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Loss of MUC2 expression correlates with progression along the adenoma-carcinoma sequence pathway as well as *de novo* carcinogenesis in the colon

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Summary. Aims: We have previously demonstrated links between clinicopathological findings and phenotypes using several gastric and intestinal phenotypic markers in stomach and pancreatic cancers. However, the clinicopathological significance of the phenotype and Cdx2 expression has hitherto remained unclear in colorectal carcinogenesis. Methods and results: We examined the correlation between gastric and intestinal phenotypic expression in 91 primary early carcinomas of the colon. MUC2 expression demonstrated a significant decrease from tubular/tubulovillous adenomas with moderate atypia, through intramucosal carcinomas, to cancers with submucosal invasion (P<0.0001). Intramucosal *de novo* carcinomas (flat type carcinomas without adenomatous components) exhibited a greater decrease of MUC2 than intramucosal lesions with adenomatous components. Expression of MUC5AC also decreased significantly with progression according to the tubular/tubulovillous adenoma-carcinoma sequence, carcinomas with villous adenomatous components having a higher level compared with their tubular adenomatous counterparts, suggesting differences in the pathway of malignant transformation. Cdx2 nuclear expression was maintained in all of the adenomas and early carcinomas examined. Conclusions: Our data suggest that the reduction of MUC2 expression may be associated with the occurrence and progression of colorectal carcinomas in both adenoma-carcinoma sequence pathway and *de novo* carcinogenesis. Tumor-suppressive effects of Cdx2 may be preserved during early stages of colorectal carcinogenesis.

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Introduction

Gastric and intestinal phenotypic expression is important for the histogenesis of the cancers of the digestive tract. We and others have previously demonstrated a relationship between clinicopathological findings and phenotypes using several gastric and intestinal phenotypic markers such as MUC5AC, MUC6, MUC2, and villin in stomach cancers (Reis et al., 2000; Mizoshita et al., 2004a,b, 2005b; Tsukamoto et al., 2005) and pancreatic tumors (Sessa et al., 1990; Matsumoto et al., 2004). Changes were also seen in intestinal metaplasia, a putative precancerous lesion for stomach cancers (Reis et al., 1999; Tatematsu et al., 2003; Tsukamoto et al., 2004). In colorectal tumors, gastric and intestinal phenotypic expression has also been evaluated in hyperplastic polyps (Bara et al., 1983, 1998; Bartman et al., 1999; Biemer-Huttmann et al., 1999; Koike et al., 2003), serrated adenomas (Jass, 1999; Yao et al., 1999; Hirono et al., 2004), adenomas with tubular components (Ajioka et al., 1997; Myerscough et al., 2001), villous adenomas (Bara et al., 1983; Buisine et al., 1996; Takata et al., 2003), and carcinomas (Chang et al., 1994; Ajioka et al., 1996; Biemer-Huttmann et al., 2000; Sylvester et al., 2001; Yao et al., 2001; Iwase et al., 2005). It is well known that colorectal cancers develop through two distinct molecular pathways, one being the adenoma-carcinoma sequence which involves activating mutations of the Wnt/APC/ß-catenin signaling pathway and is associated with chromosomal instability (Vogelstein et al., 1988). The second pathway is initiated by genetic and/or epigenetic alterations in mismatch repair (MMR) genes, which lead to microsatellite

instability (MSI) and result in inactivation of the MMR target gene (Fishel et al., 1993; Senba et al., 1998). In addition, several reports have pointed to the existence of flat type carcinomas without adenomatous components, indicative of de novo carcinogenesis (Muto et al., 1985; Kuramoto and Oohara, 1988; Shimoda et al., 1989; Blank et al., 1994; Kudo et al., 1997). However, the clinicopathological significance of phenotypic markers to these pathways of colorectal cancer development has hitherto remained unclear.

Caudal-related homeobox gene (Cdx) 2 is important for the maintenance of intestinal phenotypic expression not only in the normal small and large intestine (Silberg et al., 2000; Mizoshita et al., 2001), but also in intestinal metaplasia (Mizoshita et al., 2001; Almeida et al., 2003) and carcinomas of the stomach (Almeida et al., 2003; Mizoshita et al., 2003), and pancreas (Matsumoto et al., 2004). In fact, Cdx2 is the useful prognostic marker in stomach cancers (Mizoshita et al., 2003), invasive ductal carcinomas of the pancreas (Matsumoto et al., 2004), and carcinomas of the ampulla of Vater (Hansel et al., 2005). Several reports have pointed to tumor-suppressor potential of Cdx2 in colorectal tumorigenesis (Ee et al., 1995; Mallo et al., 1997; Mallo et al., 1998; Aoki et al., 2003; Bonhomme et al., 2003) and thus it is of interest to analyze links between Cdx2 and colorectal carcinogenesis pathways, especially in terms of alternation from adenomas to early colorectal carcinomas and with progression.

In this study, we therefore analyzed the expression of gastric and intestinal phenotypic markers by immunohistochemistry in 91 primary early colorectal carcinomas, including adjacent adenomatous components, with histological evaluation by hematoxylin and eosin (H&E) staining.

Materials and methods

Samples and tissue collection

We examined 91 primary early colorectal cancers surgically or endoscopically resected at Aichi Cancer Center Hospital between 1993 and 2005. Out of the 91 early colorectal cancer patients, 54 were men and 37 were women. All specimens were fixed in 10% buffered formalin. Colorectal carcinomas with adjacent non-neoplastic mucosa were serially cut into 5-mm slices and embedded in paraffin, and then sectioned and stained with H&E for histological examination.

Histological classification was made according to the Japanese General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum, and Anus (Japanese Society for Cancer of the Colon and Rectum, 1998). Histologically, the 91 tumors were classified as 45 well-differentiated and 46 moderately-differentiated adenocarcinomas. Out of the 91 early colorectal cancers, 23 were intramucosal (m, T1 for TNM classification) and 68 demonstrated submucosal invasion (sm, T1 for TNM classification), divided into sm1 for the layer immediately adjacent to the muscularis mucosa, sm2 for the middle layer and sm3 for the deepest area adjacent to the muscularis propria. Of the 68 lesions with submucoal invasion, 24 were sm1, 24 were sm2, and 20 were sm3. The 91 colorectal carcinomas were also divided into 72 polypoid/flat type carcinomas in/with tubular/tubulovillous adenomatous components (carcinomas with T/TV adenoma), 6 carcinomas in/with villous adenomatous components (carcinomas with villous adenoma), and 13 flat type carcinomas without adenomatous components. The 34 tubular/tubulovillous (T/TV) adenomatous lesions were divided into 2 with mild, 16 with moderate, and 16 with severe atypia, and the 32 T/TV adenomas with moderate or severe atypia were evaluated phenotypically.

Immunohistochemistry

Immunohistochemical staining of paraffin sections was carried out with monoclonal antibodies against the following antigens: Cdx2 (CDX2-88; 1:50, BioGenex, CA, USA); MUC5AC (CLH2; 1:500, Novocastra Laboratories, Newcastle upon Tyne, UK); MUC6 (CLH5; 1:500, Novocastra Laboratories); MUC2 (Ccp58; 1:500, Novocastra Laboratories,); and villin (12; 1:20000, Transduction Laboratories, Lexington, KY, USA). With regard to gastric phenotypic markers, we used normal gastric mucosa and normal ileum as positive and negative controls, and these in reverse for the intestinal phenotype. The precise procedures for immunohistochemical techniques were as previously described (Koike et al., 2003; Mizoshita et al., 2003, 2004a,b; Tsukamoto et al., 2004, 2005). Briefly, 4 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohols. After inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, antigen retrieval was conducted for detection of binding of the above-mentioned antibodies with 10 mM citrate buffer (pH 6.0) in a microwave oven for 10 minutes at 98°C. Sections were incubated with primary antibodies, thoroughly washed in phosphate-buffered saline (PBS), then incubated with biotinylated secondary antibody, followed by the avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, CA). Finally, immune complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% 3,3'-diaminobenzidine tetrachloride (DAB). Nuclear counterstaining was accomplished with Mayer's hematoxylin.

Three independent pathologists (T.M., T.T., and K-I.I.) judged the histology and immunohistochemical staining for the phenotypic markers, and Cdx2. Reactivity for the phenotypic markers and Cdx2 was scored according to the percentage of positively stained tumor cells in the section areas on a 3-point-scale: score 0, <10%; score 1, 10-33%; score 2, 34-66%; score 3, 67-100%. A result was considered positive (+) with a score of 1 or more.

The gastric and intestinal phenotypic markers

MUC5AC and MUC6 are markers of the gastric epithelial cell phenotype, whereas MUC2 and villin are typical of the intestinal epithelial cell phenotype (Koike et al., 2003; Mizoshita et al., 2003, 2004a,b, 2005a,b; Tatematsu et al., 2003; Matsumoto et al., 2004; Tsukamoto et al., 2004, 2005). In the normal alimentary tract, MUC5AC expression is observed in the cytoplasm of the gastric foveolar epithelial cells, and MUC6 expression is detected in the cytoplasm of the pyloric gland and mucous neck cells in the stomach. With regard to the distribution of intestinal epithelial cell markers, MUC2 expression is observed in the cytoplasm of goblet cells of the small intestine and colon. Villin is detected in the luminal surfaces of the absorptive cells of small intestine and colon (Mizoshita et al., 2005b). Cdx2 nuclear staining is detected in the normal epithelial cells of the colon (Silberg et al., 2000; Mizoshita et al., 2001).

Statistical analysis

The Kruskal-Wallis test was applied to establish the significance of differences between scores for each marker and the depth of tumor invasion. The relations between the positivity for each marker and the invasion of tumors in depth were assessed using the χ^2 test. The data were analyzed by the Mann-Whitney U-test or Fischer's exact test for differences between groups of adenomas with T/TV adenomatous components. The difference between villous tumors and carcinomas with T/TV adenomas was also estimated by the Mann-Whitney U-test, and Fischer's exact test. The same tests were also applied to analyze differences between carcinomas with T/TV adenomas and flat type carcinomas without adenomatous components. P-values <0.05 were considered statistically significant.

Results

The expression of gastric and intestinal phenotypic markers in the colorectal T/TV adenomatous lesions, and carcinomas with T/TV adenoma

The data for colorectal T/TV adenomatous lesions, and carcinomas with T/TV adenomas are summarized in Table 1. The scores and positivity for MUC2 expression demonstrated a significant decrease from T/TV adenomas with moderate atypia, through intramucosal carcinomas, to cancers with submucosal invasion (P<0.0001 for trends) (Fig. 1). The score and positivity of MUC5AC expression also exhibited a significant decrease from T/TV adenomas with moderate atypia, through those with severe atypia, to intramucosal carcinomas with progression (P=0.034 and P=0.032 in trend analysis for the score and positivity, respectively) (Table 1). However, there were no significant differences in the expression of villin and Cdx2. Cdx2 nuclear staining was judged to be positive in all T/TV adenomatous lesions and carcinomas with T/TV adenomas. The expression of villin was also judged to be positive in all T/TV adenomatous lesions, and all but two carcinomas with sm2 invasion. MUC6 expression was detected in only one case of TV adenoma with moderate atypia (data not shown).

Expression of gastric and intestinal phenotypic markers in colorectal carcinomas with villous adenoma

One intramucosal and 5 sm1 invasive carcinomas

Table 1. The expression of MUC2, villin, Cdx2, and MUC5AC in colorectal T/TV adenomatous lesions, and carcinomas with T/TV adenoma.

	number	MUC2		Villin		Cdx2		MUC5AC	
		score[a]	positivity	score[a]	positivity	score[a]	positivity	score[a]	positivity
Adenoma									
T adenoma with moderate atypia	n=9	2.33±0.24	9 (100%)	2.44±0.18	9 (100%)	3.00±0.00	9 (100%)	0.44±0.18	4 (44.4%)
TV adenoma with moderate atypia	n=7	2.57±0.20	7 (100%)	2.29±0.18	7 (100%)	3.00±0.00	7 (100%)	0.14±0.14	1 (14.3%)
Subtotal of moderate atypia	n=16	2.44±0.16[b]	16 (100%)[c]	2.38±0.13	16 (100%)	3.00±0.00	16 (100%)	0.31±0.12[d]	5 (31.3%)[e]
T adenoma with severe atypia	n=7	2.14±0.40	6 (85.7%)	2.71±0.18	7 (100%)	3.00±0.00	7 (100%)	0.29±0.18	2 (28.6%)
TV adenoma with severe atypia	n=9	2.22±0.22	9 (100%)	2.22±0.28	9 (100%)	3.00±0.00	9 (100%)	0.00±0.00	0 (0%)
Subtotal of severe atypia	n=16	2.19±0.21[b]	15 (93.8%)[c]	2.44±0.18	16 (100%)	3.00±0.00	16 (100%)	0.13±0.085[d]	2 (12.5%)[e]
Total of T/TV adenomatous lesions	n=32	2.31±0.13	31 (96.9%)	2.41±0.11	32 (100%)	3.00±0.00	32 (100%)	0.22±0.074	7 (21.9%)
Carcinoma									
m	n=18	0.89±0.18[b]	12 (66.7%)[c]	2.50±0.15	18 (100%)	3.00±0.00	18 (100%)	0.00±0.00[d]	0 (0%)[e]
sm1	n=16	0.75±0.19[b]	9 (56.3%)[c]	2.50±0.18	16 (100%)	2.88±0.09	16 (100%)	0.13±0.13	1 (6.3%)
sm2	n=20	0.45±0.15[b]	7 (35.0%)[c]	2.05±0.22	18 (90.0%)	2.75±0.12	20 (100%)	0.00±0.00	0 (0%)
sm3	n=18	0.17±0.09[b]	3 (16.7%)[c]	1.94±0.19	18 (100%)	2.72±0.11	18 (100%)	0.056±0.056	1 (5.6%)

"T/TV, tubular/tubulovillous; Carcinomas with T/TV adenoma, polypoid/flat type carcinomas in/with tubular/tubulovillous adenomatous components; T adenoma, Tubular adenoma; TV adenoma, Tubulovillous adenoma."; [a]: The scores of each marker are average±standard error (SE); [b]: P<0.0001 in trend analysis; [c]: P<0.0001 in trend analysis; [d]: P=0.034 in trend analysis; [e]: P=0.032 in trend analysis.

were evaluated, all 6 classified as well-differentiated adenocarcinomas. The data for villous tumors are summarized in Table 2 (Fig. 2). The average scores (P=0.0005) and positivity (P=0.0076) for MUC5AC expression in carcinomas with villous adenoma were statistically higher than those (the average score,

Table 2. The expression of MUC2, villin, Cdx2, and MUC5AC in 6 carcinomas with villous adenoma.

	number	MUC2		Villin		Cdx2		MUC5AC	
		score[a]	positivity	score[a]	positivity	score[a]	positivity	score[a]	positivity
Carcinoma with villous adenoma									
adenomatous components	n=6	2.00±0.37	6 (100%)	3.00±0.00	6 (100%)	2.83±0.17	6 (100%)	0.83±0.40	3 (50.0%)
carcinomatous components	n=6	1.50±0.50	5 (83.3%)	3.00±0.00	6 (100%)	2.67±0.33	6 (100%)	0.83±0.40	3 (50.0%)

[a]: The scores of each marker are average±standard error (SE).

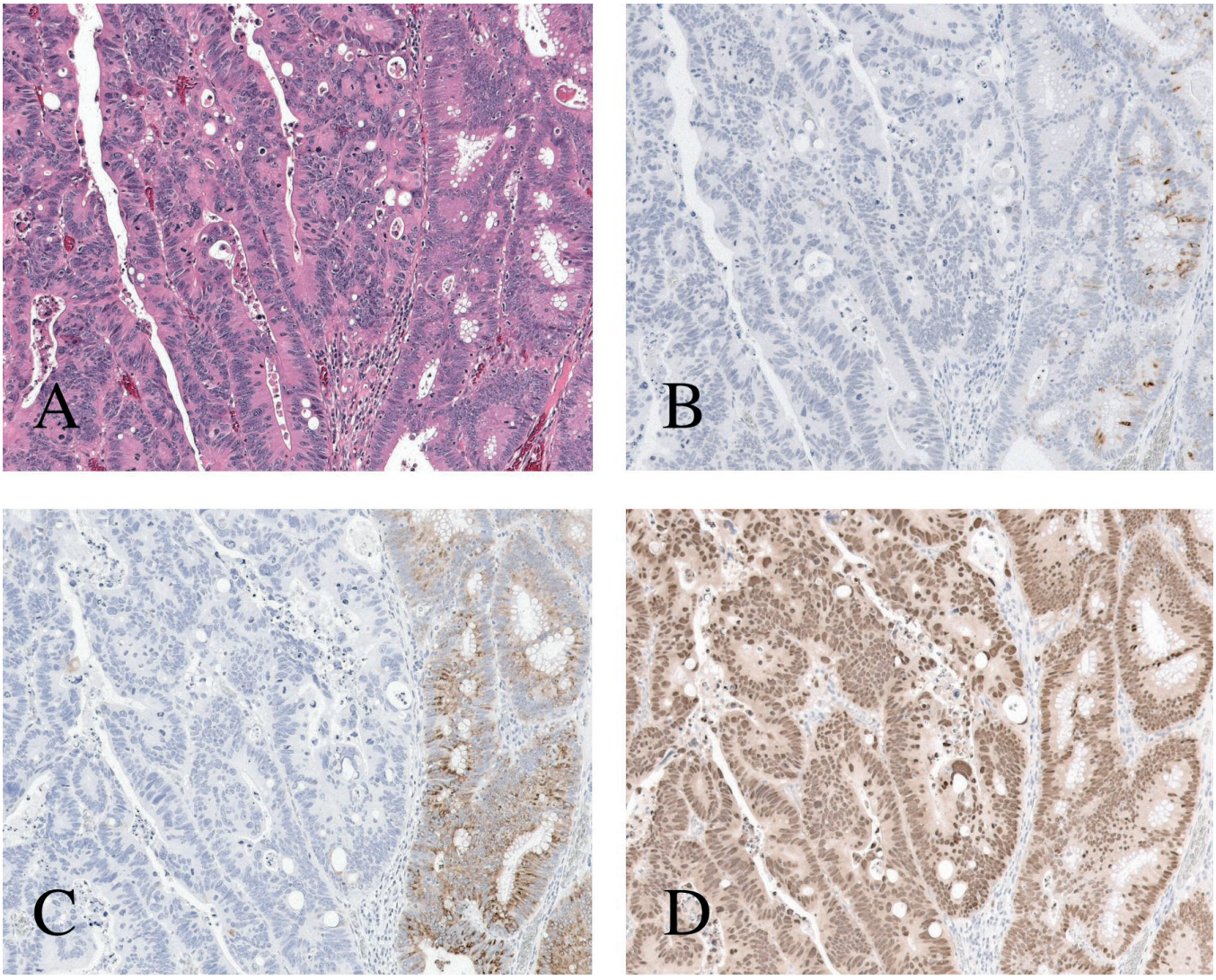

Fig. 1. A well differentiated adenocarcinoma (left), and a adjacent tubular adenoma with moderate atypia (right). **A.** H&E staining. **B.** Note partially positive MUC5AC in the cytoplasm of adenoma but not cancer cells. **C.** MUC2 is evident in the cytoplasm of adenoma but not cancer cells. **D.** Cdx2 nuclear staining is apparent in both adenoma and cancer cells. x 100

Table 3. The comparison between carcinomas with T/TV adenoma and with villous adenoma reference to the MUC5AC expression.

	number	MUC5AC	
		score[a]	positivity
Carcinomas with T/TV adenoma	"n=34 (m, n=18; sm1, n=16)"	0.059±0.059	1 (2.9%)
Carcinoma with villous adenoma	"n=6 (m, n=1; sm1, n=5)"	0.83±0.40	3 (50%)
P-value		P=0.0005	P=0.0076

[a]: The scores of each marker are average±standard error (SE).

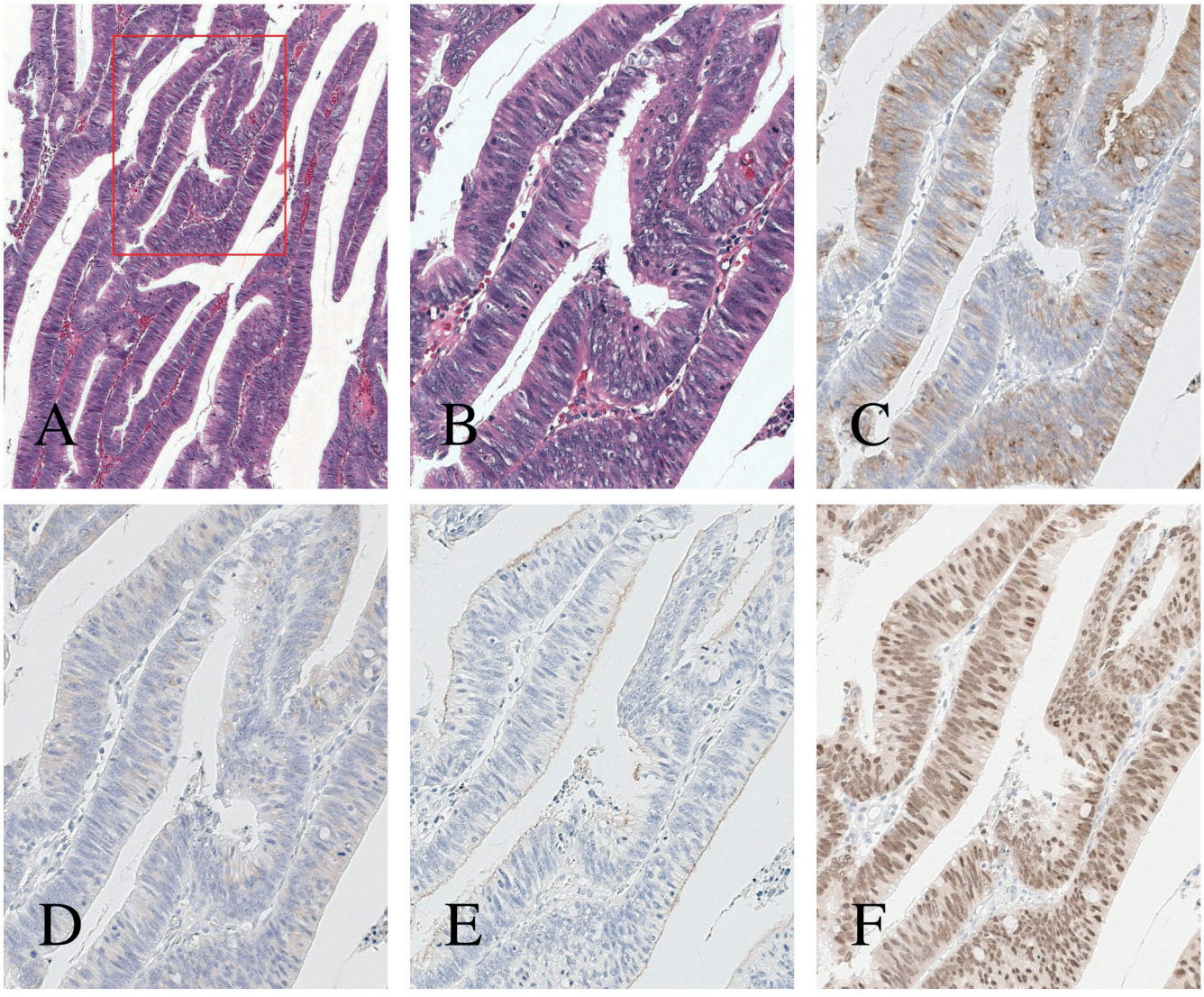

Fig. 2. Villous adenoma. **A.** H&E staining. **B.** Higher magnification of the red square in A. **C.** MUC5AC is positive in the cytoplasm of tumor cells. **D.** MUC2 is partially and weakly positive in the cytoplasm of tumor cells. **E.** Villin is positive at the luminal surfaces of tumor cells. **F.** Cdx2 nuclear staining is apparent in tumor cells. A, x 80; B-F, x 200

0.059±0.059; the positivity, 1/34 (2.9%)) in the intramucosal (m) and sm1 invasive carcinomas with T/TV adenomas (Table 3). The scores for Cdx2 in the villous adenomas were lower than in the T/TV adenomas (P=0.021, Table 4). However, regarding the expression of villin, the scores in the T/TV adenomas demonstrated a greater decrease than in the villous adenomas (P=0.020, Table 4). No MUC6 expression was observed in the villous tumors.

Expression of gastric and intestinal phenotypic markers in flat type carcinomas without adenomatous components

We defined flat type carcinomas without adenomatous components as *de novo* carcinomas, their data being summarized in Table 5 (Fig. 3). Of 13 *de novo* carcinomas, 4 intramucosal (m) lesions were classified histologically as well differentiated adenocarcinomas, while 3 sm1, 4 sm2, and 2 sm3 invasive ones were classified as moderately differentiated adenocarcinomas. No MUC2 expression was observed in 4 intramucosal de-novo carcinomas, significantly lower than the average score and positivity of the intramucosal carcinomas with T/TV adenomas (the average score, P=0.027; the positivity, P=0.029) (Table 6). The average score for Cdx2 expression in 4 intramucosal de-novo lesions was significantly lower than that of the intramucosal carcinomas with T/TV adenomas (P=0.0021). There were no significant differences in expression of markers between submucosal invasive carcinomas with and without

Table 4. The comparison between T/TV and villous adenomatous components reference to the villin and Cdx2 expression.

	number	Villin		Cdx2	
		score[a]	positivity	score[a]	positivity
T/TV adenomatous components	n=32	2.41±0.11	32 (100%)	3.00±0.00	32 (100%)
Villous adenomatous components	n=6	3.00±0.00	6 (100%)	2.83±0.17	6 (100%)
P-value		P=0.020	n.s.	P=0.021	n.s.

n.s., not significant; [a]: The scores of each marker are average±standard error (SE).

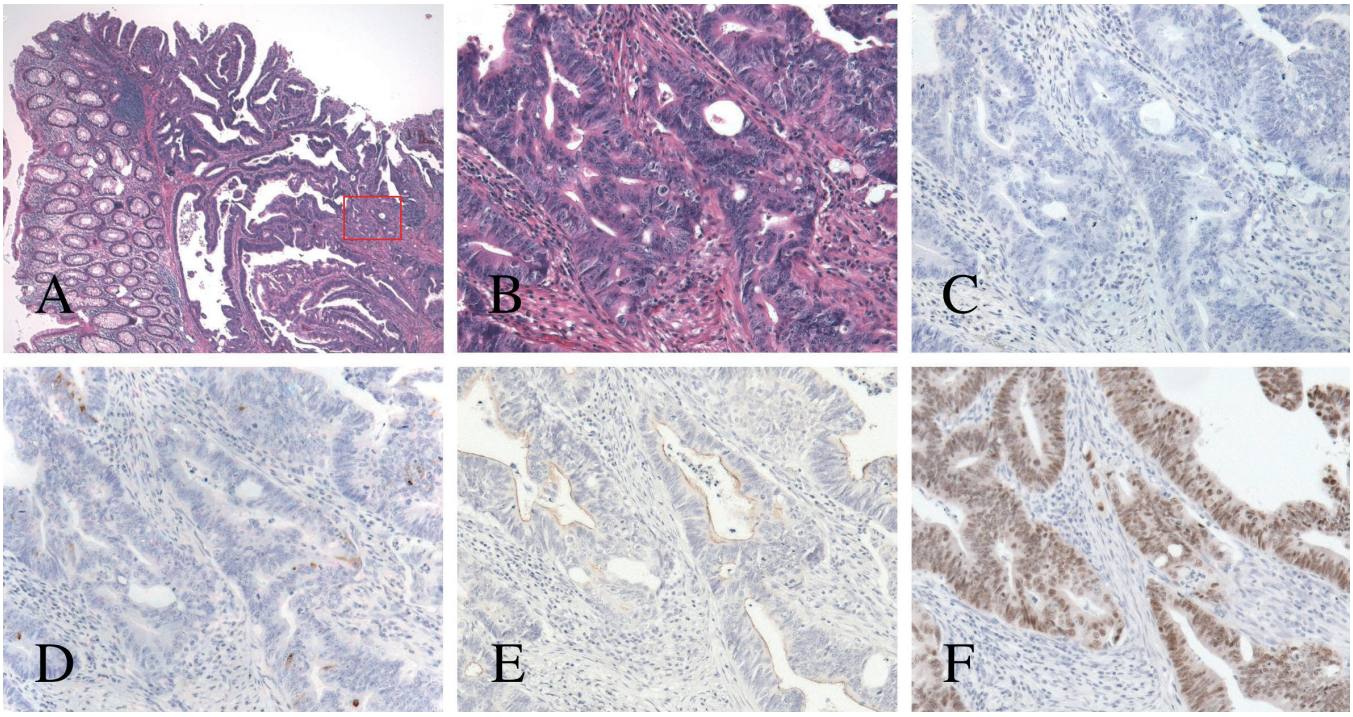

Fig. 3. A flat type moderately differentiated adenocarcinoma without adenomatous components. **A.** H&E staining. **B.** Higher magnification of the red square in A. **C.** No MUC5AC is apparent in the cytoplasm of cancer cells. **D.** MUC2 is partially positive in the cytoplasm of cancer cells. **E.** Villin is evident at the luminal surfaces of cancer cells. **F.** Cdx2 nuclear staining is apparent in cancer cells. A, x 25; B-F, x 160

Table 5. The expression of MUC2, villin, Cdx2, and MUC5AC in 13 de novo carcinomas.

	number	MUC2		Villin		Cdx2		MUC5AC	
		score[a]	positivity	score[a]	positivity	score[a]	positivity	score[a]	positivity
de novo carcinoma									
m	n=4	0.00±0.00	0 (0%)	3.00±0.00	4 (100%)	2.50±0.29	4 (100%)	0.00±0.00	0 (0%)
sm1	n=3	0.00±0.00	0 (0%)	1.67±0.88	2 (66.7%)	3.00±0.00	3 (100%)	0.00±0.00	0 (0%)
sm2	n=4	0.50±0.50	1 (25.0%)	1.50±0.65	3 (75.0%)	3.00±0.00	4 (100%)	0.25±0.25	1 (25.0%)
sm3	n=2	0.50±0.50	1 (50.0%)	2.00±1.00	2 (100%)	3.00±0.00	2 (100%)	0.00±0.00	0 (0%)

de novo carcinoma, flat type carcinoma without adenomatous components; [a]: The scores of each marker are average±standard error (SE).

Table 6. The comparisons between intramucosal carcinomas with T/TV adenoma and de vovo carcinomas reference to the MUC2 and Cdx2 expression.

	depth	number	MUC2		Cdx2	
			score[a]	positivity	score[a]	positivity
Carcinomas with T/TV adenoma	m	n=18	0.89±0.18	12 (66.7%)	3.00±0.00	18 (100%)
de novo carcinomas	m	n=4	0.00±0.00	0 (0%)	2.50±0.29	4 (100%)
P-value			P=0.027	P=0.029	P=0.0021	n.s.

[a]: The scores of each marker are average±standard error (SE).

adenoma components. No MUC6 expression was observed in the *de novo* carcinomas.

Discussion

Our present data clearly demonstrate a significant decrease of MUC2 expression from T/TV adenomas with moderate atypia, through intramucosal carcinomas, to cancers with submucosal invasion, according to the adenoma-carcinoma sequence pathway, in line with earlier findings for development and progression of colorectal carcinomas (Hanski et al., 1997; Iwase et al., 2005). Expression of MUC2 is frequently decreased with progression (Blank et al., 1994) and with an increase in the grade of epithelial dysplasia (Ajioka et al., 1997). Ajioka et al. (1996) have documented reduction of MUC2 in 53% of colorectal cancers. In the animal model, Muc2-/- mice frequently developed adenomas in the small intestine that progressed to invasive adenocarcinoma, as well as rectal tumors (Velcich et al., 2002). We thus consider that loss of MUC2 expression may be important for the occurrence and progression of colorectal carcinomas. On the other hand, Jass and Walsh (2001) have suggested that many colorectal carcinomas with MUC2 expression originate within either serrated polyps (hyperplastic polyps, mixed polyps and serrated adenomas) or villous adenomas, while those showing columnar cell differentiation lose MUC2. Several pathways may be included in the adenoma-carcinoma sequence of colorectal tumorigenesis. Regarding the regulation of MUC2 expression, Yamamoto et al. (2003) have demonstrated that Cdx2 interacts with the MUC2 promoter and activates MUC2 transcription. However, in the present study, all cases of adenomas and cancers had Cdx2 expression, while a decrease of MUC2 expression was observed in about half of the cases. Suppression of the MUC2 gene in colon carcinoma cells is associated with methylation of the promoter region (Hanski et al., 1997) and Ookawa et al. (2002) have shown that p53 directly activates transcription of the MUC2 gene in many cell lines *in vitro*. Further studies on MUC2 gene regulation will be important for elucidation of mechanisms underlying colorectal carcinogenesis.

Cdx2 has tumor-suppressive effects in carcinomas of the digestive apparatus, as shown for the large intestine (Ee et al., 1995; Mallo et al., 1997, 1998; Aoki et al., 2003; Bonhomme et al., 2003), stomach (Mizoshita et al., 2003), pancreas (Matsumoto et al., 2004) and the ampulla of Vater (Hansel et al., 2005). Our present data provide clear evidence, however, that Cdx2 nuclear expression is retained in the early stages of colorectal carcinogenesis. Nevertheless, reduced expression of CDX2 appears important in colon tumorigenesis through mTOR-mediated chromosomal instability (Aoki et al., 2003). In this regard, it should be borne in mind that Cdx2 upregulates transcription of the p21/WAF1/CIP1 gene, a cyclin-dependent kinase inhibitor (Bai et al., 2003). Analysis of mechanisms of tumor progression by which colorectal cancer cells might escape from the tumor-suppressive effects of Cdx2 is clearly important.

Several reports have previously shown aberrant gastric phenotypic expression in the colorectal hyperplastic polyps (Bara et al., 1983, 1998; Bartman et al., 1999; Biemer-Huttmann et al., 1999; Koike et al., 2003), serrated adenomas (Jass, 1999; Yao et al., 1999;

Hirono et al., 2004), adenomas with tubular components (Ajioka et al., 1997; Longman et al., 2000), and villous adenomas (Bara et al., 1983; Buisine et al., 1996; Takata et al., 2003). Regarding the villous tumors, a high incidence of malignant transformation has been well documented (Welch and Dockerty, 1958; Buisine et al., 1996; Takata et al., 2003). In the present study, colorectal carcinomas with villous adenomas had a higher level of MUC5AC expression as compared with their counterparts with T/TV adenoma. However, there was no difference in MUC5AC expression between adenomatous and carcinomatous components in the villous tumors. With regard to colorectal carcinomas, Yao et al. (2001) have demonstrated a pure gastric type phenotype in 2% of cancer cases, associated with a high histological grade, lymphatic permeation, and lympho node metastasis. We and others have also previously suggested the concept that the lack of intestinal phenotypic expression and the presence of gastric elements might be associated with a poor prognosis with stomach (Tajima et al., 2001; Baldus et al., 2002; Mizoshita et al., 2003) and pancreatic invasive ductal carcinomas (Matsumoto et al., 2004). Further studies are needed to clarify the association between gastric phenotypic expression and the occurrence/progression of carcinomas of the digestive tract, including the large intestine.

We here demonstrated significant reduction of MUC2 expression in intramucosal *de novo* carcinomas compared with intramucosal tumors with tubular adenomatous components, again in line with earlier findings (Blank et al., 1994). The *de novo* carcinomas tend to invade the submucosal layer at an earlier stage and with smaller size than the carcinomas with adenomatous components (Muto et al., 1985; Shimoda et al., 1989). We consider that early loss of MUC2 expression may be associated with the aggressive features of *de novo* colorectal carcinomas.

With regard to the distribution of intestinal epithelial cell markers such as villin and MUC2 in the normal human alimentary tract, most of them are present in both colon and small intestine. In contrast, CD10 and sucrase are present on the luminal surfaces of small intestinal absorptive cells, and rarely in the large intestine (Yao et al., 2001; Mizoshita et al., 2005b) and overexpression of CD10 may be associated with the development and progression of colorectal carcinomas (Iwase et al., 2005), as well as an increased risk of liver metastasis (Yao et al., 2002). Regarding the phenotypic expression of the pyloric glands in the stomach, no MUC6 expression was observed in our cancer cases, in agreement with the report of Iwase et al. (Iwase et al., 2005).

In the present study, most colorectal adenomas and carcinomas retained the expression of villin as the intestinal absorptive cell differentiation. However, the significant decrease of MUC2 expression as goblet cell differentiation was observed from T/TV adenomas with moderate atypia, through intramucosal carcinomas, to cancers with submucosal invasion, according to the adenoma-carcinoma sequence pathway, may be the reason why we observe colorectal mucinous carcinomas rarely.

We have previously demonstrated the expression of MUC5AC and MUC6 in stomach cancers (Reis et al., 2000; Mizoshita et al., 2004a,b, 2005b; Tsukamoto et al., 2005) and pancreatic tumors (Sessa et al., 1990; Matsumoto et al., 2004), using the monoclonal antibodies which were obtained by immunization with peptides rich in serine and threonine, but did not recognize the glycosylated mucin. In the present study of colorectal tumors, we also used the above-mentioned antibodies for the detection of MUC5AC and MUC6 expression, being independent of possible alteration of glycosylation. Regarding mucins, the threonine and/or serine rich regions are O-glycosylation sites (Reis et al., 1997). Mucins are heavily glycosylated, show viscosity, and play a major role in the protection of the epithelium from chemical and mechanical aggressions (Reis et al., 2000). The analyses of the glycosylated mucin expression are very important for the solution of carcinogenesis in the digestive apparatus, including the large intestine, using the antibodies which recognize the glycosylated mucins.

In conclusion, our data suggest that the reduction of MUC2 expression may be associated with the occurrence and progression of colorectal carcinomas in both the adenoma-carcinoma sequence pathway and de novo carcinogenesis. Gastric foveolar expression may be associated with malignant transformation, especially in the villous tumors. Furthermore, tumor-suppressive effects of Cdx2 may be maintained during early stages of colorectal carcinogenesis.

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