Gastric Cancer (2006) 9: 156–166
DOI 10.1007/s10120-006-0375-6

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Review article

Gastric-and-intestinal mixed-type intestinal metaplasia: aberrant expression of transcription factors and stem cell intestinalization

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Abstract
***Helicobacter pylori* plays a causative role in the development of chronic atrophic gastritis, intestinal metaplasia (IM), and stomach cancer. Although IM has long attracted attention as a putative preneoplastic lesion for stomach cancers, its clinicopathologic significance has yet to be clarified in detail. Using gastric and intestinal epithelial cell markers, IM was here divided into two major types: a gastric-and-intestinal (GI) mixed type and a solely intestinal (I) type. In the former, gastric and intestinal phenotypic markers appeared not only at the glandular but also at the cellular level. Furthermore, neuroendocrine cells also showed intestinalization along with their exocrine counterparts. In animal models, GI-type IM was found to appear first, followed by the solely I type. Summarizing these data, it was suggested that IM might be caused by the gradual intestinalization of stem cells from the GI to the I type. The molecular mechanisms of IM include the ectopic expression of CDX1, CDX2, OCT-1, and members of the Erk pathway. Suppression of the expression of gastric transcription factors such as SOX2, genes that are involved in the Sonic hedgehog pathway, and RUNX3, a tumor suppressor gene, could be additional relevant alterations. The expression of PDX1 may also be associated with pseudopyloric gland metaplasia and IM. Detailed analysis of gene regulation may shed light on the molecular bases of gastric lesions, leading to strategies for chemoprevention.**

Key words Gastric-and-intestinal mixed-type intestinal metaplasia · Stem cell · Transcription factor

Introduction

Since the discovery of *Helicobacter pylori* by Warren and Marshal [1] in Australia, it has been well established that this microorganism plays important roles in the development of chronic gastritis, intestinal metaplasia (IM), and stomach cancers, including malignant lymphomas [2–7]. In 1994, the World Health Organization (WHO)/International Agency for Research on Cancer (IARC) categorized *H. pylori* as a group 1 "definite carcinogen" [8]. IM has been extensively studied as a putative preneoplastic lesion in the human stomach, due to its strong association with stomach cancer development [9–18]. Although controversy exists as to its real significance [19,20], IM is considered by some to be a precancerous lesion for so-called intestinal adenocarcinomas of Lauren's classification [21]. This type comprises well- and moderately differentiated adenocarcinomas, irrespective of the presence of the intestinal properties. Clearly, the pathogenesis of IM, as well as its molecular background, needs to be detailed for the elucidation of the actual relation between IM and stomach cancer [22].

Offprint requests to: T. Tsukamoto
Received: March 10, 2006 / Accepted: March 20, 2006

Stem cells in the gastrointestinal mucosa

To investigate the cellular origin of tissues, mosaicism of cellular genetic markers is often used. One approach is to use chimeric animals, produced experimentally by the amalgamation of cells from allelically different strains. Recently, numerous histological markers have also been applied for the analysis of mosaicism in chimeric mice. Antibodies strictly recognizing C3H strain-specific antigens (CSAs) [23] enable the immunohistochemical discrimination of C3H cells in histological sections of chimeric mouse tissues. In normal gastric and intestinal mucosa of chimeric mice, each gland is composed entirely of CSA-positive or -negative cells, and no mixed glands are found, indicating that each individual gland in the adult mouse is derived from a single progenitor cell. Surface mucous cells (foveolar epithelial cells), mucous neck cells, parietal cells, and chief cells in the fundic glands thus all arise from the same cell. Similarly, surface mucous cells and pyloric gland cells arise from a single progenitor cell [24–26].

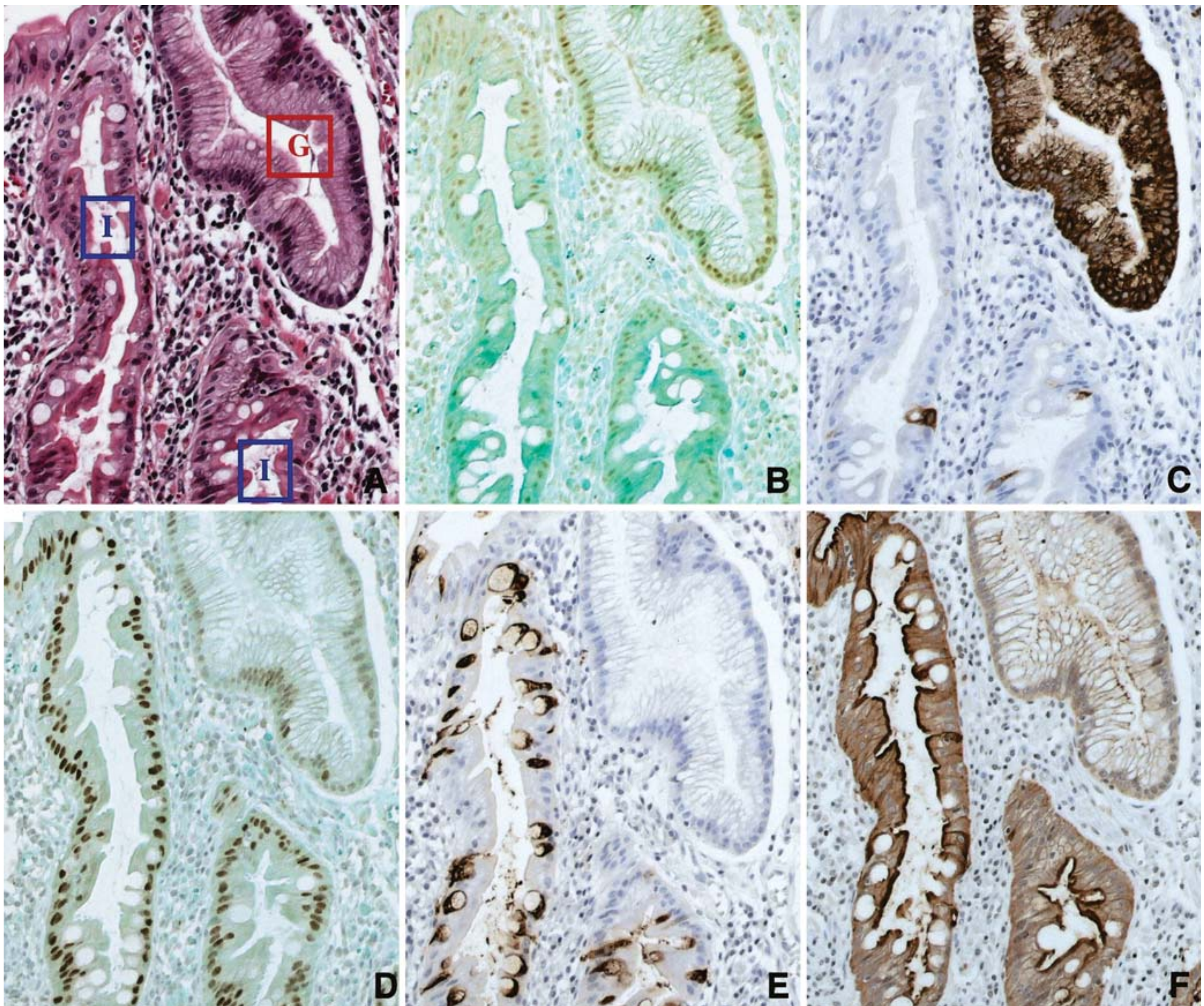

Fig. 1A–F. Immunohistochemical staining of normal human gastric mucosa and intestinal metaplasia. *G*, Pyloric gland with a gastric phenotype expressing Sox2 and MUC5AC; *I*, intestinal metaplastic gland harboring goblet cells producing Cdx2, MUC2, and villin. **A** H&E staining; **B–F** immunohistochemistry; for Sox2 (**B**), MUC5AC (**C**), Cdx2 (**D**), MUC2 (**E**), and villin (**F**). Binding was visualized with 3,3′-diaminobenzidine (DAB), and counterstaining was done with light green SF yellowish (**B**) or hematoxylin (**C–F**). ×200

Another approach to showing cellular phenotypic mosaicism in females utilizes the random inactivation of one X chromosome [27–29]. The human androgen receptor gene locus (HUMARA) has been used to assess methylation, and about half of intestinal metaplastic glands were revealed to be heterotypic (comprised of cells with differing allelic methylation), while the remainder were homotypic (cell populations with the same allelic methylation). Mouse models have also been used to show heterogeneity within a single gland/crypt, utilizing the Dlb-1 locus, which determines the expression of the binding site for the lectin *Dolichos biflorus* agglutinin (DBA) in the intestinal epithelium, in C57BL/6J x SWR F1 mice [30]. Mouse models have also shown X-linked glucose-6-phosphate dehydrogenase (G6PD) activity in C3H/Heston mice [31]. When a carcinogen, ethylnitrosourea, was administered to the C3H/Hester mice, loss of G6PD activity appeared in one side of a colonic crypt [31]. These results led to the hypothesis of the existence of four to six stem cells in one crypt [32].

The discrepancy between the concept of a single progenitor stem cell and the hypothesis of four to six stem cells in one crypt could be derived from confusion regarding the terminology used for “stem cells”. The single stem cell in glands/crypts hypothesized from the

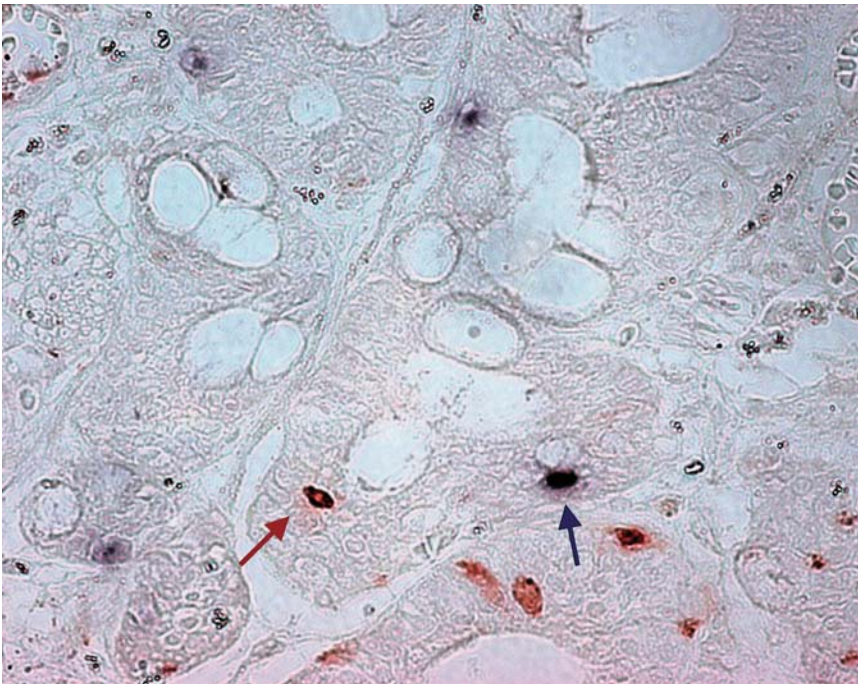

Fig. 2. An example of double staining of gastrin and glucagon-like peptide (GLP)-1 in gastric-and-intestinal mixed phenotype intestinal metaplasia (GI-IM). A mixture of gastrin- and GLP-1-positive endocrine cells is apparent in the same gland. Gastrin-positive cells (*blue arrow*) and GLP-1-positive cells (*red arrow*) are indicated. ×200

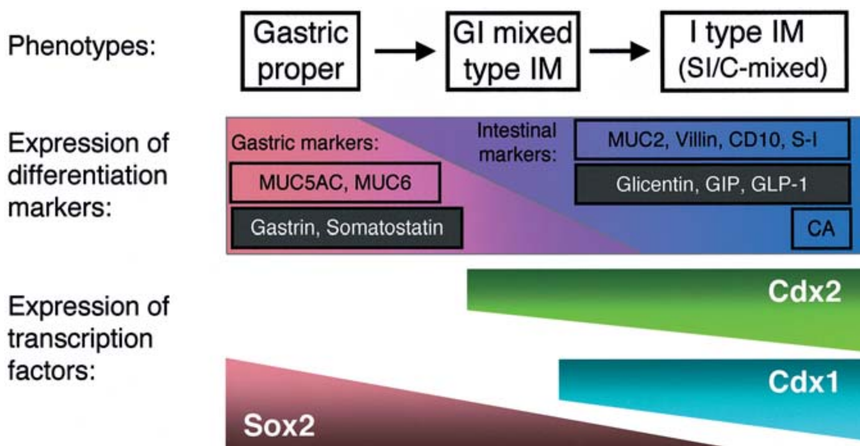

Fig. 3. Schematic view of progression of intestinal metaplasia (*IM*). Gastric mucosa changes to gastric-and-intestinal (*GI*) mixed-type IM, and then progresses to Solely intestinal (*I*)-type IM. Within the I type, small-intestinal (*SI*) type cells can colocalize with G phenotype cells, whereas colonic (*C*) type cells appear in complete I-type IM. Sox2, along with gastric markers, is decreased, and Cdx and intestinal markers emerge ectopically during the progression of IM. *S-I*, sucrase-isomaltase; *CA*, carbonic anhydrase 1; *GIP*, gastric inhibitory peptide

chimeric mouse data may be a "master stem cell" commanding the whole gland, whereas the four to six stem cells in one crypt, indicated by the latter experiments [31,32], could be "committed stem cells", obeying the master stem cell in producing their progeny.

Gastric and intestinal epithelial cell markers

Mucins in the alimentary tract can be divided into two main classes: class III mucins in mucous neck cells, pyloric gland cells, and Brunner's gland cells; and class II mucins, in surface mucous cells, goblet cells, and the surface coat of intestinal absorptive cells, as assessed utilizing paradoxical concanavalin A staining [33]. With more recent developments in mucin histochemistry and immunohistochemistry, intestinal metaplastic cells can now be clearly classified, by the analysis of phenotypic expression, into a gastric epithelial cell type (G type), resembling pyloric gland cells and surface mucous cells, and an intestinal epithelial cell type (I type), resembling goblet and intestinal absorptive cells. Gastric mucosa consists of foveolar cells in the upper two-thirds and pyloric gland cells in the lower one-third. Concerning gastric phenotypic markers, the surface mucous-cell type contains galactose oxidase-Schiff (GOS) and sialidase-GOS reactive mucin, positive for mucin core protein (MUC), MUC5AC. Cells of the pyloric gland cell type

contain class III mucin, colocalized with MUC6, and show pepsinogen reactivity. Intestinal metaplastic mucosa consists of absorptive cells with a brush border, goblet cells packed with clear rounded vacuoles containing mucin, and, sometimes, Paneth cells, harboring eosinophilic granules in their cytoplasm, which usually appear at the bottom of the glands. Regarding intestinal markers, the goblet-cell type contains mucin that is GOS-negative and sialidase-GOS reactive, possessing sialyl-Tn antigen and MUC2 core protein. Cells of the intestinal absorptive type demonstrate sucrase and intestinal-type alkaline phosphatase activity, harboring CD10 as a surface marker, and the structural protein villin. Cells of Paneth-cell type are reactive with anti-defensin antibodies [34–41] (Fig. 1, Table 1).

Classification of intestinal metaplasia (IM)

The present widely applied classification of IM, into complete and incomplete types, was first proposed by Matsukura and colleagues [10] and Kawachi and colleagues [42]. Classification based upon mucin secretion patterns as well as morphology has also allowed division into a small-intestine type and a colonic type [43,44]. Jass and Filipe [45] described three grades of IM (types I, II, and III) on the basis of morphology and classical mucin staining, using periodic acid-Schiff, Alcian blue (AB), and high iron diamine (HID) methods. Type I corresponds to the complete type and types II and III to the incomplete type. While these classifications are generally accepted, they are based only upon the intestinal properties and do not take into account the gastric properties that are still preserved in association.

We have therefore proposed a new classification, based upon the cell differentiation status, using both gastric and intestinal cell phenotypic markers [39]. With this classification, IM is divided into two major types; a gastric-and-intestinal (GI) mixed type, and a solely intestinal (I) type. To confirm this histological classification, stomach mucosa was subjected to gland isolation and classification of individual glands into gastric (G), GI mixed, and I types according to the preservation of pyloric cells and the appearance of goblet cells, as revealed with Alcian blue and paradoxical concanavalin A staining. The G type preserves the pyloric cells without the emergence of goblet cells. In the I type, intestinal metaplastic glands consist of goblet and intestinal absorptive cells, with or without Paneth cells. In the GI mixed-type, on the other hand, gastric phenotype cells are found together with intestinal phenotype cells in various-combinations. All of the subtypes of GI mixed-type IM and a subtype of the I type without Paneth cells belong to the incomplete IM category, while the I type with Paneth cells corresponds to complete-type IM. In many cases of the GI mixed type, atrophied pyloric glands are present under the intestinalized glands.

Mixtures of gastric and intestinal phenotypes occur at the cellular as well as the glandular level. Intestinal metaplastic glands are easily found on hematoxylin and eosin (H&E) staining, by the presence of goblet cells and brush border lining the apical side of the epithelium. Goblet cells have been confirmed to show an intestinal phenotype, as shown with MUC2 immunostaining, which is not present in gastric epithelium. The brush border is positive for villin, as shown in normal intestinal epithelium. However, gastric mucin sometimes remains in both goblet and absorptive cells, as revealed by MUC5AC immunohistochemistry, with villin expression being weaker in MUC5AC-positive cells as compared to those without MUC5AC expression. Thus, IM subtypes should not be considered as independent entities, but, rather, as a sequence of pathological states with a gradual change from gastric to intestinal character. GI mixed-type IM may be composed of mixtures of cells with various degrees of intestinal phenotypic shift, rather than being just a random mixture of gastric- and intestinal-type cells. This allows us to introduce the notion that IM may be due to abnormal stem cell differentiation, but with some stem cells still obeying certain orders.

It is believed that stem cells (multipotent progenitor cells) are present in the proliferative cell zone in the isthmus region of gastric glands, giving rise to all the various cell types by differentiation, so that, consequently, gastric glands are monoclonal in the adult stage [46,47]. In the environment of a normal gastric gland, cells derived from stem cells undergo complex bipolar migration from the isthmus, either upward or downward. In the pyloric mucosa, surface mucous cells move upward, while pyloric gland cells migrate downward [48]. In the crypts of the small intestine, on the other hand, stem cells would be expected to be present in the proliferative cell zone at the bottom of the crypts. In the normal intestinal gland, cells that will become absorptive and goblet cells move up, and only those differentiating into Paneth cells migrate lower from the proliferative cell zone. In GI mixed-type IM, gastric surface mucous cells, intestinal absorptive cells, and goblet cells are found in the glandular portions above the proliferative zone, while pyloric gland cells and Paneth cells are found in the lower glandular portions, below the proliferative zone [40]. GI mixed-type IM may be the consequence of the abnormal differentiation of stem cells that can produce both gastric- and intestinal-type cells, with the normal cell migration pattern preserved. Because epithelial cell differentiation and the migration of gastric glands are thought to be closely linked, it is not clear why only the former is disturbed.

Table 1. Differentiation markers for the human gastrointestinal tract

Differentiation markers	Gastric				
	Foveolar cell	Pyloric cell	Fundic mucous neck cell	Fundic chief cell	Fundic parietal cell
Structural proteins					
Functional proteins					Proton pump alpha subunit[c] Proton pump beta subunit[c]
Enzymes		Pepsinogen II[e]		Pepsinogen I[f] Pepsinogen II	
Mucin core proteins	MUC5AC[a]	MUC6[a]	MUC6[a]		
Mucins	HGM[a] SH9[k] GOS staining PAS staining	PCS			
Neuroendocrine hormone					
Transcription factors	Sox2[j]	Pdx1[n]		RUNX3[o]	

HGM, human gastric mucin; GOS, galactose oxidase Schiff staining; PAS, periodic acid Schiff staining; PCS, paradoxical concanavalin A staining; I-ALP, intestinal alkaline phosphatase; CA1, carbonic anhydrase 1; SIMA, small intestinal mucious antigen; S-GOS, sialidase GOS; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1

Sources of available antibodies

[a] Novocastra (Newcastle upon Tyne, UK)
[b] Transduction Laboratory (Lexington, KY, USA)
[c] Medical and Biological Laboratories (MBL) (Nagoya, Japan)
[d] Dr. E. M. Porter, University of California, Los Angeles
[e] Biogenesis (Poole, England, UK)
[f] Dr. M. Ichinose, Wakayama Medical College
[g] Dr. T. Irimura, Tokyo University
[h] DAKO (Glostrup, Denmark)
[i] Dr. K. Hirano, Gifu Pharmaceutical University
[j] Chemicon (Temecula, CA, USA)
[k] Dr. Imai, Nara Medical College
[l] Dr. S. Hakomori (Tokyo University)
[m] Yanaihara Institute (Fujinomiya, Shizuoka, Japan)
[n] Dr. Y. Yuasa, Tokyo Medical and Dental University
[o] Dr. Y. Ito, Institute of Molecular and Cell Biology, Singapore
[p] Biogenex (San Ramon, CA, USA)

	Intestinal			
Neuroendocrine cell	Absorptive cell	Goblet cell	Paneth cell	Neuroendocrine cell
	CD10[a] Villin[b]			
			Defensin 5[d]	
	Sucrase-isomaltase[g] I-ALP[i] CA1[j]		Lysozyme[h]	
		MUC2[a]		
		SIMA[a] TKH2[l] Sialyl-Tn antigen[l] 91.1H[g] S-GOS staining Alcian blue staining		
Chromogranin A[h] Gastrin[l] Somatostatin[h]				Chromogranin A[hl] Glicentin[l] GIP[l] GLP-1[l]
	Cdx1 Cdx2[p]	Cdx1 Cdx2	Cdx1 Cdx2	

Sequential analysis using animal models

Experimentally, the shift from GI mixed-type IM to I-type IM can be observed in sequential observations in animal models. The occurrence of IM in rats gradually increases with time after X-ray irradiation; the number of GI mixed-type IMs is relatively high at 2–4 weeks, becoming lower thereafter. On the other hand, the number of I-type IMs is extremely low at 2 weeks, and then increases with time. These observations suggest that the phenotype of IM sequentially changes from the GI mixed-type to the I type [49].

H. pylori infection in Mongolian gerbils causes IM in their glandular stomachs [50]. Twenty-five weeks after inoculation with *H. pylori*, the glandular stomach epithelium becomes hyperplastic, and heterotopic proliferating glands (HPGs) penetrate the muscularis mucosae. Fifty weeks after infection, intestinal metaplastic cells appear among gastric epithelial cells, including goblet cells possessing Alcian blue-positive mucins and/or absorptive cells with a striated brush border, so that the lesions are characterized as GI-mixed-type IM. At 75 weeks, HPGs with gastric phenotype decrease and most animals possess solely I-type HPGs. Paneth cells appear by 100 weeks.

The *N*-methyl-*N*-nitrosourea-induced mouse stomach carcinogenesis model also provides support for the conclusion that intestinalization of the stomach epithelium occurs in late stages, as assessed by monitoring intestinal alkaline phosphatase expression [51].

Coexistence of gastric- and intestinal-type endocrine cells in gastric-and-intestinal mixed-type intestinal metaplasia (IM) of the human stomach

Gastrointestinal glands possess neuroendocrine cells, usually in their bottom regions, among the mucous and absorptive cells. Gastrin-positive endocrine cells are predominantly detected in the normal pyloric mucosa, with some detected in the duodenal mucosa. Somatostatin-positive cells are also mainly detected in the normal pyloric mucosa, with some detected in the fundic and duodenal mucosae. Glicentin, gastric inhibitory peptide (GIP)-, and glucagon-like peptide 1 (GLP-1)-positive endocrine cells are detected exclusively in the duodenum, small intestine, and colon, but not in the normal gastric mucosa. Therefore, gastrin and somatostatin could be gastric-predominant endocrine cell markers, whereas glicentin, GIP, and GLP-1 characterize the intestinal phenotype.

In GI mixed-type IM glands, both gastric and intestinal endocrine markers have been found to be present in endocrine cells, correlating with the phenotypic expression of the glands. Thus, in I-type IM glands harboring only intestinal mucous cell markers, endocrine cells demonstrate only intestinal endocrine peptides. However, double immunostaining for gastrin and GLP-1 has revealed the existence of both gastric and intestinal endocrine cells in the same glands of the GI-mixed-IM type. Furthermore, at the single cell level, quite a few glands harbored endocrine cells that were positive for both gastrin and GLP-1 (Fig. 2).

All of the different types of mucous, absorptive, and endocrine cells in normal as well as intestinal metaplastic glands may be derived from a single progenitor cell. In the light of the clonal findings with C3H/HeN↔BALB/c chimeric mice, we consider that the alteration from gastric to intestinal metaplastic glands must be controlled at the stem-cell level.

Expression of transcription factors in intestinal metaplasia (IM)

CDX homeobox gene family

Caudal-type homeobox (*Cdx*) 1 and *Cdx2* are mammalian members of the caudal-related homeobox gene family [52]. In the adult mouse, and in humans, expression is strictly confined to the gut, from the duodenum to the rectum. Silberg et al. [53] reported the presence of Cdx1 protein in intestinal metaplastic lesions of the human stomach, and Mizoshita et al. [54] demonstrated the expression of *Cdx1* and *Cdx2* in both the small and large intestine, and in intestinal metaplastic mucosa of the human stomach. Eda et al. [55] found that the expression of *Cdx2* preceded that of *Cdx1* during the progression of IM. Satoh et al. [56] described *Cdx2* expression in the gastric epithelium of *H. pylori*-infected patients, with or without obvious IM. *Cdx2* plays an important role in the intestine-specific expression of carbonic anhydrase 1 [57]. Furthermore, it stimulates the intestine-specific expression of sucrase-isomaltase [58], lactase-phlorizin hydrolase [59], and guanylyl cyclase C [60]. More recently, *Cdx2* has been revealed to induce the expression of MUC2 mucin in goblet cells [61]. Cdx1 has been reported to appear in intestinal metaplastic glands, as described by Silberg et al. [53]. Its expression is strong in regenerating epithelial foci, but not in quiescent sterilized crypts after irradiation-induced damage [62], and recent analyses have shown that Cdx1 is a direct transcriptional target of the Wnt β-catenin signaling pathway during mouse gut development [63] and that Cdx1 is stimulated by oncogenic β-catenin in human colon cancer cells [64]. Dietary factors may be involved in the suppression of *Cdx2* via its promoter methylation [65] (Fig. 3).

Sox gene family

To analyze the shift from a gastric to an intestinal phenotype, one should also focus on gastric transcription factors, including the *Sox* gene family [66], which consists of ten subgroups, divided according to Sry-like high-mobility group (HMG) box homology. The *Sox* genes in group B1, including *Sox1*, *Sox2*, and *Sox3*, are important for gut development in mice [67]. In-situ analysis of the chicken *cSox2* gene demonstrated localized expression in the embryonic endoderm, with transcripts appearing before the commencement of morphogenesis, and cytodifferentiation in the rostral gut epithelium from the pharynx to the stomach. The caudal limit of *cSox2* expression coincides with that of the region competent for proventricular differentiation and with the rostral limit of the domain of *CdxA* [68]. In the human digestive tract, *Sox2* expression is found in stomach epithelium, including the fundic and pyloric mucosae, but is very low in the intestine, as observed in the chicken. However, in IM, *Sox2* transcripts begin to decrease and gradually disappear as IM progresses from the GI-mixed-type to the I type, with *Sox2* showing an inverse correlation with *Cdx1* and *Cdx2*. *Sox2* may regulate the expression of gastric differentiation markers, including MUC5AC, as suggested in the chicken system [69]. The expression patterns of *Sox2* and *Cdx1*/*Cdx2* are inversely related, and downregulation of *Sox2* could thus be an important mechanism in IM, in addition to the ectopic expression of *Cdx1*/*Cdx2* [70]. Specificity of the expression pattern of these transcription factors also persists in stomach cancers [71,72] (Fig. 3).

PDX1

Pancreatic-duodenal homeobox 1 (*PDX1*), a *ParaHox* gene which contributes to the genesis and development of the pancreas, duodenum, and antrum, has been found to be frequently expressed in pseudopyloric glands and IM. MUC6 is more abundant than MUC5AC in pseudopyloric glands, while higher levels of MUC5AC than MUC6 are evident in IM. In carcinomas, *PDX1* expression is closely associated with MUC6, whereas no link is apparent between *PDX1* and MUC5AC reactivity. Thus, *PDX1* may play an important role in the development of pseudopyloric glands and subsequent IM [73,74].

OCT-1

OCT-1 is a member of the POU homeodemain family of transcription factors [75]. This protein recognizes the canonical octamer motif (ATGCAAAT) and is implicated in the activation of the mouse Cdx2 promoter in pancreatic and intestinal cell lines. OCT-1 is expressed in chronic gastritis, particularly when it is adjacent to IM, and it is also expressed in 87% of IM foci. Furthermore, 74% of gastric carcinomas in one series were found to be positive for OCT-1, and a strong association was observed between OCT-1 expression and an intestinal-type phenotype. OCT-1 is able to bind to the CDX2 promoter, although transactivation of CDX2 has not been demonstrated [76].

Sonic hedgehog (Shh) pathway

High levels of *Shh* are expressed in the fundic glands of the stomach in the normal gastrointestinal tract, but *Shh* expression is lost in IM of the human stomach [77], resulting in a glandular phenotype of intestinal transformation and overgrowth. Hedgehog-related transcription factors, Gli2 and Gli3, may be involved in *Shh* signaling. While disruption of Gli2 (the principal factor mediating the activator function of Shh), leads to minimal changes in glandular development in the mutant mouse, knockout of Gli3, functioning as a repressor of the Hedgehog signal, causes a striking phenotype of glandular expansion and intestinal transformation. A reduction in apoptotic events was seen in the stomachs of all Gli3 mutants, without affecting proliferation [78]. In humans, impaired expression of the gastric morphogenic factor *Shh* by parietal cells, and the increased expression of transcriptional activators of intestinal and pancreatic differentiation; namely, *CDX2* and *PDX1*, seem to be crucial for the development of gastric atrophy and for intestinal, endocrine, and pancreatic transdifferentiation processes [74].

Erk pathway

The increased expression of villin is one of the earliest changes seen in *H. pylori* infection [70]. These bacteria have been found to stimulate the villin promoter in a human gastric adenocarcinoma cell line (AGS) via activation of the Erk pathway, where Elk-1 and the serum response factor (SRF) are downstream transcriptional targets. Inducible binding of Elk-1 and the SRF to the proximal promoter of villin after 3 and 24 h of treatment with *H. pylori* suggests that these bacteria alone are sufficient to initiate a cascade of signaling events responsible for villin expression.

Runt-related transcription factor gene 3 (RUNX3)

The RUNX family of transcription factors plays pivotal roles during normal development and in neoplasias [79], and *RUNX3* is reported to be a tumor suppressor gene for stomach cancer [80]. The loss of *RUNX3* expression due to aberrant methylation of its CpG island (evident in gastric cancer cell lines) suggests that this factor is a

target for epigenetic gene silencing in gastric carcinogenesis. *RUNX3* methylation has also been found in mucosa with chronic gastritis or IM [81]. Immunohistochemistry disclosed RUNX3 protein in most chief cells and a few gastrin-containing G cells in normal mucosa, but not in IM or carcinoma cells [82]. Furthermore, in vitro studies have shown that gastric epithelial cells can differentiate into intestinal-type cells, probably due to the expression of *Cdx2*, when the function of *Runx3* is impaired in *Runx3*-knockout mice [83].

Expression of small-intestinal and colonic phenotypes in complete intestinal metaplasia (IM)

Jass and Filipe [45] described three grades of IM, in terms of small-intestinal sialomucin and colonic sulfomucin expression, shown by high-iron diamine alcian blue (HID-AB) staining. Type I glands have no mucins in columnar cells, but feature goblet cells. Type II glands have blue-stained columnar cells possessing sialomucins, while type III glands harbor brown-stained columnar cells producing sulfomucins, with type II and III glands characterized by slight distortion. To discriminate small-intestinal and colonic differentiation in IM, molecular markers, including sucrase and carbonic anhydrase 1 (CA1) could be utilized in comparison with MUC5AC mucin core protein. CA1 expression is detectable in the cytoplasm of colon epithelial cells (especially on the luminal side of the colonic mucosa), but not in the jejunum. Sucrase, on the other hand, is present on the luminal surfaces of mature small-intestinal absorptive cells, but not in the colon. In IM, gastric MUC5AC expression is higher in CA1-negative mucous cells of GI-mixed-type IM glands, compared with CA1-positive I-type IM, in line with levels of MUC5AC mRNA. In contrast, the expression of sucrase is more strongly detected on the luminal surfaces of CA1-positive IM gland cells than in CA1-negative IM glands. MUC2, villin, and *Cdx2* expression is observed in intestinal metaplastic cells, irrespective of CA1 expression. The number of glands with CA1 expression is higher in type I complete IM compared to types II and III incomplete IM. Furthermore, there appear to be no differences between types II and III in terms of CA1 expression, and no correlation of colonic sulfomucin expression. In short, the expression of gastric and colonic markers may be regulated in a different manner, although both can be colocalized with small-intestinal markers [84] (Fig. 3).

Conclusion

Atrophic gastritis and IM of the stomach mucosa are generally considered to be precancerous lesions, and chronic *H. pylori* infection is one of the most important factors in their development. However, *H. pylori* strains show a wide variety at the genome level, especially regarding the *cag* and *vac* genes, and this variation may underlie the observed large differences in stomach cancer incidence and mortality around the world, including the "Asian paradox" and "African enigma". In addition to bacterial factors, polymorphisms in host genes (for example, for cytokines that modulate inflammatory responses) are believed to exert synergistic effects. For the prevention of detrimental changes in the stomach mucosa, it is necessary to elucidate the pathogenetic mechanisms of mucosal atrophy and IM due to *H. pylori* infection.

Acknowledgments The authors thank colleagues in their laboratory for their expert technical assistance and valuable discussion. This work was supported in part by a Grant-in-Aid for the Third-term Comprehensive 10-year Strategy for Cancer Control, and a Grant-in-Aid for Cancer Research, both from the Ministry of Health, Labour, and Welfare, Japan; and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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