UEA-1 and CEA Localization and Nuclear DNA Content in Adenoma and Carinoma Cells of the Human Colorectum

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UEA-1 and CEA Localization and Nuclear DNA Content in Adenoma and Carinoma Cells of the Human Colorectum

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In order to elucidate the relationship between adenoma and carcinoma, intracellular localization of UEA-1 and CEA and the quantity of nuclear DNA content were examined in 54 adenomas and 29 carcinomas of the human colorectum. UEA-1 localization was confirmed in 77% of the adenoma with mild dysplasia, 84% of the adenomas with moderate dysplasia and in 100% of the carcinomas; the localized rates increased in accordance with the advance of cellular dysplasia. CEA was found in 40% and 60%, respectively, of the adenomas with mild and moderate dysplasias and in all of the carcinomas. In particular, CEA which was diffusely distributed in the cytoplasm was found in 9% and 26%, respectively, of the adenomas with mild and moderate dysplasias and in 79% of the carcinomas. Nuclear DNA content also increased in parallel with the increase of dysplasia. In all the carcinomas, the proportion of polyploidy cells over 4C were higher than 8%. However, 10% and 50%, respectively, of the adenomas with mild and moderate dysplasias also showed the same proportion of polyploidy cells as that of the cacrinomas. These results suggest that changes of UEA-1 and CEA localization and of nuclear DNA content occur in adenoma cells preceding any structural malignant changes and, further, that colorectal adenomas have already acquired malignant potential, thereby, supporting the "adenoma-carcinoma sequence".

Key Words
Colorectal adenoma,
Adenoma-carcinoma sequence,
UEA-1 (Urex europaeus agglutinin-1),
CEA (carcinoembryonic antigen),
Nuclear DNA content.

INTRODUCTION

The relationship between adenoma and carcinoma has been gradually clarified because of increasing information from recent advances in the diagnosis of colorectal lesions, and also because of the recent increase in the occurrence of colorectal adenoma and carcinoma. In general, the concept presently known as the "adenoma-carcinoma sequence" has widely accepted in which colorectal adenoma has malignant potential and the majority of all the carcinomas may originate from adenomas (1–5), though some investigators have reported that "*de novo* carcinomas" may play an important role also in the colorectum as well as the stomach (6–8).

On the other hand, changes of rectin localization specifically binding to glycoconjugate and also changes of glycoprotein localization in neoplastic cells have been recently demonstrated in many studies (9–14). It has been also clarified that nuclear DNA content is different from non-neoplastic cells and that it generally increases in neoplastic cells including

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adenoma and carcinoma cells (15–17). However, few studies in regard to the changes of neoplastic cells have yet performed simultaneously from the point of the above 3 factors. Therefore, in order to elucidate the relationship between adenoma and carcinoma in the colorectum, in the present study the localized pattern of a rectin (Urex europaeus agglutinin-1, UEA-1) and a glycoprotein (carcinoembryonic antigen, CEA), and the nuclear DNA content were simultaneously examined in the cells of adenomas with different grades of dysplasia and in carcinomas.

MATERIALS AND METHODS

Surgically or endoscopically extirpated specimens were examined from 54 patients with adenoma and 27 patients with carcinoma of the rectum and left-sided colon. The classification of adenoma authorized by the Japanese Research Society for Cancer of the Colon and Rectum was employed as a measure of the dysplasia in adenoma: mild, moderate and severe dysplasias corresponded to the groups 2, 3 and 4–5 respectively. Examined adenomas consisted of 35 mild, 19 moderate and 2 severe dysplasias in this study. Because adenoma with severe dysplasia was clinicopathologically treated as "carcinoma *in sisu*" (5), 2 adenomas were also classified into carcinoma in this study. The specimens were fixed in 10% formalin solution and embedded in paraffin. Every section with 3–4 μm was stained with hematoxylin and eosin (HE) in the line with general procedures. UEA-1 and CEA localization in the sections and nuclear DNA content were examined by staining with the following methods.

1. UEA-1 Stanining Method

In every specimen, immunohistochemical UEA-1 localization was examined in the same serial as the HE staining sections. For the immunohistochemical demonstration, rectin-antirectin PAP technique was employed (18). Sections were deparaffinized, dehydrated and washed in methanol with 0.03% H_2O_2 for 30 minutes, in order to block endogenous peroxidase and to avoid nonspecific reaction. After being washed in physiological buffered saline (PBS, pH 7.2), sections were immersed in BSA solution (PBS with 1% cow serum, E–Y Laboratories Inc., San Matro, CA) for 15 minutes, and then they were allowed to react in BSA solution with UEA-1 (40 μg/ml) for 1 hour at room temperature followed by washing 3 times PBS. Further, the sections were allowed in BSA solution containing 1% goat anti-rabbit IgG (Dakopatts, Copenhagen, Denmark) for 30 minutes and then washed 3 times in PBS. After the reaction on 1% rabbit PAP complex (Dakopatts) for 30 minutes, the sections were washed 3 times in PBS and then stained by AEC (3-amino-9-ethyl carbazole) solution containing 0.3% H_2O_2 and stained with hematoxylin for nuclear stain.

2. CEA Staining Method

The same serial sections as the histopathologic specimens were used also for CEA stain. To demonstrate CEA, unlabelled PAP technique was employed. The staining chemicals and procedures were the same as those previously described precisely by Hamada et al (19) and us (20). The absorption of primary CEA antibody (Dakopatts, Copenhagen, Denmark) with perchloric acid extracts of the normal spleen, and tests for the specificity of the antibody were also performed as previously reported by Nagura and co-

workers (14).

3. Feulgen Staining Method and Measurement of Nuclear DNA Content

The diameter of 50 nuclei in 20 adenomas and 10 carcinomas was measured in the HE staining sections, and then the sections 1.2 times thicker than the average diameter were sliced from the portions just adjacent to the sections used for histopathologic study. The sections were stained with Feulgen reaction described precisely by Naora (21). Using a microspectrophotometer (MSPA, type IV, Olympus Co., Tokyo, Japan) with two-wave length method (22), nuclear DNA content was measured in 50 cells of every adenoma and carcinoma. Nuclear DNA content of 50 stromal lymphocytes was used as the control for the normal diploid content (2C), in order to determine variation in the DNA content of the neoplastic cells. By way of the measurement, only the whole nuclei were selected and measured with exception overlying and/or partially cut nuclei.

4. Statistical Analysis

The data were analyzed by the χ^2 test and Student's t test.

RESULTS

1. UEA-1 Localization

The UEA-1 localized pattern in cells was classified into 2 types; apical and diffuse types according to the difference in the localization within the cytoplasm. In the former, UEA-1 was localized only in the supra-nuclear cytoplasm. In the latter, UEA-1 was found in the supra-nuclear and also in the infra-nuclear portion of the cytoplasm (Fig. 1).

UEA-1 localization was seen in all of the carcinomas, which consisted of 7 (24.1%) apical and 22 (75.9%) diffuse types. The apical type was found only in early carcinomas and not observed in advanced carcinomas. Statistically, the apical type was found significantly ($P < 0.05$) more frequently in the former than

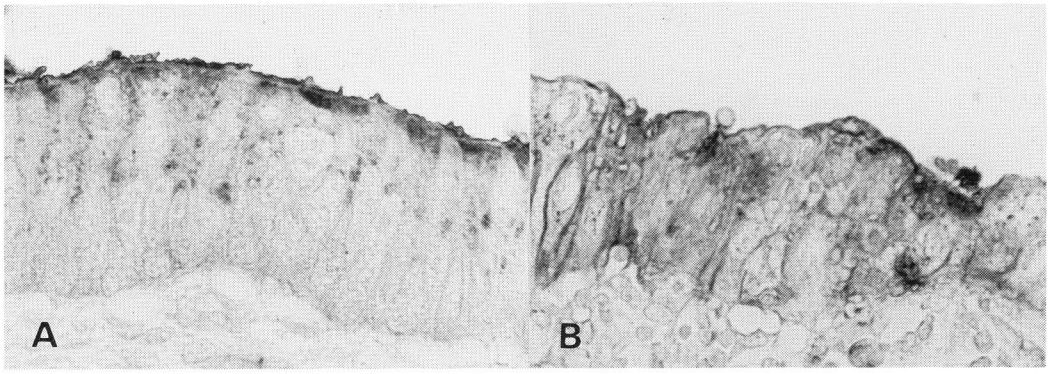

Figure 1. UEA-1 localized pattern in the cytoplasm of neoplastic cells. In the apical type (A), UEA-1 is found only in the supranuclear portion of the cytoplasm. However, in the diffuse type (B), it is diffusely localized in the cytoplasm and/or basolateral membrane. UEA-1 stain, × 800.

Table 1. UEA-1 localized pattern in the cytoplasm of adenoma and carcinoma cells.

Neoplastic lesion		No	Apical type (%)		Diffuse type (%)		Total (%)
Adenoma	mild dysplasia	35	23 (65.7)	36 (66.7) †1	4 (11.4)	7 (13.0) †2	27 (77.1) †3
	moderate dysplasia	19	13 (68.4)		3 (15.8)		16 (84.2) †3
Carcinoma	early	19	7 (36.8) †2	7 (24.1)	12 (63.2)	22 (75.9)	19 (100) †3
	advanced	10	0		10 (100)		10 (100) †3

†1 Significant difference from carcinoma ($P < 0.005$).
†2 Significant difference from advanced carcinoma ($P < 0.05$).
†3 Significant correlation among 4 neoplasms ($P < 0.005$).

in the latter. In adenomas, UEA-1 was confirmed in 43 (79.6%) adenomas and not seen in 11 (20.4%) adenomas; and these rectin localized adenomas consisted of 36 (66%) apical and 7(13%) diffuse types. Although no differences in the localized pattern was found between mild and moderate dysplasias, the apical type was noted significantly ($P<0.005$) more frequently in adenomas than in carcinomas. On the contrary, the diffuse type was found significantly ($P<0.005$) more frequently in carcinomas than in adenomas (Table 1).

2. CEA Localization

The CEA localized pattern in the cells, as well as the UEA-1 pattern, was also classified into the apical and diffuse types

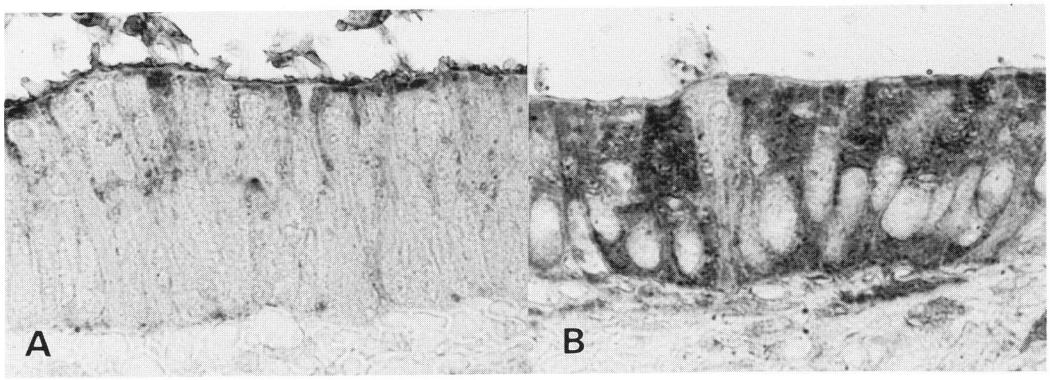

Figure 2. CEA localized pattern in the cytoplasm of neoplastic cells. In the apical type (A), CEA is shown only in the supranuclear portion. In the diffuse type (B), CEA is diffusely found in the cytoplasm and/or basolateral membrane. CEA stain, × 800.

Table 2. CEA localized pattern in the cytoplasm of adenoma and carcinoma cells.

Neoplastic lesion		No	Apical type (%)		Diffuse type (%)		Total (%)
Adenoma	mild dysplasia	35	11 (31.4)	19 (35.2)	3 (8.6) †2	8 (14.8) †1	14 (40.0) †3
	moderate dysplasia	19	8 (42.1)		5 (26.3) †2		13 (68.4) †3
Carcinoma	early	19	4 (21.1)	6 (20.7)	15 (78.9) †2	23 (79.3) †2	19 (100) †3
	advanced	10	2 (20.0)		8 (80.0)		10 (100) †3

†1 Significant difference from carcinoma ($P < 0.001$).
†2 Significant correlation among 4 neoplasms ($P < 0.005$).
†3 Significant correlation among 4 neoplasms ($P < 0.001$).

according to the localization in the cytoplasm; in the former, CEA was found only in the supa-nuclear portion and in the latter, CEA was diffusely observed in the cytoplasm including the supra-and infa-nuclear portions (Fig. 2).

CEA localization was found in 14(40%) adenomas with mild dysplasis, in 13(68.4%) adenomas with moderate dysplasia and in all 29 (100%) carcinomas. The frequency of the occurrence of localization significantly ($P<0.005$) increased in parallel with the increase of cellular dysplasia (Table 2). The relationship between the CEA localized types and cellular dysplasia is also shown in Table 2. The diffuse type was found in 3 (8.6%) adenomas with mild dysplasia, 5(26.3%) adenomas with moderate dysplasia and in 23(79.3%) carcinomas. This type of CEA localization was significantly ($P< 0.005$) correlated with the cellular dysplasia. On the other hand, the apical type was observed in 11 (31.4%) adenomas with mild dysplasia, 8 (42.1%) adenomas with moderate dysplasia and in 8 (21.1%) early carcinomas and 2 (20.0%) advanced carcinomas. The apical type was more frequently found in adenomas than in carcinomas. However, there was no significant difference among adenomas with mild and moderate dysplasias and carcinomas (Table 2).

3. Nuclear DNA Content

Twenty adenomas and 10 carcinomas were examied according to 3 variables in the cells which consisted of the main mode, the average of nuclear DNA content, and the proportion of cells over 4C. They are summalized in Table 3. No statistical difference in the main mode was found between adenomas with mild and moderate dysplasias or between early and advanced carcinomas. However, the main mode (2.3 ± 0.35C) in adenomas with moderate dysplasia was significantly ($P<0.005$) lower than that (3.3 ± 0.84C) in early carcinomas. The average DNA content and proportion of the cells over 4C were, respectively, 2.4 ± 0.27C and 1.8 ± 2.47 (range 0.0 – 8.0)% in the adenomas

Table 3. Nuclear DNA content in adenoma and carcinoma cells.

Neoplastic lesion		No	Main mode (C)*	Average DNA (C)*	Polyploidy cells over 4C (%)* and range [ ]
Adenoma	mild dysplasia	10	2.2 ± 0.26	2.4 ± 0.27	1.8 ± 2.74 [0.0-8.0]
	moderate dysplasia	10	2.3 ± 0.35 †1	2.6 ± 0.39 †2	4.6 ± 5.08 [0.0-14.0] †2
Carcinoma	early	10	3.3 ± 0.84	3.7 ± 0.52	32.0 ± 14.90 [8.0-42.0]
	advanced	10	3.9 ± 0.82	3.9 ± 0.96	38.0 ± 51.50 [10.0-88.2]

* mean ± standard deviation.
†1 significant difference from carcinoma ($P < 0.005$).
†2 significant difference from carcinoma ($P < 0.0005$).

with mild dysplasia, 2.6 ± 0.93C and 4.6 ± 5.08 (range 0.0 – 14.0)% in the adenomas with moderate dysplasia, 3.7 ± 0.52C and 32.0 ± 14.9 (range 8.0 – 42.0)% in the early carcinomas and 3.9 ± 0.96C and 38.0 ± 31.5 (range 10.0 – 88.0)% in the advanced carcinomas. The tendency for increase was noted in parallel with the advance in cellular dysplasia, and the proportion of polyploidy cells over 4C was higher than 8% in all the carcinomas. Although the average DNA content and proportion of the cells over 4C were not statistically noted between the adenomas with mild and moderate dysplasis or between the early and advanced carcinomas, these were significantly ($P<0.005$) higher in carcinomas than in adenomas. However, 1(10%) of 10 adenomas with mild dysplasia and 5(50%) of 10 adenomas with moderate dysplasia also showed the same proportion of polyploidy cells as that of carcinomas (Table 3).

DISCUSSION

In general, there has been wide acceptance of the concept presently known as the "adenoma-carcinoma sequence" in the human colorectum (1–5). The causes supporting the sequence are based on indications such as the presence of microscopical malignant foci in adenomas (1–5), the increasing malignancy rate in parallel with the growth of adenomas (1–3), the concurrence in adenomas and carcinomas (3, 4, 23), the common occurrence of carcinomas in adenomatosis or in muptiple adenomas (1, 22) *et cetra* (23). As a matter of fact, cases have been reported in which carcinomas have developed from pre-existing adenomas during the follow-up of patients (1, 24). In addition to human studies, experimental evidence also supports the adenoma-carcinoma sequence whereby adenomas frequently developed into carcinomas (25, 26).

On the other hand, changes of glycoconjugates in the cell membrane of malignant cells and also changes of glycoprotein

or protein production in malignant or neoplastic cells have been recently demonstrated in many studies (9–14). Nuclear DNA content, as well as glycoconjugates or glycoprotein, also has been clarified as different in the non-neoplastic cells and generally increase in neoplastic and malignant cells (15–17). However, few studies on the neoplastic cells including adenoma and carcinoma cells have been performed yet simultaneously from the point of view of the 3 varibbles above. Thus, in the present study, in order to elucidate the relationship between adenoma and carcinoma a rectin "UEA-1" specifically binding to glycoconjugates, CEA production or localization in the cells and nuclear DNA content were simultaneously examined in adenomas with different grades of dysplasia and in carcinomas of the human colorectum.

Glycoconjugate binding with UEA-1 is not seen in almost all the non-neoplastic mucosal cells of the left-sided colon and rectum (10). However, it is frequently demonstrated in neoplastic cells including those of carcinoma and adenoma, because a neoplastic glycoprotein with α-1-fucosyl residue may be produced or because the terminal carbohydrate structure of glycoprotein present in non-neoplastic mucosal cells may be altered to bind easily with UEA-1 after the neoplastic transformation (10). In the present study, UEA-1 location was confirmed in 77.1% of the adenomas with mild dysplasia, 84.2% of the adenomas with moderate dysplasia and in 100% of the carcinomas, and the positive rates increased in accordance with the advance of cellular dysplasia. Regarding the UEA-1 localized pattern, the diffuse and apical types were found, respectively, 11.4% and 65.7% in the adenomas with mild dysplasia, and 15.8% and 68.4% in the adenomas with moderate dysplasia; these type were found, respectively, in 63.2% and 36.8% of the early carcinomas and 0% and 100% of the advanced carcinomas. The result indicates that chemical changes of glycoconjugate binding with UEA-1 take place in carcinomas and even in adenomas as already reported by some investigators (10, 18), even though adenomas are not structurally confirmed to be malignant. This suggests that adenomas may biochemically have malignant potential, supporting the adenoma-carcinoma sequence.

In general, CEA is widely accepted as one of the tumor or neoplastic markers. In non-neoplastic mucosal cells, CEA is reported to exist only in the microvillus surface (13, 14). However, in carcinoma cells it is clarified to be characteristic that the antigen exists in the cytoplasm and also in the basolateral membrance (14, 14, 19, 20). Thus, the existence of CEA in the cytoplasm is thought to indicate carcinoma cells or cells with the same biological charateristics as carcinoma cells. Therefore, in the present study, the relationship between carcinoma and adenoma was examined through the CEA localization and the localized pattern in the cytoplasm. CEA localization was found in 40% of the adenomas with mild dysplasia, 68.4% of the adenomas with moderate dysplasia and in 100% of the early and advanced carcinomas, and the location in the cytoplasm increased in rate in accordance with the advance of cellular dysplasia. The apical and diffuse types were, respectively, 31.4% and 8.6% in adenomas with mild dysplasia, 42.1% and 26.3% in adenomas with moderate dysplasia, 21.1% and 78.9% in early carcinomas and 20.0% and 80.0% in advanced carcinomas. No difference of the CEA localized pattern was found between early and advanced carcinomas. However, significant difference

was seen in adenomas, and the diffuse type was significantly higher in adenomas with moderate dysplasia than in those with mild dysplasia. The result suggests that some of colorectal adenomas, even with mild dysplasia, have already acquired a biochemical characteristic of carcinoma preceding the structural carcinomatous change, as already proposed by some investigators (1–5, 23).

In human and experimentally induced neoplasms, analysis of nuclear DNA content has been frequently used as an indicator of malignant changes. Furthermore, it has been reported to be also an indicator of malignant potential in precarcinomatous lesions such as adenomatosis and ulcerative colitis, *etc* (15–17, 27). When the proportion of polyploidy cells over 4C is more 5.0% of all the cells, the lesions are generally thought to be malignant cells (28, 29). Additionally, when the proportions are between 2.0% and 4.0%, the lesions are reported to be borderline lesions having malignant potential (28, 29). In the present study, no statistical difference of main mode and average DNA content was found between early and advanced carcinomas or between adenomas with mild and moderate dysplasias, though a tendency to increase in parallel with the advance in cellular dysplasia was noted. However, the main mode and the average content were, respectively, 3.2C and 2.6C in adenomas with moderate dysplasia and 3.5C and 3.7C in early carcinomas. They were significantly higher in the latter in the former. The average proportion of polyploidy cells over 4C were, respectively, 1.8% (range 0–8)% in adenomas with mild dysplasia, 4.6 (range 0–14)% in adenomas with moderate dysplasia, 32.0 (range 8–42)% in early carcinomas and 38.0 (range 10–88)% in advanced carcinomas. Both early and advanced carcinomas showed the characteristic polyploidy of malignant cells. On the other hand, the proportion of polyploidy cells over 4C was distributed between 2% and 8% in 4 of the 10 adenomas with mild dysplasia and between 2% and 14% in 4 of the 10 adenomas with moderate dysplasia. Ten percents and 50%, respectively, of adenomas with mild and moderate dysplasias showed the same proportion of polyploidy cells as that of carcinomas. Therefore, from the point of view of nuclear DNA content in the cells, it is suggested that some of the adenomas have already acquired a carcinomatous characteristic and that some of them have been revealed to hold malignant potential, though they reveal no structual malignancy.

In conclusion, although changes of UEA-1 and CEA localization and nuclear DNA content in the cells are regarded as representing only some of the characteristics among many biological and/or biochemical changes which may occur in carcinoma cells, these changes are thought to occur prior to any structural or histological malignant changes. Furthermore, it is also suggested that colorectal adenomas have already acquired malignancy and/or a malignant potential, supporting the adenoma-caocinoma sequence.

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