

## Phenotypic Expression of Colorectal Adenocarcinomas with Reference to Tumor Development and Biological Behavior

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**The purpose of this study is to clarify the correlation between cell differentiation and tumor development, including tumor aggressiveness and biological behavior. Eighty-three cases of advanced colorectal adenocarcinoma were randomly selected. Using immunohistochemical staining with antibodies to CD10, MUC2 and human gastric mucin (HGM), the colorectal adenocarcinomas could be classified into five types (18 small intestinal, 27 large intestinal, 2 gastric, 9 mixed and 27 unclassified). Each type had characteristic features. The small-intestinal type showed a relatively lower incidence of lymphatic permeation and higher venous invasion. The large-intestinal type showed a low incidence of venous invasion and lymph node metastasis. The mixed type revealed female and right-side-dominant distribution, large tumor size, high incidence of mucinous carcinoma, and low incidence of venous invasion. Gastric type was seen in only two cases (2%), which exhibited high histologic grade, lymphatic permeation and lymph node metastasis with no venous invasion. Such phenotypic classifications are considered to be useful not only for evaluation of the biological behavior of the carcinoma, but also for analysis of tumorigenesis.**

Key words: Colorectal carcinoma — Phenotype — MUC2 — CD10 — Human gastric mucin

On the basis of the Lauren classification,<sup>1)</sup> gastric carcinomas were classified into two types, intestinal type and diffuse type. Following recent advances in mucin histochemistry and immunohistochemistry, it has been clarified that differentiated adenocarcinomas can be classified into two subtypes, gastric and intestinal phenotypes.<sup>2,3)</sup> Some authors reported that the phenotypic expression was related to the tumor growth pattern and aggressiveness.<sup>4,5)</sup> We have already suggested that gastric carcinomas could be classified into four types (small intestinal, incomplete intestinal, gastric, and undifferentiated) by analogy with intestinal metaplasia.<sup>6,7)</sup>

It has been suggested that MUC2-positive colorectal adenocarcinomas, which exhibit goblet cell differentiation, have a relatively good prognosis or weaker liver-colonizing activity,<sup>8,9)</sup> so cell differentiation may be inversely correlated with tumor aggressiveness. However, the correlation has not yet been fully evaluated in colorectal tumors. We also reported an unusual type of colorectal carcinoma, “serrated adenocarcinoma,” which showed gastric-phenotypic expression.<sup>10)</sup> However, its incidence has not been elucidated.

The purposes of this study are 1) to clarify the correlation between cell differentiation and tumor development, including biological behavior, and 2) to investigate the incidence of colorectal “serrated adenocarcinoma” with gastric differentiation.

### MATERIALS AND METHODS

Eighty-three cases of colorectal adenocarcinomas were randomly selected for this study from the surgical files of the Graduate School of Medical Sciences, Kyushu University (Fukuoka), which were collected from its affiliated hospitals.

The histological classification is based on the Japanese Classification of Colorectal Carcinoma.<sup>11)</sup> In order to evaluate venous invasion, representative sections of all lesions were stained with Elastica van Gieson stain.

Paraffin-embedded blocks from these lesions were available for immunohistochemistry. We selected three immunohistochemical markers for cell differentiation: MUC2 for intestinal goblet cells,<sup>12–14)</sup> CD10 for small intestinal brush border<sup>15–18)</sup> and human gastric mucin (HGM) for gastric differentiation.<sup>19,20)</sup> A representative section from each tumor was processed for immunohistochemical staining with monoclonal antibodies against MUC2 (Novocastra, Newcastle-upon-Tyne, UK, diluted 1:200), CD10 (Novocastra, diluted 1:200) and HGM (45M1, Novocastra, diluted 1:50). Four-micron-thick sections were cut, deparaffinized in xylene and dehydrated in an ethanol series. For the antigen retrieval of CD10 and MUC2, slides were treated with microwave heating in citrate buffer (pH 6.0) for 30 min. Endogenous peroxidase activity was blocked by 30 min of incubation with 0.3% hydrogen peroxidase in absolute methanol. Background staining was minimized by incubation with 1% normal goat serum for 10 min. Sections were incubated with a pri-

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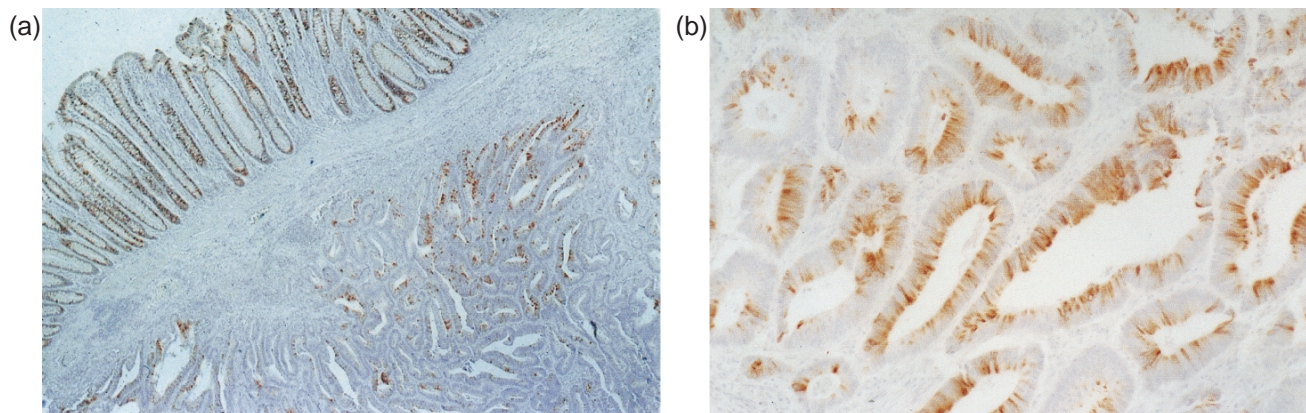


Fig. 1. Large-intestinal type. (a) MUC2 is diffusely positive. MUC2 is also positive in goblet cells of normal colonic mucosa (MUC2,  $\times 30$ ). (b) High-power view reveals that the cytoplasm of carcinoma cells is stained by MUC2 (MUC2,  $\times 100$ ).

Table I. Correlation between MUC2 Expression and Clinicopathologic Features

		MUC2(+) (n=41)	MUC2(-) (n=42)
Age (yrs)	(average)	64	66
Sex	(male:female)	20:21	22:20
Location	(right:left)	17:24	13:24
Size (mm)	(average)	54	55
Histologic grade	(well:mod/poor:muc)	20:15:6	22:20:0
ly permeation (%)	(positive rate)	76	60
v invasion (%)	(positive rate)	15	31
LN metastasis (%)	(positive rate)	37	41

well, well differentiated; mod, moderately differentiated; poor, poorly differentiated; muc, mucinous; ly, lymphatic; v, venous; LN, lymph node.

mary antibody overnight at 4°C. This was followed by testing with a streptavidin-biotin-peroxidase kit (Nichirei, Tokyo). Diaminobenzidine tetrahydrochloride was used as the chromogen. Finally, sections were counterstained with Mayer's hematoxylin or methyl green.

Immunopositivity was designated as sporadically positive (1+) when <5% of cells were positive, focally positive (2+) when 5–50% cells were positive, and diffusely positive (3+) when >50% cells were positive. Immunoreactivity of 2+ or greater was regarded as positive, and that of 1+ or less, as negative.

**RESULTS**

**MUC2 immunohistochemistry** Forty-one (49%) of the 83 cases were positively stained with MUC2, within the

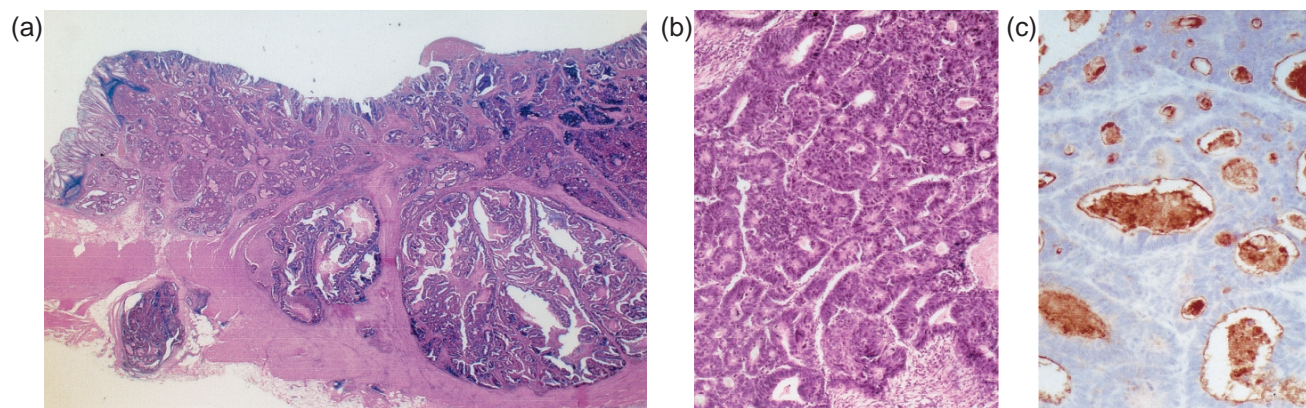


Fig. 2. Small-intestinal type. (a) Low-power view of the carcinoma. The carcinoma involves the whole thickness of the colonic wall with prominent vascular invasion (H&E,  $\times 7$ ). (b) High-power view of the carcinoma reveals moderately differentiated adenocarcinoma (H&E,  $\times 70$ ). (c) CD10 is positive along the luminal surface of the carcinoma glands (CD10,  $\times 70$ ).



cytoplasm around the nuclei (Fig. 1). The correlation between MUC2 expression and clinicopathologic features is summarized in Table I. The incidence of venous invasion was lower in the MUC2(+) group than in the MUC2(-) group, but the difference was not significant. There was no difference in clinicopathologic factors between the MUC2(+) and MUC2(-) groups. In addition, all six mucinous adenocarcinomas were strongly positive for MUC2.

**CD10 immunohistochemistry** Eighteen (22%) of the 83 cases were positively stained with CD10, along the apical portion of the carcinomatous tubules (Fig. 2). In the CD10(+) cases, CD10 tended to be more frequently expressed in the center and deep areas of the tumors than in their periphery. The correlation between CD10 expression and clinicopathologic features is summarized in Table II. The incidence of venous invasion was significantly

Table II. Correlation between CD10 Expression and Clinicopathologic Features

		CD10(+) (n=18)	CD10(-) (n=65)
Age (yrs)	(average)	69	64
Sex	(male:female)	11:7	31:34
Location	(right:left)	4:14	26:39
Size (mm)	(average)	47	56
Histologic grade	(well:mod/poor:muc)	6:12:0	36:23:6
ly permeation (%)	(positive rate)	56	71
v invasion (%)	(positive rate)	50	15
LN metastasis (%)	(positive rate)	47	37

well, well differentiated; mod, moderately differentiated; poor, poorly differentiated; muc, mucinous; ly, lymphatic; v, venous;

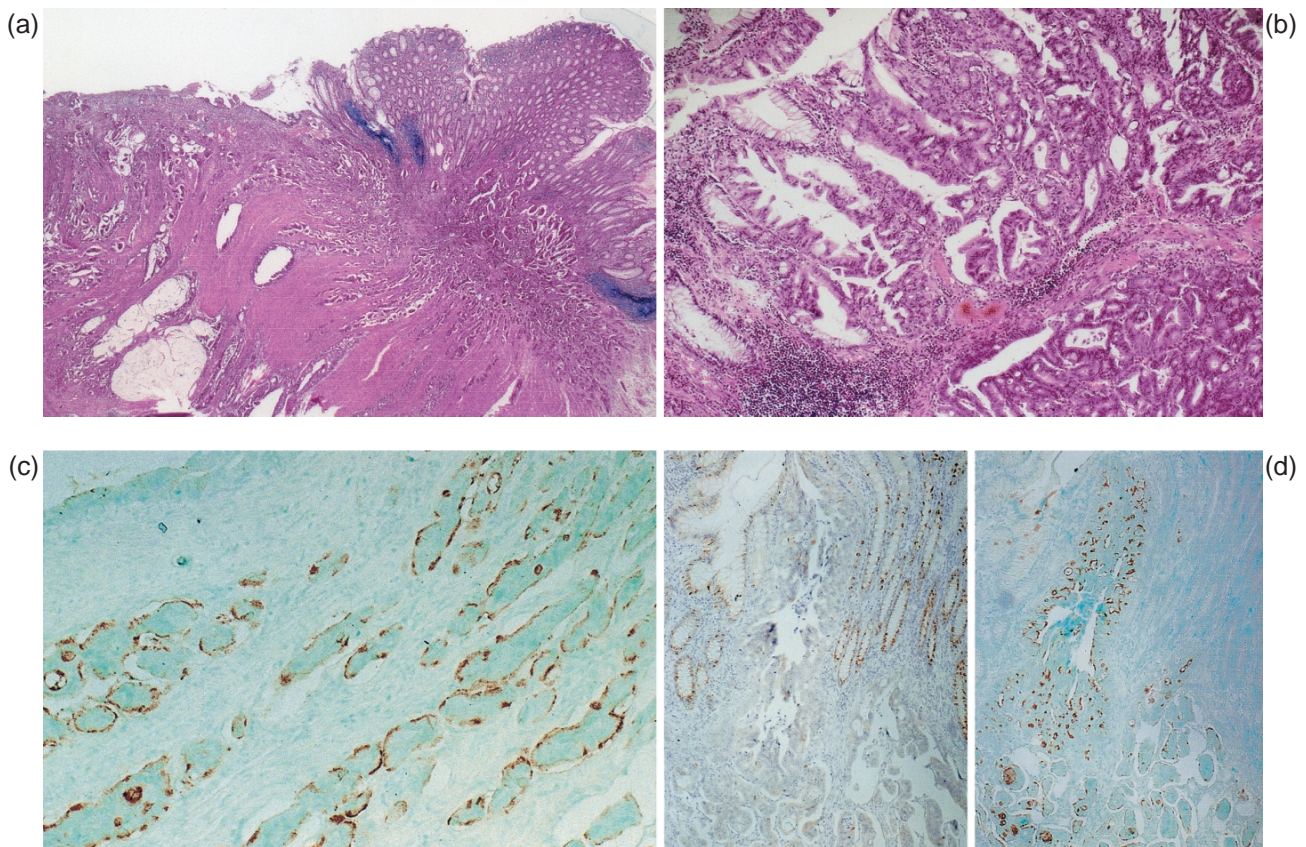


Fig. 3. Gastric type. (a) Low-power view of the carcinoma. The moderately to poorly differentiated adenocarcinoma diffusely infiltrates throughout the colonic wall (H&E,  $\times 10$ ). (b) Intramucosal component focally shows a serrated structure resembling that of serrated adenoma (H&E,  $\times 70$ ). (c) In the area of invasion into the muscularis propria, HGM is also positive in cytoplasm of the carcinoma cells (HGM,  $\times 100$ ). (d) On the left, MUC2 is positive in goblet cells in the surrounding mucosa, but negative in carcinoma cells (MUC2,  $\times 35$ ). On the right, HGM is diffusely positive in carcinoma cells, and is also positive in a few goblet cells in the surrounding mucosa (HGM,  $\times 35$ ).

higher in the CD10(+) group than in the CD10(-) group. There was no significant difference in other clinicopathologic factors between CD10(+) and CD10(-) groups.

**HGM immunohistochemistry** Twelve (14%) of the 83 cases were positively stained with HGM, in the cytoplasm of both goblet-like cells and nongoblet-like cells (Fig. 3). The correlation between HGM expression and clinicopathologic features is summarized in Table III. The tumor size was significantly larger in the HGM(+) group than in

Table III. Correlation between HGM Expression and Clinicopathologic Features

		HGM(+) (n=12)	HGM(-) (n=71)
Age (yrs)	(average)	68	64
Sex	(male:female)	3:9	39:32
Location	(right:left)	7:5	23:48
Size (mm)	(average)	66	52
Histologic grade	(well:mod/poor:muc)	5:2:5	37:33:1
ly permeation (%)	(positive rate)	75	66
v invasion (%)	(positive rate)	17	24
LN metastasis (%)	(positive rate)	50	37

well, well differentiated; mod, moderately differentiated; poor, poorly differentiated; muc, mucinous; ly, lymphatic; v, venous; LN, lymph node.

Table IV. Classification of Phenotypes

		Human gastric mucin (HGM)	
		(-)	(+)
CD10 (+)	MUC2 (+)	small intestinal	mixed
	MUC2 (-)	small intestinal	mixed
CD10 (-)	MUC2 (+)	large intestinal	mixed
	MUC2 (-)	unclassified	gastric

the HGM(-) group. Female sex and right-sided location were seen more frequently in the HGM(+) group than in the HGM(-) group, but the difference was not significant. There was no significant difference in other clinicopathologic factors between the HGM(+) and HGM(-) groups.

**Phenotypic subclassification of the carcinomas** Based on immunoreactivity to the three antibodies (MUC2, CD10 and HGM), the carcinomas were subclassified into five groups. Those with CD10(+) and HGM(-) were classified as small-intestinal type (S-type), regardless of MUC2 positivity. Those with CD10(-), MUC2(+) and HGM(-) were classified as large-intestinal type (L-type). Those with CD10(-), MUC2(-) and HGM(+) were classified as gastric type (G-type). Those with CD10(+)/MUC2(+) and HGM(+) were classified as mixed intestinal and gastric type (M-type). The remaining carcinomas with CD10(-), MUC2(-) and HGM(-) were unclassified (U-type). The schema of the subclassification is shown in Table IV.

Of the 83 cases, 18 (22%) were classified as S-type, 27 (33%) as L-type, 2 (2%) as S-type, 9 (11%) as M-type and 27 (33%) as U-type. The correlation between the subtypes and clinicopathologic features is summarized in Table V. Characteristics of each type were as follows. The S-type was characterized by relatively lower incidence of lymphatic permeation and higher venous invasion. The incidence of venous invasion of S-type was significantly higher than that of L-type. The L-type was characterized by low incidence of venous invasion and lymph node metastasis. M-type was characterized by female and right-sided predominance, large tumor size, and low incidence of venous invasion. It is noteworthy that five of six mucinous adenocarcinomas belonged to the M-type, being strongly positive for both MUC2 and HGM. Only two cases of G-type were seen, with high histologic grade, lymphatic permeation and lymph node metastasis, but no venous invasion. Serrated structures and infiltrative

Table V. Correlation between the Clinicopathological Features and Phenotypes of Carcinoma

		Small int. type (n=18)	Large int. type (n=27)	Gastric type (n=2)	Mixed type (n=9)	Unclassified (n=27)
Age (yrs)	(average)	69	62	64	69	65
Sex	(male:female)	11:7	15:12	1:1	2:7	13:14
Location	(right:left)	3:15	8:19	1:1	5:4	9:18
Size (mm)	(average)	45	50	53	69	59
Histologic grade	(well:mod/poor:muc)	6:12:0	15:11:1	0:2:0	3:2:5	16:11:0
ly permeation (%)	(positive rate)	56	78	100	78	59
v invasion (%)	(positive rate)	50	11	0	22	22
LN metastasis (%)	(positive rate)	47	29	100	33	40

well, well differentiated; mod, moderately differentiated; poor, poorly differentiated; muc, mucinous; ly, lymphatic; v, venous; LN, lymph node.

growth were characteristic of G-type. The U-type was characterized by female predominance, with relatively lower incidence of lymphatic permeation and higher venous invasion.

## DISCUSSION

It has been reported that MUC2 is specific for core protein of intestinal goblet cells.<sup>12-14</sup> CD10 was originally used as a marker for common acute lymphoblastic leukemia antigen (CALLA), but it proved to react with brush border of the small intestine as well as germinal centers of lymphoid follicles and microvilli of kidney.<sup>15-18</sup> HGM is a specific marker for core protein of gastric foveolar cells.<sup>19, 20</sup> The correlations between positivity to these antibodies and clinicopathologic features suggested that cell differentiation is inversely associated with tumor growth and aggressiveness.

Using these three antibodies, the gastric carcinomas could be classified into four types (small intestinal, incomplete intestinal, gastric, and unclassified).<sup>6, 7</sup> This classification is based on that of intestinal metaplasia, and the phenotype of the carcinoma showed a good accordance with that of the background mucosa. This method is considered to be simple and convenient for the classification of phenotypes of carcinoma, although it may be insufficient for complete evaluation of phenotypic expression. Using the same method, the current cases of colorectal carcinomas could be roughly subclassified into five types (large intestinal, small intestinal, gastric, mixed, and unclassified) on the basis of cell differentiation.

We reported that serrated adenoma and hyperplastic (metaplastic) polyp shared a common cell lineage, with gastric differentiation.<sup>21</sup> Subsequently, we also reported a case of multiple colonic adenocarcinomas with gastric differentiation, which occurred in association with hyperplastic (metaplastic) polyp and serrated adenoma. These carcinomas were characterized by serrated and lace-like structures, infiltrative growth, poorly differentiated components and immunopositivity for HGM.<sup>10</sup> In the current study, pure gastric-type carcinomas were recognized in only 2 cases (2%). Serrated structure, infiltrative growth and HGM(+) were recognized in both, while a poorly differentiated component was recognized in one of them. These features are compatible with those of the previously reported case of "serrated adenocarcinoma" of the colorectum. The current study revealed the existence of gastric-type carcinoma in the colorectum, though its incidence was low. It is suggested that the serrated polyp-carcinoma sequence is relatively rare in colorectal carcinogenesis.

Some authors indicated that the histologic features and biological behavior of gastric carcinomas were different between the intestinal and gastric types.<sup>2, 5</sup> For example, the gastric-type carcinoma tends to show less glandular

formation and diffuse infiltration with higher incidence of lymphatic permeation, and intestinal-type carcinoma tends to show glandular formation and expansive growth with a higher incidence of vascular invasion. In our cases, a higher incidence of venous invasion was recognized in the small-intestinal type than in other types. The gastric type revealed lower incidence of vascular and higher incidence of lymphatic invasion and lymph node metastasis. It was also reported that MUC2(+) colorectal carcinomas had a relatively good prognosis and a low incidence of liver metastasis.<sup>8</sup> Our cases with MUC2(+) revealed lower incidence of venous invasion and lymph node metastasis, in accordance with the previous reports. Most mucinous carcinomas revealed positivity for both MUC2 (strongly positive) and HGM, and HGM was usually positive for goblet-like cells in such cases. These findings suggest that mucin in the goblet cells of mucinous carcinomas might change to gastric-type mucin. Further examination is required to clarify this phenomenon.

The adenoma-carcinoma sequence, which is seen in familial adenomatous polyposis, has been considered to be the main mechanism for colorectal carcinogenesis. At the molecular level, Fearon and Vogelstein proposed a genetic model for colorectal carcinogenesis.<sup>22</sup> In addition to the usual adenoma-carcinoma sequence, other pathways of colorectal carcinogenesis have also been proposed, such as hereditary non-polyposis colorectal cancer (HNPCC),<sup>23</sup> hereditary mixed polyposis syndrome<sup>24</sup> and serrated adenomatous polyposis.<sup>25</sup>

Morphologic features and phenotypic expression of tumors are considered to be associated with genetic changes. Biemer-Hüttmann *et al.* reported the phenotype of MUC2+/MUC5AC+ in mucinous carcinoma of the colorectum, and suggested a relationship between microsatellite instability (MSI) and mucinous phenotype.<sup>26</sup> The mucinous phenotype (MUC2+/MUC5AC+) is also common in serrated polyps including hyperplastic polyps and serrated adenomas,<sup>27</sup> and the importance of MSI in histogenesis of serrated polyps was reported by several authors.<sup>28, 29</sup> In addition, Uchida *et al.* mentioned that mutations other than APC mutations might play an important role in the development of serrated polyps.<sup>30</sup> Thus, phenotypic classification of epithelial tumors, as well as histologic classification,<sup>31</sup> might be useful for clarifying the carcinogenetic pathway in the colorectum.

In conclusion, the current study has demonstrated that some colorectal carcinomas revealed small-intestinal or gastric differentiation, and also suggests that the cell differentiation is correlated with the histologic features and biological behavior of the tumor. Such subclassification will be useful not only for evaluation of the prognosis, but also for analysis of tumorigenesis. In addition, the existence of colorectal "serrated adenocarcinoma" was confirmed, although its incidence was low (2% in this series).

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