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Original article

Expression of *Cdx1* and *Cdx2* mRNAs and relevance of this expression to differentiation in human gastrointestinal mucosa — with special emphasis on participation in intestinal metaplasia of the human stomach

Tsutomu Mizoshita[1,2], Ken-ichi Inada[1], Tetsuya Tsukamoto[1], Yasuhiro Kodera[3], Yoshitaka Yamamura[3], Takashi Hirai[3], Tomoyuki Kato[3], Takashi Joh[2], Makoto Itoh[2], and Masae Tatematsu[1]

[1] Division of Oncological Pathology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan
[2] First Department of Internal Medicine, Nagoya City University Medical School, Nagoya, Japan
[3] Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya, Japan

Abstract

Background. **The caudal-type homeobox genes, *Cdx1* and *Cdx2*, are candidates for directing intestinal development, differentiation, and maintenance of the intestinal phenotype. The aims of this study were: (1) to assess the normal tissue expression patterns of *Cdx1* and *Cdx2* in the human gastrointestinal tract and (2) to ascertain levels in intestinal metaplasia (IM) of the stomach associated with gastritis.**

Methods. **Fresh human tissues were collected by surgical resection from 39 patients after informed consent had been received. RNAs were extracted from 11 distinct sites in the gastrointestinal mucosa (gastric body, gastric antrum, duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum), and Northern hybridization was performed for *Cdx1* and *Cdx2* mRNAs. In addition, RNAs were also extracted from normal gastric mucosa, and gastric mucosa with mild to severe IM, confirmed histopathologically. Reverse-transcriptase polymerase chain reaction (RT-PCR) was then carried out for *Cdx1* and *Cdx2*.**

Results. **The expression of *Cdx1* mRNA increased gradually from the duodenum to the distal colon, with no expression detected in the stomach. Compared with the distribution of *Cdx1* mRNA in the mouse gastrointestinal tract, the expression of *Cdx1* mRNA in the human gastrointestinal tract showed greater predominance in the jejunum and ileum. The expression of *Cdx2* mRNA increased gradually from the duodenum to the proximal colon and decreased from the ascending colon to the rectum. Compared with the expression pattern of *Cdx2* mRNA in the mouse gastrointestinal tract, the expression of *Cdx2* mRNA in the human gastrointestinal tract showed greater predominance in the ileum. By RT-PCR, both *Cdx1* and *Cdx2* mRNAs were detected in the mild and severe types of IM. However, neither of these mRNAs was identified in normal gastric mucosa without IM.**

Conclusions. ***Cdx1* and *Cdx2* mRNAs are widely present in the human intestinal and colonic mucosae, but not in the gastric mucosa, suggesting that their expression may contribute to the intestinal phenotype. The high levels of these mRNAs in IM mucosa associated with chronic atrophic gastritis point to an association with this phenotypic shift in the gastric mucosa.**

Key words Caudal-type homeobox gene (*Cdx*) 1 · *Cdx2* · Intestinal metaplasia

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Introduction

Homeobox genes are essential for the control of normal embryonic development. Above all, *Cdx1* and *Cdx2*, members of the caudal-related homeobox family [1], are believed to be important in the early differentiation and maintenance of intestinal epithelial cells. The expression of these genes has been widely investigated both in the mouse and the human. In the mouse fetus, the longitudinal patterns of both genes are already established, and are maintained during the steps of fetal and postnatal development [2–8]. In the adult, the anterior limit of *Cdx1* and *Cdx2* expression corresponds to the beginning of the duodenum. Both genes exhibit graded expression from the small intestine to the colon. In the colon, in particular, the level of *Cdx1* increases in the posterior direction, whereas the *Cdx2* level is higher in the proximal region than in the distal. No expression is normally detected in the stomach or esophagus.

Recently, the detailed expression patterns of both gene products in the mouse gastrointestinal (GI) tract were demonstrated by immunohistochemical methods [9]. In the human, on the other hand, probably because of the difficulties entailed in the collection of samples, there have been no studies in which the expression of *Cdx1* and/or *Cdx2* was examined precisely in the various regions of the GI tract. Consequently, it is unclear whether species-specific differences in their tissue expression may exist between the human and the mouse.

Evidence of a role for *Cdx* genes in the maintenance of the intestinal phenotype has accumulated from both in-vivo and in vitro studies. Findings obtained from experiments using *Cdx* knockout mice included several developmental abnormalities in null and heterozygous mutants. Moreover, an important role of *Cdx* genes in intestinal differentiation is suggested by the decreased expression of both genes in colorectal carcinomas [1,10,11] and by the abnormal expression of *Cdx1* in furan-induced rat cholangiocellular carcinomas with an intestinal phenotype [12]. Mallo et al. [1] have reported the abnormal expression of *Cdx1* in gastric and esophageal mucosa undergoing intestinal metaplasia (IM). However, to our knowledge, no studies have focused upon *Cdx2* expression in any type of IM.

In the human gastric mucosa, IM is commonly observed in frequent association with chronic atrophic gastritis, peptic ulcers, and gastric carcinomas. Recently, it has been clearly demonstrated that *Helicobacter pylori* infection causes severe mucosal damage and subsequent IM appearance during mucosal regeneration in the glandular stomach of Mongolian gerbils [13]. While the pathogenesis of IM remains to be clarified, many gene products have been investigated, including enzymes, growth factors, and structural proteins, to characterize the epithelium and to elucidate the pathogenesis of IM [10,14–19]. We have investigated IM in the human stomach not only from morphological aspects but also with regard to the cellular differentiation status [20]. We revealed that a large proportion of intestinal metaplastic gastric glands consisted of both gastric and intestinal type cells, named gastric and intestinal mixed type IM [20].

On the basis of our studies until the present, we have hypothesized that IM is a pathological state caused by abnormal stem cell differentiation. We also presume that the genes which normally control intestinal epithelial cell differentiation, such as *Cdx* genes, could participate in this abnornal differentiation. To clarify whether this is indeed the case, it is necessary to have a detailed understanding of the tissue expression patterns of *Cdx* genes throughout the human GI tract. In this article, we have documented findings for the mRNA expression of *Cdx* genes, discussed with a comparison between human and mouse tissues. In addition, we examined the state of *Cdx* gene expression in IM lesions associated with mild to severe gastritis in the human stomach.

Patients and methods

Samples

Forty-nine fresh human mucosal samples from 11 distinct regions of the GI tract (gastric antrum, gastric body, duodenum, jejunum, ileum, cecum, ascending colon, transverse colon, descending colon, sigmoid colon, and rectum) were collected from the surgically resected specimens of 37 patients with cancer and 2 with benign tumor, after informed consent had been obtained. Three to six specimens, which were at least 2 cm apart from tumor tissues and were macroscopically diagnosed as normal mucosa, were obtained from each GI region. Detailed profiles of the patients are provided in Table 1. Of the 39 patients, 21 were men and 18 were women; they ranged in age from 23 to 83 years (mean, 59.8 ± 13.4 years).

After the luminal contents had been removed, the specimens were cut into 5 × 5-mm squares and stored at −80 °C. Adjacent small portions of the mucosa, used for the RNA assay from each GI region, were fixed in 10% buffered formalin solution and embedded in paraffin for histological examination. They were microscopically confirmed as normal on hematoxylin and eosin (H&E) staining. With the stomach, mucosa adjacent to lesions was also fixed in 10% buffered formalin solution and embedded in paraffin for histological examination. Sections were stained with H&E, and then assessed by microscopy to determine the degree of IM, which was classified as mild, moderate, and severe, as previously described [21]. Five specimens were obtained from each IM region with mild IM and four specimens were obtained from each IM region with severe IM. The mucosa of regions with mild to severe IM was used for RNA analysis.

Northern blotting

Total RNAs from frozen tissues were isolated with TRIzol Reagent (GIBCO BRL, Life Technologies, Tokyo, Japan) as previously described [22]; 20-μg aliquots were electrophoresed in 0.8% agarose-formaldehyde gels and transferred to Hybond N (−) nylon membranes (Amersham Japan, Tokyo, Japan). An approximately 535-bp *Not*I/*Eco*RI fragment of *Cdx1* cDNA, corresponding to the 3′-untranslated region, and a 468-bp *Eco*RI/*Apa*I fragment of *Cdx2* cDNA, corresponding to the 5′-coding region without the homeobox domain, were used as probes and labeled with [α-^{32}P] dCTP, using the Megaprime DNA labeling system (Amersham Japan, Tokyo, Japan). Membranes were hybridized with ^{32}P-labeled *Cdx1* or *Cdx2* cDNA in QuikHyb solution (Stratagene, La Jolla, CA, USA) for 1 h and washed with 2 × standard sodium citrate (SSC) and 0.1% sodium dodecyl-sulfate (SDS) at room temperature and then with 0.1 × SSC and 0.1% SDS, followed by the exposure of Image Plates at room temperature for 3 h [22]. Each band was quantitatively analyzed by densitometry. We defined the average amounts of *Cdx1* and *Cdx2* mRNAs in the ascending colon as 1.0 in each case.

Table 1. Detailed profiles of the patients

Age (years), Sex	Tumor site	Pathological diagnosis	Stage	Operation	Region of samples
63, M	Rectum	Endocrine cell carcinoma	IIIb	Low anterior resection	Rectum
67, M	Rectum	Moderately diff. adenocarcinoma	I	Low anterior resection	Rectum
61, F	Rectum	Moderately diff. adenocarcinoma	IV	Low anterior resection	Rectum
65, M	Rectum	Well diff. adenocarcinoma	IIIa	Low anterior resection	Rectum
59, F	Sigmoid	Moderately diff. adenocarcinoma	IV	Sigmoidectomy	Sigmoid colon
63, M	Sigmoid	Moderately diff. adenocarcinoma	IIIa	Sigmoidectomy	Sigmoid colon
73, F	Descending	Moderately diff. adenocarcinoma	IIIa	Left hemicolectomy	Sigmoid colon
77, F	Sigmoid	Moderately diff. adenocarcinoma	II	Sigmoidectomy	Descending colon
45, F	Transverse	Moderately diff. adenocarcinama	IIIb	Left hemicolectomy	Descending colon
77, F	Descending	Moderately diff. adenocarcinoma	I	Left hemicolectomy	Descending colon
63, F	Transverse	Moderately diff. adenocarcinoma	I	Transverse colectomy	Transverse colon
36, M	Transverse	Poorly diff. adenocarcinoma	IIIb	Right hemicolectomy	Transverse colon
36, M	Transverse	Mucinous adenocarcinoma	IIIb	Transverse colectomy	Transverse colon
67, M	Ascending	Moderately diff. adenocarcinoma	0	Right hemicolectomy	Ascending colon
43, M	Ascending	Moderately diff. adenocarcinoma	II	Right hemicolectomy	Ascending colon
55, F	Ascending	Moderately diff. adenocarcinoma	IV	Right hemicolectomy	Ascending colon
54, F	Ascending	Moderately diff. adenocarcinoma	IV	Right hemicolectomy	Cecum, ileum
70, F	Ascending	Moderately diff. adenocarcinoma	I	Right hemicolectomy	Cecum
61, M	Ascending	Well diff. adenocarcinoma	0	Ileocecalectomy	Cecum
75, F	Ascending	Tubulovillous adenoma[a]		Right hemicolectomy	Ileum
57, M	Ascending	Moderately diff. adenocarcinoma	IV	Right hemicolectomy	Ileum
44, F	Stomach	Poorly diff. adenocarcinoma	IIIA	Total gastrectomy	Jejunum
65, M	Pancreas	Intraductal papillary mucinous tumor[a]		PpPD	Jejunum, duodenum
64, F	Stomach	Poorly diff. adenocarcinoma	IB	Proximal gastrectomy	Jejunum
59, M	Stomach	Poorly diff. adenocarcinoma	IIB	Distal gastrectomy	Duodenum
59, F	Duodenum	Moderately diff. adenocarcinoma	III	PpPD	Duodenum
23, F	Stomach	Signet-ring cell carcinoma	IA	Distal gastrectomy	Gastric body and antrum
32, F	Stomach	Poorly diff. adenocarcinoma	IV	Distal gastrectomy	Gastric body and antrum
53, M	Stomach	Poorly diff. adenocarcinoma	IV	Total gastrectomy	Gastric body and antrum
62, F	Stomach	Poorly diff. adenocarcinoma	IA	Distal gastrectomy	Gastric antrum
68, M	Stomach	Poorly diff. adenocarcinoma	IV	Distal gastrectomy	Gastric antrum, IM (mild)
68, M	Stomach	Poorly diff. adenocarcinoma	IV	Total gastrectomy	Gastric antrum, IM (mild and severe)
75, M	Stomach	Moderately diff. adenocarcinoma	IA	Total gastrectomy	Gastric body, IM (severe)
68, M	Stomach	Well diff. adenocarcinoma	IA	Total gastrectomy	Gastric body
74, M	Stomach	Poorly diff. adenocarcinoma	IA	Total gastrectomy	Gastric body, IM (mild)
64, M	Stomach	Poorly diff. adenocarcinoma	IA	Distal gastrectomy	IM (mild)
44, F	Stomach	Poorly diff. adenocarcinoma	IIIA	Total gastrectomy	IM (mild)
60, M	Stomach	Well diff. adenocarcinoma	IA	Distal gastrectomy	IM (severe)
83, M	Stomach	Poorly diff. adenocarcionma	IIIA	Distal gastrectomy	IM (severe)

Diff, Differentiated; PpPD, pylorus-preserving pancreatoduodenectomy; IM, intestinal metaplasia
[a] Benign

Reverse-transcriptase polymerase chain reaction (RT-PCR)

First-strand cDNAs were prepared from isolated RNAs, using a cDNA Synthesis System (GIBCO BRL, Life Technologies) according to the manufacturer's instructions. PCR amplification was performed at 95 °C for 10 min, then 30 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and 72 °C for 10 min. cDNA was PCR-amplified in 1 × PCR buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl2,) containing 200 μM dNTP, 0.2 μM of each primer, and 1.25 units of AmpliTaq polymerase (Applied Biosystems, Branchburg, NJ, USA) in a final

volume of 50 μl. The following oligonucleotides were synthesized for PCR amplification: 5′-AGCGCAAA GTGAACAAGAAGAAACAG-3′ (sense) and 5′-GGGGCTATGGCAGAAACTCCTCT-3′ (antisense) for the 3′-untranslated region of *Cdx1* mRNA, and 5′-TCAGCCAGGTCCTCTGAGAA-3′ (sense) and 5′-GCCTGGAATTGCTCTGCCGC-3′ (antisense) for the 3′-untranslated region of *Cdx2* mRNA. As an internal control for RT-PCR analysis, beta-actin transcripts were amplified from the same cDNA samples with the following primer pair: 5′-TGGCACCCAGCACAAT GAAG-3′ (sense) and 5′-GATGGAGGGGCCGGAC TC-3′ (antisense). The PCR products were separated in 1.5% agarose gels.

Results

Expression of Cdx1 mRNA in normal GI tract

A single RNA species of about 2.0 kb hybridized to the *Cdx1* 3′-untranslated region probe (Fig. 1). We used 36B4 as a loading control [22]. The relative expression of *Cdx1* mRNA increased gradually from the duodenum to the sigmoid colon and was slightly decreased in the rectum (Fig. 2). It exhibited a peak in the region from the transverse to the sigmoid colon. In normal gastric mucosa without IM, confirmed by examination of histological sections, no obvious expression of *Cdx1* mRNA was detected.

Expression of Cdx2 mRNA in normal GI tract

A single mRNA species of 1.9 kb hybridized to the *Cdx2* 5′-coding region probe (Fig. 1). We used 36B4 as a loading control [22]. The expression of *Cdx2* mRNA increased gradually from the duodenum to the proximal colon and decreased gradually in the vicinity of the rectum (Fig. 3). The level from the cecum to the ascending colon was maximal. As found with *Cdx1*, no *Cdx2* mRNA was apparent in gastric mucosa without IM.

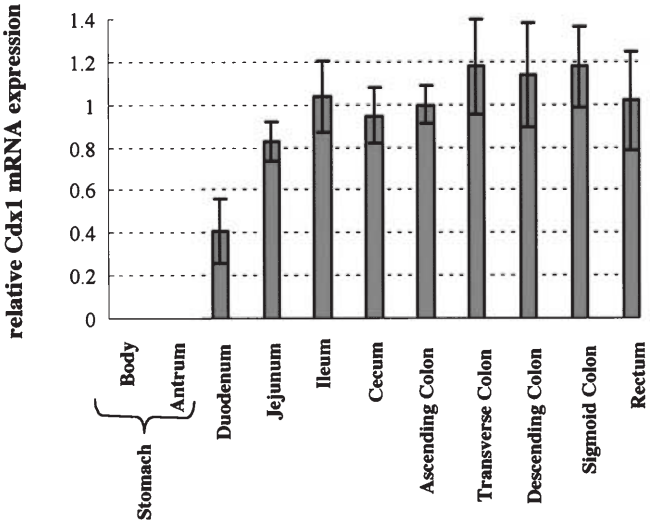

Fig. 2. Relative *Cdx1* mRNA expression in human GI tract mucosa. *Cdx1* mRNA expression relative to that of the ascending colon in 11 distinct regions (stomach to rectum) is shown. The expression of *Cdx1* mRNA increased gradually from the duodenum to the sigmoid colon and was slightly decreased in the rectum. Data values are averages from three independent experiments (means ± SD)

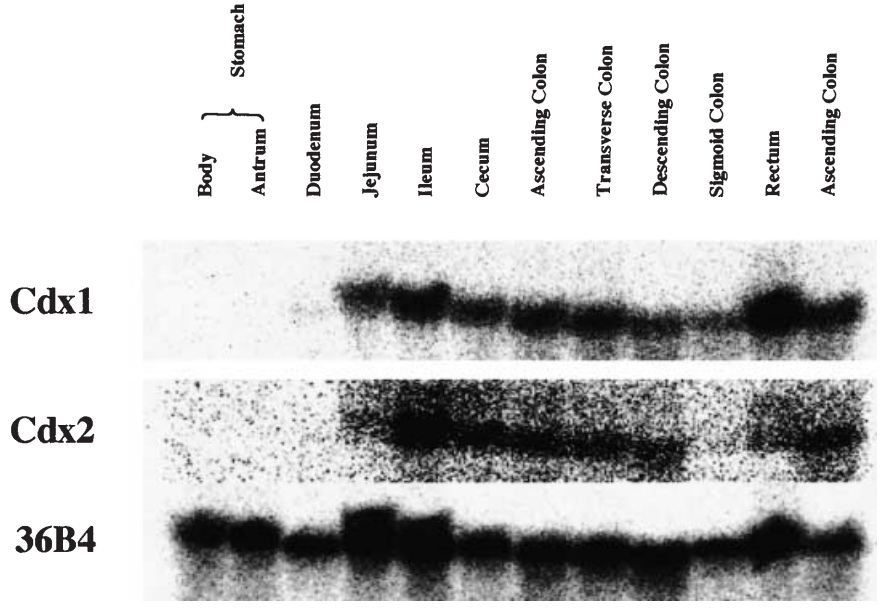

Fig. 1. *Cdx1* and *Cdx2* mRNA expression in human GI tract mucosa. The *Cdx1* and *Cdx2* mRNAs are detected as 2.0-kb and 1.9-kb bands; 36B4, a 1.5-kb band, was used as a loading control. Data values shown are representative of three independent experiments

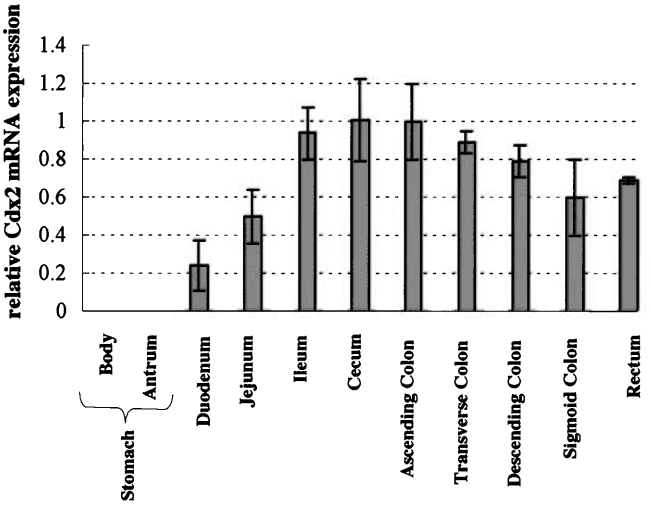

Fig. 3. Relative *Cdx2* mRNA expression in human GI tract mucosa. *Cdx2* mRNA expression relative to that of the ascending colon in 11 distinct regions (stomach to rectum) is shown. The expression of *Cdx2* mRNA increased gradually from the duodenum to the proximal colon and decreased in the vicinity of the rectum. The level from the cecum to the ascending colon is maximal. Data values are averages from three independent experiments (means ± SD)

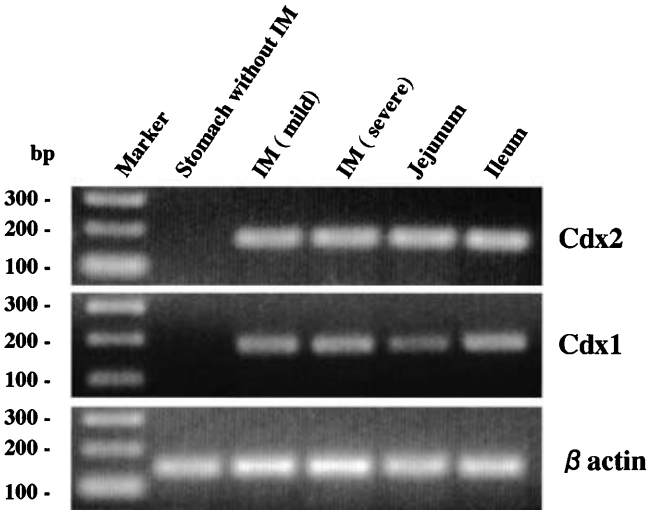

Fig. 4. *Cdx1* and *Cdx2* mRNA expression in IM of the human stomach. Polymerase chain reaction (PCR) products are derived from cDNAs reverse transcribed from RNAs of the normal stomach, jejunum, and ileum and regions with mild to severe IM. In stomach, note obvious products only in samples with IM

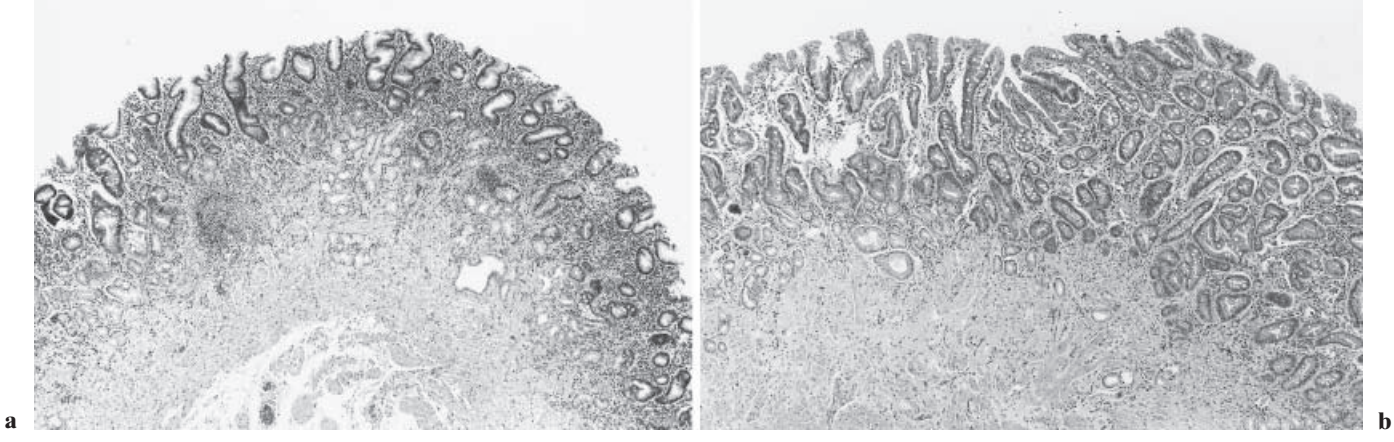

Fig. 5a,b. Examples of IM lesions adjacent to the site of samples used for RNA extraction. **a** Mild IM; **b** severe IM. **a** and **b** H&E, ×46

Expression of Cdx1 and Cdx2 mRNAs in IM

By RT-PCR analysis, *Cdx1* and *Cdx2* PCR products were obviously detected, as 177-bp and 170-bp bands, respectively, both in the jejunum and ileum, compatible with our results from Northern blotting (Fig. 4). Products of beta-actin, detected as 149-bp bands, were used as an internal control. Although both *Cdx1* and *Cdx2* PCR products were detected in gastric mucosa with mild and severe IM in all cases tested, neither *Cdx1* nor *Cdx2* bands were observed in the normal gastric mucosa without IM. Figure 5 shows examples of IM lesions adjacent to the site of samples used for RNA extraction.

Discussion

Our data clearly demonstrated the expression of *Cdx1* and *Cdx2* in both the small and large intestine, but not in the stomach (Figs 1, 2, and 3). As with the tissue expression pattern of *Cdx1* mRNA in the mouse, a ten-

dency for gradual increase along the anterior-posterior intestinal axis from the proximal to the distal regions was evident [6]. In the mouse, however, the expression was of *Cdx1* mRNA was rather lower in the small than in the large intestine, while in the human samples, *Cdx1* mRNA was rather high in both the small and the large intestine. Similar findings were obtained for *Cdx2*. The resemblance of these tissue expression patterns of both *Cdx1* and *Cdx2* along the anterior-posterior intestinal axis in the human and mouse suggests that these genes, equally, have very critical roles in morphological and functional epithelial cell development and differentiation throughout the intestine.

We also demonstrated the expression of *Cdx1* and *Cdx2* mRNAs in mucosa with IM in the stomach. Silberg and colleagues [10] and Ren and colleagues [12] previously reported the presence of *Cdx1* protein in IM lesions of the human stomach, and in epithelium from intestinal metaplastic glands within furan-induced hepatic cholangiofibrosis; associated intestinal type cholangiocarcinomas were, similarly, found to be positive by immunohistochemistry with anti-*Cdx1* antibody. Our results of the RT-PCR analysis of mucosa with IM are in line with these data, suggesting involvement in intestinal phenotypic differentiation in the human stomach. To our knowledge, this is the first such demonstration for *Cdx2*. Because we examined the mRNA expression of both the *Cdx1* and *Cdx2* genes in mucosa with IM by the RT-PCR method, it remains to be clarified which gene is predominant. Analysis by Northern blotting needs to be performed to answer this question, but there are difficulties to overcome or consider. The amounts of RNAs extracted from intestinal phenotype cells constituting intestinalized glands vary from individual to individual and are often insufficient for Northern blotting analysis. Moreover, IM has several subtypes, with the combination of gastric and intestinal phenotype cells in intestinalized gastric glands, as we have demonstrated [20]. Furthermore, *Cdx1* and *Cdx2* expression demonstrates gradients along the crypt-villus axis, with *Cdx1* primarily in the crypts and *Cdx2* in the villi [9]. In vitro, it is well known that *Cdx1* regulates the expression of villin and aminopeptidase N, while *Cdx2* regulates sucrase-isomaltase, lactase-phlorigen hydrolase, calbindin-D9K and glucagon, and carbonic anhydrase 1, suggesting that both genes may play important roles as regulators of intestinal phenotype expression in vivo [7,23–30]. Villin and sucrase-isomaltase are commonly demonstratable immunohistochemically in intestinalized gastric glands, and we have concentrated attention on subtypes as intestinal epithelial differentiation markers. Taken together, the above findings, as well as our present results, suggest that the amounts and/or patterns of both *Cdx1* and *Cdx2* gene expression might be expected to differ among IM subtypes and, indeed, have an influence on the phenotype observed. Analysis of *Cdx1* and *Cdx2* mRNA expression by a semi-quantative RT-PCR method, using crypt-isolated and subdivided intestinalized gastric glands, is now in progress to provide more information regarding this question.

Several studies have focused upon the interrelationship of *Cdx* gene expression and colon carcinogenesis in humans [1,10,11,31–34]. It is possible that *Cdx1* may act as a tumor accelerator, while *Cdx2* is reported to be oncosuppressive [10,12,29,34–36], although these findings are controversial. We believe that the idea that *Cdx1* acts as a tumor accelerator is reasonable, considering its physiological function in inducing a differentiated epithelial phenotype in the upper part of the crypts. However, only limited information is available with reference to gastric carcinogenesis. Bai et al. [37] reported *Cdx2*, but not *Cdx1*, expression in 11 gastric cancer cell lines, stating that *Cdx2* levels progressively decreased with the transition from a well differentiated to a poorly differentiated state. Ren et al. [12] reported *Cdx1* expression in furan-induced IM lesions and in intestinal type cholangiocarcinoma in the liver. Although they did not examine *Cdx2* expression, they suggested that the ratio of *Cdx2* to *Cdx1* transcriptional factors might be important for determining the degree of proliferation versus differentiation. In the rat furan model, they considered the preexisting IM glands to be a precancerous lesion. Also, in the human, IM has long been regarded as a precancerous state, but no obvious consensus regarding its role in carcinogenesis has thus far been achieved. To solve this impasse, it will be necessary to investigate the *Cdx* gene expression pattern not only in human gastric cancers but also in IM lesions in the background mucosa, a pattern which we are currently studying.

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References

1. Mallo GV, Rechreche H, Frigerio JM, Rocha D, Zweibaum A, Lacasa M, et al. Molecular cloning, sequencing and expression of the mRNA encoding human *Cdx1* and *Cdx2* homeobox genes. Down-regulation of *Cdx1* and *Cdx2* mRNA expression during colorectal carcinogenesis. Int J Cancer 1997;74:35–44.
2. Freund JN, Demon-Dell C, Kedinger M, Duluc I. The *Cdx1* and *Cdx2* homeobox genes in the intestine. Biochem Cell Biol 1998;76:957–69.

3. Duluc I, Lorentz O, Fritsch C, Leberquier C, Kedinger M, Freund JN. Changing intestinal connective tissue interactions alters homeobox gene expression in epithelial cells. J Cell Sci 1997;110: 1317–24.
4. Freund JN, Boukamel R, Benazzouz A. Gradient expression of *Cdx* along the rat intestine throughout postnatal development. FEBS Lett 1992;314:163–6.
5. James R, Erler T, Kazenwadel J. Structure of the murine homeobox gene *cdx-2*. Expression in embryonic and adult intestinal epithelium. J Biol Chem 1994;269:15229–37.
6. James R, Kazenwadel J. Homeobox gene expression in the intestinal epithelium of adult mice. J Biol Chem 1991;266:3246–51.
7. Suh E, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. Mol Cell Biol 1994;14:7340–51.
8. Bonner CA, Loftus SK, Wasmuth JJ. Isolation, characterization, and precise physical localization of human *Cdx1*, a caudal-type homeobox gene. Genomics 1995;28:206–11.
9. Silberg DG, Swain GP, Suh ER, Traber PG. *Cdx1* and *Cdx2* expression during intestinal development. Gastroenterology 2000;119:961–71.
10. Silberg DG, Furth EE, Taylor JK, Schuck T, Chiou T, Traber PG. *Cdx1* protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. Gastroenterology 1997;113: 478–86.
11. Ee HC, Erler T, Bhathal PS, Young GP, James RJ. *Cdx2* homeodomain protein expression in human and rat colorectal adenoma and carcinoma. Am J Pathol 1995;147:586–92.
12. Ren P, Silberg DG, Sirica AE. Expression of an intestine-specific transcription factor (*Cdx1*) in intestinal metaplasia and in subsequently developed intestinal type of cholangiocarcinoma in rat liver. Am J Pathol 2000;156:621–7.
13. Shimizu N, Ikehara Y, Inada K, Nakanishi H, Tsukamoto T, Nozaki K, et al. Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. Cancer Res 2000;60:1512–4.
14. Stemmermann GN, Hayashi T. Intestinal metaplasia of the gastric mucosa: a gross and microscopic study of its distribution in various disease states. J Natl Cancer Inst 1968;41:627–34.
15. Wu GD, Beer DG, Moore JH, Orringer MB, Appleman HD, Traber PG. Sucrase-isomaltase gene expresion in Barrett's esophagus and adenocarcinoma. Gastroenterology 1993;105:837–44.
16. Osborn M, Mazzoleni G, Santini D, Marrano D, Martinelli G, Weber K. Villin, intestinal brush border hydrolases and keratin polypeptides in intestinal metaplasia and gastric cancer: an immunohistologic study emphasizing the different degrees of intestinal and gastric differentiation in signet ring cell carcinomas. Virchows Arch A Pathol Anat Histopathol 1988;413:303–12.
17. Brito MJ, Filipe MI, Linehan J, Jankoowski J. Association of transforming growth factor alpha (TGF-α) and its precursors with malignant change in Barrett's epithelium: biological and clinical variables. Int J Cancer 1995;60:27–32.
18. Filipe MI, Osborn M, Linehan J, Sanidas E, Brito MJ, Jankowski J. Expression of transforming growth factor alpha, epidermal growth factor receptor and epidermal growth factor in precursor lesions to gastric carcinoma. Br J Cancer 1995;71:30–6.
19. Uchida Y, Matsuda K, Sasahara K, Kawabata H, Nishioka M. Immunohistochemistry of gap junctions in normal and diseased gastric mucosa of humans. Gastroenterology 1995;109:1492–6.
20. Inada K, Nakanishi H, Fujimitsu Y, Shimizu N, Ichinose M, Miki K, et al. Gastric and mixed and solely intestinal types of intestinal metaplasia in the human stomach. Pathol Int 1997;47: 831–41.
21. Inada K, Tanaka H, Nakanishi H, Tsukamoto T, Ikehara Y, Tatematsu K, et al. Identification of Paneth cells in pyloric glands associated with gastric and intestinal mixed-type intestinal metaplasia of the human stomach. Virchows Arch 2001;439:14–20.
22. Tsukamoto T, Yoo J, Hwang SIH, Guzman RC, Hirokawa Y, Chou YC, et al. Expression of MAT1/PEA-15 mRNA isoforms during physiological and neoplastic changes in the mouse mammary gland. Cancer Lett 2000;149:105–13.
23. Suh E, Traber PG. An intestine-specific homeobox gene regulates proliferation and differentiation. Mol Cell Biol 1996;16:619–25.
24. Troelsen JT, Mitchelmore C, Spodsberg N, Jensen AM, Noren O, Sjostrom H. Regulation of *lactase-phlorigen hydrolase* gene expression by the caudal-related homeodomain protein *Cdx2*. Biochem J 1997;322:833–8.
25. Colnot S, Romagnolo B, Lambert M, Cluzeaud F, Porteu A, Vandewalle A, et al. Intestinal expression of the calbindin-D9K gene in transgenic mice. Requirement for a *Cdx2*-binding site in distal activator region. J Biol Chem 1998;273:31939–46.
26. Jin T, Drucker DJ. Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein cdx2/3. Mol Cell Biol 1996;16:19–28.
27. Drummond F, Sowden J, Morrison K, Edwards YH. The caudal-type homeobox protein *Cdx2* binds to the colon promoter of the carbonic anhydrase 1 gene. Eur J Biochem 1996;236:670–81.
28. Lorentz O, Suh ER, Taylor JK, Boudreau F, Traber PG. cAMP response element-binding protein interacts with the homeodomain protein *Cdx2* and enhances transcriptional activity. J Biol Chem 1999;274:7196–99.
29. Soubeyran P, Andre F, Lissitzky JC. *Cdx1* promotes differentiation in a rat intestinal epithelial cell line. Gastroenterology 1999; 117:1326–38.
30. Fang R, Santiago NA, Olds LC, Sibley E. The homeodomain protein *Cdx2* regulates lactase gene promoter activity during enterocyte differentiation. Gastroenterology 2000;118:115–7.
31. Mallo GV, Soubeyran P, Lissitzky JC, Andre CF, Farnarier C, Marvaldi J, et al. Expression of *Cdx1* and *Cdx2* homeotic genes leads to reduced malignancey in colon cancer-derived cells. J Biol Chem 1998;273:14030–6.
32. Yagi OK, Akiyama Y, Yuasa Y. Genomic structure and alterations of homeobox gene *CDX2* in colorectal carcinomas. Br J Cancer 1999;79:440–4.
33. Wicking C, Simms LA, Evans T, Walsh M, Chawengsaksophak K, Beck F, et al. CDX2, a human homologue of *Drosophila caudal*, is mutated in both alleles in a replication error positive colorectal cancer. Oncogene 1998;17:657–9.
34. da Costa LT, He TC, Yu J, Sparks AB, Morin PJ, Polyak K, et al. *CDX2* is mutated in a colorectal cancer with normal APC/beta-catenin signaling. Oncogene 1999;18:5010–4.
35. Chawengsaksophak K, James R, Hammond VE, Kontgen F, Beck F. Homeosis and intestinal tumours in *Cdx2* mutant mice. Nature 1997;386:84–7.
36. Lorentz O, Cadoret A, Duluc I, Capeau J, Gespach C, Freund JN. Downregulation of the colon tumor-suppressor homeobox gene *Cdx2* by oncogenic ras. Oncogene 1999;18:87–92.
37. Bai YQ, Akiyama Y, Nagasaki H, Yagi OK, Kikuchi Y, Saito N, et al. Distinct expression of *CDX2* and *GATA4/5*, development-related genes, in human gastric cancer cell lines. Mol Carcinog 2000;28:184–8.