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Original Paper

p53 Immunoreactive Stain and Early Colorectal Adenocarcinomas

S. Hirota,[1] S. Kudo,[1] S. Hosobe,[2] T. Kobayashi,[1] M. Himori[1], M. Ikeda,[1] Y. Takemoto,[1] M. Nomoto,[3] Y. Aoyagi[3] and H. Asakura[3]

[1]Department of Gastroenterology, Akita Red Cross Hospital, 1-4-36 Nakadouri, Akita City; [2]Department of Pathology, Akita Red Cross Hospital; and [3]Department of Third Internal Medicine, Niigata University, Japan

565 cases of early colorectal adenocarcinomas were used in this study to examine mechanisms of carcinogenesis. Specimens were paraffin embedded and histological sections were stained with haematoxylin–eosin and p53. Macroscopically, early colorectal adenocarcinomas could be classified into two types: protruding and depressed. The former were composed of branching glands, while the latter were composed of straight glands which opened to the surface. The p53 positive ratio was similar for protruding tumours but was higher in depressed submucosal invasive adenocarcinomas than in depressed intramucosal adenocarcinomas. These results raise the possibility of at least two pathways for colorectal carcinogenesis, adenoma–carcinoma lesions and *de novo* carcinoma lesions.

Key words: p53, colorectum, early adenocarcinoma
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INTRODUCTION

BETWEEN 1987 and 1993, 14 023 patients were investigated by colonoscopy at Akita Red Cross Hospital. Among them, special attention was paid to early adenocarcinomas, which could be divided into two morphological types: protruding and depressed. In this study, we examined the histopathological and immunohistochemical differences between the two types in order to determine whether there exist different pathways of carcinogenesis in colorectal cancer.

PATIENTS AND METHODS

Of the 14 023 cases, 565 were diagnosed as early colorectal adenocarcinomas and used in this study. These were divided morphologically into two types: protruding and depressed (Figure 1). Specimens were fixed in 20% formaldehyde solutions and embedded in paraffin wax. Each specimen was cut in half: one half was cut into 3 μm sections on the vertical axis, and the other half into 3 μm sections on the horizontal axis, from mucosal surface to muscularis mucosae to analyse the structure of glands opening on to the surface. They were stained with haematoxylin–eosin (H&E) and p53 immunoreactive stain (p53) (DAKO-p53, DO-7, Copenhagen, Denmark). 454 cases (334 male; 120 female, aged 33–83 years) were diagnosed as intramucosal adenocarcinoma; 111 cases (80 male, 31 female cases, aged 41–85 years) were diagnosed as submucosal invasive adenocarcinoma. Histological diagnoses were determined according to the WHO criteria [1].

Correspondence to S. Hirota.
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RESULTS

Of the 454 intramucosal adenocarcinomas, 374 cases (82.4%) were protruding and 80 cases (17.6%) were depressed. Of 111 submucosal invasive adenocarcinomas, 92 cases (82.9%) were protruding and 19 (17.1%) were depressed. Size and form are shown in Table 1.

Protruding lesions were mainly composed of branching glands (Figure 2), while depressed lesions were almost entirely composed of straight glands which connected with the surface (Figure 3). In both morphological forms of submucosal invasive adenocarcinoma, and/or some intramucosal adenocarcinomas, carcinoma glands existed below glands connected to the surface, and showed no connection with the surface, independent growth, loss of polarity and progression in all directions. Various atypical cytology and architecture were also seen.

The results of p53 immunostaining are shown in Table 2. In protruding lesions, positive glands were all branching (Figure 3) while in depressed lesions, all positive glands which opened to the surface were straight (Figure 3).

DISCUSSION

As colorectal carcinomas originate from the mucosal surface, it is not appropriate to diagnose cell and structural atypia in sections of the submucosal or deeper layers. To investigate the

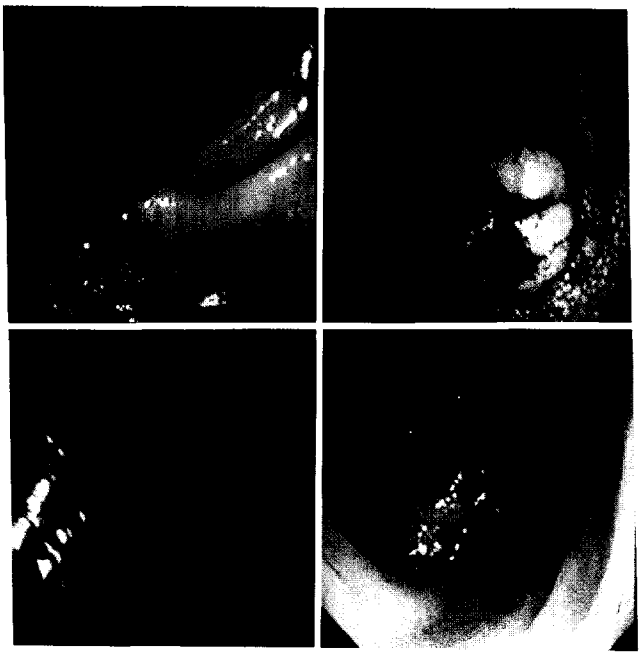

Figure 1. (A) Endoscopic view: protruding lesion, diagnosed as an intramucosal adenocarcinoma. (B) Endoscopic view with contrast method: protruding lesion diagnosed as a submucosal invasive adenocarcinoma. (C) Endoscopic view with contrast method: depressed lesion diagnosed as an intramucosal adenocarcinoma. (D) Endoscopic view: depressed lesion diagnosed as submucosal invasive adenocarcinoma.

Table 1. Size and form of early colorectal adenocarcinomas

	5 mm	10 mm	⩾ 11 mm
Intramucosal adenocarcinoma			
Protruding (n = 374)	33 (8.8%)	167 (44.7%)	174 (46.5%)
Depressed (n = 80)	56 (70.0%)	19 (23.8%)	5 (6.3%)
Submucosal invasive adenocarcinoma			
Protruding (n = 92)	3 (3.3%)	23 (25.0%)	66 (71.7%)
Depressed (n = 19)	8 (42.1%)	9 (47.4%)	2 (10.5%)

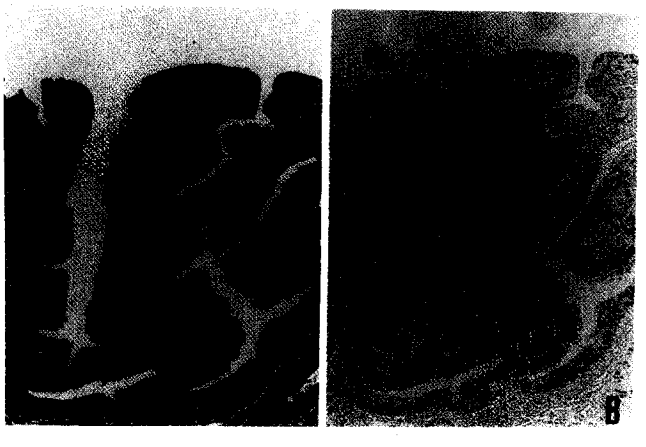

Figure 2. (A) A carcinoma gland branching and opening on the surface into an intramucosal adenocarcinoma (protruding type) (H&E ×100); (B) stained by p53 immunoreactive stain (×100).

Table 2. p53 immunoreactive stain and early colorectal adenocarcinomas

	Positive	Negative
Intramucosal adenocarcinoma		
Protruding type (n = 374)	254 (67.9%)	120 (32.1%)
Depressed type (n = 80)	19 (23.8%)	61 (76.3%)
Submucosal invasive adenocarcinoma		
Protruding type (n = 92)	59 (64.1%)	33 (35.9%)
Depressed type (n = 19)	14 (73.7%)	5 (26.3%)

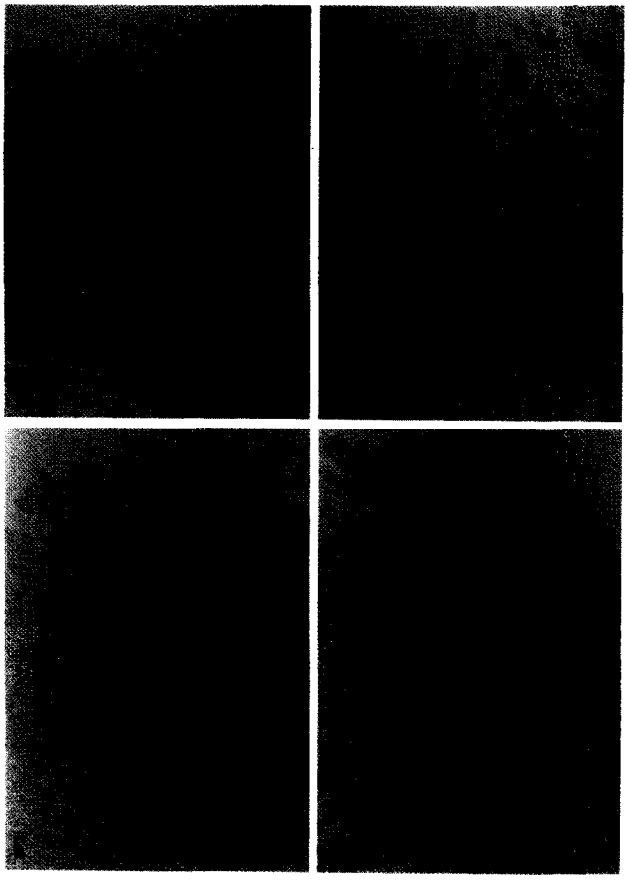

Figure 3. (A) Carcinoma glands are straight and open to the surface in submucosal invasive adenocarcinoma (depressed type). Beneath these glands, the carcinoma glands exist which show no connection with the surface, independent growth, loss of polarity and bearings, and progression in all directions (H&E ×100). (B,C,D) These glands are stained by p53 immunoreactive stain (×100, ×200, ×300, respectively).

spontaneous development of colorectal carcinomas, it is better to examine the mucosal layer, giving special attention to the glands which open to the surface [2].

A normal gland is straight and opens to the surface. An adenoma gland is branching [1]. The branching carcinoma gland which opens to the surface is thought to be a result of malignant transformation of the adenoma gland (Figure 2). As the area occupied by adenoma glands is greater than that of normal glands (if the adenoma glands concentrate), it takes time for carcinoma glands to replace adenoma glands. Accordingly, focal

carcinoma cells and glands have been seen in adenoma lesions [2].

The straight carcinoma gland which opens to the surface is considered to result from the normal gland becoming malignant without becoming adenomatous. It is the "*de novo* carcinoma gland". Beneath the glands, isolated carcinoma glands which are not apparently connected with the surface are often seen. These are thought to be rapidly invasive carcinoma glands which separated from the bottom of the straight carcinoma glands [2] (Figure 3).

Expression of p53 is associated with malignant transformation in colorectal tumours [3], and it is a late event in colorectal tumorigenesis [4]. In protruding intramucosal and submucosal invasive adenocarcinomas, all positive glands were branching, and the positive ratio was almost equal in both. This indicates that transformation from adenoma to malignancy is a slow process. In depressed intramucosal and submucosal invasive adenocarcinomas, all positive glands were straight but the positive ratio for submucosal invasive adenocarcinomas was much higher than that of intramucosal adenocarcinomas. It is, therefore, thought that intramucosal lesions change to submucosal invasive adenocarcinoma within a short time. This must occur before p53 expression since this is a late event [4].

The depressed morphological type is thought to penetrate the deeper layer more rapidly than the protruding type. Protruding lesions are mainly composed of branching glands which originate from adenoma glands. Depressed lesions are almost completely composed of straight glands which correspond to *de novo* carcinoma glands. Straight carcinoma glands appear to invade more rapidly than branching carcinoma glands.

The results of this study suggest to us that there may be at least two pathways involved in colorectal carcinogenesis.

1. Morson BC, Sobin LH. *Histological Typing of Intestinal Tumours*. Geneva, World Health Organization, 1976.
2. Kudo, S, Hirota S, Nakajima T, *et al.* Colorectal tumours and pit pattern. *J Clin Pathol* 1994, **47**, 880–885.
3. Purdie CA, O-Grady J, Piris J, Wyllie AH, Bird CC. p53 Expression in colorectal tumors. *Am J Pathol* 1991, **138**, 807–813.
4. Baker SJ, Preisinger AC, Jessup JM, *et al.* p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 1990, **50**, 7717–7722.