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Masafumi Nomura · Jiro Watari · Kinichi Yokota
Yusuke Saitoh · Takeshi Obara · Yutaka Kohgo

Morphogenesis of nonpolypoid colorectal adenomas and early carcinomas assessed by cell proliferation and apoptosis

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Abstract Nonpolypoid neoplasms, as well as ordinary polypoid tumours, are occasionally found in the colorectum. To clarify whether cell kinetic status affects the macroscopic morphology of colorectal neoplasms, we investigated proliferative indices (PI), apoptotic indices (AI), and the expression of apoptosis-related gene products. We examined 110 colorectal neoplasms comprised of 36 polypoid, 38 flat elevated and 36 depressed tumours. According to WHO's criteria these tumours consisted of 61 adenomas with low grade dysplasia (LGD), 30 adenomas with high grade dysplasia (HGD) and 19 carcinomas with submucosal invasion. Apoptotic cells were detected by TUNEL staining. Proliferating cells and apoptosis-related gene products were assessed by immunohistochemistry for Ki-67, p53, Bcl-2, and Bax antigens. AI were closely associated with macroscopic morphology in adenomas but not in carcinomas. PI were relatively constant among the three macroscopic types in adenomas and carcinomas. Median AI values of polypoid, flat elevated and depressed tumours were 1.8%, 2.1% and 4.6% for adenomas with LGD, 0.8%, 2.4% and 6.2% for adenomas with HGD and 2.9%, 4.0% and 3.6% for carcinomas, respectively. Overall PI were significantly higher in carcinomas than in adenomas with LGD, whereas AI were not different. Although the incidence of expression was significantly higher in carcinomas for p53 and in adenomas for Bcl-2 than the others, the expression of apoptosis-related gene products (p53, Bcl-2 and Bax) was similar among polypoid, flat elevated and depressed tumours. Macroscopic morphology of colorectal adenomas is determined by the apoptosis not by proliferation, and high apoptosis found in depressed adenomas implies their low net growth.

M. Nomura · J. Watari (✉) · K. Yokota · Y. Saitoh · T. Obara
Y. Kohgo
Third Department of Internal Medicine,
Asahikawa Medical College, 2-1-1-1, Midorigaoka-Higashi,
Asahikawa 078-8510, Japan
e-mail; jiro@asahikawa-med.ac.jp
Tel.: +81-166-682462, Fax: +81-166-682469

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Introduction

The vast majority of colorectal carcinomas are widely believed to arise from polypoid adenomas, a phenomenon known as the polyp cancer sequence [21] or adenoma carcinoma sequence [22]. However, recent reports have indicated that a number of nonpolypoid neoplasms exist in the colorectum, the morphology of which show either flat elevation [23, 43] or slight depression [19]. These nonpolypoid neoplasms have a unique nature when compared with polypoid tumours: they tend to be de novo carcinomas without having adenomatous component; they easily invade the submucosal layer [19, 32, 35, 36, 39, 45]; and they do not show K-*ras* mutation [6, 44] which is common in polypoid neoplasms. This reports suggest that these nonpolypoid neoplasms are important precursors for advanced colorectal carcinomas. To our knowledge, however, there is little data on the reasons for the differences of macroscopic morphology in colorectal tumours, which remain unclear.

Currently, polypoid adenomas are believed to be formed when the rate of cell proliferation exceeds that of the adjacent normal tissue [27]. Moreover, the reduction of polyps in patients with adenomatous polyposis coli after sulindac ingestion suggests that polypoid adenomas could regress when the rate of apoptotic cell death exceeds that of cell production [26]. Apoptosis is a process whereby cells die in a controlled manner in response to specific stimuli and apparently according to an intrinsic and specific program [11]. This process plays an important role in cell-deletion in normal homeostasis and embryogenesis [30], and contributes to retardation of tumour growth [12]. Apoptosis is regulated by members of the Bcl-2 protein family, some of which such as Bcl-2, Bcl-X_L and Mcl-1, suppress apoptosis, whereas others, including Bax, Bak and Bcl-X_S, promote it [16]. The tumour suppresser p53, which is often mutated in colorectal carcinomas [8], represses Bcl-2 expression but enhances Bax expression [20, 31].

It would be reasonable to assume that cell proliferation and naturally occurring apoptosis affect the morphology of colorectal tumours. Therefore, in this study, we investigated the proliferative indices (PI), apoptotic indices (AI), and the expression of apoptosis-related gene products, p53, Bcl-2, and Bax, and compared them among polypoid, flat elevated and depressed colorectal tumours.

Materials and methods

A total of 110 cases of colorectal tumours were selected from the colonoscopy reports of the Third Department of Internal Medicine, Asahikawa Medical College Hospital and the Department of Gastroenterology, Asahikawa Kosei General Hospital, by referring to hospital pathology reports. Since the aim of the present study was to determine the relationship between macroscopic morphology and cell kinetic status, the initial numbers of each macroscopic type of adenomas and carcinomas were adjusted to be roughly equal. The tumours selected consisted of 36 polypoid, 38 flat elevated and 36 depressed types. The definition of macroscopic types used here was the same as that previously reported [28, 41]. Briefly, the polypoid tumour indicates pedunculated or subpedunculated spheroid lesion. The flat elevated tumour was defined as a flat and slightly elevated lesion. The depressed tumour was defined as a lesion composed mainly of depressed surface with or without marginal elevation.

Because the WHO's criteria [10] for colorectal carcinomas was adopted after the sample selection, intramucosal carcinomas in Japanese criteria were simply reclassified as adenoma with high grade dysplasia (HGD). The term "carcinoma" used in the present study was only applied to the cases with submucosal carcinomatous invasion. Consequently the histological diagnosis of the materials consisted of 61 adenomas with low grade dysplasia (LGD) (15 polypoid, 27 flat elevated, 19 depressed), 30 adenomas with HGD (14 polypoid, 7 flat elevated, 9 depressed) and 19 carcinomas (7 polypoid, 4 flat elevated, 8 depressed). These tumours were treated by surgical operation (n=16) or the endoscopic mucosal resection method (n=94), all of which included normal mucosa surrounding tumours.

The specimens were fixed in 10% formalin, embedded in paraffin wax, and 4 μm consecutive sections were used for histological examination, in situ detection of apoptotic cells, and immunostaining of proliferating antigen Ki-67, p53, Bcl-2 and Bax proteins.

Apoptotic cells in situ were detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) method described by Gavrieli et al. [7]. The slides were dewaxed, rehydrated through a graded alcohol series, and washed with distilled water. The tissues were digested with 20 μg/ml proteinase K (Boehringer, Mannheim, Germany) for 30 min at 37°C, followed by washing in running tap water. The sections were then treated with a 2% H_2O_2 solution and washed in distilled water. The sections were preincubated with 100 mM potassium cacodylate, 2 mM cobalt chloride, and 0.2 mM dithiothreitol at pH 7.2 for 3 min, and then incubated with the same buffer containing 0.3 U/μm terminal deoxynucleotidyl transferase (TdT, GIBCO BRL Gaithersburg, Md.) and 0.04 nmol/μl biotinylated dUTP (Boehringer, Mannheim, Germany) in a humid chamber at 37°C for 1 h. The slides were rinsed in 30 mM sodium citrate and 300 mM sodium chloride for 30 min at room temperature, and then washed in phosphate-buffered saline (PBS). After blocking with 10% rabbit serum for 10 min and rinsing briefly in PBS, sections were incubated with the avidin–biotin peroxidase complex (Vector Laboratories, Burlingame, Calif.) for 30 min at room temperature, and again washed in PBS. Labelled cells were visualized with diaminobenzidine–H_2O_2 solution. The sections were then counterstained with hematoxylin.

The avidin–biotin peroxidase complex method was employed for the detection of Ki-67 antigen, p53, Bcl-2 and Bax proteins using the following antibodies: MIB-1 against Ki-67 antigen of proliferating cells (mouse IgG, Immunotech, Marseilles, France); DO7 against p53 (mouse IgG diluted at 1:100, Dako, Glostrup, Denmark); clone 124 against Bcl-2 (mouse IgG diluted at 1:60, Dako); and P-19 against Bax (rabbit IgG diluted at 1:100; Santa Cruz Biotechnology, Santa Cruz, Calif.). After deparaffinization and rehydration, endogenous peroxidase activity was blocked with 0.6% H_2O_2 in methanol for 25 min. The slides were then treated with the antigen-retrieval technique based on microwave oven heating in 10 mM citrate buffer (pH 6.0) five times for 5 min each. The container was allowed to cool at room temperature for 20 minutes. After blocking any nonspecific reaction with 5% horse serum, sections were incubated with primary antibody (MIB-1 and DO-7, for 1 h at room temperature; clone 124 and P-19, overnight at 4°C), followed by the biotinylated second antibody diluted at 1:100 for 30 min at room temperature, visualized with diaminobenzidine-H_2O_2 solution, and counterstained with haematoxylin.

PI and AI of tumours were obtained as the percentages of Ki-67-positive cells and TUNEL-positive cells relative to the total number of tumour cells. Positive cells were counted among 1,000 tumour cells on the photographs, which covered the total height of representative tumour glands and were taken with high power magnification (×200). TUNEL-positive cells located in the stroma and lumen were excluded because these labelled cells may have originated from other cell types. PI and AI were also obtained separately in upper third, middle third and lower third of the representative tumour glands in each case. These indices were presented as mean ± standard error (SE), or median with interquartile range in figures using box plots.

The sections for p53 were judged positive when 20% or more tumour nuclei were stained; others were judged negative [3]. Those for Bcl-2 and Bax were regarded as positive when tumours showed stronger cytoplasmic immunoreaction than adjacent normal epithelium [3]. The results of these immunohistochemical staining were evaluated independently by two observers (M.N. and J.W.).

Statistical differences were assessed by the Mann-Whitney U-test between two independent groups, by the Kruskal-Wallis test among three independent groups, by the Friedman test among serial data groups, and by Chi-square test between two proportions. Statistical significance was defined as P<0.05.

Results

Ki-67-positive cells were confined to the lower third of the normal crypts corresponding to the proliferative zone in normal colonic mucosa. In contrast, those of tumours were present in significant numbers in the upper third of the neoplastic glands of both adenomas and carcinomas, irrespective of their macroscopic morphology (Fig. 1). TUNEL positive cells frequently exhibited the morphological features of apoptosis, such as condensation and margination of nuclear chromatin, relatively small and roundish nuclei, and clustering of two or three fragments. However, some TUNEL positive cells lacked these morphological features, suggesting that these cells were present before the morphology changed but DNA fragmentation had already occurred (Fig. 2). TUNEL positive cells lay scattered throughout tumour area of both adenomas and carcinomas, whereas they were negligible in normal colonic mucosa.

PI of carcinomas were significantly higher than those of adenomas with LGD while PI of adenomas with HGD were in between the two values and were not statistically significant. There were no differences in AI among the three histological categories (Table 1). PI of the tumours were not significantly different among the three macro-

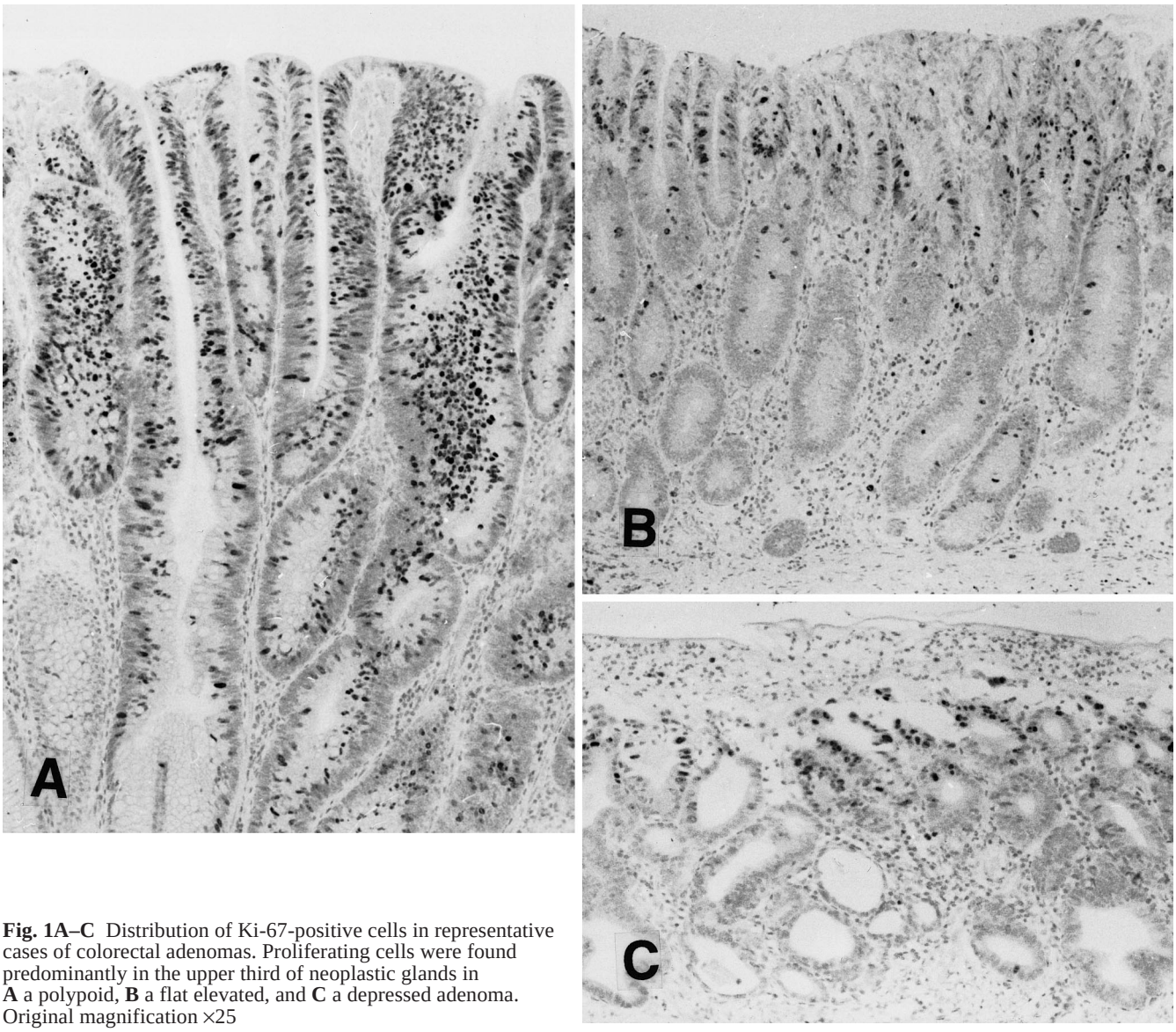

Fig. 1A–C Distribution of Ki-67-positive cells in representative cases of colorectal adenomas. Proliferating cells were found predominantly in the upper third of neoplastic glands in **A** a polypoid, **B** a flat elevated, and **C** a depressed adenoma. Original magnification ×25

scopic types (Fig. 3A,B, and C), whereas AI were closely associated with macroscopic morphology of tumours except in carcinomas. Depressed tumours showed highest AI among three macroscopic types in adenomas with LGD and HGD (Fig. 3D, E).

With regard to the distribution of proliferating cells within tumours, PI were increased in areas closer to the luminal surface irrespective of histological category. This tendency, however, decreased with the development of histological grade (Fig. 4A–C). When comparing the distribution of proliferating cells among the three macroscopic types, the tendency was also found in each type of adenomas with LGD, HGD, and carcinomas, except in the flat elevated carcinomas which showed no alteration of the distribution (data not shown). In contrast, the distribution of apoptotic cells within tumours was roughly constant among the three separate areas of neoplastic glands irrespective of histological category (Fig. 4D–F) or of macroscopic morphology (data not shown).

As shown in Table 2, carcinomas and adenomas with HGD often expressed p53 (58% and 47%), whereas it was relatively rare in adenomas with LGD (8%). Significant differences were found between adenomas with LGD and adenomas with HGD, and between adenomas with LGD and carcinomas, but not between adenomas with HGD and carcinomas. In adenomas with HGD, p53 positive cases showed significantly higher PI (45.3±4.40%) than negative cases (29.8±4.50%) ($P<0.05$), but the difference was not found in adenomas with LGD and carcinomas. No association was noted between p53 expression and AI of tumours in each histological category. Adenomas with LGD and HGD expressed Bcl-2 more frequently (51% and 47%) than carcinomas (11%). Significant differences were found between carcinomas and adenomas with LGD, and between carcinomas and adenomas with HGD, but not between adenomas with LGD and adenomas with HGD. No association was detected between Bcl-2 expression and AI in each

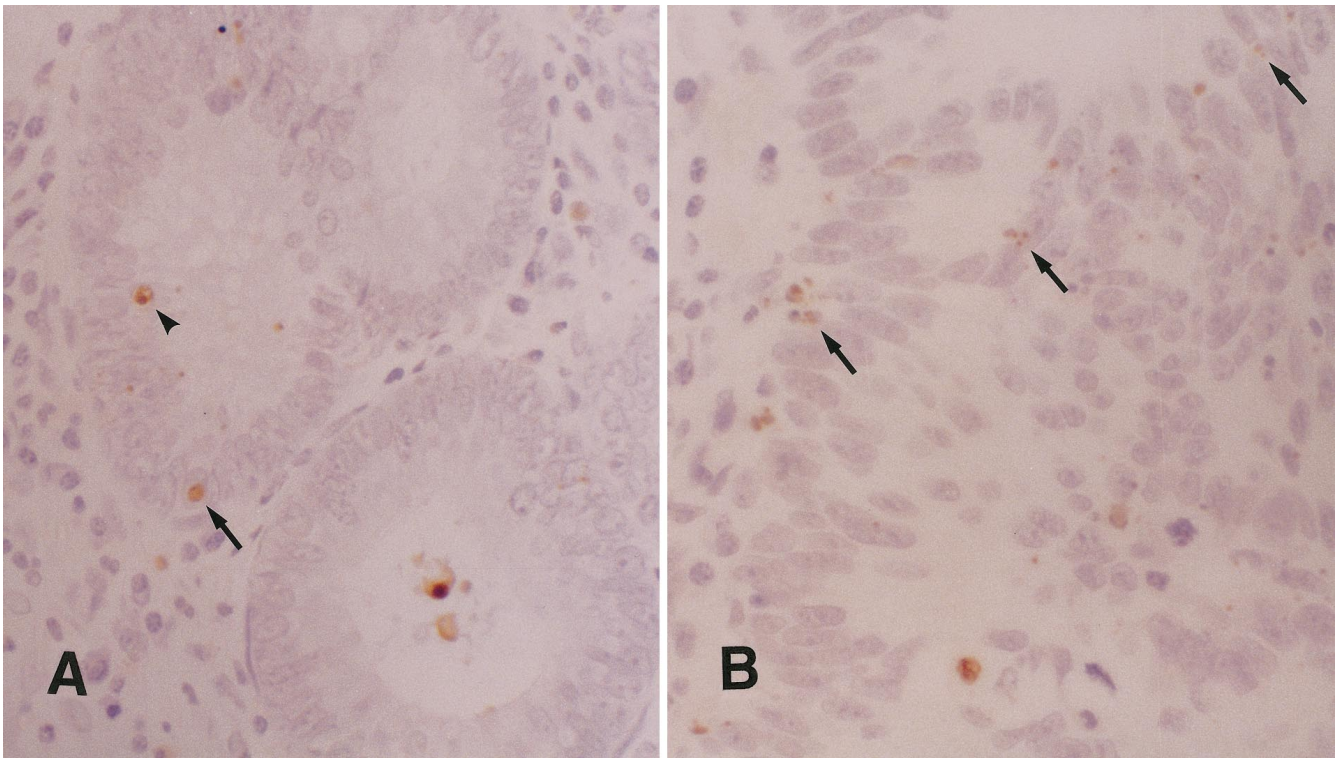

Fig. 2A, B Morphological features of apoptosis found in a representative case of depressed carcinoma. TUNEL positive cells show **A** condensed (*arrow*) and marginated nuclear chromatin (*arrowhead*) and **B** roundish nuclei and clusters of two or three fragments (*arrows*). Original magnification ×100)

Table 1 Relationship between histological category and proliferative index (*PI*) or apoptotic index (*AI*). Numbers are mean ± standard error. (*LGD* adenoma with *low grade* dysplasia, *HGD* adenoma with *high grade* dysplasia)

	No.	PI	*P*-value[a]	AI
Adenoma				
LGD	61	30.54±1.59	<0.05	2.81±0.36
HGD	30	37.02±3.42		2.78±0.62
Carcinoma	19	43.19±5.08		3.41±0.56

[a] Evaluation by Mann-Whitney U- test

histological category. As for the expression of Bax, no differences were found among adenomas with LGD, adenomas with HGD, or carcinomas (51%, 50%, and 53%, respectively). No association was detected between macroscopic morphology of tumours and expression of these apoptosis-related gene products (data not shown).

Discussion

The present study clearly shows that the amount of naturally occurring apoptosis determines macroscopic morphology of colorectal adenomas with LGD and adenomas with HGD but not of carcinomas. To our knowledge, very little has been reported about the association between macroscopic morphology and apoptosis in colorectal tumours. Arai and Kino [1] reported that the apoptosis rate of villous adenomas was significantly lower than that of tubular adenomas, thus the villous adenomas developed into larger tumours than the tubular adenomas. Their study suggested that reduced apoptosis increased the mucosal tumour volume when compared with other polypoid adenomas. Tsujitani et al. [38] examined colorectal carcinomas with submucosal invasion, and failed to discover any difference of apoptosis between polypoid and flat type carcinomas, the latter of which included both the flat elevated and depressed tumours of our definition. Their results, together with ours shown in Fig. 3F, suggest that colorectal tumours show no differences in apoptosis among the three macroscopic types when submucosal carcinomatous invasion has already occurred.

The present study also indicates that the overall apoptotic rate was similar between colorectal adenomas and carcinomas (Table 1), which was consistent with previous reports [3, 24, 40]. However, a number of discrepancies between different authors is noted in this regard. While some reports described higher apoptosis in adenomas than carcinomas [13, 34, 38] of the colorectum, some researchers have found the opposite [37]. The diagnostic criteria for carcinoma of the colorectum are quite different between Japanese and Western pathologists [29]. Since intramucosal carcinomas in Japan are not accepted as carcinomas in the West, these different results could be, at least in part, derived from the differences in distinction between adenomas and carcinomas. Another reason for the discrepancy could be the tumour

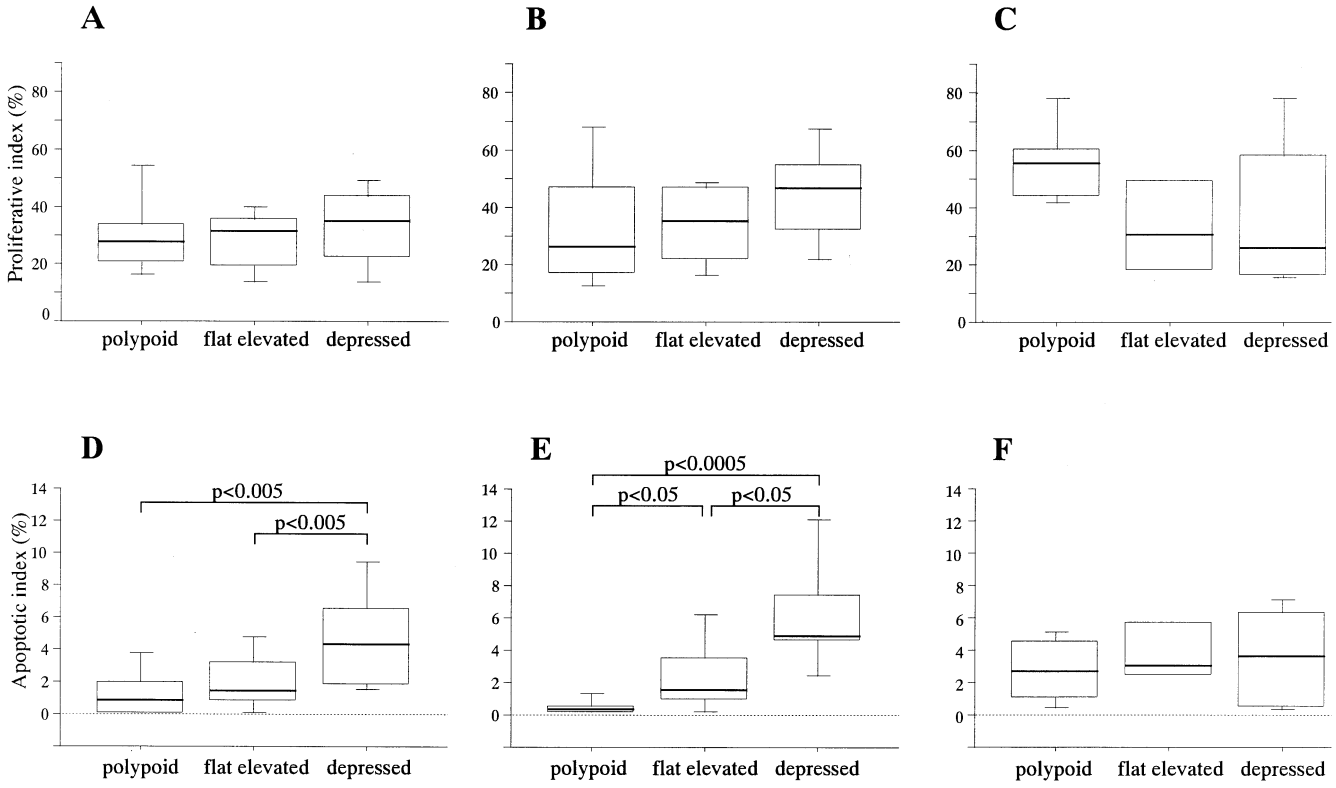

Fig. 3A–F Relationship between macroscopic morphology and **A–C** proliferative indices (*PI*) or **D–F** apoptotic indices (*AI*). PI are relatively constant among the three macroscopic types in both of adenomas and carcinomas, whereas AI are closely associated with macroscopic morphology in adenomas (**D, E**; $P<0.005$, $P<0.001$, $P<0.05$, Kruskal-Wallis test) but not in carcinomas (**F**). Depressed adenomas show highest AI among the three macroscopic types. Box plots show median values, interquartile ranges, and 90% ranges

stage of the patients: since apoptosis decreases with the progression of cancer stage [9, 37], apoptotic counts could vary depending on whether early or advanced carcinomas were used as the material for colorectal carcinomas. In addition, the results could be affected by whether the study sample included the depressed adenomas showing high apoptosis.

Some authors reported the relationship between proliferating cells and macroscopic morphology of colorectal tumours. Kobayashi et al. [14] reported that proliferating cells of depressed tumours were predominantly found in superficial portion, whereas those of polypoid tumours were diffusely distributed, but we were not able to confirm such a tendency. Yao et al. [46] reported that proliferating cells were predominantly found in the upper portion of both depressed adenomas and polypoid adenomas, but were randomly distributed in carcinomas. Although the conclusion was somewhat different, we observed a similar tendency of proliferating cell distribution, namely, the feature was prominent in adenomas with LGD and HGD of any macroscopic types, but less marked in carcinomas (Fig. 4). However we need to take account of factors other than the proliferating cells. Because it is still unknown as to why the difference of macroscopic morphology arises, whether the distribution of proliferating cells differs or not among the three macroscopic types is not an issue.

The present study offers one answer to this question: it is the amount of naturally occurring apoptosis in tumours which causes the difference of intramucosal tumour volume. It is demonstrated here that cell loss by apoptosis

Table 2 Relationship between histological category and expression of p53, Bcl-2 and Bax (*NS* not significant)

	p53		*P*-value		Bcl-2		*P*-value		Bax		*P*-value
	(+)	(–)			(+)	(–)			(+)	(–)	
Adenoma											
LGD	5	56	**	**	31	30	*	*	31	30	
HGD	14	16			14	16			15	15	
Carcinoma	11	8			2	17			10	9	NS

***P*<0.0001, LGD vs HGD and carcinoma, **P*<0.005, LGD and HGD vs carcinoma (Chi-square test)

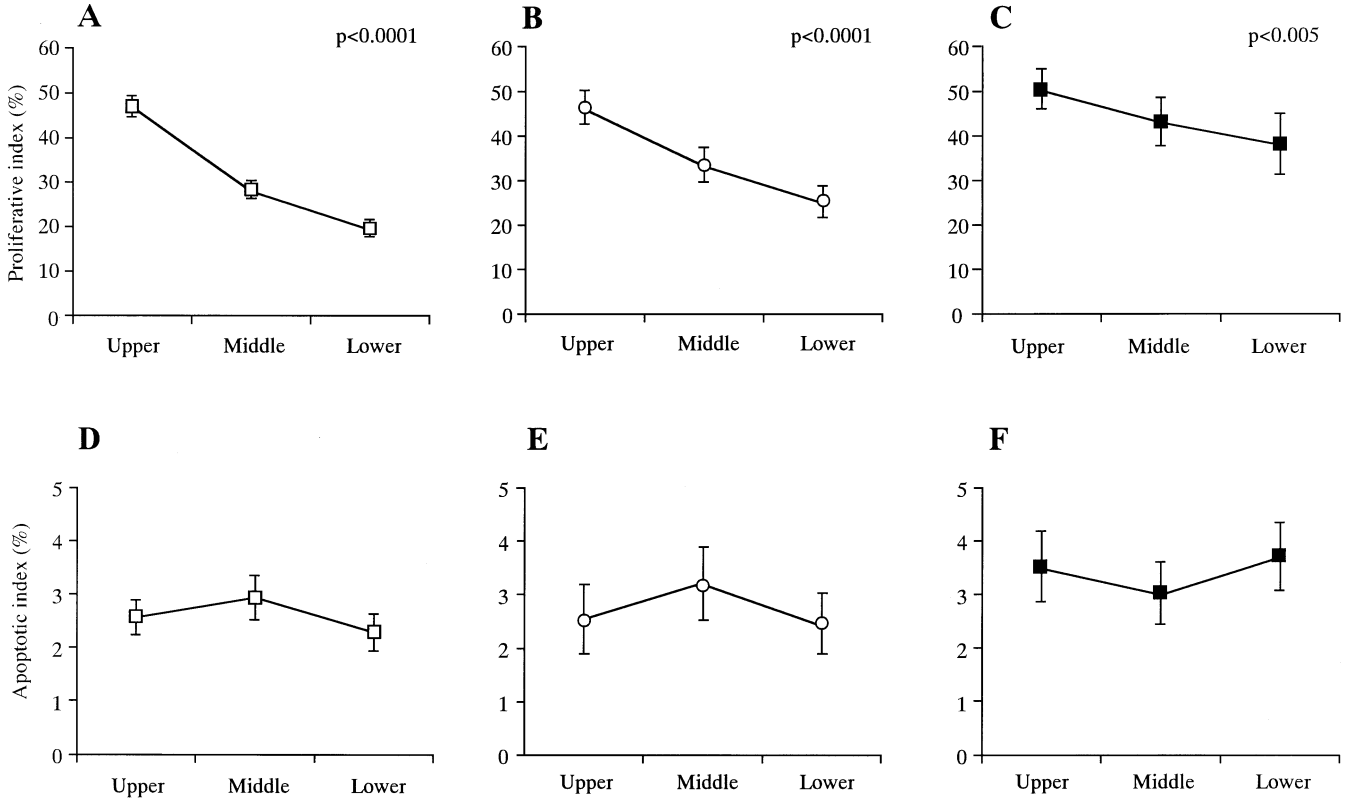

Fig. 4 PI and AI in the three separate areas of tumour glands. PI are increased as the area is nearing the luminal surface in both adenomas with **A** *LGD*, **B** *HGD* and **C** carcinomas ($P<0.0001$, $P<0.0001$, $P<0.005$, Friedman test). However, AI are roughly constant among the three separate areas of neoplastic glands of both **D, E** adenomas and **F** carcinomas

was largest in depressed adenomas, moderate in flat elevated adenomas, and smallest in polypoid adenomas, although cell increase by proliferation was constant among these three groups (Fig. 3). Therefore, apoptotic cell loss could be an important factor in the morphogenesis from LGD to HGD of adenomas. However, once carcinomatous invasion has occurred, the steady state of cell kinetics found in each macroscopic type might be lost. Other factors besides the cell kinetics might affect the morphogenesis of colorectal carcinomas, such as submucosal tumour mass, necrosis, and so forth.

We found that the expression of apoptosis-related gene products was not related to macroscopic morphology. Yao et al. [47] reported that the incidence of p53 expression was higher in depressed adenomas than polypoid adenomas, but Yukawa et al. [49] and ourselves failed to detect any such association. Yao et al. regarded sporadic staining of p53 less than 5% as positive, whereas we and Yukawa et al. judged such cases as negative. Even if we had adopted Yao's criterion, our results would not have been different because cases of sporadic staining of p53 were rare in any macroscopic type of adenoma. Concerning the p53 overexpression in the colorectal neoplasms, a consensus of results is that the incidence is markedly higher in carcinomas than adenomas (Table 2) [3, 13, 33, 34, 40, 42, 47, 49], and that its overexpression does not correlate with apoptotic index [3, 13, 24, 33, 37, 38, 40]. It is therefore suggested that apoptosis of colorectal tumours is not enhanced by the functional loss of p53, which is derived from mutational inactivation and detected by immunohistochemical overexpression [2], although wild-type p53 is believed to sensitize tumour cells to apoptosis [48]. This discrepancy is explained by the evidence that apoptosis can be induced by p53-dependent and/or p53-independent pathways [4], and that concordance between p53 overexpression and gene mutation is only 69% in colorectal carcinomas [5].

In contrast to p53, the incidence of Bcl-2 expression is generally higher in adenomas than carcinomas (Table 2) [3, 13, 15, 24, 40, 42]. Many studies, including ours, have shown that apoptotic count was not related to Bcl-2 positivity of colorectal tumours [13, 24, 40], but there have been reported carcinomas which described lower apoptosis in Bcl-2-positive cases than negative cases [3, 34]. Krajewska et al. [15] examined a series of Bcl-2 family expressions in colorectal adenomas and carcinomas, and concluded that a shift of expression from Bcl-2 and Mcl-1 to Bcl-X_L occurs during the progression of colorectal tumours. They also showed a relatively constant expression of Bax between adenomas and carcinomas, which was confirmed in the present study (Table 2). Therefore Bcl-2 and Bax system [16, 25] does not seem to play an important role on the expression of apoptosis in colorectal tumours.

Nonpolypoid colorectal neoplasms, especially depressed carcinomas are believed to develop rapidly. This

assumption is confirmed by the histological findings that they frequently invade the submucosa even when small [17, 19, 32, 39]. Furthermore, a frequent lack of adenomatous component gives the impression of aggressive behaviour by carcinomas [17, 19, 32, 35, 36, 39]. These findings were taken at only one point during the developmental process, and thus the speed of growth has not been confirmed. Matsui et al. [18] investigated the growth of 9 depressed and 12 elevated (sessile and semipedunculated) colorectal carcinomas, by doing a retrospective radiographic study. They diagnosed these tumours as intramucosal carcinomas on first examination, which corresponded to adenomas with HGD in the present study. They found that depressed tumours developed more slowly than elevated tumours; the average doubling times were 32.3 and 8.6 months, respectively. This observation strongly supports the present study because depressed adenomas with HGD have the highest apoptotic indices among three macroscopic types, suggesting a slow net growth of this type tumours. Therefore, it is suggested that depressed adenomas with HGD develop not rapidly but slowly when they exist in the mucosal layer, and thus take longer to reach a given size than polypoid tumours. This would account for a higher incidence of submucosal invasion when compared with polypoid tumours of same size. Once carcinomatous invasion has occurred, however, depressed tumours will develop similarly with polypoid carcinomas with submucosal invasion because of a equivalent cell kinetic state.

In conclusion, the present study shows that the apoptotic rate against cell proliferation determines macroscopic morphology of colorectal adenomas with LGD and HGD, where p53 and bcl-2 participate in the progression, but do not do so in the morphogenesis of tumours, and that high apoptosis found in depressed adenomas implies their low net growth.

References

1. Arai T, Kino I (1995) Role of apoptosis in modulation of the growth of human colorectal tubular and villous adenomas. J Pathol 176:37–44
2. Baas IO, Mulder JW, Offerhaus GJ, Vogelstein B, Hamilton SR (1994) An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. J Pathol 172:5–12
3. Baretton GB, Diebold J, Christoforis G, Vogt M, Muller C, Dopfer K, Schneiderbanger K, Schmidt M, Lohrs U (1996) Apoptosis and immunohistochemical bcl-2 expression in colorectal adenomas and carcinomas. Aspects of carcinogenesis and prognostic significance. Cancer 77:255–264
4. Clarke AR, Purdie CA, Harrison DJ, Morris RG, Bird CC, Hooper ML, Wyllie AH (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature 362: 849–852
5. Dix B, Robbins P, Carrello S, House A, Iacopetta B (1994) Comparison of p53 gene mutation and protein overexpression in colorectal carcinomas. Br J Cancer 70:585–590
6. Fujimori T, Satonaka K, Yamamura-Idei Y, Nagasako K, Maeda S (1994) Non-involvement of ras mutations in flat colorectal adenomas and carcinomas. Int J Cancer 57: 51–55
7. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493–501
8. Hamelin R, Laurent-Puig P, Olschwang S, Jego N, Asselain B, Remvikos Y, Girodet J, Salmon RJ, Thomas G (1994) Association of p53 mutations with short survival in colorectal cancer. Gastroenterology 106:42–48
9. Ikenaga M, Takano Y, Saegusa M, Ohtani Y, Hiki Y, Kakita A, Okayasu I (1996) Apoptosis of colon cancers assessed by in situ DNA nick end-labeling method. Pathol Int 46:33–37
10. Jass JR, Sobin LH (1989) Histological typing of intestinal tumours. World Health Organization International Classification of Tumours. Springer, Berlin Heidelberg New York
11. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257
12. Kerr JF, Winterford CM, Harmon BV (1994) Apoptosis. Its significance in cancer and cancer therapy. Cancer 73:2013–2026
13. Kikuchi Y, Dinjens WN, Bosman FT (1997) Proliferation and apoptosis in proliferative lesions of the colon and rectum. Virchows Arch 431:111–117
14. Kobayashi M, Watanabe H, Ajioka Y, Honma T, Asakura H (1996) Effect of K-ras mutation on morphogenesis of colorectal adenomas and early cancers: relationship to distribution of proliferating cells. Hum Pathol 27:1042–1049
15. Krajewska M, Moss SF, Krajewski S, Song K, Holt PR, Reed JC (1996) Elevated expression of Bcl-X and reduced Bak in primary colorectal adenocarcinomas. Cancer Res 56: 2422–2427
16. Kroemer G (1997) The proto-oncogene Bcl-2 and its role in regulating apoptosis. Nat Med 3:614–620
17. Kudo S (1993) Endoscopic mucosal resection of flat and depressed types of early colorectal cancer. Endoscopy 25: 455–461
18. Matsui T, Yao T, Yao K, Takenaka K, Sakurai T, Iwashita A, Fuchigami T, Aoyagi K, Date H (1996) Natural history of superficial depressed colorectal cancer: retrospective radiographic and histologic analysis. Radiology 201:226–232
19. Minamoto T, Sawaguchi K, Ohta T, Itoh T, Mai M (1994) Superficial-type adenomas and adenocarcinomas of the colon and rectum: a comparative morphological study. Gastroenterology 106:1436–1443
20. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA, Hoffman B, Reed JC (1994) Tumour suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene 9:1799–1805
21. Morson BC (1974) The polyp cancer sequence in the large bowel. Proc R Soc Med 67:451–457
22. Muto T, Bussey HJ, Morson BC (1975) The evolution of cancer of the colon and rectum. Cancer 36:2251–2270
23. Muto T, Kamiya J, Sawada T, Konishi F, Sugihara K, Kubota Y, Adachi M, Agawa S, Saito Y, Morioka Y, et al (1985) Small "flat adenoma" of the large bowel with special reference to its clinicopathologic features. Dis Colon Rectum 28:847–851
24. Nakamura T, Sakai T, Nariya S (1995) Cell death in colorectal polyps as evaluated by in situ 3'-tailing reaction and its relationship to BCL-2 expression. Pathol Int 45:721–728
25. Oltvai ZN, Milliman CL, Korsmeyer SJ (1993) Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609–619
26. Pasricha PJ, Bedi A, O'Connor K, Rashid A, Akhtar AJ, Zahurak ML, Piantadosi S, Hamilton SR, Giardiello FM (1995) The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. Gastroenterology 109:994–998
27. Risio M, Lipkin M, Candelaresi G, Bertone A, Coverlizza S, Rossini FP (1991) Correlations between rectal mucosa cell proliferation and the clinical and pathological features of nonfamilial neoplasia of the large intestine. Cancer Res 51: 1917–1921
28. Saitoh Y, Obara T, Einami K, Nomura M, Taruishi M, Ayabe T, Ashida T, Shibata Y, Kohgo Y (1996) Efficacy of high-

frequency ultrasound probes for the preoperative staging of invasion depth in flat and depressed colorectal tumors. Gastrointest Endosc 44:34–39
29. Schlemper RJ, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, Sipponen P, Stolte M, Watanabe H (1998) Differences in the diagnostic criteria used by Japanese and Western pathologists to diagnose colorectal carcinoma. Cancer 82:60–69
30. Searle J, Kerr JF, Bishop CJ (1982) Necrosis and apoptosis: distinct modes of cell death with fundamentally different significance. Pathol Annu 17:229–259
31. Selvakumaran M, Lin HK, Miyashita T, Wang HG, Krajewski S, Reed JC, Hoffman B, Liebermann D (1994) Immediate early up-regulation of bax expression by p53 but not TGF beta 1: a paradigm for distinct apoptotic pathways. Oncogene 9: 1791–1798
32. Shimoda T, Ikegami M, Fujisaki J, Matsui T, Aizawa S, Ishikawa E (1989) Early colorectal carcinoma with special reference to its development de novo. Cancer 64:1138–1146
33. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin B (1995) bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 55:237–241
34. Sinicrope FA, Roddey G, McDonnell TJ, Shen Y, Cleary KR, Stephens LC (1996) Increased apoptosis accompanies neoplastic development in the human colorectum. Clin Cancer Res 2: 1999–2006
35. Spjut HJ, Frankel NB, Appel MF (1979) The small carcinoma of the large bowel. Am J Surg Pathol 3:39–46
36. Spratt JS, Ackerman LV (1962) Small primary adenocarcinomas of the colon and rectum. JAMA 179:337–346
37. Takano Y, Saegusa M, Ikenaga M, Mitomi H, Okayasu I (1996) Apoptosis of colon cancer: comparison with Ki-67 proliferative activity and expression of p53. J Cancer Res Clin Oncol 122:166–170
38. Tsujitani S, Shirai H, Tatebe S, Sugamura K, Ohfuji S, Gomyo Y, Maeta M, Ito H, Kaibara N (1996) Apoptotic cell death and its relationship to carcinogenesis in colorectal carcinoma. Cancer 77 [Suppl 8]:1711–1716
39. Wada R, Matsukuma S, Abe H, Kuwabara N, Suda K, Arakawa A, Kitamura S (1996) Histopathological studies ofsuperficial-type early colorectal carcinoma. Cancer 77: 44–50
40. Ward RL, Todd AV, Santiago F, O'Connor T, Hawkins NJ (1997) Activation of the K-ras oncogene in colorectal neoplasms is associated with decreased apoptosis. Cancer 79: 1106–1113
41. Watari J, Saitoh Y, Obara T, Fujiki T, Taniguchi M, Nomura M, Ayabe T, Ohta T, Orii Y, Kohgo Y (1997) Early non-polypoid colorectal cancer: radiographic diagnosis of depth of invasion. Radiology 205:67–74
42. Watson AJ, Merritt AJ, Jones LS, Askew JN, Anderson E, Becciolini A, Balzi M, Potten CS, Hickman JA (1996) Evidence of reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br J Cancer 73:889–895
43. Wolber RA, Owen DA (1991) Flat adenomas of the colon. Hum Pathol 22:70–74
44. Yamagata S, Muto T, Uchida Y, Masaki T, Sawada T, Tsuno N, Hirooka T (1994) Lower incidence of K-ras codon 12 mutation in flat colorectal adenomas than in polypoid adenomas. Jpn J Cancer Res 85:147–151
45. Yao T, Tada S, Tsuneyoshi M (1994) Colorectal counterpart of gastric depressed adenoma. A comparison with flat and polypoid adenomas with special reference to the development of pericryptal fibroblasts. Am J Surg Pathol 18:559–568
46. Yao T, Tsuneyoshi M, Matsumoto T, Tada S, Kuwano Y (1994) Depressed adenoma of the colorectum: analysis of proliferative activity using immunohistochemical staining for proliferating cell nuclear antigen (PCNA). Pathol Int 44:520–527
47. Yao T, Utsunomiya T, Nagai E, Oya M, Tsuneyoshi M (1996) p53 expression patterns in colorectal adenomas and early carcinomas: a special reference to depressed adenoma and non-polypoid carcinoma. Pathol Int 46:962–967
48. Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M (1991) Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. Nature 352: 345–347
49. Yukawa M, Fujimori T, Maeda S, Tabuchi M, Nagasako K (1994) Comparative clinicopathological and immunohistochemical study of ras and p53 in flat and polypoid type colorectal tumours. Gut 35:1258–1261