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Relationship between morphological diversity and AgNORs or cathepsin B expression in colorectal cancers

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Abstract: The biological characteristics associated with the morphological diversity of colorectal cancers were investigated to elucidate the causes of this diversity. We examined the proliferative and infiltrating activity of tumor cells, indicated by the mean number of Ag nucleolar organizer region associated proteins (NORs) per nucleus (MNA) and the immunohistochemical response to cathepsin B(CB), in various morphological types of early and advanced colorectal cancers. We examined 73 colorectal cancers obtained by endoscopic and surgical resection. MNA values for sessile and flat-elevated cancers were greater than the values for pedunculate, subpedunculate, and flat-or-depressed early cancers (sessile, $P < 0.05$). In advanced cancers invading the muscularis propria, protruding cancers showed significantly higher MNA values than small ulcerative cancers ($P < 0.01$). CB expression increased significantly with the progression of colorectal cancers ($P < 0.01$), but was not related to morphological diversity in early and advanced cancers. In both sessile and flat cancers, CB expression was higher in moderately differentiated than in well differentiated adenocarcinomas. These results indicate that, in colorectal cancers, protruding early cancers without stalks and protruding advanced cancers have higher proliferative activity than pedunculate or flat early cancers and small ulcerative advanced cancers, respectively, and that CB expression is not associated with morphological diversity, but with depth of invasion and histological differentiation.

Key words: colorectal cancer, morphological diversity, flat type cancer, AgNORs, cathepsin B

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Introduction

The flat type of colorectal cancer exhibits clinicopathological and biological features that differ from those of polypoid cancers.[1–3] Flat type cancers often invade the submucosal layer while they are very small and the incidence of accompanying adenoma is very low. A molecular-biological assessment of flat type cancer has shown that the incidence of K-*ras* mutation, a frequent genetic change in common colorectal cancers, was very rare.[4,5]

Nucleolar oganizer regions (NOR), looped structures of ribosomal DNA located on acrocentric chromosomes,[6] are very important in protein synthesis.[7] NOR-associated proteins are argyrophilic, and silver-stained NOR associated proteins (AgNORs) are detectable as black dots of various sizes in the nucleolus and nucleus.[8] The number of AgNORs is both good indicator of cellular proliferative activity and a useful diagnostic tool with which to assess the malignancy of tumors.[8,9]

Proteases secreted by cancer cells have been investigated because of their possible role in cancer infiltration and metastasis. Cathepsin B (CB), a cysteine endopeptidase contained in the lysosome,[10] is implicated in invasion by cancer cells.[11,12] The expression of CB is closely associated with the staging of gastric and colorectal cancers.[13,14]

We considered that the proliferative and infiltrating activities of tumor cells would be associated with the development of tumors and that the morphology and pathway of progression during the early stage of colorectal cancers might be affected by these biological characteristics. In this study, we investigated the mean number of AgNORs per nucleus (MNA), and the immunohistochemical responses to CB and assessed the relationship between morphological diversity and these features in early and advanced colorectal cancers.

Materials and methods

Materials

Clinical materials, consisting of 73 colorectal cancers obtained by endoscopic and surgical resection, were retrospectively and specifically selected by the morphology. Specimens were obtained from the First Department of Surgery-Nagasaki University School of Medicine (Nagasaki, Japan), Sasebo City General Hospital, Isahaya Health Insurance General Hospital, and Fukuda Yutaka Clinic between 1984 and 1994. Two pathologists performed the histologic examinations, and the morphological classification was determined by their pathological findings in accordance with the *General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum* and *Anus* of the Japanese Research Society for Cancer of the Colon and Rectum.[15] We used the criteria for diagnosing flat type cancer proposed by Nagasako[16]: (1) Tubular cancer glands are arranged horizontally; (2) the top of elevation and the bottom of the depression are parallel to the normal mucosa; (3) the tumor height is less than double that of the normal mucosa; and (4) villous cancers are excluded. Specimens included 28 polypoid cancers (13 pedunculate [Ip], 6 subpedunculate [Isp], and 9 sessile [Is]) and 25 flat type cancers (13 flat-elevated [IIa] and 12 flat-or-depressed [IIb, IIc]). Further, 20 advanced cancers invading the muscularis propria (10 protruding [type 1] and 10 small ulcerative [type 2]) were examined. Seven adenomas and 16 adenomatous components with low-grade dysplasia[17] from Ip and Isp cancers were selected as controls. Representative sections of each tumor that had been fixed in 10% formalin neutral buffer solution for 24 h and paraffinized were stained.

Methods

AgNORS staining. We performed one-step silver staining, as described by Proton et al.[18] in 1986. The tissue was cut into 3 μ-m sections and deparaffinized. The reagent was prepared in the dark by combining one volume of 2% gelatin solution in 1% formic acid with two volumes fo 50% silver nitrate, then spreading the mixture over the sections at room temperature for 30 min.[18] The sections were then washed and destained with 5% sodium thiosulfate to detect AgNOR dots. The MNA[8] was counted in 100 nuclei in the superficial layer and in the deepest layer of each tumor, except for intramucosal cancers, under a light microscope (× 1000).

Cathepsin B staining. Each sample was cut into 5 μ-m sections and deparaffinized. The sections were immersed in 1% H_2O_2 with phosphate-buffered saline (PBS) for 10 min to inactivate endogenous peroxidase, then exposed for 1 h at 37°C to 1:100 diluted, sheep anti-human cathepsin B antiserum (Binding Site, Birmingham, UK) as the primary antibody. The sections were reacted with biotinylated rabbit anti-sheep immunoglobulin and ABC reagent, using a Vectastatin ABC kit (Vector Laboratories Inc., Burlingame, Calif.). After the peroxidase reactions were visualized with 0.01% H_2O_2 and 3,3′-diaminobenzidine, the sections were observed under a light microscope (× 400). The response to staining was assessed according to the criteria of Higashiyama et al.,[19] and sections containing 10% or more stained cells were regarded as positive.

Statistical analysis

All MNA values were expressed as means ± SD and data were tested by one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test. The incidence of CB expression was tested using the χ^2 test for differences in percentages. A two-tailed P value below 0.05 was regarded as significant in both tests.

Results

Histopathological features of specimens

The adenomas and adenomatous components with cancer consisted of 6 tubular adenomas, 16 tubulovillous adenomas, and 1 villous adenoma.[17] The histopathological features of the cancers are summarized in Table 1. All early colorectal cancers had a tumor diameter less than 20 mm. All samples of Ip and Isp involved well differentiated adenocarcinomas, while other early cancers included well and moderately differentiated adenocarcinomas. Neither lymphatic (ly) nor venous (v) invasion was found in the polypoid cancers, although these modes of invasion were seen in some flat type cancers. There were no lymph node metastases in any of the early cancers. In the advanced cancers, the tumor size in all type 1 specimens exceeded 20 mm, while that of type 2 was less than 20 mm. The rates of ly and v invasion in each of these types were higher than those of early cancers, but lymph node metastasis was not frequent.

AgNOR staining

AgNOR sites were visualized as granular black dots, of various sizes, in the nucleolus and the nucleus (Fig. 1). The MNA value for each morphological group is shown in Fig. 2. The MNA values for adenomas and adenomatous components with cancer were 4.7 ± 0.6, and 5.5 ± 1.0, respectively. The latter was significantly greater than that for normal epithelium (3.9 ± 0.9) ($P < 0.01$). In relation to the histological subtype of the

Table 1. Histopathological features of various morphological types of colorectal cancer examined

Morphological type	Tumor size (mm)	Histological grade of differentiation		Depth of invasion		ly(+)[a]	v(+)[b]	n(+)[c]
		Well	Moderately	m	sm			
Early cancers								
Polypoid type								
Ip (n = 13)	13.8 ± 4.5	13	0	10	3	0	0	0
Isp (n = 6)	10.5 ± 3.5	6	0	4	2	0	0	0
Is (n = 9)	15.1 ± 3.3	6	3	5	4	0	0	0
Flat type								
IIa (n = 13)	10.9 ± 3.0	6	7	4	9	2	1	0
IIb, IIc (n = 12)	8.8 ± 3.9	5	7	4	8	0	1	0
Advanced cancers								
Type 1 (n = 10)[d]	23.9 ± 2.6	2	8			8	5	1
Type 2 (n = 10)[d]	17.0 ± 2.5	3	7			8	4	1

[a] Lymph duct invasion
[b] Venous invasion
[c] Lymph node metastasis
[d] All tumors invaded the muscularis propria

adenomas, the MNA for villous adenomas and villous components with cancer (6.2 ± 1.1) was slightly greater than the value for tubular adenomas and tubular components with cancer (5.1 ± 0.7) (data not shown). The MNA for all types of colorectal cancer was elevated compared to that for adenomas and adenomatous components with cancer. The MNA values in the early cancers were 8.6 ± 2.0 for Is, 8.2 ± 2.2 for IIa, 6.7 ± 1.7 for Ip, Isp and 6.6 ± 1.3 for IIb, IIc. The MNA values for Is and IIa were similar as were those for Ip, Isp and IIb, IIc. The MNA value for Is was significantly greater than the values for Ip, Isp and IIb, IIc ($P < 0.05$), whereas in advanced cancers, the MNA for type 1 (10.4 ± 3.3) was significantly greater than that for type 2 (7.8 ± 1.7) ($P < 0.01$). The MNA values for the intramucosal cancers and for the superficial and the deepest layers of submucosal and advanced cancers are shown in Fig. 3. There were no significant differences in the MNA values among the three layers in any of the cancers. In advanced cancers, the MNA value for the deepest layer in type 1 was significantly greater than that for the deepest layer in small type 2 ($P < 0.05$).

The correlation between MNA and histological grade is shown in Fig. 4. As Ip and Isp were well differentiated adenocarcinomas, as described above, the MNA for all

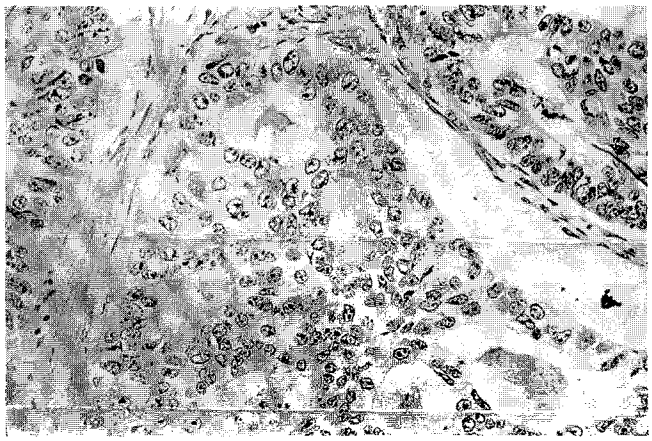

Fig. 1. Argyrophil nucleolar organizer region (*AgNORs*) staining of colorectal cancer. Most cancer cells contained several black dots of various sizes in the nucleolus and nucleus. Silver stain, × 100

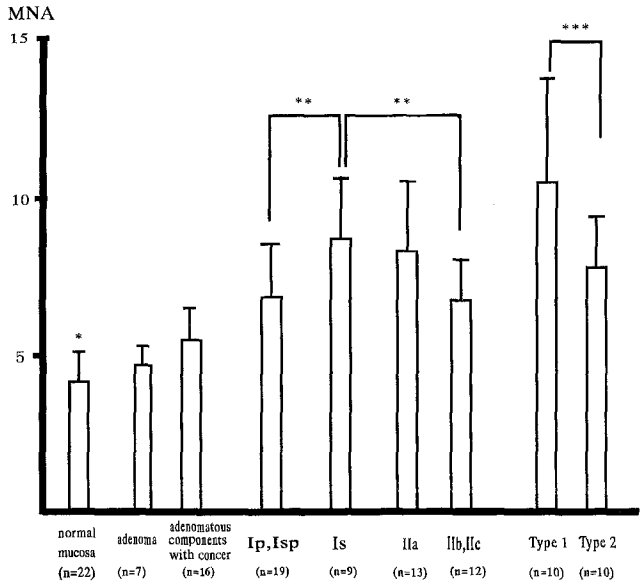

Fig. 2. Mean number of AgNORs per nucleus (*MNA*) in relation to morphological subtype of colorectal cancer. Early cancers consisted of polypoid type (*Ip, Isp, and Is*) and flat type (*IIa and IIb, IIc*). Advanced cancers consisted of protruding (*type 1*) and small ulcerative (*type 2*) cancers. Data were analyzed by Dunnett's multiple comparison test (two-tailed). * $P < 0.01$ relative to all cancers; * $P < 0.05$; ** $P < 0.01$

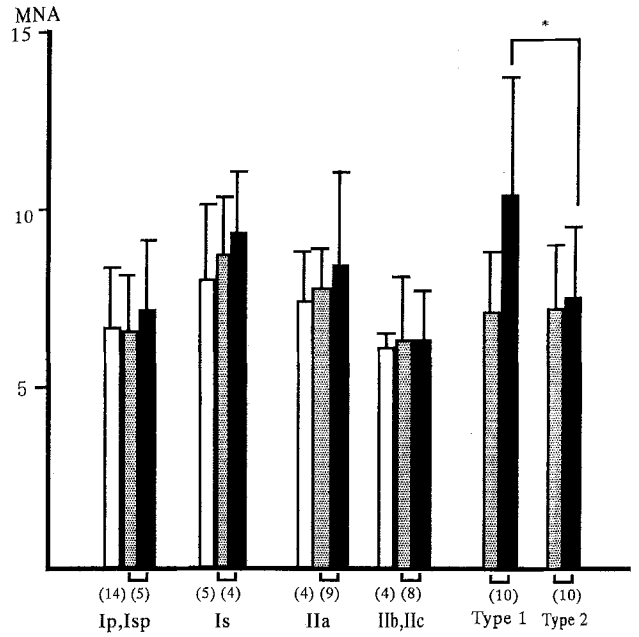

Fig. 3. Mean numbers of AgNORs in intramucosal cancers, and in each layer of submucosal and advanced colorectal cancers. *White bars*, *dotted bars*, and *black bars* indicate intramucosal cancers, the superficial layer of submucosal cancers, and the deepest layer of advanced cancers, respectively. Data were analyzed by Dunnett's multiple comparison test (two-tailed). $*P < 0.05$

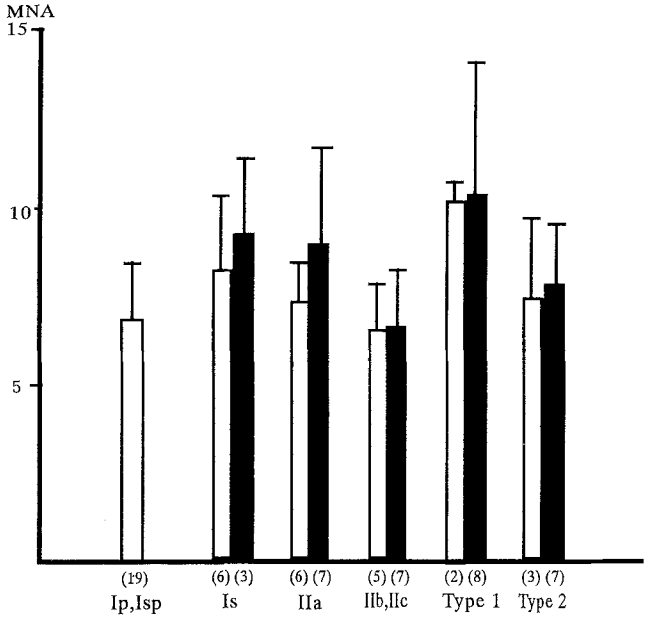

Fig. 4. Mean number of AgNORs in relation to histological differentiation in each morphological type of colorectal cancer. The *white bars* and *black bars* represent well and moderately differentiated adenocarcinomas, respectively

Table 2. Morphological type and cathepsin B expression

Morphological type	Incidence of cathepsin B expression		Significance
	Number	Percentage	
Adenoma (n = 23)	5/23	22	
Adenoma only (n = 7)	2/7	29	
Adenomatous components with cancer (n = 16)	3/16	19	
Early cancer (n = 53)	33/53	62	**
Ip, Isp (n = 19)	12/19	63	
Is (n = 9)	6/9	67	
IIa (n = 13)	8/13	62	
IIb, IIc (n = 12)	7/12	58	
Advanced cancer (n = 20)	20/20	100	
Type 1 (n = 10)	10/10	100	
Type 2 (n = 10)	10/10	100	

** $P < 0.01$
Data were analyzed by the χ^2 test

groups of well differentiated cancers were compared. However, there were no significant differences among any early cancers.

Immunohistochemical response of cathepsin B

CB staining in the cytoplasm of cancer cells was granular or diffuse (Fig. 5a). A comparison of responses to cathepsin B staining in the different morphological types is shown in Table 2. The CB positive rate was significantly elevated in the order of adenomas (22%), early cancers (63%), and advanced cancers (100%) ($P < 0.01$). However, there was no difference in CB expression between any early cancers or between the two types of advanced cancer. All advanced cancers were CB-positive. The incidence of CB expression in

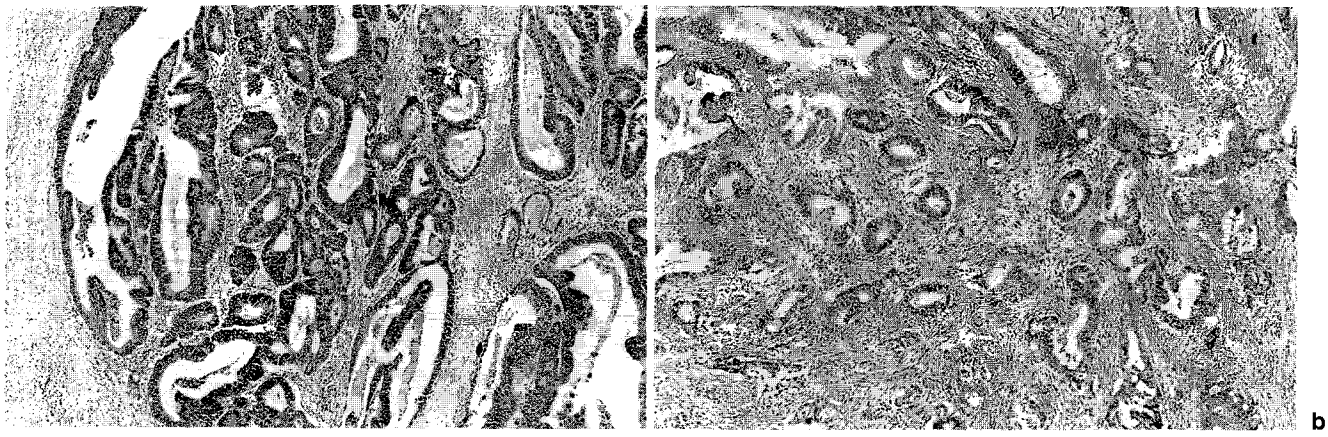

Fig. 5a,b. Expression of cathepsin B in colorectal cancers. **a** Strong positive response to cathepsin B is observed in the cytoplasma of cancer cells. **b** Negative response to cathepsin B. DAB, × 100

Table 3. Cathepsin B expression in each morphological type of cancer in the various layers examined

Morphological type		Cathepsin B expression		Significance
		Number	Percentage	
Intramucosal cancers (n = 27)		10/27	37	
Ip, Isp (n = 14)		7/14	50	
Is (n = 5)		2/5	40	
IIa (n = 4)		0/4	0	**
IIb, IIc (n = 4)		1/4	25	**
Submucosal cancers (n = 26)	SP	21/26	81	
	DP	23/26	89	
Ip, Isp (n = 5)	SP	5/5	100	
	DP	5/5	100	
Is (n = 4)	SP	3/4	75	
	DP	4/4	100	
IIa (n = 9)	SP	7/9	78	
	DP	8/9	89	
IIb, IIc (n = 8)	SP	6/8	75	
	DP	6/8	75	
Advanced cancers (n = 20)	SP	15/20	85	*
	DP	20/20	100	**
Type 1 (n = 10)	SP	8/10	80	
	DP	10/10	100	
Type 2 (n = 10)	SP	7/10	70	
	DP	10/10	100	

* $P < 0.05$; ** $P < 0.01$
Data were analyzed by the χ^2 test
SP, Superficial layer; *DP*, deepest layer

intramucosal cancer and in each layer of submucosal and advanced cancers is shown in Table 3. In all early cancers, CB expression in the superficial (21/26: 81%) and the deepest layers (23/26: 89%) of submucosal cancers was significantly greater than that in intramucosal cancers (10/27: 37%) ($P < 0.01$), and this tendency was similar in each type of cancer. In submucosal and advanced cancers, CB expression was similar in the superficial and the deepest layers.

The correlation between histological grade and CB expression is shown in Table 4. The CB-positive rate of moderately differentiated adenocarcinomas in Is, IIa and IIb, IIc (14/17: 82%) was significantly greater than that of well differentiated cancers (7/17: 41%) ($P < 0.05$). In each histological grade of sessile and flat cancers, however, there was no significant difference in CB expression among each morphological types of cancers.

Table 4. Histological grade and cathepsin B expression

Morphological type	Cathepsin B expression		Significance
	Number	Percentage	
Ip, Isp (n = 19)[a]	12/19	63	
Sessile and flat early cancers			
Well differentiated adenocarcinomas (n = 17)	7/17	41	
Is (n = 6)	3/6	50	
IIa (n = 6)	2/6	33	*
IIb, IIc (n = 5)	2/5	40	
Moderately differentiated adenocarcinomas (n = 17)	14/17	82	
Is (n = 3)	3/3	100	
IIa (n = 7)	6/7	86	
IIb, IIc (n = 7)	5/7	71	

* $P < 0.05$
Data were analyzed by the χ^2 test
[a] All Ip and Isp were well differentiated adenocarcinomas. All advanced cancers expressed cathepsin B

Discussion

Although polypoid cancers originating from protruding adenomas may develop into protruding advanced cancers, and finally become large ulcerative cancers, flat type cancers may develop de novo[20] or from flat micro adenomas[21,22] and directly develop into small ulcerative cancers.[23] The morphological differences in cancer tissues could depend on different biological characteristics, including the proliferative and infiltrating activity of the various tumor cells.

Bromodeoxyuridine,[24] DNA polymerase α,[25] Ki-67,[26] and proliferating cell nuclear antigen[27] are indicators of cellular proliferation activity. The number and the size of AgNORs is also related to cellular proliferative activity,[28–30] as well as to cellular differentiation.[31] A few studies of the proliferative activity in the flat type of colorectal adenomas and cancers have been published.[32–34] Mitomi et al.[32] have reported that the PCNA labelling index (LI) is higher in the order of polypoid, flat elevated, and flat or depressed adenomas. Yamada and Iwashita[33] have reported similar results for the Ki-67 LI in adenomas. Murata[34] evaluated flat type neoplasms by means of AgNORs, and reported that the score in IIc was higher than that in Is or IIa. We postulated that the proliferative activity for flat type cancers, especially flat-or-depressed (IIb, IIc) types, would be higher than that for other types of early colorectal cancers, since flat cancers readily invade the deeper layer while still very small.[2,3] However, contrary to our notion and the findings of Murata et al.,[34] we found higher MNA values in early Is and IIa cancers. The MNA values they reported (less than 3.0) were considerably lower than ours and there seemed to be no significant differences among Is, IIa and IIc. We also counted all small clear dots in the nucleolus and nucleus, following the procedure of Crocker and McGovern.[8] If these dots are not counted, it is impossible to distinguish malignant from benign cells. Our results and those of others[32,33] indicate that flat or depressed cancers do not necessarily have high proliferative activitiy. Although Ip and Isp cancers also protrude into the lumen, the MNA value for these cancers has smaller than the values for Is and IIa types. It is possible that Is and IIa, which protrude widely, without adenomas, are essentially different from Ip or Isp and have higher proliferative activity. Is and IIa may be similar with regard to proliferative activity.

As mentioned above, it is thought that protruding cancers develop into protruding advanced cancers, whereas flat type cancers develop directly into ulcerative cancers when their diameter is less than 20 mm.[16,23,35] In this study, we found the MNA for type 1 was significantly greater than that for small type 2 and that, protruding cancers had high proliferative activity in advanced cancers, as well as in early cancers. Moreover, the MNA value for small type 2 was not greater than the values for Is and IIa. These results indicate that there are similarities among protruding and depressed cancers, which may support the theory that Is and IIa develop into protruding advanced cancers, whereas IIb, IIc develop into ulcerative advanced cancers during the progression from early to advanced colorectal cancers.

Watari and Yokota[36] examined cells proliferation by Ki-67 staining. They reported that, although Ki-67-positive cells were noted mainly in the upper third of neoplastic glands in superficial elevated lesions, there was no difference in Ki-67 staining in whole glands of clear and wide depression neoplasma without marginal

elevation. In this study, we examined AgNORs staining in each layer of the cancer lesions. However, the MNA value in early cancers did not differ markedly among the intramucosal cancers and each layer of submucosal cancer in any of the cancer groups and it seemed that all layers had similar AgNOR activity. We also examined the relationship between histological grade and MNA. Rayter et al.[37] reported that the number of AgNORs was not related to histologic differentiation in colorectal cancer. We also found that the MNA was not associated with histological grade for any cancers and that there were no differences among groups with the same histological grade.

Three steps—attachment, migration, and local proteolysis—have been proposed to constitute the process of tumor cell invasion. Several classes of proteinase have been implicated in this process. Of these, CB is an important indicator of tumor invasion in various cell lines[11,12] and solid tumors.[38–41] According to a report by Buck et al.,[38] CB induces the denaturation and destruction of collagen, fibronectin, laminin, proteoglycans, and other substances contained in the extracellular matrix, and abnormal CB activity may be related to the development of human cancers. Some investigators have reported that increased CB activity is also related to the progression and prognosis of colorectal cancers.[14,42,43] To date, to our knowledge the infiltrative activity of flat colorectal cancers has not been reported. In this study, we found the incidence of CB expression increased significantly from adenomas to advanced colorectal cancers. However, there was no significant difference in CB expression among each group of early cancers, or between the two advanced cancers. CB expression in colorectal cancers has been shown to be correlated with tumor progression.[14,42] In this study, CB expression was increased in all layers of submucosal compared with intramucosal cancers. As there were no significant differences between layers in the submucosal or advanced cancers, it appears that all cancer layers may have similar proteinase content, as well as similar proliferative activity.

Regarding the relationship between CB expression and histological grade, although Sheahan et al.[42] have reported that the CB-positive rate was higher in less differentiated cancers, Campo et al.[14] did not find any correlation with the histological classification. Our results showed that, in early cancers, CB expression in moderately differentiated adenocarcinomas was more frequent than in well differentiated adenocarcinomas, findings that support the results of Sheahan et al.[42]

In this study, we investigated the morphological diversity of early colorectal cancers and, however, the clinical implications can not be sufficiently answered. However, we suggest that if activities of AgNORs and CB are high on biopsied or surgical cancer specimens, the degree of malignancy and invasion of cancers may be predicted.

In conclusion, high MNA values were associated with the protruding growth of Is, IIa, and type 1 colorectal cancers and these early and advanced cancers showed similar proliferative characteristics. Although CB expression was related to tumor invasion from adenomas to advanced cancers and to histological differentiation, it was not related to the morphological diversity of cancer. We speculate that other specific features of cancer cells also affect the morphology of colorectal adenomas or cancers and we believe that further examinations are necessary to clarify the biological characteristics of the flat type of colorectal cancers.

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References

1. Shimoda T, Ikegami M, Fujisaki J, et al. Early colorectal carcinoma with special reference to its development de novo. Cancer 1989;64:1138–1146.
2. Muto T. Endoscopic and histological features of small early carcinomas of the colon with special reference to flat elevation and type IIa carcinoma (in Japanese with English abstract). Ito Cho (Stomach Intestine) 1987;22:397–406.
3. Tanaka S, Tatsuta S, Ohtsu N, et al. Assessment for development of superficial colorectal neoplasm—endoscopic and clinicopathologic analysis of 149 submucosal invasive carcinomas (in Japanese with English abstract). Nippon Shokakibyo Gakkai Zasshi (Jpn J Gastroenterol). 1994;91:1182–1189.
4. Satonaka K, Nagasako K, Fujimori T, et al. The study of K-*ras* point mutation in superficial type of colorectal cancer (in Japanese with English abstract). Shoukakiganno Hasseito Shinten (J Jpn Res Soc Gastroenterol Cacinogenesis) 1991;3:181–184.
5. Yamagata S, Muto T, Uchida Y, et al. Lower incidence of K-*ras* codon 12 mutation in flat colorectal adenomas than in polypoid adenomas. Jpn J Cancer Res 1994;85:147–151.
6. Kurvink K, Monica K, Porzucek L. Acrocentric interconnections and NOR variants in human lymphocytes. Cancer Genet Cytogenet 1990;50:207–226.
7. Crocker J, Nar P. Nucleolar organizer regions in lymphomas. J Pathol 1987;151:111–118.
8. Crocker J, McGovern J. Nucleolar organiser regions in normal, cirrhotic, and carcinomatous livers. J Clin Pathol 1988;41:1044–1048.
9. Derenzini M, Romagnoli T, Mingazzini P, et al. Interphasic nucleolar organizer region distribution as a diagnostic parameter to differentiate benign from malignant epithelial tumors of human intestine. Virchows Arch [B] 1988;54:334–340.
10. Greenhaum LM, Fruton JS. Purification and properties of beef spleen cathepsin B. J Biol Chem 1957;226:173–180.
11. Maciewics RA, Wardale JR, Etherington DJ, et al. Immunodetection of cathepsins B and L present in and secreted from human premalignant and malignant colorectal tumour cell lines. Int J Cancer 1989;43:478–486.
12. Honn KV, Timar J, Rozhin J, et al. A lipoxygenase metabolite, 12-(S)-HETE, stimulates protein kinase C-mediated release of

cathepsin B from malignant cells. Exp Cell Res 1994;214:120–130.
13. Watanabe M, Higashi T, Watanabe A, et al. Cathepsin B and L activities in gastric cancer tissue: Correlation with histological findings. Biochem Med Metabol Biol 1989;42:21–29.
14. Campo E, Munoz J, Miquel R, et al. Cathepsin B expression in colorectal carcinomas correlates with tumor progression and shortended patient survival. Am J Pathol 1994;145:301–309.
15. Japanese Research Society for Cancer of the Colon and Rectum. General rules for clinical and pathological studies on cancer of the colon, rectum and anus. 5th ed. Tokyo: Kanehara, 1994;5–6.
16. Nagasako K. Natural history of superficial colorectal early cancers—from superficial to ulcerated lesions (in Japanese with English abstract). Nippon Shokaki Naishikyou Gakkai Zasshi (Gastroenterol Endosc) 1993;5:289–296.
17. Jass JR, Sobin LH. Large intestine. World Health Organization's histological typing of intestinal tumours. 2nd ed. Berlin Heidelberg New York London Paris Tokyo Hong Kong: Springer 1989;29–40.
18. Proton D, Menager M, Jeannesson P, et al. Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer region at the optic level. Histochem J 1986;18:5–14.
19. Higashiyama M, Doi O, Kodama K, et al. Cathepsin B expression in tumour cells and laminin distribution in pulmonary adenocarcinoma. J Clin Pathol 1993;46:18–22.
20. Spratt JS, Ackerman LV. Small primary adenocarcinomas of the colon and rectum. JAMA 1962;179:337–346.
21. Adachi M, Muto T, Morioka Y, et al. Flat adenoma and flat mucosal carcinoma (IIb Type)—a new precursor of colorectal carcinoma? Dis Colon Rectum 1988;31:236–243.
22. Rubio C, Shetye J. Flat adenoma-adenocarcinoma sequence in the colon of rats. Dis Colon Rectum 1994;37:1300–1306.
23. Shimoda T. Pathological features of early colorectal carcinomas originating from superficial type (in Japanese with English abstract). Ito Cho (Stomach Intestine) 1995;30:141–147.
24. Raza A, Ukar K, Preisler HD. Double labeling and in vitro versus in vivo incorporation of bromodeoxyuridine in patients with acute nonlymphocytic leukemia. Cytometry 1985;6:633–640.
25. Mushika M, Miwa T, Suzuoki Y, et al. Detection of proliferative cells in dysplasia, carcinoma in situ, and invasive carcinoma of the uterine cervix by monoclonal antibody against DNA polymerase α. Cancer 1988;61:1182–1186.
26. Gerdes J, Schwab U, Lemke H, et al. Production of a mouse monoclonal antibody reactive with human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13–20.
27. Bravo R, Macdonald-Bravo H. Changes in the nuclear distribution of cyclin (PCNA) but not its synthesis depend on DNA replication. EMBO J 1985;655–661.
28. Janmohamed RMI, Murray PG, Crocker J, et al. Sequential demonstration of nucleolar organizer regions and Ki67 immunolabeling in non-Hodgkin's lymphomas. Clin Lab Haematol 1990;12:395–399.
29. Trere D, Pession A, Derenzini M. The silver-stained proteins of interphasic nucleolar organizer regions as a parameter of cell duplication rate. Exp Cell Res 1989;184:131–137.
30. Derenzini M, Pession A, Trere D. Quantity of nucleolar silver-stained proteins is related to proliferating activity in cancer cells. Lab Invest 1990;63:137–145.
31. Reeves BR, Casey S, Honeycombe JR, et al. Correlation of differentiation and silver staining of nucleolar organizers in the promyelocytic cell line HL-60. Cancer Genet Cytogenet 1984; 13:159–166.
32. Mitomi H, Atari E, Uesugi H, et al. Pathlogical study of flat and polypoid type adenomas of the colon (in Japanese with English abstract). Nippon Daichou Koumonbyo Gakkai Zasshi (J Jpn Soc Coloproctol) 1994;47:401–412.
33. Yamada Y, Iwashita A. Immunohistochemical study of minute adenomas of the large intestine (in Japanese with English abstract). Ito Cho (Stomach Intestine). 1995;30:1543–1550.
34. Murata N. Relationship between macroscopic appearance and objective atypism of Ag-NORs in minute colorectal epithelial neoplasms (in Japanese with English abstract). Nippon Daichou Koumonbyou Gakkai Zasshi (J Jpn Soc Coloproctol). 1995; 48:281–288.
35. Kasumi A, Kratzer GL. Fast-growing cancer of the colon and rectum. Am Surg 1992;58:383–386.
36. Watari J, Yokota K. Macroscopic classification and kinetic alterations in the superficial lesions of colorectal neoplasmas (in Japanese with English abstract). Nippon Shokakibyo Gakkai Zasshi (Jpn J Gastroenterol) 1994;91:2040–2048.
37. Rayter Z, Surtees P, Tildsley G, et al. The prognostic value of argyrophil nucleolar organiser regions (AgNORs) in colorectal cancer. Eur J Surg Oncol 1992;18:37–40.
38. Buck MR, Karustis DG, Day NA, et al. Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumor tissues. Biochem J 1992;282:273–278.
39. Visscher DW, Sloane BF, Sameni M, et al. Clinicopathologic significance of cathepsin B immunostaining in transitional neoplasia. Mod Pathol 1994;7:76–81.
40. Sukoh N, Abe S, Nakajima I, et al. Immunohistochemical distributions of cathepsin B and basement membrane antigens in human lung adenocarcinoma: Association with invasion and metastasis. Virchows Arch [A]. 1994;424:33–38.
41. Terada T, Ohta T, Minato H, et al. Expression of pancreatic trypsinogen/trypsin and cathepsin B in Human cholangiocarcinomas and hepatocellular carcinomas. 1995;26:746–752.
42. Sheahan K, Shuja S, Murnane MJ. Cysteine protease activities and tumor development in human colorectal carcinoma. Caner Res 1989;49:3809–3814.
43. Corticchiato O, Cajot J-F, Abrahamson M, et al. Cystatin and cathepsin B in human colon carcinoma: Expression by cell lines and matrix degradation. Int J Cancer 1992;52:645–652.