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Morphometric analysis of cytological atypia in colonic adenomas

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Summary. A total of 29 colorectal polyps were classified into those with mild, moderate, and severe dysplasia, and carcinoma. Morphometric analyses were performed on each group, determining; mean nuclear area (N-area), nuclear cytoplasmic (N/C) ratio, nuclear crowding index (Cx) and mean cellular area (C-area). N-area and N/C ratio increased in accordance with the progress of the atypical changes. Cx showed a gradual increase up to moderate dysplasia and a decline from severe dysplasia to carcinoma. N-area in severe dysplasia and carcinoma was distinctively larger than that in mild and moderate dysplasia. As for C-area, the value from normal to moderate dysplasia remained about the same, in contrast to the much higher levels in severe dysplasia and carcinoma. These results clearly indicated that the rise in N/C ratio from normal to moderate dysplasia depends mainly on nuclear crowding, but that from severe dysplasia to carcinoma on nuclear enlargement.

Key words: Colorectal polyps – Cytologic atypism – Morphometry

Introduction

The term "dysplasia" in colonic adenomas may comprise structural and cytological abnormalities, and their atypia has been determined based on these two components. While the structural atypia is easily recognizable, exact morphological evaluation (Cook and Goligher 1975; Ekelund and Lindstrom 1974; Enterline 1976; Muto et al. 1975) of the latter is difficult because of a paucity of reliable methods. Although grading of dysplasia is a subjective assessment, it would be of interest to us to know what cytological changes might be subconciously regarded as important in grading of colonic adenomas. In the present study, we tried to analyze quantitatively the variable changes seen in the adenomatous glands by using plastic embedded semi-thin sections. On the basis of the results obtained, we hoped to determine which parameters would had a close association with the progress of atypia of colonic adenomas.

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Materials and methods

During the period from 1981 to 1985, a total of 169 colorectal polyps were surveyed in our laboratory. Of these, 116 were obtained by polypectomy; the remaining 53 were from colons resected for carcinoma. For normal controls, 15 cases were selected that had normal colonic mucosa adjacent to colonic carcinoma or polyps. All specimens were fixed in 10% formalin and divided into two blocks. One was embedded in paraffin in the usual manner and the other was preserved for JB-4 embedding. Specimens embedded in paraffin were cut at 4–6 μm and stained with haematoxylin and eosin (H & E).

The grade of glandular atypia in the adenomas was determined according to the criteria of Konishi and Morson (1982) which consist of the normal epithelium, mild to severe dysplasia, and carcinoma.

For morphometry the blocks were directly embedded in JB-4 medium (Polysciences, USA), and were sectioned at 0.6 μm with a JB-4 microtome. The sections were stained with some modified Weigert haematoxylin (20 min) and then with a mixture of 0.5 phloxin and 0.5 eosin Y (5 min). Histological changes seen in paraffin and plastic sections were compared with one another and a corresponding area was selected from the latter specimens for morphometry. A preliminary study showed that the rate of shrinkage in JB-4 embedded tissues was negligible (less than 0.6%) while the value was about 11% in paraffin embedded specimens. Photographs of the glands presenting features consistent with those seen in paraffn sections were taken on 35 mm film and appropriately enlarged (×960). Much attention was paid for selecting those glands which had been cut vertically or horizontally to their longitudinal axis as precisely as possible. Using these photographs, morphometric analysis was performed with the aid of an image analyser (Muto, MGC-1000, Tokyo), which consists of a magnet tablet and a connected microcomputer. By outlining perimeters of objects with a scribing cursor, the traced area or length could be calculated automatically and recorded.

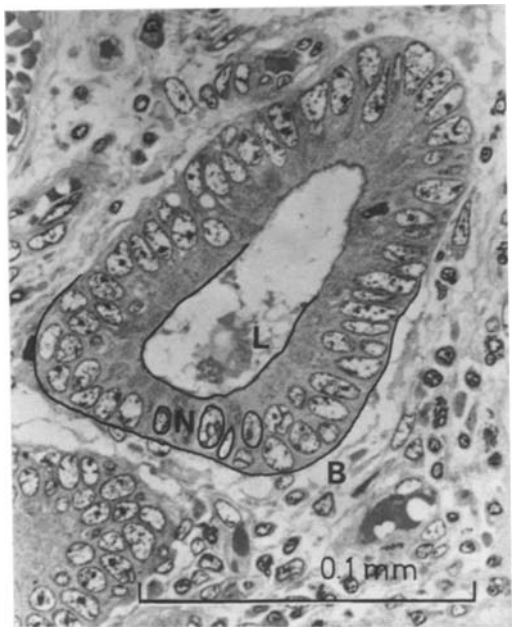


Fig. 1. By tracing the margin of each nucleus (N), luminal surface (L) and glandular base (B), total glandular area and total nuclear counts were calculated (plastic section, ×440, carcinoma gland)

Morphometric analysis was performed to define the following items; mean nuclear area, nuclear cytoplasmic ratio, mean cellular area, and nuclear crowding index. For determination of the nuclear area (N-area) [μm^2], outlines of nuclei contained in a certain glandular area were accurately traced. A glandular area was measured by tracing luminal surface and basal border and then deducting the former value from the latter value (Fig. 1). In a longitudinal section of a gland, the area was determined by tracing a glandular portion corresponded to basal distance of 150 μm.

Nuclear cytoplasmic (N/C) ratio was calculated by the following formula:

$$N/C = \frac{\text{Total nuclear area included in glandular area}}{\text{Glandular area}}$$

Mean cellular area (C-area) which represents an area per one cell was determined by:

$$\text{C-area} = \frac{\text{Glandular area}}{\text{Total number of nuclei in glandular area}} \ (\mu m^2)$$

Nuclear crowding index (Cx) was expressed here by nuclear counts located on a constant length (10 μm) of the basal cell border. It was calculated by the following formula:

$$Cx = \frac{\text{Nuclear counts in a gland}}{\text{Length of basa cell border}} \times 10$$

The total number of glands and nuclei thus examined are presented in Table 1. The results of the measurements of each parameter are shown in Table 2. All results were given as mean values ± SEM. One way analysis of variance was used to compare arithmetic means and Scheffe's multiple-comparison was used to compare the results among five groups ranging from normal to carcinoma. The results were considered to be significant if the probability of error, p, was lower than 0.05.

Results

Histological changes in the colonic epithelium as viewed by plastic sections were basically consistent

Table 1. Number of glands and nuclei investigated

	Normal	Dysplasia			Carcinoma	Total
		Mild	Moderate	Severe		
Glands	80	81	101	95	102	459
Nuclei	4807	8829	8717	5828	5609	33790

Table 2. Changes in each parameter correlated with grades of atypism

	Normal	Dysplasia			Carcinoma	Statistics
		Mild	Moderate	Severe		
N-area	18.9 ±0.67	29.0 ±0.69	32.4 ±0.59	51.4 ±0.95	64.0 ±1.2	X1*X2=X3*X4*X5
N/C	0.115±0.005	0.215±0.004	0.267±0.005	0.286±0.005	0.338±0.004	X1*X2*X3*X4*X5
Cx	1.54 ±0.05	2.50 ±0.07	3.18 ±0.11	2.11 ±0.07	1.60 ±0.05	X1*X2*X3*X4*X5 X1*X5, X2=X4
C-area	147.8 ±4.5	149.0 ±5.2	150.8 ±2.6	190.3 ±6.4	189.5 ±4.0	X1=X2=X3*X4=X5 X3*X5

Mean ± SEM. N-area: Mean nuclear area; N/C: Nuclear cytoplasmic ratio; Cx: Crowding index; C-area: Cellular area. The means of five groups from normal to carcinoma were tested by one way analysis of variance, and the results between the groups were tested by the Scheffe's multiple-comparison method. X1–5 are the mean values from one parameter from left to right. The sign * means statistical differences whereas the sign = mean not significant. (A result was considered to be significant if p is less than 0.05)

with those observed in paraffin sections (Figs. 2, 3, 4, and 5). Elimination of nuclear overlapping in the semi-thin sections provided a great advantage for revealing detailed cytological changes, a prerequisite for morphometrical studies. The characteristic features of each grade of dysplasia will be briefly described below.

In mild dysplasia (Figs. 2A and B), the nuclei were more crowded than normal and enlarged with a pencil-shaped appearance, but they were basally situated and arranged parallel to the cell axis. Mucin production was relatively well maintained. Architectural deformity of glands, if present, was mild.

In moderate dysplasia (Figs. 3A and B), the nuclei were present at various locations elevated from the cell base, with some pseudostratifications and abnormalities in their polarities. Intraluminal budding and "back to back" arrangement were seen in some glands. Mucin secretion was minimal.

Severely dysplastic glands (Figs. 4A and B) displayed markedly reduced mucin secretion. The glands were closely packed one another and their structural atypia, e.g., "back to back" arrangement became more prominent. Nuclei were plump but still uniform and smaller than those in carcinomatous glands. These abnormalities seemed to be yet insufficient to warrant a diagnosis of carcinoma.

In carcinomatous glands (Figs. 5A and B), the nuclei were enlarged and pleomorphic with variable loss of their polarity. The glandular structure was distorted and resembled that seen in overt colonic carcinoma.

Accordingly, 169 adenomas were classified into 100 mild dysplasia, 29 moderate dysplasia, 36 severe dysplasia, and 4 carcinoma of well differenciated type.

Twenty-nine polyps were available for morphometric analysis, including 9 mild dysplasia, 9 moderate dysplasia, 7 severe dysplasia and 4 carcinoma. These cases used for morphometry were selected from well preserved and properly processed tissue samples that contained a certain area showing representative grade of atypia with uniform distribution. Samples showing marked structural distortion were excluded. Four polyps classified into "carcinoma" showed unequivocal evidence of invasive growth across the line of the lamina muscularis mucosa. In these cases, the glands were selected from such a portion.

The results of the morphometric analysis are shown in Table 2.

As indicated in Table 2 the mean nuclear area (N-area) tended to increase together with the progress of dysplastic changes. The N-area in mild and moderate dysplasia was 1.5 times and 1.7 times larger than that of the normal gland, respectively. The difference was slight with no significance. By contrast, there was a more distinct increase of the N-area in severe dysplasia and carcinoma, each value of which was 2.7 times and 3.4 times as large as that of the normal gland with a statistical significance ($P<0.05$).

Although the nuclear cytoplasmic ratio (N/C ratio) increased in proportion to the development of epithelial atypia as in the case of N-area, the pattern was somewhat different (Table 2). The N/C ratio in moderate dysplasia was more than two times when compared with the normal value. The difference was not so prominent between moderate and severe dysplasia but the carcinoma glands again showed a high N/C ratio. Each value demonstrated a significant difference. If the increase in N/C ratio was based solely on the nuclear enlargement, the N-area and N/C ratio should have shown a similar trend line. However, this could not be demonstrated, as will be discussed later.

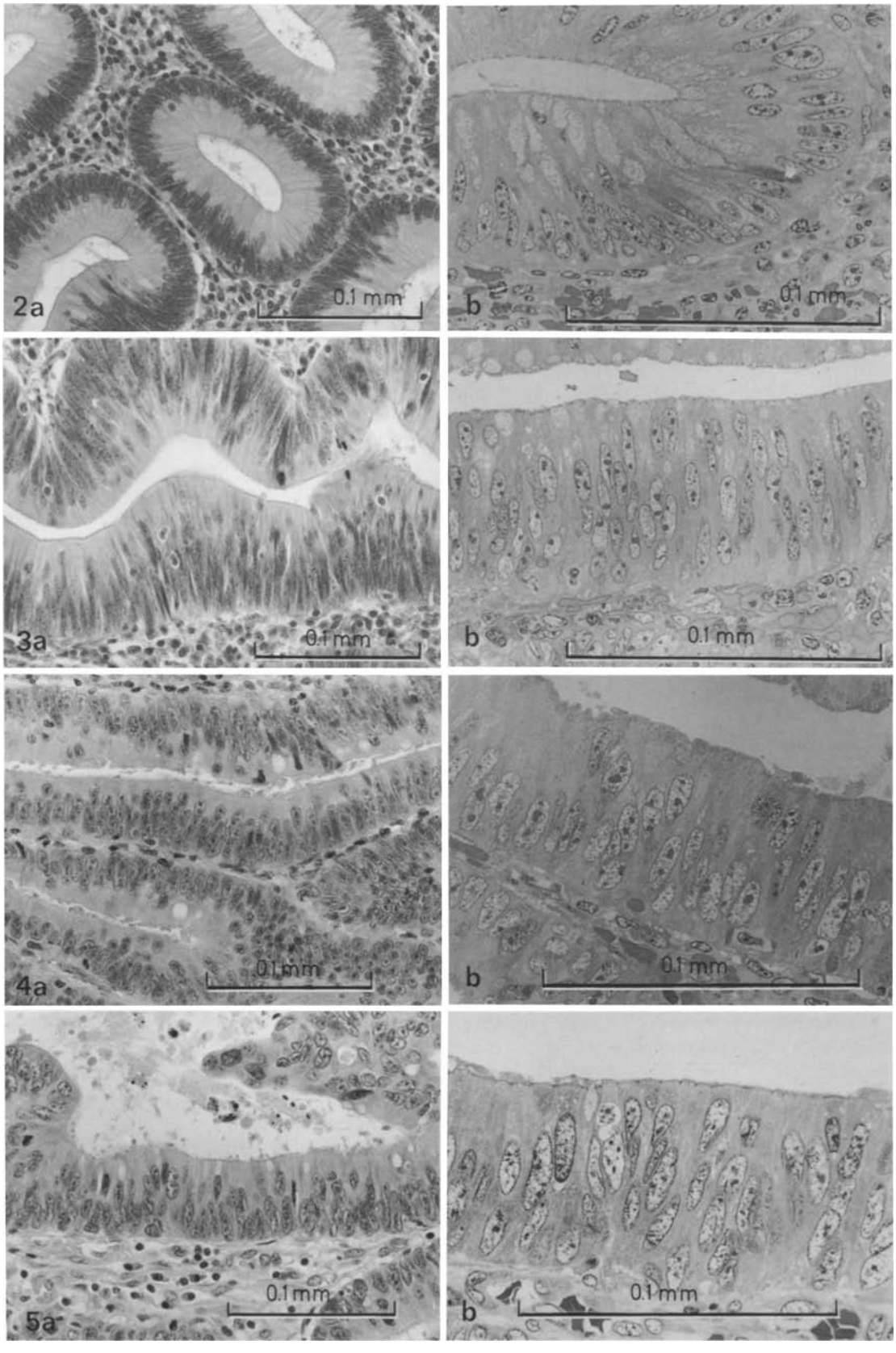
The degree of nuclear crowding of a gland (Cx) in relationship to the grade of atypia is summarized in Table 2. The trend line was distinctively different from that of the N/C ratio and N-area. Cx first increased in proportion to the advance of atypia from normal to moderate dysplasia, but subsequently decreased. In carcinomatous glands it returned to the normal level.

Levels of Mean cellular area (C-area) from normal to moderate dysplasia showed very little change. In contrast, in severe dysplasia and carcinoma, much higher levels were revealed (Table 2).

Discussion

It is well understood that colonic adenomatous polyps are of a dysplastic nature, a predisposing condition resulting in the development of carcinoma (Castleman and Krickstein 1966; Enterline et al. 1962; Hill et al. 1978; Morson 1968; Shinya and Wolff 1979).

Many attempts have been made to elucidate the process of the malignant transformation by focusing on the structural and cytological atypia occurring in the adenomas with the aid of various techniques (Kaye et al. 1973; Kikuchi et al. 1984; Togo and Nakamura 1983; Wiebeck et al. 1974). Some authors have suggested that changes in the labelling index or DNA content of adenoma cells were intimately related to cellular atypia (Goh and Jass 1986; Kanemithu et al. 1985). However, little has been known with regard to quantitative alter-


2a
0.1 mm
b
0.1 mm
3a
0.1 mm
b
0.1 mm
4a
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b
0.1 mm
5a
0.1 mm
b
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ations of the nuclei and cells in dysplasia-carcinoma sequence.

Our morphometric analysis of colonic adenomas demonstrated that histolgical atypism was correlated well with an increase of N-area and N/C ratio. It should be emphasized such a change occurred in two steps; in the first step, nuclear increase, rather than nuclear enlargement, played an important role in the elevation of the N/C ratio, as demonstrated by a high Cx level. In contrast, in the second step, a prominent increase in N-area was mainly responsible for a rising N/C ratio while the Cx level returned toward the normal. As a whole, nuclear enlargement seems to be recognized as an important parameter in grading dysplasia.

The present investigation was conducted primarily based on the subjective assessment of the grading of adenomas. The samples were carefully selected but relatively small in number. The results are, therefore, not quite objective although they possibly reflect the pathologist's view on grades of cellular atypia done subconciously on colonic adenomas.

In the present study, severely dysplastic glands were seen to be confined to the mucosa. Carcinomatous glands were selected from those invading into the submucosa to prevent a mixture of these two lesions, as far as possible in order to ensure that the results concerning these two lesions would be less subjective. Unfortunately, however, only 4 such carcinoma cases were available in our series. In any case, from morphometric viewpoint, both severe dysplasia and carcinoma were included in the same category when compared with mild or moderate dysplasia, suggesting that severe dysplasia has a close resemblance to cancer glands. Strictly speaking, however, the values in N-area, N/C ratio, and Cx were distinguishable with statistical significance between severe dysplasia and carcinoma. In conventional paraffin sections, such a difference might sometimes be a minute variation beyond our visual recognition.

In brief, our morphometric study indicated that nuclear enlargement has a close association with the progress of atypia in the dysplasia-carcinoma sequence and that severe dysplasia does share a property with carcinoma. The biological significance of these findings remains unknown.

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Fig. 2. Mild dysplasia. **(A)** Elongated nuclei are situated basally and mucin production is well maintained (H & E, ×300). **(B)** Nuclei are not quite uniform containing one or two prominent nucleoli. Nuclear parallelism, however, is still preserved (plastic section, ×580)

Fig. 3. Moderate dysplasia. **(A)** Nuclei show various elevation from the cell base with some pseudostratifications. Mucin is decreased (H & E, ×300). **(B)** Nuclei show mild enlargement, considerable crowding and some loss of polarity (plastic section, ×580)

Fig. 4. Severe dysplasia. **(A)** Mucin is markedly reduced. The glands are packed with conspicuous nuclei and show "back to back" arrangement (H & E, ×150). **(B)** Plump nuclei contain enlarged nucleoli and exhibit some irregularities in their contour (plastic section, ×580)

Fig. 5. Carcinoma. **(A)** Nuclei are further enlarged and pleomorphic with some disarragement (H & E, ×150). **(B)** Nuclear enlargement and irregularity are prominent (plastic section, ×580)

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