THE POSSIBLE ASSOCIATION BETWEEN EXPRESSION OF p53 AND DEVELOPMENT IN DEPRESSED TYPE COLORECTAL CARCINOMA

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We evaluated the possible association between the morphometrical findings of the microtumors with a diameter of less than 5 mm and the expression of p53, using 61 depressed epithelial minute neoplasias of large bowel. The nucleus/cytoplasm area ratio was associated with the frequency of positive expression of p53 in these tumors.

Also we examined the expression of p53 in early and advanced carcinomas of large bowel. Although 6 lesions of the 12 minute adenocarcinomas showed positive reaction for p53, the positive-stained carcinoma cells could be recognized only in a part of the lesion. On the other hand, in early (except minute carcinoma) and/or advanced carcinomas lesions 100% of adenocarcinoma cells showed positive for p53.

These findings suggest that the expression of p53 in the colorectal neoplasia have a relationship to both the morphological atypia of these lesion and the development of large bowel carcinoma.

Although carcinoma in their early stage and adenoma of large bowel are said to form polyp type, the depressed type of the early carcinoma of large bowel was sometimes encountered (7). Such type of lesions has been rather frequently encountered in Japan (8, 9) (Fig. 1).

Recent studies made clear various kinds of genes have been expressed in the development of large bowel carcinoma (12, 18). Among these genes, phosphoprotein p53 (10, 11), a cancer suppressor gene (4), has been frequently confirmed in large bowel cancer by immunohistochemical staining but not in adenoma (3).

This study demonstrated p53 immunohistochemically in various sizes of the depressed type of large bowel carcinomas, suggesting the possible association between the expression of p53 and the development in these tumors.

MATERIALS AND METHODS

61 samples were obtained from depressed epithelial minute neoplasias in large bowel of 61 patients, the diameter of which was less than 5 mm after endoscopic mucosal resection and 15 samples of depressed type of moderately and well differentiated adenocarcinomas in large bowel were also obtained from 15 patients after surgery or endoscopic mucosal resection between May of 1990 and March of 1992. The size of these lesions was between 6 and 20 mm. Among them 9 samples were found to be early cancers, 6 cases invaded into the submucosal layer and 6 cases were advanced cancer.

The depressed lesions in this study were of IIc, IIa+Iic, Iic+Iia (superficially depressed early cancer), and Borrmann 2 and 3 (depressed advanced cancer) according to the Japanese Research Society for Cancer of Colon and Rectum (6).

All these materials were fixed in 10% formalin solution for 1 day. The minute tumors were cut 1 ~ 1.5 mm, and the other tumors were cut as large as possible. Each section was embedded in paraffin and stained with hematoxylin-eosin and anti-p53 oncoprotein (CM1, rabbit polyclonal antibody for p53, Novocastra laboratories) immunohistochemically by the avidine-biotin peroxidase complex method (CM1 at a dilution of 1 : 500, for 1 hr at room temperature).
Expression of p53 was classified into 4 groups when the nucleus of tumor cell was definitely stained (G0: negative, G1: positive only in a part of the lesion as shown in Fig. 2, G3: positive in almost the whole area of the lesion as shown in Fig. 3, G2: intermediate pattern between G1 and G3).

As to minute neoplasias, the nucleus/cytoplasm area ratio (N/C, %) and the ratio of the area occupied by tumor tubules (T/A, %) were counted by the method of Ishido et al. (5) using an image analyzer (IBAS, Zeiss). Finally, the microscopic views (enlargement ×20) with hematoxylin-eosin staining were input to the color image analyzer. The outline of all tumor tubuli in the TV camera were drawn by hand on the image analyzer and calculating percentages of tumor tubules occupying a unit area (T/A, %). The lumen line of all tumor tubuli in the TV camera were also drawn by hand, and as tumor nuclei

Fig. 1. One of the depressed type micro-neoplasias of large bowel, obtained the endoscopic mucosal resection. Hematoxylin-eosin staining. ×20

Fig. 2. Adenocarcinomas, limited to the lamina propria of the mucosa, showed positive in only a part of the lesion for p53 immunohistochemically (G1, Arrows). Anti-p53 oncoprotein, counter stain with hematoxylin. ×400
were brightened in image processing and the ratio of the total area of the nucleus to the total area of the cytoplasm of the tumor tubuli \((N/C, \%)\) was calculated. With this equation, using both N/C and T/A, we diagnosed 61 depressed minute neoplasias as 12 cases of early carcinomas (well differentiated adenocarcinomas), including 2 cases invading the submucosal layer and as tubular adenomas following the criteria by Nakamura (15) in 49 cases.

RESULTS (TABLE 1)

1. Expression of p53 in carcinomas in relation to size and depth of carcinomas

Of the 12 minute adenocarcinomas, 6 lesions showed positive reaction for p53 immunohistochemically, all of which belongs to G1. On the other hand 6 lesions were found to be negative. All lesions of the 9 early carcinomas, other than minute carcinomas, showed positive (2 lesions: G1, 3 lesions: G2, 4 lesions: G3).

Among these 21 early carcinomas including 12 minute adenocarcinomas, 53.9\% (7 of 13 cases) of the
Wada et al.

2. Expression of p53 in minute neoplasias and morphometric findings

All minute tumors exhibited a positive reaction for p53 in G1 level. The N/C ratio was higher in the positive tumors in p53 than that in the negative tumors without a statistical significance [G1: 56.5±12.8%, G0: 38.4±13.1%; t-test, p < 0.1]. The T/A ratio was not associated with the presence or absence of the expression of p53 [G1: 78.9±7.3%, G0: 71.4±9.4%].

3. Expression of p53 in minute depressed tubular adenomas

All lesions of the 49 depressed tubular adenomas were found to be negative for p53 immunohistochemically.

**DISCUSSION**

Phosphoprotein p53 has attracted attention as a cancer suppressor gene (4). P53 is located on the short arm of chromosome 17 and is mutated in various types of cancers (1, 2, 14, 16), Baker et al. (1) have suggested that the mutation of p53 is a late event in colorectal carcinogenesis. Rodrigues et al. (16) showed a good correlation between the immunohistochemical localization of the nuclear p53 and the gene mutation of p53. This study on this particular gene showed its positive expression in many early and advanced carcinomas of large bowel and negative expression in adenomas. Our results were similar to the description of p53 expression of several authors (3). However, p53 expression in this study has a relationship to a degree of morphological atypia and the development of large bowel carcinoma.

In this study, all minute carcinomas which are positive for p53 showed only G1 (positive only in a part of the lesion). Many other early and advanced carcinomas showed a higher positive grade. The early carcinomas with G2 and G3 in the expression of p53 had a diameter of more than 5 mm or the carcinomas with invasion to the submucosa. These findings suggest that the expression of p53 has a relationship to the development of large bowel carcinoma.

Now there are two theories concerning the development of large bowel cancer: the adenoma-carcinoma sequence theory (13), in which the development of this cancer is mediated by adenoma, and the *de novo* theory (17), in which it directly develops from the normal mucosa. Though the former has been more widely supported, the latter has recently been considered to be also important in Japan. The predominant mode of the development of large bowel cancer may depend on whether the intramucosal microtumors with severe histological atypia are diagnosed as malignant or benign. Usually in the Atlantic criteria (13) the intramucosal neoplasias of large bowel, even if these lesion showed severe histological atypia as well as the adenocarcinoma with invasion to the deeper zone than the lamina propria of mucosa or with metastasis to other organs, are diagnosed as adenoma. On the other hand, many tumors of them are diagnosed as adenocarcinoma by the criteria of Nakamura (15) using the morphometrical analysis, which was used in this study. We evaluated the possible association between the morphometrical findings of these microtumors and the expression of p53. Therefore, in this study, many lesions of tubular adenomas with severe dysplasia, as classified by the Atlantic criteria, were diagnosed as adenocarcinoma. In this study, the N/C ratio was associated with the expression of p53 in minute depressed type neoplasias of large bowel. These findings suggest that the morphological atypia, determined as a result of many previous studies, reflects gene abnormalities of the tumor to some degrees.

Today, the depressed early carcinomas of large bowel, used in this study were not rare findings (8, 9). At what stage, should the neoplastic epithelium be
regarded as carcinoma? After submucosal infiltration of the tumor, or after destruction of blood vessels by the tumor? Even tumors limited to the lamina propria of the mucosa should be regarded as carcinomas when they have specific gene abnormalities similar to those of carcinoma showing distant metastasis.

We consider that the further genetic studies, according to the morphologic atypia of the large bowel neoplasia, will strengthen the diagnosis and the therapy for the large bowel carcinoma.

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REFERENCES


p53 in Depressed Colorectal Carcinoma