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Diagnostic p53 Expression in Gastric Endoscopic Mucosal Resection

Endoscopic mucosal resection (EMR) has been standardized for the treatment of intestinal type of intramucosal gastric carcinomas, and careful histological examination of the resected specimen is important for further treatment. To evaluate the diagnostic utility of p53 expression in gastric EMR samples, using immunohistochemical staining, we examined 24 gastric carcinomas (22 intestinal types and two diffuse types) and 20 adenomas removed by EMR. Intestinal type of adenocarcinomas revealed strong p53 expression in 13 cases (59%), weak in four cases (18%), and negative in five cases (23%). Resection margins of 11 carcinomas were involved in the carcinoma cells, which showed the same p53 expression pattern with main carcinoma cells. Squeezed carcinoma cells, remaining in resection margins, were definitely identified by strong p53 expression in seven cases of which the main tumor strongly expressed p53. Microscopic in situ carcinoma could be easily detected in p53 immunostaining. Multifocal involvement and submucosal invasion of carcinomas could be demarcated easily and definitely by strong p53 expression of carcinoma cells. All adenomas showed diffuse weak p53 expression. The difference of p53 expression ($p<0.001$) could be used as a differential diagnosis between adenomas and carcinomas. According to these results, we propose that for careful histological examination in hospital diagnosis, both histological evaluation and p53 immunostaining are important diagnostic parameters in EMR samples of the intestinal type of gastric carcinomas.

Key Words: *Protein p53; Immunohistochemistry; Surgical procedure, endoscopic, mucosal resection; Adenoma, gastric; Early gastric carcinoma*

Jeong-Hee Cho, Im Hwan Roe*, Young Joo Jin*

Departments of Pathology and Internal Medicine*, College of Medicine, Dankook University, Chunan, Korea

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Address for correspondence
Jeong-Hee Cho, M.D.
Department of Pathology, College of Medicine, Dankook University, 29 Anseo-dong, Chunan, Chungnam 330-715, Korea
Tel: +82.417-550-3896, Fax: +82.417-561-9127
E-mail: jhcho@anseo.dankook.ac.kr
http://www.anseo.dankook.ac.kr/~jhcho

INTRODUCTION

Endoscopic mucosal resection (EMR) has been standardized for the treatment of intestinal type intramucosal adenocarcinoma of a superficial elevated lesion (diameter <2 cm) and a superficial depressed lesion (diameter <1 cm) without any ulcer or scar inside (1-4). Careful histological examination of the resected specimen is important in repeat EMR, laser therapy, and surgical gastrectomy in case of incomplete resection. In EMR specimen of the stomach, however, pathologic diagnosis of carcinoma is occasionally difficult because of the following: (a) Intestinal type of early carcinomas is frequently associated with adjacent mucosal changes (dysplasia, adenoma, or regenerative changes). (b) In cases of microscopic in situ carcinomas, chances of skipping over the lesion increase. (c) Due to the squeezed carcinoma cells by cauterization, it is also not easy to determine the involvement of resection margins by the cancer cells. (d) Microscopic stromal invasion is not easy to distinguish from pseudoinvasion. Therefore, the definite pathologic diagnosis of these early gastric cancers (EGCs) in EMR samples needs a diagnostic marker for carcinoma cells, which represents morphological and genetic changes.

Compared to other oncogenes or tumor suppressor genes found, only *p53* tumor suppressor gene shows that gene mutation produces increased protein expression, which can be detected by immunohistochemical staining. Mutant and wild-type p53 proteins are found in oligomeric protein complexes in genetically altered cancer cells (5, 6). The stability of mutant p53 proteins is much greater than that of wild-type p53 proteins. Wild-type p53 protein has a half-life in cells of only 20 min (7), whereas all mutant p53 alleles produce proteins with half-lives of hours. This results in much higher concentrations of p53 proteins in tumor tissue (8).

Although heterogeneous p53 expression was present, a previous study demonstrated that the pattern of intra-

tumoral p53 mutation and expression was the same in over 80% of intestinal type of advanced gastric cancer (AGC) (9). Mutation of *p53* gene was found in less than 5% of precancerous lesions but detected in over 40 to 50% of carcinomas (10). These genetic changes were manifested by either weak p53 expression in precancerous lesions or strong p53 expression in over 50% of intestinal type of gastric carcinomas (10, 11). On the other hand, intratumoral expression of p53 mediators such as p21 and bcl2 showed no correlation between morphological changes and protein expression in gastric neoplasm (10). These results suggest that intestinal type of small or microscopic early carcinomas might have the same p53 gene mutation, which is manifested as the same pattern of p53 expression in immunohistochemical staining. Therefore, to evaluate the diagnostic utility of p53 expression in gastric EMR samples, using immunohistochemical staining, we examined 24 gastric carcinomas and 20 adenomas removed by EMR.

MATERIALS AND METHODS

Twenty-four early gastric carcinomas, which were resected by EMR from 1995 to 1998 at Dankook University Hospital, were examined. Early gastric carcinomas included 22 intestinal types and two diffuse types of carcinomas and were less than 2 cm in size. Tumor infiltration was in mucosa (18 cases), or in submucosa (two cases), or in situ (four cases). Synchronous presence of adenoma was noted in seven of 22 patients. Positive resection margin was found in 11 cases at the first EMR. Atrophic gastritis and intestinal metaplasia were present, adjacent to both type of all carcinomas. For the comparative analysis, 20 tubular adenomas resected by EMR were examined. These included low-grade (mild and moderate dysplasia, 12 cases) and high-grade dysplasia (severe dysplasia, eight cases). All had a single lesion and less than 1 cm in size.

The first 4 μm were stained by Hematoxylin-Eosin and the next section was immunostained. Deep and lateral margins were marked by black ink. Histological evaluation with H&E staining was performed first and correlation with immunostaining followed. Normal mucosa adjacent main lesions was used as a negative control.

The immunoperoxidase method, using the avidin-biotinylated horseradish peroxidase complex (Dako LSAB kit, Los Angeles, CA, U.S.A.), was carried out with formalin-fixed paraffin-embedded tissue sections. The microwave antigen retrieval procedure in 0.01 M sodium citrate buffer (pH 6.0) was performed. Anti-p53 monoclonal antibody (mab) (DO-7, Novocastra, Inc, Manhasset, NY, U.S.A.), which detected both wild and mutant p53 recognizes a amino-terminal residues 19-26 of p53 protein. Staining was done by immersing slides in diaminobenzidine as chromogen and was counterstained in hematoxylin. Positive tumor cells were quantified twice, expressed as intensity and proportion of the positive tumor cells, and assigned to one of four categories: −, negative; +, weak and focal (<50%) or diffuse (>50%); ++, moderate and focal or diffuse; +++, strong and diffuse.

A colonic cancers which confirmed the presence of p53 mutation with a high level of nuclear p53 immunoreactivity, was used as a positive control for this protein (+++). A negative control was processed with each slide, excluding the primary antibody but including all other steps in the procedure. The difference of p53 expression between intestinal type of carcinomas and adenomas was analyzed by Fisher's exact test. Statistical significance was defined as $p<0.001$.

RESULT

Intestinal type of adenocarcinomas revealed strong p53 expression in 13 cases (59%), weak in four cases (18%), and negative in five cases (23%) (Table 1). Two diffuse

Table 1. p53 Expression in early gastric cancers resected by EMR

Gross	Histologic type	p53 expression			RM+
		Ca	TA	Ca in RM	
EGC (IS)	I	+++			−
EGC (SM)	I	+++		+++	+
EGC (M)	I	+++	+	+++	+
EGC (M)	I	+++		+++	+
EGC (M)	I	+++		+++	+
EGC (M)	I	+++			−
EGC (M)	I	+++		+++	+
EGC (M)	I	+++			−
EGC (IS)	I	+++	+		−
EGC (M)	I	+++		+++	+
EGC (M)	I	+++	+	+++	+
EGC (IS)	I	+++			−
EGC (IS)	I	+++	+		−
EGC (M)	I	++			−
EGC (M)	I	++	+		−
EGC (SM)	I	+			−
EGC (M)	I	+			−
EGC (M)	I	−			−
EGC (M)	I	−	+	−	+
EGC (M)	I	−			−
EGC (M)	I	−	+	−	+
EGC (M)	I	−		−	+
EGC (M)	D	−			−
EGC (M)	D	+		+	+

EGC, early gastric carcinoma; M, mucosa; SM, submucosa; IS, in situ; I, intestinal type; D, diffuse type; Ca, carcinoma; TA, concurrent tubular adenoma; RM+, positive involvement of resection margin

types of carcinomas showed negative or weak p53 expression (Table 1). Compared to the intestinal type of carcinomas, p53 expression in all 27 tubular adenomas was diffusely weak both in concurrent adenomas (seven cases) and in adenomas resected by EMR (20 cases) ($p<0.001$) (Table 2, Fig. 1AB). There was no difference in p53 expression according to grade of dysplasia. A few cells, showing strong p53 expression, were scattered in a few adenomas. Adjacent atrophic gastritis and intestinal metaplasia also showed weak p53 expression, mainly in

Table 2. Comparison of p53 expression between intestinal type of carcinomas and adenomas

p53 expression+	Carcinoma (I)	Tubular adenoma
-	5	0
+-++	4	27*
+++	13	0
Total	22	27

* Cases of both concurrent adenomas (7) and adenomas resected by EMR (20); I, intestinal type

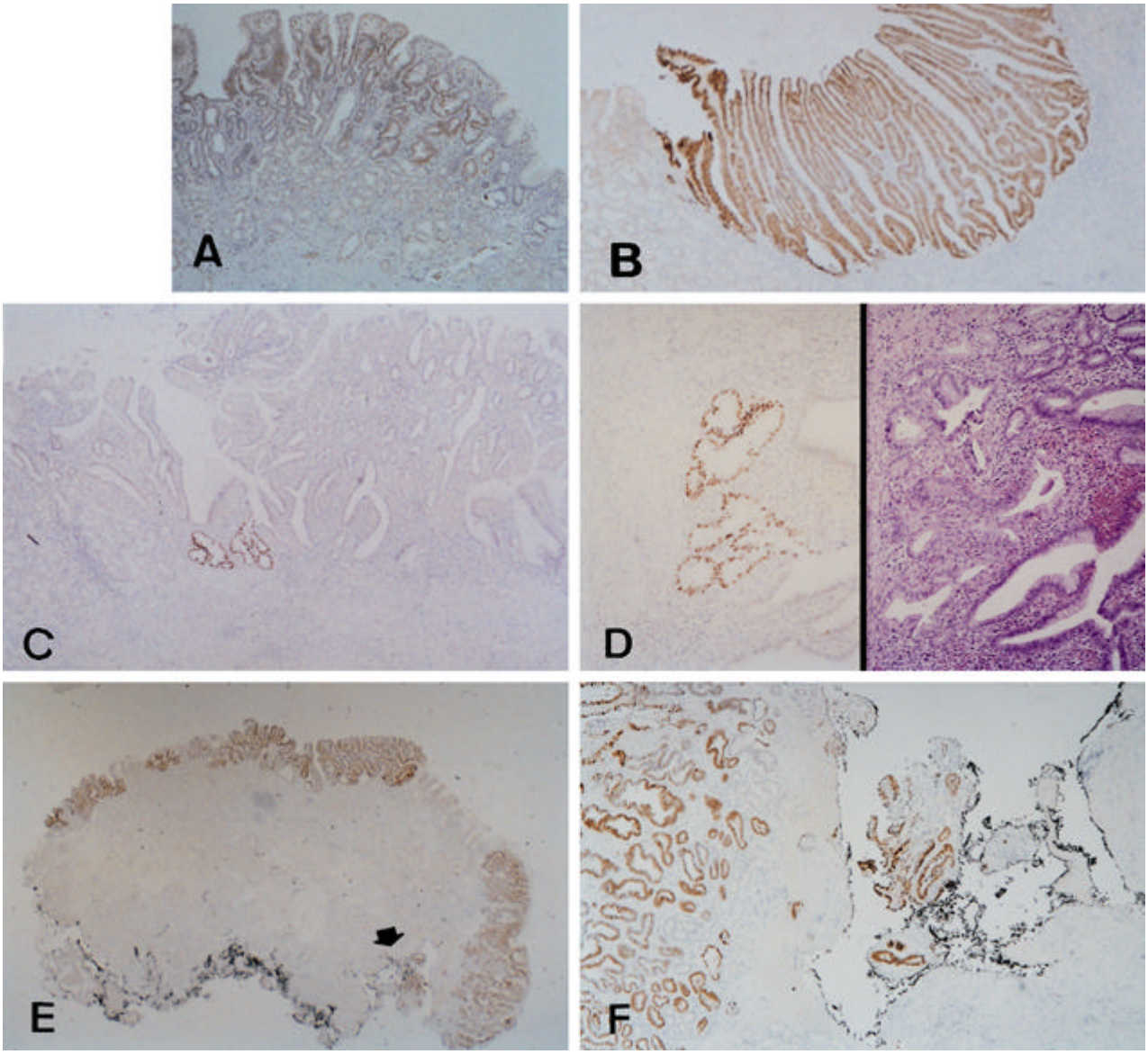

Fig. 1. A) p53 expression was diffusely weak in adenomatous epithelial cells compared to adjacent normal mucosa (ABC staining, ×40). B) Intestinal type of early gastric carcinoma revealed diffuse and strong p53 expression, which was delineated from adjacent normal mucosa (ABC staining, ×100). C-D) In situ carcinoma could be detected easily by strong p53 expression in a few carcinoma glands (ABC and H&E staining, ×10 and ×400). E-F) In the intestinal type of early gastric carcinoma, strong p53 expression in the multiple main carcinomas was the same as in the carcinoma cells involving resection margins (arrow, F) (ABC and H&E staining, ×4 and ×400).

the regenerative region. Microscopic in situ carcinoma, which showed strong p53 expression could be easily detected in p53 immunostaining (Fig. 1CD). Resection margins of 11 cases were involved in the carcinoma cells, which showed the same pattern of p53 expression with the main carcinoma cells (Table 1). Although carcinoma cells were squeezed by cauterization, they remained in the resection margins which were definitely identified by strong p53 expression in seven cases. The main tumor of those cases also expressed p53 strongly (Fig. 1E-G). Multifocal involvement and submucosal invasion of carcinomas could be demarcated easily and definitely by strong p53 expression of carcinoma cells.

DISCUSSION

The inactivation of p53 gene is considered to play a role in the early stage of gastric carcinogenesis, especially in intestinal type of gastric carcinoma (10-13). Mutation of *p53* gene has been reported in less than 5% of precancerous lesions (adenoma or dysplasia) but detected in 40-60% of intestinal type of advanced gastric carcinomas as well as in 20% of early gastric carcinomas (10-12). Stability of mutant p53 proteins results in much higher concentrations of p53 proteins in tumor tissue, which can be detected by immunostaining (5, 6). Our previous data showed that the p53 mutation occurred in the process of transformation from adenoma to intestinal type carcinoma during gastric carcinogenesis. We proposed that strong p53 expression in intestinal type of carcinomas could be used as a differential diagnosis between adenoma or dysplasia and early carcinoma (10).

The study not only supported but also extended the diagnostic meaning of p53 expression in gastric EMR samples because of the following: (a) It is technically less cumbersome and more widely available than genetic assays, and immunostaining of the p53 protein provides an opportunity to correlate specific morphological and genetic changes in over 50% of intestinal types of gastric carcinomas. (b) The trend for adenoma, dysplasia or regenerative cells to express p53 weakly (8-10, 14), and for in situ or early carcinomas to express p53 strongly, suggests that p53 immunostaining can be a useful diagnostic marker for differential diagnosis between precancerous lesions (adenoma or dysplasia or regnerative atypia) and early carcinomas. (c) The data highlight the hypothesis that intratumoral p53 gene mutations in intestinal type of small and early gastric carcinomas are the same because the clonal heterogeneity of carcinoma cells has still not progressed, compared to large and advanced cancers (9, 15, 16). Therefore, carcinoma cells separated from the main mass have the same pattern of *p53* gene mutation, which is manifested by the same pattern of p53 expression. EMR is an indication of only intestinal type of small intramucosal gastric carcinoma. Carcinoma cells spreading to either resection margins or stroma can be detected clearly by the same pattern of p53 expression.

The data, however, raises the question as to whether only one positive p53 immunostaining represents the carcinoma change and is if the best marker for gastric cancer. In our previous report, we examined p53 and its mediators, p21 and bcl2, in precancerous lesions and carcinomas of the stomach, using immunostaining and molecular methods. Expression of p53 showed histological correlation between carcinoma changes and protein expression in over 50% of intestinal type carcinomas, but its mediators had no correlation (10). Therefore, we think that a single evaluation of p53 expression can represent carcinoma changes in intestinal type of gastric carcinomas strongly expressing p53.

Immunohistochemistry of p53 also has some disadvantages (9, 17): (a) All *p53* gene mutations do not appear as p53 overexpression by immunohistochemistry; nonsense mutation of *p53* gene does not produce expressions of p53 protein. The reason for this is still unclear and may considered as hiding an epitope by shortening and structural disconformation of mutant protein (8, 9). In this data, 23% of intestinal types of carcinoma and one diffuse type showed no p53 expression. (b) The interpretation of weak expression of p53, admixed with carcinoma cells strongly expressing p53, is in dilemma because these cases either have p53 mutation or not. In this study as well as in other studies (8-10), 20% of intestinal types of gastric carcinomas showed weak protein expression. That is, without genetic study, weak p53 expression does not reflect the presence of *p53* gene mutation in these carcinomas and therefore can not be used as a diagnostic marker.

Obviously, both immunohistochemical and genetic evaluation of p53 are ideal approaches to determine carcinoma changes. However, immunohistochemistry of p53 protein provides an effective and convenient means to determine carcinoma changes in intestinal type of gastric cancers, which are removed by EMR and express p53 strongly. Therefore, we propose, for the careful histological examination in hospital diagnosis, both histological evaluation and p53 immunostaining are important diagnostic parameters in EMR samples of the intestinal type of gastric carcinomas.

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