbreviations used are: GED, gastric epithelial dysplasia; GC, gastric carcinoma.

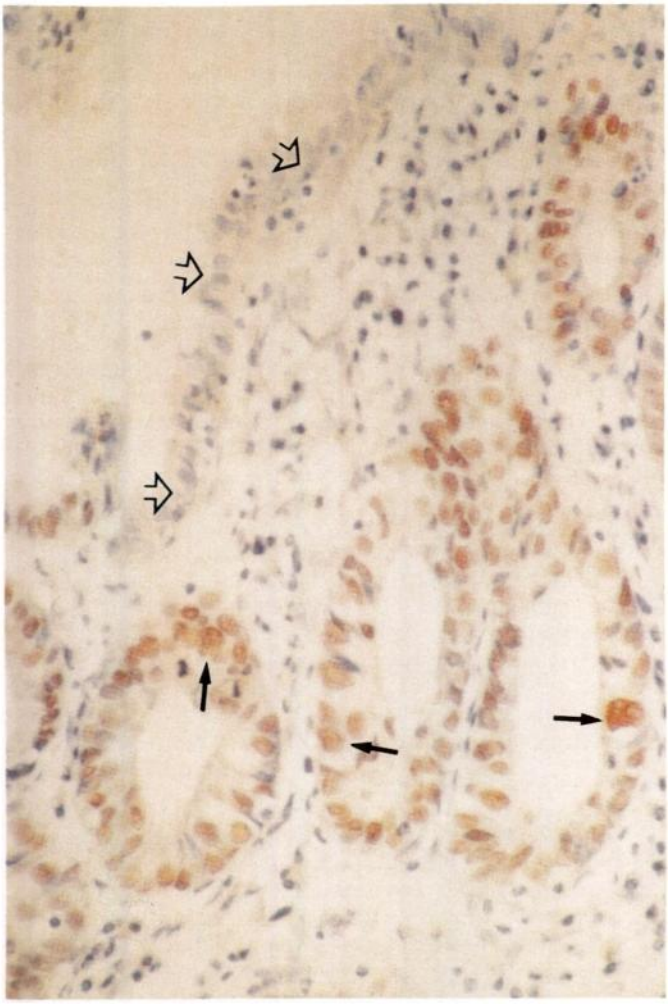

Fig. 1. Gastric biopsy showing moderate dysplasia, stained with antibody to p53 protein (CM-1). The *solid arrows* point to positively stained nuclei in the dysplastic glands. The *open arrows* point to the surface epithelium, negative for CM-1 antibody.

malin-fixed paraffin-embedded colonic cancers were cut and prepared in the same manner as the specimens. In serial sections of the same tissue, primary antibody was omitted as the negative control. Each histological sample was examined for the presence of areas of mild (G1), moderate (G2), and severe (G3) GED (16) and gastric cancer following previously published criteria (15). In the resected stomachs, multiple specimens were available from normal, dysplastic, and neoplastic areas. The stage of each neoplasia was defined according to the classification system of the International Union Against Cancer (17), and early (stage I) and advanced (stages II, III, and IV) cancers were analyzed separately. In each lesion the nuclear immunoreactivity for p53 protein was independently scored by two investigators. When more than one histological lesion was present in the same specimen (coexistence of G1/2/3 and/or GC), the p53 immunoreactivity was stated independently for each. Nuclear reactivity for p53 protein was estimated to distinguish whether there was more than or less than 25% involvement of the nuclei in the dysplastic or neoplastic field. Regardless of the percentage of involvement of positive nuclei, two different patterns of immunoreactivity were distinguished: the presence of multiple, isolated immunoreactive nuclei was described as "scattered," while the pattern was described as "clustered" when the nuclear reactivity was detected in glands close to each other.

Table 1 Anti-p53 reactivity in GED and gastric cancer

Morphology	Biopsy		Gastrectomy	
	No. cases	Positive p53 (%)	No. cases	Positive p53 (%)
GED				
G1	15	1 (6.70)	6	3 (50.0)
G2	7	0 (0.00)	4	3 (75.0)
G3	8	4 (50.00)	7	6 (85.7)
EGC[a]			8	5 (62.5)
AGC			2	1 (50.0)
Cancer[b]	3	2 (66.7)		

[a] EGC, superficial "early" gastric cancer; AGC, advanced gastric cancer.
[b] Superficial (early) or advanced cancer cannot be classified by biopsy only.

Results

Nuclear p53-positive reaction was found in 12 of 33 cases (36.4%) of GED (Fig. 1). No positive immunostaining was detected in normal gastric epithelium (repre-

Table 2 Semiquantitative score of p53 reactivity in GED and gastric cancer

Morphology	<25% nuclei		>25% nuclei	
	Clustered	Scattered	Clustered	Scattered
Biopsy				
GED				
G1		1		
G2				
G3	1	2	1	
Cancer[a]	2			
Gastrectomy				
GED				
G1	1		2	
G2		1	2	
G3			6	
EGC[b]			5	
AGC			1	

[a] Superficial (early) or advanced cancer cannot be classifed by biopsy only.
[b] EGC, superficial (early) gastric cancer; AGC, advanced gastric cancer.

sented in all cases studied) or in stromal cells. In biopsy samples as well as in the surgical specimens of each patient, more than one histological lesion was present because of the coexistence of various grades of GED and/or GC. The histological lesions detected in biopsies and surgical specimens are shown in Table 1, in which the percentage of p53 protein-positive foci is also reported. When biopsy and surgical specimens were considered together, the percentage of positive lesions in the combined groups was 19.0% for GED-G1, 27.3% for GED-G2, 64.3% for GED-G3, and 61.5% for the carcinomatous lesions. Table 2 illustrates the results of the semiquantitative score of the positive nuclei; in addition, the pattern of the nuclear positivity, described as clustered and scattered, is also shown. A clustered pattern was found more frequently both in biopsy and in surgical specimens. In the surgical samples, the dysplastic lesions surrounding infiltrating carcinoma showed the highest number of reactive nuclei.

Discussion

The present study is the first to report positive nuclear staining for p53 protein in gastric epithelial dysplasia. Twelve of the 33 (36.4%) patients with GED (with or without GC) were positive, suggesting a relationship between the gastric precancerous lesions and p53 protein overexpression. In the field of the gastrointestinal precancerous lesions, colonic dysplasia has been tested. In one study of 10 tubulovillous adenomas, no p53 overexpression was detected by immunohistochemistry (7). In a larger series, Purdie *et al.* (11) detected mutant p53 protein in 4 of 46 (8.7%) colonic adenomas, and positive nuclei were found most frequently "restricted" to a few glands. In the present study various morphological lesions were detected in the same patient, and the p53 reactivity was evaluated in each of them. Five of 30 (16.7%) foci of GED, detected in biopsies from 24 patients, showed positive nuclei (Table 1). On the other hand, a positive reaction was present in 12 of 17 (70.5%) foci of GED in 11 stomachs resected for GC or severe dysplasia (Table 1). In addition, in all the surgical specimens, a clustered immunoreaction in more than 25% of the nuclei was present, and a similar pattern was detected in the surrounding carcinomatous tissue (Table 2). Although it is not possible to rule out that the higher percentage of immunoreactive lesions detected in association with GC was the result of the extensive sampling in the surgical specimens, it would appear that the higher percentage of p53-positive GED detected in gastrectomy is related to a more advanced stage of the neoplastic disease. This is supported by the finding of a greater proportion of G3 in gastrectomy specimens (41%) than in biopsy samples (27%).

Recent reports have pointed to frequent alterations of the *p53* gene and its products in gastric carcinoma (18). Overexpression of the p53 proteins detected with immunohistochemical methods is more frequent in advanced tumors, suggesting some relationship with prognosis (13). Our studies detect increasing prevalence of such abnormalities in the late stages of the precancerous process (dysplasia). These two studies suggest an incremental change in p53 protein abnormalities throughout the late precancerous and the cancerous process. Since the p53 protein is suspected to play a role in the regulation of the cell cycle, it may be suggested that in the gastric precancerous and cancerous processes such abnormalities result in a failure to block the progression of cell division (19).

Overexpression of p53 protein in lung cancer has been linked to mutations primarily within exons 5–8 (20). Our results may indicate that in gastric cancer the p53 protein abnormalities detected by immunochemistry with CM-1 polyclonal antibody represent lesions in specific regions of chromosome 17. It is hoped that future studies including DNA sequence analysis will clarify the molecular mechanisms of the gastric carcinogenesis process.

Acknowledgments

We wish to thank Dr. I. Meli and Dr. E. Venza from the Interdisciplinary Group of Gastric Epithelial Dysplasia, who provided the histological material of two of the cases in the present study.

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Cancer Epidemiol Biomarkers Prev 1992;1:551-554.