Hyperplastic polyps and sessile serrated 'adenomas' of the colon and rectum display gastric pyloric differentiation

Akiyoshi Mochizuka, Takeshi Uehara, Takamichi Nakamura, Yukihiro Kobayashi, Hiroyoshi Ota

A. Mochizuka, T. Nakamura
Department of Laboratory Medicine, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Japan

T. Uehara, Y. Kobayashi
Department of Laboratory Medicine, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto, Japan

H. Ota
Department of Biomedical Laboratory Sciences, School of Health Sciences, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Japan

Send correspondence to:
Hiroyoshi Ota, MD
Professor
Department of Biomedical Laboratory Sciences
School of Health Sciences, Shinshu University School of Medicine
3-1-1 Asahi
Matsumoto, Nagano 390-8621
Japan
E-mail: hohta@shinshu-u.ac.jp
Tel.:+81-263-37-2389
Fax: +81-263-37-2389

Abstract

The serrated polyp-neoplasia pathway is a novel concept that has been demonstrated to differ from the conventional adenoma-carcinoma pathway. To characterize the phenotypic patterns of differentiation in colorectal serrated polyps, we examined the immunohistochemical expression profile of gastric (MUC5AC, TFF1, MUC6, GlcNAcα1→4Gal→R, and PDX1) and intestinal (MUC2, TFF3, and CDX2) epithelial markers in 15 hyperplastic polyps (HPs), 29 sessile serrated adenomas (SSAs),12 traditional serrated adenomas (TSAs), and 16 conventional adenomas (CAs). MUC5AC and TFF1 were upregulated in the HPs, SSAs, and TSAs. MUC6 was expressed in the HPs and SSAs. GlcNAcα1→4Gal→R was expressed only in the SSAs. Although MUC2 expression was preserved, TFF3 was downregulated in the HPs, SSAs, and TSAs. PDX1 was upregulated in the HPs, SSAs, and TSAs. On the other hand, CDX2 was downregulated in the HPs and SSAs. The colorectal serrated polyps showed higher expression of gastric makers than CAs. The HPs and SSAs showed gastric and intestinal mixed phenotype expression with gastric pyloric organoid differentiation and almost identical, but different from the TSAs, in marker profile. PDX1 up-regulation and CDX2 down-regulation could be an important for the induction of a gastric pyloric pattern of cell differentiation in colorectal serrated polyps.

Key words:

homeodomain protein, hyperplastic polyp, mucin core protein, serrated adenoma, TFF

Introduction

Hyperplastic polyps (HPs) of the colorectum, characteristically display elongated crypts and an irregular serrated configuration of the crypt lining cells (Arthur 1968). Traditionally, these polyps have been considered to be benign and non-neoplastic; however, there have been reports of HPs in association with colorectal adenomas or adenocarcinoma. In 1990, Longacre and Fenoglio-Preiser proposed the term “serrated adenoma” for colorectal serrated mucosal lesions comprising dysplastic adenomatous cells (architecturally hyperplastic but cytologically adenomatous) (Longacre and Fenoglio-Preiser 1990). Torlakovic *et al.* (Torlakovic et al. 2003) and, subsequently, Snover *et al.* (Snover et al. 2005) subdivided colorectal serrated polyps into HPs, sessile serrated adenomas (SSAs, serrated lesions with architecturally distorted crypts and cytologically bland cytological features of the cells lining the crypts), and traditional serrated adenomas (TSAs, serrated lesions comprising cells with adenomatous cytological features). Phenotypically, the cells lining crypts of HPs and serrated adenomas show differentiation of the gastric surface and pyloric gland mucous cells (Biemer-Huttmann et al. 1999; Hirono et al. 2004; Koike et al. 2003; Yao et al. 1999). Recently, the concept of a serrated polyp-neoplasia pathway has emerged, but, as yet consensus for the nomenclature of serrated polyps in general, and SSA in particular, has not been reached and interobserver variability is most pronounced for SSAs (Glatz et al. 2007).This pathway may account for up to 15% of sporadic colorectal carcinomas that are characterized by microsatellite instability and DNA mismatch repair deficiency (Iino et al. 1999; Jass et al. 2002). In addition, progress in understanding the molecular basis of serrated colorectal polyps has suggested the origin of the serrated polyp neoplasia pathway in HP and its stepwise progression from HP to SSA, to serrated adenoma, and ultimately to invasive adenocarcinoma (Kambara et al. 2004; O'Brien et al. 2006).

The gastrointestinal mucosa is covered by mucous cells, which produce cell-specific mucins. Mucins are highly glycosylated glycoproteins, and their core proteins (mucin core proteins: MUC) have been named according to the corresponding genes (mucin gene) (Moniaux et al. 2001). MUC5AC, MUC6, and MUC2 are expressed in gastric surface mucous cells, gastric gland mucous cells, and intestinal goblet cells, respectively (Buisine et al. 2000a; Buisine et al. 2000b; Nakajima et al. 2003). In addition, N-acetyl-glucosaminα1$\rightarrow$4galactose$\rightarrow$R (GlcNAcα1$\rightarrow$4Gal$\rightarrow$R), which is recognized by the monoclonal antibody HIK1083 (Kanto Chemical, Tokyo, Japan) (Ishihara et al. 1996) is a sugar residue specific for mucins produced by the gastric gland mucous cells (gastric gland mucous cell-type mucin) (Nakajima et al. 2003; Ota et al. 2001). The gastric gland

mucous cell type mucin has previously been defined by paradoxical concanavalin A staining (Katsuyama and Spicer 1978).

Besides mucins, mucous cells secrete the trefoil factor family (TFF) peptides (TFF1, TFF2, and TFF3), which are mucin-associated peptides that share a structural mucosal barrier function with mucins in addition to participating in the repair and healing of damaged mucosa (Hoffmann 2004). TFF1 (formerly designated as pS2) is produced by gastric surface mucous cells (Ruchaud-Sparagano et al. 2004); TFF2 (human spasmolytic peptide: HSP), by gastric gland mucous cells (Hanby et al. 1993); and TFF3 (intestinal trefoil factor: ITF), mainly by intestinal goblet cells (Podolsky et al. 1993).

Cell growth and differentiation are controlled by homeobox gene-encoded transcription factors. The development of the gastric antrum along with the pancreas and duodenum is regulated by the pancreatic-duodenal homeobox 1 gene (*PDX1*) (Gannon et al. 2001; Stoffers et al. 1999). In mice and humans, PDX1 is expressed in the nuclei of the epithelial cells of the gastric antrum, pancreatic islets and the duodenum (Gannon et al. 2001; Nomura et al. 2005; Sakai et al. 2004; Stoffers et al. 1999). Recently, PDX1 expression was confirmed in the pseudopyloric glands of the gastric mucosa in mice and humans (Nomura et al. 2005; Sakai et al. 2004). In contrast, intestinal development is regulated by the caudal homeobox 2 gene (*CDX2*), and CDX2 is expressed in the nuclei of normal intestinal epithelial cells, intestinal metaplastic cells, and neoplastic cells with intestinal differentiation (Bai et al. 2002; Eda et al. 2002).

The expression of these mucins, mucin-associated peptides and homeobox gene-encoded transcription factors are cell-type specific; hence, they are useful phenotypic indicators of direction of cell differentiation in normal, metaplastic, or neoplastic cells in the gastrointestinal tract (Tatematsu et al. 2003).

In the present study, to promote an understanding of the phenotypic differences among HPs, SSAs, and TSAs and their histogeneses, we examined the characteristics of the epithelial cells of HPs, SSAs, and TSAs diagnosed based on previously described criteria (Parfitt and Driman 2007; Snover et al. 2005) by using immunostaining for cell-type specific mucins, TFFs, and homeobox gene-encoded transcription factor proteins.

Materials and Methods

Histological diagnosis of colorectal polyps.

In this study, we compared HPs, SSAs, and TSAs with normal colon mucosa and conventional adenoma (CA). The diagnosis of HP, SSA, and TSA were diagnosed based on previously described criteria (Parfitt and Driman 2007; Snover et al. 2005), and we included representative cases that fulfilled the following criteria in our study (Figure 1). In brief, HP was diagnosed when a serrated polyp without overt cytological atypia showed narrowed crypt bases predominantly lined with immature cells. In the HPs, crypt branching and dilatation or horizontal orientation of the crypt base were not observed, and serration was noted only in the upper half to one-third of the crypts. SSA was diagnosed when a sessile polyp with epithelial serration demonstrated the following architectural features: crypt branching, dilatation or horizontal orientation of the crypt bases, and the presence of a disordered proliferative zone (presence of mature mucous cells in the bottom of crypts). In cases diagnosed as SSA, some cell populations showed cytoplasmic eosinophilia (Snover et al. 2005). SSA generally showed minimal cytological atypia; however, some cells expressing cytoplasmic eosinophilia showed cytological atypia (cuboidal to short columnar cells with round to oval enlarged nuclei with small nucleoli), as previously reported (Higuchi et al. 2005; Snover et al. 2005). The TSA was diagnosed when a protuberant polyp with epithelial serration was demonstrated to possess a uniform population of columnar cells with cytoplasmic eosinophilia and elongated, polarized, and pencillate nuclei. In this study, serrated polyp included HP, SSA and TSA. CA with low-grade dysplasia was diagnosed based on the criteria of the World Health Organization classification (Hamilton et al. 2000).

Tissue sample selection

Archived pathology reports at Shinshu University Hospital and its affiliated hospitals were searched for cases diagnosed as hyperplastic polyp or serrated adenoma. The hematoxylin and eosin (H&E)-stained slides of the retrieved cases were reviewed and representative cases of HP, SSA and TSA that fulfilled the above mentioned criteria were randomly selected. This yielded 15 HPs (from 15 patients), 29 SSAs (from 27 patients), and 12 TSAs (from 12 patients). In addition, 16 CAs with low grade dysplasia (from 16 patients) were randomly selected for comparison. These lesions were endoscopically excised or collected from the colectomy specimens of patients who had undergone surgery for colorectal cancers. Histologically normal appearing colonic mucosa (5 cases of right-sided colonic lesions and 5 cases of left-sided colonic lesions) was also randomly selected from colectomy specimens for colorectal cancer and served as normal colonic mucosa. The

lesions were considered right sided if they occurred proximal to the splenic flexure and left sided if they occurred distal to the splenic flexure. Polyp size was determined at the time of pathological analysis.

As the positive control for gastric markers, histologically normal appearing pyloric mucosa with no intestinal metaplasia and minimal mononuclear cell infiltration (5 cases) were randomly obtained from pancreatoduodenectomy specimens resected for pancreatic cancer or bile duct cancer.

Preparation of tissue sections, immunohistochemisty, and histochemistry

Serial paraffin sections of 3-μm thickness were stained with H&E for histological examination or subsequent immunohistochemical staining to investigate the phenotype of the cells lining the crypts of the serrated polyps and CAs. To investigate the expression of the characteristics of gastrointestinal epithelial cells, the following indicators of direction of cell differentiation were used: anti-MUC5AC (mouse monoclonal antibody, 45M1, x100, Novocastra, Newcastle-upon-Tyne, UK) and anti-TFF1 (mouse monoclonal antibody, BC04, x50, DAKO, Carpenteria, CA, USA) for gastric surface mucous cells; anti-MUC6 (mouse monoclonal antibody, CLH5, x100, Novocastra) and anti-N-acetyl-glucosaminα1→4galactose→R (GlcNAcα1→4Gal→R) (mouse monoclonal antibody, HIK1083, x5, Kanto Chemical, Tokyo, Japan) for gastric gland mucous cells: anti-PDX1 (guinea pig polyclonal antibody, generously provided by Prof. Christopher V.E. Wright, Vanderbilt University Medical School, Nashville, TN, USA) for gastric epithelial cells; anti-MUC2 (mouse monoclonal antibody, Ccp58, x100, Novocastra) and anti-TFF3 (mouse monoclonal antibody, 15C6, x200, NanoTools Antikoerpertechnik, Teningen, Germany) for intestinal goblet cells; and anti-CDX2 (mouse monoclonal antibody, CDX2-88, x400, BioGenex Inc. San Ramon, CA, USA) for intestinal epithelial cells. Immunohistochemical staining was performed using the immuno-enzyme polymer method (Histofine Simple Stain MAX PO, Nichirei Biosciences, Tokyo, Japan) with 3, 3'-diaminobenzidone as the chromogen. For the immunostaining of PDX1, rabbit polyclonal anti-guinea pig immunoglobulin (DAKO) was used as the secondary antibody prior to using the Histofine detection system. Prior to immunostaining, antigen retrieval was performed using a Pascal pressurized heating chamber (DAKO) (0.01M citrate buffer, pH 6.0) for MUC5AC, MUC6, PDX1, MUC2, and CDX2 or microwave (1 mM EDTA solution, pH 8.0) for TFF1, GlcNAcα1→4Gal→R, and TFF3.

Histologically normal pyloric mucosa and colonic mucosa were used as positive controls for the differentiation markers of gastric and intestinal epithelial cells, respectively.

Negative controls were performed by omitting the primary antibody or substituting it with a non-immune mouse or guinea pig serum.

Evaluation of immunostaining

Immunoreactivity of the phenotypic markers was semiquantitatively estimated as follows: 0, negative; 1, less than one-third of the cells were positive; 2, one-third to two-third of the cells were positive; or 3, more than two third of the cells were positive. Staining scores are nonparametric and are thus expressed as median scores with an interquartile range rather than mean values.

Histology and the immunoreactivity were independently evaluated by 2 pathologists (A. M. and H. O.). Differences in evaluation were resolved after discussion.

Statistics

The Mann-Whitney *U* test was used to compare the clinical data and staining scores between the groups. Differences were considered significant when the *p* value was less than 0.05.

This study was approved by the Ethics Committee of Shinshu University, Japan.

Results

Clinical and histopathological findings

The clinical and histopathological characteristics of the serrated polyps and CAs examined are summarized in Table 1.

The SSAs were more likely to be right sided than the HPs and TSAs. Patients with right-sided HP and SSA tended to be older, and right-sided HPs and SSAs tended to be larger, although age and size differences between right-sided HPs and SSAs and left-sided HPs and SSAs were proved to be statistically not significant. The SSAs and TSAs were larger than the HPs. The TSAs tended to be larger than the SSAs, although size differences between SSAs and TSAs were proved to be statistically not significant.

Expression of gastric epithelial cell markers

In normal gastric pyloric mucosa, MUC5AC was expressed in the mucous granules of the surface mucous cells, MUC6 and GlcNAcα1→4Gal→R were expressed in the mucous granules and cytoplasm of the pyloric gland cells, and PDX1 was expressed in the nuclei of normal gastric epithelial cells in all cases examined (data not shown).

Comparison between normal colonic mucosa and HP, SSA, and TSA

MUC5AC was expressed in the normal colonic mucosa (40%) and in all the serrated polyps examined (Table 2). MUC5AC was expressed in the mucous granules in the goblet cells of normal mucosa and the crypt lining cells of serrated polyps (Figure 2).The staining scores for MUC5AC were higher in the HPs, SSAs, and TSAs than in the normal colonic mucosa (Table 2, Figure 2). In the normal colonic mucosa, goblet cells were generally negative for MUC5AC, and in MUC5AC-positive cases, MUC5AC-positive goblet cells were only sporadically distributed on the surface and in the upper crypts (Figure 2A). In the serrated polyps, MUC5AC was generally expressed throughout the length of the crypt, but some reduction in expression was observed in the basal portion of the crypts (Figures 2B–E).

TFF1 was expressed in the normal colonic mucosa (70%) and in all the serrated polyps examined (Table 2). TFF1 was expressed in the mucous granules and cytoplasm in the goblet cells in normal mucosa and the crypt lining cells in serrated polyps (Figure 3). The staining scores for TFF1 were higher in the HPs, SSAs, and TSAs than in the normal colonic mucosa (Table 2, Figure 3). In the normal colonic mucosa immunoreactive for TFF1, TFF1-positive goblet cells were only sporadically distributed on the surface and in the upper crypts (Figure 3A). In the serrated polyps, TFF1 was expressed in an increasing concentration gradient toward the upper crypts (Figures 3B–E).

MUC6 was expressed in the HPs (26.7%), and SSAs (75.9%), but not in the normal colonic mucosa or TSAs (Table 2, Figure 4). MUC6 was expressed in the mucous granules and cytoplasm in the crypt lining cells localized at the crypt base or with an increasing concentration gradient toward the crypt base (Figures 4B–D).

GlcNAcα1→4Gal→R was expressed only in 3 cases of the SSA (10.3%) (Table 2, Figure 5). GlcNAcα1→4Gal→R-positive cells were cuboidal mucous cells with flattened nuclei at the cell base and formed a gland-like structure similar to the pyloric gland at the base of the crypts (Figures 1D and 5D). In the SSAs with the gland-like structure immunoreactive for GlcNAcα1→4Gal→R, the crypt epithelium comprised columnar cells with eosinophilic cytoplasm (Figure 1D). These eosinophilic crypt cells showed low grade nuclear atypia in 1 case (Figure 1D).

The nuclear expression of PDX1 was detected in the crypt lining cells in the HPs (93.3%), SSAs (96.6%), and TSAs (100%), but not in the normal colonic mucosa (Table 2, Figure 6).

Comparison of between CA and HP, SSA, and TSA

The CAs were immunoreactive for MUC5AC (68.8%), TFF1 (50%), MUC6 (6.3%), and PDX1 (12.5%) but not for GlcNAcα1→4Gal→R (Table 2), and the staining scores for MUC5AC, TFF1, and PDX1 were higher in the HPs, SSAs, and TSAs than in CAs (Table 2). The staining scores for MUC6 were higher only in the SSAs than in CAs (Table 2).

Comparison among serrated polyps

The HPs and SSAs showed similar staining scores for all gastric markers, except for MUC6 (Table 3). Compared to the HPs, the SSAs showed higher staining scores for MUC6 (Tables 2 and 3), and right-sided SSAs showed higher staining scores for MUC6 than the left-sided ones ($p < 0.05$). There were no significant differences between right-sided HPs and left-sided HPs on gastric marker expressions. Compared to TSAs, the HPs showed higher staining scores for MUC5AC, and the SSAs showed higher staining scores for MUC5AC, TFF1, and MUC6 (Table 3).

Expression of intestinal epithelial cell markers

Comparison between normal colonic mucosa and HP, SSA, and TSA

MUC2, TFF3, and CDX2 were expressed in all the specimens of normal colonic mucosa and serrated polyps examined (Table 2). MUC2 and TFF3 were expressed in the mucous granules and cytoplasm in the goblet cells in normal mucosa (Figure 7A) and the crypt

lining cells in serrated polyps (Figures 7B-E). CDX2 were localized in the nuclei of epithelial cells in normal colonic mucosa (Figure 8A) and serrated polyps (Figures 8B-E). The staining scores for MUC2 were not significantly different between the normal colonic mucosa and all 3 types of serrated polyps (Table 2). The staining scores for TFF3 were lower in all the 3 types of serrated polyps (Table 2) and those for CDX2 were lower in the HPs and SSAs than in normal colonic mucosa (Table 2).

Comparison between CA and HP, SSA, and TSA

The results were identical to those obtained by comparing the normal colonic mucosa with HP, SSA, and TSA (Table 2).

Comparison among serrated polyps

The staining scores for all intestinal epithelial markers in the HPs were similar to those in SSAs (Table 2). The TSAs showed higher staining scores for CDX2 than either the HPs or SSAs (Tables 2 and 3). Additionally, compared to the SSAs, the TSAs showed higher staining scores for TFF3 (Tables 2 and 3).

Discussion

The present study demonstrates the clinical and phenotypic characteristics of colorectal serrated polyps: HPs, SSAs, and TSAs.

In this study, the SSAs occurred at a higher frequency in the left-side than the right-side of the colon and showed a predilection for the right-side of the colon compared with the HPs and TSAs: these observations were consistent with prior reports that used a consecutively accrual methodology (Higuchi et al. 2005) or used a case-selected methodology (Torlakovic et al. 2003). Contrastingly, in another study, which used a consecutive methodology (Parfitt and Driman 2007), it was noted that SSAs were predominantly located in the right-side of the colon. These discrepancies among reports could be explained by differences in the criteria of SSA as well as case selection bias. However, there is agreement that SSAs show a predilection for the right-side of the colon compared with HPs and TSAs (Higuchi et al. 2005; Parfitt and Driman 2007; Torlakovic et al. 2003). In addition, we found the SSAs and TSAs were larger than the HPs. This is generally in good agreement with the previous study that used consecutive samples (Higuchi et al. 2005).

A comparison of the cellular phenotypes of these 3 types of serrated polyps revealed that the HPs and SSAs were characterized by a higher expression of gastric markers than the TSAs and had essentially similar cellular phenotypes. On the other hand, TSAs were characterized by a higher expression of intestinal markers than the HPs and SSAs. These observations demonstrate that HPs and SSAs are closely related lesions with regard to phenotype. Some recent reports have noted molecular events to account for the transition in the serrated polyp neoplasia pathway from HP to SSA (Kambara et al. 2004; O'Brien et al. 2006). Thus, high prevalence of *BRAF* mutation among serrated colorectal polyps, its origin in HP, and increase of CpG island methylation in SSA have been reported (Kambara et al. 2004; O'Brien et al. 2006). Additional molecular features may serve as more selective markers for high-risk serrated polyps.

In HPs, SSAs, and TSAs, MUC5AC (gastric surface mucous cell marker) was generally expressed throughout the length of the crypts, and TFF1 (gastric surface mucous cell marker) was expressed in an increasing concentration gradient toward the upper crypts. In contrast, MUC6 (gastric gland mucous cell marker) was expressed in an increasing concentration gradient toward the crypt base in the HPs and SSAs but not in the TSAs. Moreover, GlcNAcα1$\rightarrow$4Gal$\rightarrow$R (gastric gland mucous cell marker) was expressed in mucous cells of a gland-like structure similar to the pyloric gland at the crypt base in the SSAs. The results of this study are generally in agreement with previous reports regarding

the cellular phenotype characteristics of serrated polyps (Biemer-Huttmann et al. 1999; Hirono et al. 2004; Koike et al. 2003; Yao et al. 1999). Yao *et al.* demonstrated the presence of MUC5AC and TFF1 (formerly termed pS2) in HPs and serrated adenomas and suggested the differentiation of the mucous cells in these serrated polyps toward gastric surface mucous cells (Yao et al. 1999). Biemer-Huttmann *et al.* and Koike *et al*. also demonstrated the aberrant expression of MUC5AC in HPs and serrated adenomas (Biemer-Huttmann et al. 1999; Koike et al. 2003). More recently, Hirono *et al.* noted the expression of MUC5AC and MUC6 in HPs and serrated adenomas and the expression of GlcNAcα1→4Gal→R in serrated adenomas, suggesting bidirectional differentiation of mucous cells toward gastric surface mucous cells and gastric gland mucous cells in HPs and serrated adenomas (Hirono et al. 2004).These findings indicate that organoid differentiation simulating the gastric pyloric mucosa occurs in HPs and SSAs, and gastric surface mucous cell differentiation but not gastric gland mucous cell differentiation occurs in TSAs. Similar organoid differentiation simulating the gastric pyloric mucosa has been reported in pyloric metaplasia of the pancreatic duct (Arakura et al. 2001; Matsuzawa et al. 1992; Ota et al. 2001), ileum (Kushima et al. 1997) and the uterine cervix (Ishii et al. 1998), and in adenocarcinoma of the stomach (Fujimori et al. 1995; Ota et al. 2001), pancreas (Arakura et al. 2001; Matsuzawa et al. 1992; Ota et al. 2001), lung (Honda et al. 1998; Ota et al. 2001), ovary (Shiozawa et al. 1992), and uterine cervix (Ishii et al. 1998). Importantly, the pyloric metaplasia has been suggested to be a precursor lesion for adenocarcinoma in which gastric mucins are produced in the pancreas (Matsuzawa et al. 1992) and uterine cervix (Ishii et al. 1998).

Our results show an increased expression of PDX1 in all 3 types of serrated polyps and a decreased expression of CDX2 in the HPs and SSAs. These findings may be related to the gastric pyloric pattern of cell differentiation in serrated polyps. PDX1 is a transcription factor that plays an essential role in the genesis and development of the gastric antrum as well as of the pancreas and duodenum (Gannon et al. 2001). In normal human or mice gastrointestinal mucosa, PDX1 expression has been reported in the nuclei of the epithelial cells of the gastric pyloric mucosa as well as the pancreatic islet and the duodenal mucosa (Gannon et al. 2001; Nomura et al. 2005; Sakai et al. 2004). Interestingly, the presence of ectopic PDX1 expression in the gastric fundic mucosa was reported in pseudopyloric gland metaplasia (antralization) in humans (Nomura et al. 2005; Sakai et al. 2004) and in mice (Nomura et al. 2005). In contrast, CDX2 is the decisive differentiation factor for the development and homeostasis of the intestinal epithelium. Recent studies have reported that the loss of CDX2 function in the intestine leads to the transformation of intestinal

mucosa to gastric mucosa in the human duodenum (gastric meteplasia) (Faller et al. 2004) and the *Cdx2*+/– mouse colon (Beck et al. 1999). Thus, it appears that PDX1 up-regulation and CDX2 down-regulation could be important for the induction of a gastric pyloric pattern of cell differentiation in colorectal serrated polyps.

In this study, the mixed phenotype expression in the colorectal serrated polyps was confirmed and elaborated (Biemer-Huttmann et al. 1999; Higuchi et al. 2005; Koike et al. 2003). Importantly, a similar disturbance of cellular differentiation, i.e., differentiation resulting in a gastric and intestinal mixed-phenotype, has been observed in lesions showing heterogeneous proliferations with a malignant potential, namely, gastric intestinal metaplasia (Inada et al. 1997) and in pancreatic intraductal papillary-mucinous neoplasms (Terris et al. 2002). Interestingly, colorectal serrated polyps, particularly SSAs, have been reported to be candidates for precursor lesions of colorectal carcinoma with high microsatellite instability, for which a gastric and intestinal mixed mucinous phenotype has been reported (Biemer-Huttmann et al. 2000; Goldstein et al. 2003). These observations support the concept that the progression of serrated polyps to colorectal carcinoma differs from the conventional adenoma-carcinoma sequence (Hawkins and Ward 2001; Jass et al. 2002), particularly for SSAs that might present with abnormal mismatch repair (Torlakovic et al. 2003) and develop into adenocarcinoma with microsatellite instability (Goldstein et al. 2003).

In summary, here, we confirmed and elaborated the characteristics of the gastric and intestinal mixed phenotype in colorectal serrated polyps, particularly HPs and SSAs that showed organoid differentiation simulating the gastric pyloric mucosa. The PDX1 up-regulation and CDX2 down-regulation could be important for inducing a gastric pyloric pattern of cell differentiation in colorectal serrated polyps. Further research on the regulation of PDX1 and CDX2 expressions may provide useful information for investigating the mechanisms involved in aberrant phenotypic expression and the histogeneses of colorectal serrated polyps.

Acknowledgments

We gratefully thank Professor Tsutomu Katsuyama (Department of Laboratory Medicine, Shinshu University School of Medicine) for his advice and encouragement, and Fujie Miyazawa, Reiko Horiuchi, and Hiroko Kobayashi (Maruko Central Hospital) for their excellent technical assistance.

Figure legends

Figure 1. Morphological appearance of normal colonic mucosa (A) (original magnification, ×50), hyperplastic polyp (HP) (B) (original magnification, ×30), sessile serrated adenoma (SSA) (C and D) (original magnification, ×40; inset: original magnification, ×130), and traditional serrated adenoma (TSA) (E) (original magnification, ×25).

HP shows serrated crypt epithelium comprising cells without overt cytological atypia and crypts with narrowed bases (B). SSA shows serrated crypt epithelium comprising cells without overt cytological atypia with dilatation and horizontal orientation of the crypt base (C). Further, SSA shows serrated crypt epithelium comprising cells with eosinophilic cytoplasm and gland-like structure at the base of crypts (arrows and inset) (D). TSA shows epithelial serration comprising a uniform population of columnar cells with cytoplasmic eosinophilia and elongated nuclei.

Figure 2. Immunostaining for MUC5AC.

MUC5AC immunoreactivity is found only sporadically in goblet cells on the surface and in the upper crypts in normal colonic mucosa (A) and is increased in HP (B), SSA (C and D), and TSA (E).

Figure 3. Immunostaining for TFF1.

TFF1 immunoreactivity is found only sporadically in goblet cells on the surface and in the upper crypts in normal colonic mucosa (A) and is increased with an increasing concentration gradient toward the upper crypts in HP (B), SSA (C and D), and TSA (E).

Figure 4. Immunostaining for MUC6 (inset: original magnification, ×100).

MUC6 is not expressed in normal colonic mucosa (A), or TSA (E). It is expressed at the crypt base in HP (B) (arrows and inset) or with an increasing concentration gradient toward the crypt base in SSA (C and D).

Figure 5. Immunostaining for GlcNAcα1→4Gal→R (inset: original magnification, ×130).

GlcNAcα1→4Gal→R is not expressed in normal colonic mucosa (A), HP (B), SSA without gland-like structure (C), or TSA (E) but is expressed in the cells with gland-like structure at the crypt base (arrows and inset) of SSA (D).

Figure 6. Immunostaining for PDX1 (inset: original magnification ×150)

PDX1 immunoreactivity is not detected in normal colonic mucosa (A), instead, it is detected in the nuclei (inset) of HP (B), SSA (C and D), and TSA (E).

Figure 7. Immunostaining for TFF3

TFF3 immunoreactivity is found throughout the length of the crypts in normal mucosa (A), and is decreased in HP (B), SSA (C and D), and TSA (E).

Figure 8. Immunostaining for CDX2 (inset: original magnification ×150)

Nuclear expression of CDX2 is found throughout the length of the crypts in normal mucosa (A) and TSA (E); however, its expression is decreased in HP (B) and SSA (C and D).

References

Arakura N, Hayama M, Honda T, Matsuzawa K, Akamatsu T, Ota H (2001) Histone H3 mRNA in situ hybridization for identifying proliferating cells in human pancreas, with special reference to the ductal system. Histochem J 33:183-191.

Arthur JF (1968) Structure and significance of metaplastic nodules in the rectal mucosa. J Clin Pathol 21:735-743.

Bai YQ, Yamamoto H, Akiyama Y, Tanaka H, Takizawa T, Koike M, Kenji Yagi O, Saitoh K, Takeshita K, Iwai T, Yuasa Y (2002) Ectopic expression of homeodomain protein CDX2 in intestinal metaplasia and carcinomas of the stomach. Cancer Lett 176:47-55.

Beck F, Chawengsaksophak K, Waring P, Playford RJ, Furness JB (1999) Reprogramming of intestinal differentiation and intercalary regeneration in Cdx2 mutant mice. Proc Natl Acad Sci U S A 96:7318-7323.

Biemer-Huttmann AE, Walsh MD, McGuckin MA, Ajioka Y, Watanabe H, Leggett BA, Jass JR (1999) Immunohistochemical staining patterns of MUC1, MUC2, MUC4, and MUC5AC mucins in hyperplastic polyps, serrated adenomas, and traditional adenomas of the colorectum. J Histochem Cytochem 47:1039-1048.

Biemer-Huttmann AE, Walsh MD, McGuckin MA, Simms LA, Young J, Leggett BA, Jass JR (2000) Mucin core protein expression in colorectal cancers with high levels of microsatellite instability indicates a novel pathway of morphogenesis. Clin Cancer Res 6:1909-1916.

Buisine MP, Devisme L, Maunoury V, Deschodt E, Gosselin B, Copin MC, Aubert JP, Porchet N (2000a) Developmental mucin gene expression in the gastroduodenal tract and accessory digestive glands. I. Stomach. A relationship to gastric carcinoma. J Histochem Cytochem 48:1657-1666.

Buisine MP, Devisme L, Degand P, Dieu MC, Gosselin B, Copin MC, Aubert JP, Porchet N (2000b) Developmental mucin gene expression in the gastroduodenal tract and accessory digestive glands. II. Duodenum and liver, gallbladder, and pancreas. J Histochem Cytochem 48:1667-1676.

Eda A, Osawa H, Yanaka I, Satoh K, Mutoh H, Kihira K, Sugano K (2002) Expression of homeobox gene CDX2 precedes that of CDX1 during the progression of intestinal metaplasia. J Gastroenterol 37:94-100.

Faller G, Dimmler A, Rau T, Spaderna S, Hlubek F, Jung A, Kirchner T, Brabletz T (2004) Evidence for acid-induced loss of Cdx2 expression in duodenal gastric metaplasia. J Pathol 203:904-908.

Fujimori Y, Akamatsu T, Ota H, Katsuyama T (1995) Proliferative markers in gastric carcinoma and organoid differentiation. Human Pathology 26:725-734.

Gannon M, Gamer LW, Wright CV (2001) Regulatory regions driving developmental and tissue-specific expression of the essential pancreatic gene pdx1. Dev Biol 238:185-201.

Glatz K, Pritt B, Glatz D, Hartmann A, O'Brien MJ, Blaszyk H (2007) A multinational, internet-based assessment of observer variability in the diagnosis of serrated colorectal polyps. Am J Clin Pathol 127:938-945.

Goldstein NS, Bhanot P, Odish E, Hunter S (2003) Hyperplastic-like colon polyps that preceded microsatellite-unstable adenocarcinomas. Am J Clin Pathol 119:778-796.

Hamilton SR, Volgelstein B, Kudo S, Riboil E, Nakamura S, Hainaut P, Rubio CA, Sobin LH, Fogt F, Winawer SJ, Goldgar DE, Jass JR, editors. 2000. Carcinoma of the colon and rectum. Lyon: IARC Press. 105-119 p.

Hanby AM, Poulsom R, Singh S, Elia G, Jeffery RE, Wright NA (1993) Spasmolytic polypeptide is a major antral peptide: distribution of the trefoil peptides human spasmolytic polypeptide and pS2 in the stomach. Gastroenterology 105:1110-1116.

Hawkins NJ, Ward RL (2001) Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. J Natl Cancer Inst 93:1307-1313.

Higuchi T, Sugihara K, Jass JR (2005) Demographic and pathological characteristics of serrated polyps of colorectum. Histopathology 47:32-40.

Hirono H, Ajioka Y, Watanabe H, Baba Y, Tozawa E, Nishikura K, Mukai G, Honma T, Aoyagi Y (2004) Bidirectional gastric differentiation in cellular mucin phenotype (foveolar and pyloric) in serrated adenoma and hyperplastic polyp of the colorectum. Pathol Int 54:401-407.

Hoffmann W (2004) Trefoil factor family (TFF) peptides: regulators of mucosal regeneration and repair, and more. Peptides 25:727-730.

Honda T, Ota H, Ishii K, Nakamura N, Kubo K, Katsuyama T (1998) Mucinous bronchioloalveolar carcinoma with organoid differentiation simulating the pyloric mucosa of the stomach: clinicopathologic, histochemical, and immunohistochemical analysis. American Journal of Clinical Pathology 109:423-430.

Iino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y, Watanabe H (1999) DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? J Clin Pathol 52:5-9.

Inada K, Nakanishi H, Fujimitsu Y, Shimizu N, Ichinose M, Miki K, Nakamura S, Tatematsu

M (1997) Gastric and intestinal mixed and solely intestinal types of intestinal metaplasia in the human stomach. Pathol Int 47:831-841.

Ishihara K, Kurihara M, Goso Y, Urata T, Ota H, Katsuyama T, Hotta K (1996) Peripheral alpha-linked N-acetylglucosamine on the carbohydrate moiety of mucin derived from mammalian gastric gland mucous cells: epitope recognized by a newly characterized monoclonal antibody. Biochemical Journal 318:409-416.

Ishii K, Hosaka N, Toki T, Momose M, Hidaka E, Tsuchiya S, Katsuyama T (1998) A new view of the so-called adenoma malignum of the uterine cervix. Virchows Archiv 432:315-322.

Jass JR, Whitehall VL, Young J, Leggett BA (2002) Emerging concepts in colorectal neoplasia. Gastroenterology 123:862-876.

Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, Young J, Jass JR, Leggett BA (2004) BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut 53:1137-1144.

Katsuyama T, Spicer SS (1978) Histochemical differentiation of complex carbohydrates with variants of the concanavalin A-horseradish peroxidase method. Journal of Histochemistry & Cytochemistry 26:233-250.

Koike M, Inada K, Nakanishi H, Matsuura A, Nakamura S, Tatematsu M (2003) Cellular differentiation status of epithelial polyps of the colorectum: the gastric foveolar cell-type in hyperplastic polyps. Histopathology 42:357-364.

Kushima R, Borchard F, Hattori T (1997) A new aspect of gastric metaplasia in Crohn's disease: bidirectional (foveolar and pyloric) differentiation in so-called 'pyloric metaplasia' in the ileum. Pathol Int 47:416-419.

Longacre TA, Fenoglio-Preiser CM (1990) Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. Am J Surg Pathol 14:524-537.

Matsuzawa K, Akamatsu T, Katsuyama T (1992) Mucin histochemistry of pancreatic duct cell carcinoma, with special reference to organoid differentiation simulating gastric pyloric mucosa. Hum Pathol 23:925-933.

Moniaux N, Escande F, Porchet N, Aubert JP, Batra SK (2001) Structural organization and classification of the human mucin genes. Front Biosci 6:D1192-1206.

Nakajima K, Ota H, Zhang MX, Sano K, Honda T, Ishii K, Nakayama J (2003) Expression of gastric gland mucous cell-type mucin in normal and neoplastic human tissues. J Histochem Cytochem 51:1689-1698.

Nomura S, Settle SH, Leys CM, Means AL, Peek RM, Jr., Leach SD, Wright CV, Coffey RJ, Goldenring JR (2005) Evidence for repatterning of the gastric fundic epithelium associated with Menetrier's disease and TGFalpha overexpression. Gastroenterology 128:1292-1305.

O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA (2006) Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol 30:1491-1501.

Ota H, Hayama M, Nakayama J, Hidaka H, Honda T, Ishii K, Fukushima M, Uehara T, Kurihara M, Ishihara K, Hotta K, Katsuyama T (2001) Cell lineage specificity of newly raised monoclonal antibodies against gastric mucins in normal, metaplastic, and neoplastic human tissues and their application to pathology diagnosis. Am J Clin Pathol 115:69-79.

Parfitt JR, Driman DK (2007) Survivin and hedgehog protein expression in serrated colorectal polyps: an immunohistochemical study. Hum Pathol 38:710-717.

Podolsky DK, Lynch-Devaney K, Stow JL, Oates P, Murgue B, DeBeaumont M, Sands BE, Mahida YR (1993) Identification of human intestinal trefoil factor. Goblet cell-specific expression of a peptide targeted for apical secretion. J Biol Chem 268:6694-6702.

Ruchaud-Sparagano MH, Westley BR, May FE (2004) The trefoil protein TFF1 is bound to MUC5AC in human gastric mucosa. Cell Mol Life Sci 61:1946-1954.

Sakai H, Eishi Y, Li XL, Akiyama Y, Miyake S, Takizawa T, Konishi N, Tatematsu M, Koike M, Yuasa Y (2004) PDX1 homeobox protein expression in pseudopyloric glands and gastric carcinomas. Gut 53:323-330.

Shiozawa T, Tsukahara Y, Ishii K, Ota H, Nakayama J, Katsuyama T (1992) Histochemical demonstration of gastrointestinal mucins in ovarian mucinous cystadenoma. Acta Pathologica Japonica 42:104-110.

Snover DC, Jass JR, Fenoglio-Preiser C, Batts KP (2005) Serrated polyps of the large intestine: a morphologic and molecular review of an evolving concept. Am J Clin Pathol 124:380-391.

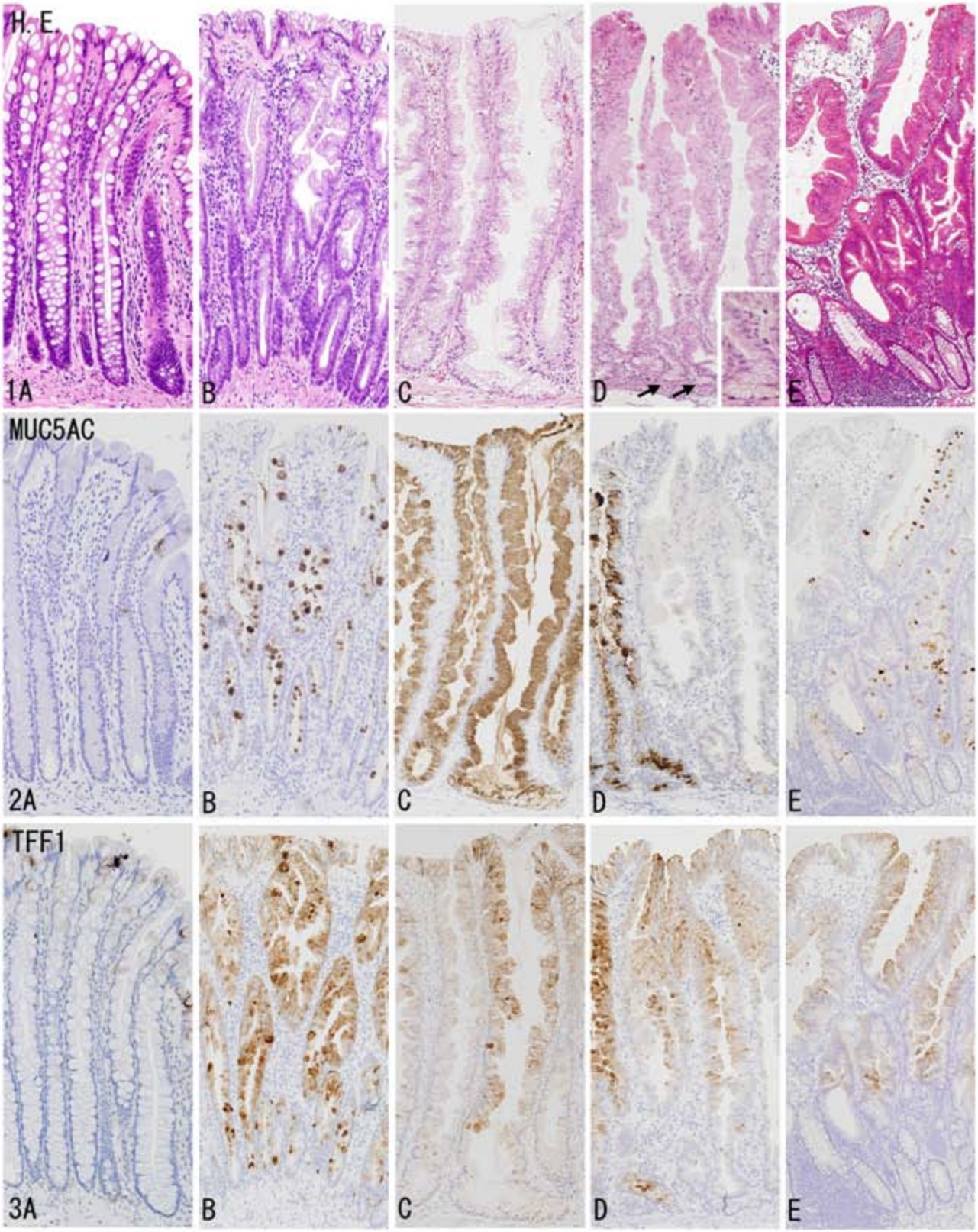
Stoffers DA, Heller RS, Miller CP, Habener JF (1999) Developmental expression of the homeodomain protein IDX-1 in mice transgenic for an IDX-1 promoter/lacZ transcriptional reporter. Endocrinology 140:5374-5381.

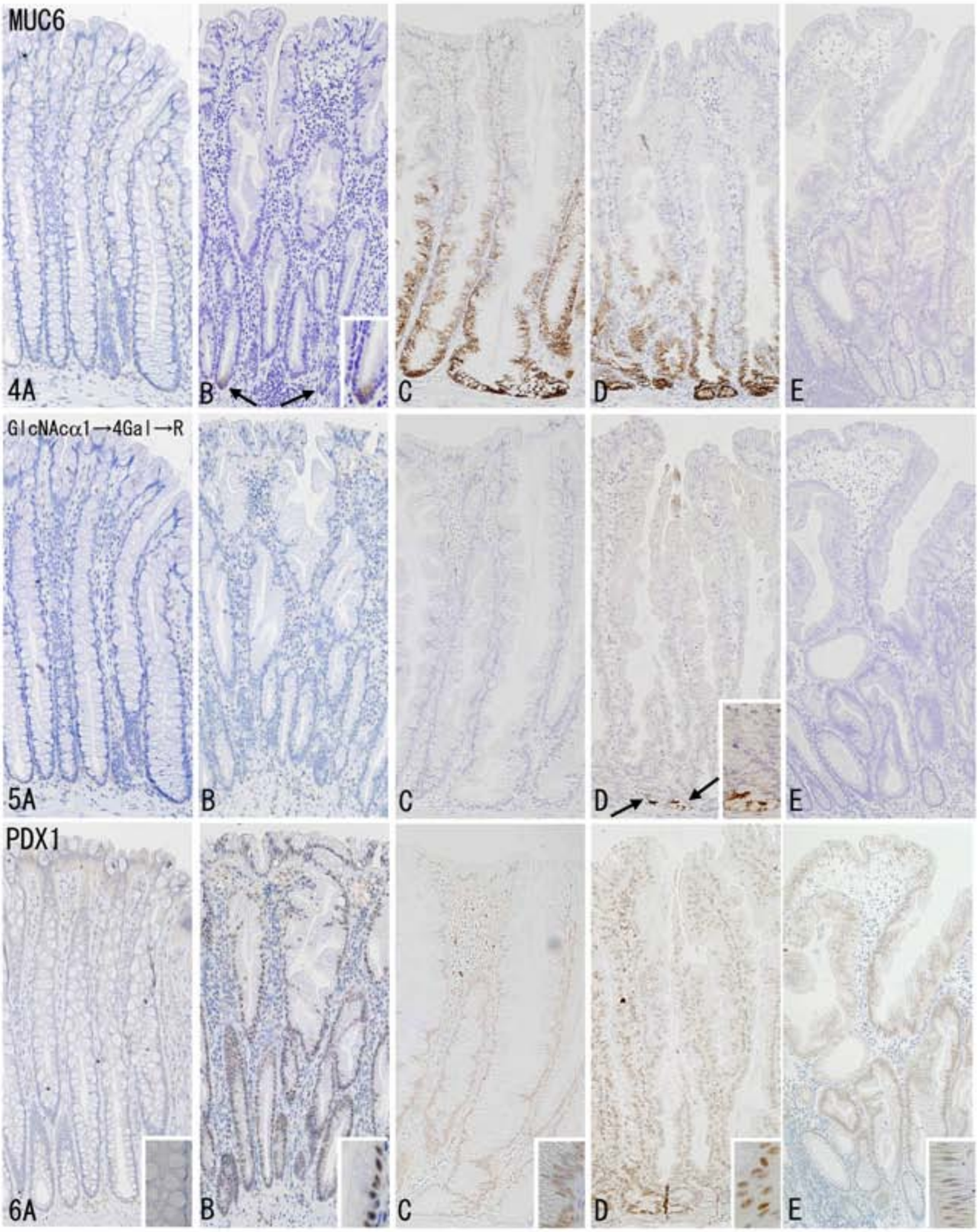
Tatematsu M, Tsukamoto T, Inada K (2003) Stem cells and gastric cancer: role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci 94:135-141.

Terris B, Dubois S, Buisine MP, Sauvanet A, Ruszniewski P, Aubert JP, Porchet N, Couvelard A, Degott C, Flejou JF (2002) Mucin gene expression in intraductal papillary-mucinous pancreatic tumours and related lesions. J Pathol 197:632-637.

Torlakovic E, Skovlund E, Snover DC, Torlakovic G, Nesland JM (2003) Morphologic reappraisal of serrated colorectal polyps. Am J Surg Pathol 27:65-81.

Yao T, Kouzuki T, Kajiwara M, Matsui N, Oya M, Tsuneyoshi M (1999) 'Serrated' adenoma of the colorectum, with reference to its gastric differentiation and its malignant potential. J Pathol 187:511-517.


H. E.
1A
B
C
D
E
MUC5AC
2A
B
C
D
E
TFF1
3A
B
C
D
E


MUC6
4A
B
C
D
E
GlcNAcα1→4Gal→R
5A
B
C
D
E
PDX1
6A
B
C
D
E

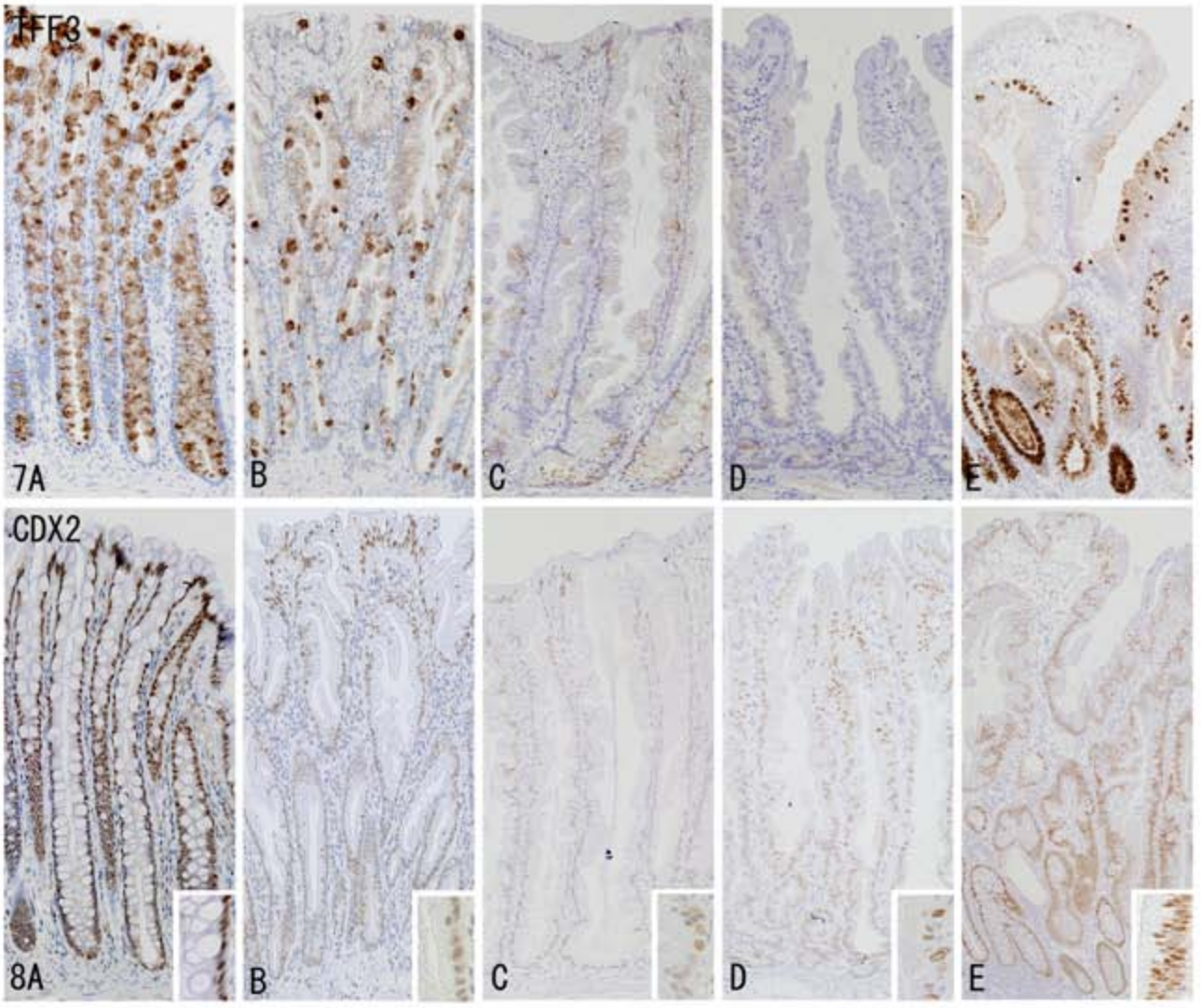

TFF3
7A
B
C
D
E
CDX2
8A
B
C
D
E

Table 1. Clinical and pathology data of normal mucosa, conventional adenoma, hyperplastic polyp, sessile serrated adenoma, and traditional serrated adenoma

	Normal mucosa (n = 10)	CA (n = 16)	HP (n = 15)	SSA (n = 29)	TSA (n = 12)
Distribution					
Right (%)	5	4 (25)	4 (26.7)	12 (41.4)	0 (0)
Left	5	12	11	17	12
Mean age ± SD (y)					
Right	68.4 ± 9.4	62.8 ± 8.3	68.5 ± 7.6	68.8 ± 8.3	NA
Left	72.2 ± 6.4	60.9 ± 9.8	60.7 ± 12.4	58.7 ± 13.1	60.5 ± 12.4
Right + Left	70.3 ± 8.2	61.4 ± 9.5	62.8 ± 11.4	63.2 ± 12.3	60.5 ± 11.9
Size ± SD (mm)					
Right		4.0 ± 2.9	3.3 ± 0.8	8.3 ± 3.7*	NA
Left		5.3 ± 1.8	3.2 ± 0.7	6.8 ± 2.3*	8.8 ± 3.5*
Right + Left		4.9 ± 2.2	3.2 ± 0.7	7.4 ± 3.0*	8.8 ± 3.5*

CA, conventional adenoma; HP, hyperplastic polyp; SSA, sessile serrated adenoma;
TSA, traditional serrated adenoma; NA, not available
*$p < 0.05$ vs HP

Table 2. Expression of each phenotypic marker in normal mucosa, conventional adenoma, hyperplastic polyp,
sessile serrated adenoma, and traditional serrated adenoma

	MUC5AC	TFF1	MUC6	HIK	PDX1	MUC2	TFF3	CDX2
Nor (N = 10)	40[a] (0/0–1)[b]	70 (1/0–1)	0 (0/0–0)	0 (0/0–0)	0 (0/0–0)	100 (3/3–3)	100 (3/3–3)	100 (3/3–3)
CA (N = 16)	68.8 (1/0–1)	50 (0.5/0–1)	6.3 (0/0–0)	0 (0/0–0)	12.5 (0/0–0)	100 (3/3–3)	100 (3/3–3)	100 (3/3–3)
HP (N = 15)	100 (2/2–3)*#	100 (3/2–3)*#	26.7 (0/0–0.75)	0 (0/0–0)	93.3 (3/2–3)*#	100 (3/3–3)	100 (2/1–2.75)*#	100 (2/1–3)*#
SSA (N = 29)	100 (3/2–3)*#	100 (3/2–3)*#	75.9 (1/0.75–1) *#	10.3 (0/0–0)	96.6 (2/1–3) *#	100 (3/3–3)	100 (2/1.75–2) *#	100 (1/1–2) *#
TSA (N = 12)	100 (1/0–1) *#	100 (2/2–3) *#	0 (0/0–0)	0 (0/0–0)	100 (3/3–3) *#	100 (3/3–3)	100 (2.5/2–3) *#	100 (3/3–3)

Nor, normal mucosa; CA, conventional adenoma; HP, hyperplastic polyp; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma.

[a] Frequency (%) of positive specimens

[b] Median score with the interquartile range in parentheses

Scores for staining were analyzed by the Mann-Whitney U-test

* $p < 0.05$ vs normal mucosa.

Table 3. Comparison of marker expression among hyperplastic polyp, sessile serrated adenoma, and traditional serrated adenoma

	HP vs SSA	HP vs TSA	SSA vs TSA
MUC5AC	NS	<0.01	<0.01
TFF1	NS	NS	<0.05
MUC6	<0.05	NS	<0.01
HIK	NS	NS	NS
PDX1	NS	NS	NS
MUC2	NS	NS	NS
TFF3	NS	NS	<0.01
CDX2	NS	<0.01	<0.01

HPP, hyperplastic polyp; SSA, sessile serrated adenoma; TSA, traditional serrated adenoma; NS, not statistically significant.