Human intestinal-type gastric carcinoma is associated with gastric atrophy and intestinal metaplasia that are caused mainly by Helicobacter pylori infection. Correa presented a hypothesis with respect to the mechanism of gastric carcinogenesis due to H pylori infection. H pylori infection is involved in the progression from normal gastric mucosa to superficial gastritis, chronic active gastritis, atrophic gastritis, and finally to intestinal metaplasia. The terminal stage of this process is gastric carcinoma.

We and others have reported that the intestinal specific transcription factor Cdx2 is expressed in human gastric intestinal metaplastic mucosa. Furthermore, we established Cdx2 transgenic mice expressing the transcription factor Cdx2 exclusively in the gastric epithelium. The gastric fundic mucosa of the Cdx2 transgenic mouse was completely changed into intestinal metaplastic mucosa. However, whether intestinal metaplasia is limited to the epithelium or influences the underlying mesenchyme has not been elucidated as it is difficult to discriminate intestinal mesenchyme from gastric mesenchyme.

The normal intestinal crypt of Lieberkühn is invested by a mesenchymal sheath, a specialised part of the lamina propria consisting of fibroblasts tightly surrounding the epithelium and of collagen fibres oriented circumferentially to the crypt. The mesenchymal sheath is a distinct and highly organised system of fibroblasts immediately subjacent to the epithelial basement membrane located at the epithelial-mesenchymal interface. The highly specialised fibroblasts, that were reported as pericryptal fibroblasts by Kay and colleagues, form the pericryptal fibroblast sheath (PCFS). PCFS consists of a network of fibroblast cells and extracellular matrix immediately subjacent to the crypt epithelial cells. Such pericryptal fibroblasts under the basement membrane envelop the glands of the intestine, are seen as elongated cells tightly appliqued to the base of the epithelial cells of the crypt, have a fusiform appearance, and follow the contours of the wall of the crypt. Fibroblasts of the PCFS have an important role in colonic fluid absorption. Colonic absorptive function depends not only on crypt luminal cells but also on the fibroblast cells of the surrounding pericryptal sheath.

There is a close interactive epithelial-mesenchymal relationship between the epithelial cell system and the underlying pericryptal fibroblast system to maintain the normal structure and function of the crypts of Lieberkühn. Pericryptal fibroblasts have been shown to play a fundamental role in epithelial differentiation via epithelial-mesenchymal cell interactions during both fetal and adult life. The PCFS is a self renewing population of mesenchymal cells in close contact with the intestinal epithelium; its cells maintain a parallel relationship in replication, migration, and differentiation with the overlying epithelium suggesting that it is involved in the maintenance of the normal structure and function of the intestinal mucosa. Autoradiographic studies in rabbit colon after 3H-thymidine injection demonstrate steady state renewal of pericryptal fibroblasts and migration to upper portions of the synchrony with epithelial migration. The fibroblast progenitor population, like that of the epithelium, is in the deep one third of the crypt. The kinetics of this portion of the normal intestinal mucosa suggest that the pericryptal fibroblasts and the epithelium act as a unit to maintain the normal structure, maturation, and function of the crypt of Lieberkühn.

These findings prompted us to investigate (1) whether intestinal metaplasia influences the epithelium as well as the formation of the PCFS and (2) the relationship between PCFS and intestinal-type gastric carcinoma.

**Background and aims:** In the progression of chronic gastritis, gastric mucosal cells deviate from the normal pathway of gastric differentiation to an intestinal phenotype which is closely related to gastric carcinoma. However, to date, it has not been elucidated whether the intestinal metaplasia is merely a change in the epithelium or whether the underlying mesenchyme also changes from gastric type to intestinal type. We have investigated the relationship between intestinal metaplasia and the pericryptal fibroblast sheath (PCFS) in the mesenchyme. In addition, we also examined PCFS in gastric carcinoma.

**Methods:** We determined the existence of PCFS in the intestinal metaplastic mucosa and carcinoma of both human and Cdx2 transgenic mouse stomach. PCFS was determined using the antibody against α-smooth muscle actin and electron microscopic observations.

**Results:** PCFS formed an almost complete layer around the small and large intestinal crypts while it did not exist around the normal gastric glands in both mice and humans. PCFS was seen around the glands of intestinal metaplastic mucosa in both Cdx2 transgenic mouse and human stomachs. However, PCFS was virtually absent in the intestinal-type gastric adenocarcinoma area.

**Conclusion:** We successfully demonstrated that the epithelium as well as the mesenchyme changed from the gastric type to the intestinal type in intestinal metaplasia and that PCFS disappeared in intestinal-type gastric carcinoma.

**Abbreviations:** PCFS, pericryptal fibroblast sheath; α-SMA, α-smooth muscle actin; PBS, phosphate buffered saline
MATERIALS AND METHODS

Cdx2 transgenic mice

We used Cdx2 transgenic mice with stomach specific expression of Cdx2 using the β-subunit gene promoter of rat H⁺/K⁺-ATPase. The gastric mucosa of Cdx2 transgenic mice was completely changed to intestinal metaplastic mucosa.

Histology

Stomach tissue specimens were fixed in neutral buffered 10% formalin for 12–24 hours, washed in 70% ethanol, processed by standard methods, embedded in paraffin, sectioned at 3 μm, and stained with haematoxylin and eosin for histological evaluation.

Immunohistochemistry

Thick sections (3 μm) were cut, deparaffinised, rehydrated in phosphate buffered saline (PBS), placed in 10 mM citrate buffer (pH 6.0), and heated in an 850 W microwave for 15 minutes to recover antigenicity. Endogenous peroxidase activity was blocked by incubation for 30 minutes in methanol containing 0.3% H₂O₂. After washing twice with PBS, including 0.1% Triton X-100, sections were preincubated with blocking buffer (Dako, Carpinteria, California, USA) for 15 minutes at room temperature. Primary antisera, anti-α-smooth muscle actin (α-SMA) (1:100; Dako), or anti-Cdx2 (1:100; BioGenex, San Ramon, California, USA) were diluted in PBS and incubated overnight at 4°C. Slides were then washed in PBS and incubated with Envision (Dako). After

Figure 1  Mouse gastric glands (A), small intestinal crypts (B), and large intestinal crypts (C) shown in cross section. Cross sections of crypts of mouse small (B) and large (C) intestines show pericryptal fibroblast nuclei (arrows) immediately subjacent to the epithelial cells. The long axis of each pericryptal fibroblast is perpendicular to that of the crypt. Pericryptal fibroblast nuclei are not seen forming a sheath round the glands of mouse gastric mucosa (A). Magnification ×400.

Figure 2  Normal mouse small (A, B) and large (C, D) intestinal mucosa. Immunohistochemical stain for α-smooth muscle actin. The pericryptal fibroblast sheath formed by pericryptal fibroblasts is closely embracing epithelial cells of normal intestinal crypts. Magnification ×100 (A, C); ×400 (B, D).
development with 3,3'-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Osaka, Japan), slides were counterstained with haematoxylin and viewed under a light microscope.

**Fixation and preparation of tissue for electron microscopy**

Intestinal metaplastic mucosa from Cdx2 transgenic mouse stomach was fixed at 4°C in 2% glutaraldehyde in PBS, followed by six washes in PBS with post fixation in 1% osmium tetroxide. Sections were examined under a Hitachi H-7500 scanning electron microscope.

**RESULTS**

To examine whether intestinal metaplasia is merely epithelial cell metaplasia or influences the underlying mesenchyme, we focused our attention on the PCFS in the mesenchyme. Light microscopic examination of normal mouse small and large intestine revealed fibroblasts subtending the epithelial basement membrane in the crypt (fig 1B, 1C). In contrast, fibroblasts were not seen forming a sheath round the glands of normal mouse gastric mucosa (fig 1A). These fibroblasts subjacent to the epithelium were easily distinguished from the rest of the mesenchymal elements of the lamina propria, which consist of a loose meshwork of collagen, fibroblasts, haematogenous cells, and capillaries.

α-SMA is present in pericryptal fibroblasts and is used as a marker for pericryptal fibroblast cells. Pericryptal fibroblasts labelled by α-SMA formed single cell layers that embraced the whole length of the crypts in the normal small and large intestine mucosa of the mouse (fig 2) and humans (fig 3). α-SMA positive PCFS was seen around many glands. Areas of abutment were broad and the sheath appeared continuous.

We previously generated Cdx2 transgenic mice expressing intestine specific transcription factor Cdx2 gene exclusively in the gastric epithelium under the control of the β-subunit gene promoter of rat H+/K+-ATPase. Cdx2 transgenic mice developed normally into superficially healthy adults and showed intestinal metaplasia in the stomach up to 12 weeks of age. The gastric mucosa of Cdx2 transgenic mouse was completely replaced by intestinal metaplastic mucosa. Cdx2 induced intestinal metaplastic mucosa consisted of terminally differentiated intestinal epithelial cells, including absorptive enterocytes, goblet cells, and enteroendocrine cells. To clarify whether Cdx2 expression in gastric epithelium affects the underlying mesenchyme in vivo, the intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach was stained for α-SMA. The PCFS expressing α-SMA was not seen around the glands of the normal gastric mucosa (fig 4A, B) whereas PCFS was easily recognised around the crypts of intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach (fig 4C, 4D). In addition to mouse intestinal metaplastic mucosa, PCFS was also recognised around the glands of human intestinal metaplastic mucosa (fig 5C, D) while it was not seen around normal human gastric glands (fig 5A, B). Electron microscopy revealed an even closer association between pericryptal fibroblasts and the epithelium than was revealed by light microscopy. In intestinal metaplastic mucosa of Cdx2 transgenic mouse stomach, the PCFS was in intimate contact with the epithelial basal lamina (fig 6). Fibroblasts were seen surrounding the base of the crypts in the intestinal metaplastic mucosa. These cells had large areas of contact with the epithelial basal lamina and had a plum fusiform shape (fig 6B, C).

As it is reported that the PCFS is significantly reduced in colorectal epithelial neoplasms, we examined the relationship between PCFS and gastric adenocarcinoma. We observed Cdx2 transgenic mice periodically without carcinogens or H pylori infection. Cdx2 transgenic mice at 50 weeks of age indicated preservation of intestinal metaplasia and no gastric polyp formation, similar to those at 12 weeks (unpublished data). Cdx2 transgenic mice developed gastric polyps in the intestinal metaplastic lesion at two years after birth. Gastric polyps developed from intestinal metaplastic mucosa in all stomachs of 10 Cdx2 transgenic mice examined. The polyps consisted of adenocarcinoma that invaded the submucosa or beyond (unpublished data). Using the adenocarcinoma, we examined the relationship between PCFS and adenocarcinoma. PCFS detected by immunohistochemical stain for α-SMA was virtually absent in the area of gastric adenocarcinoma.
adenocarcinoma of both humans and mice (fig 7C, G) while PCFS was easily recognised in the intestinal metaplastic areas (fig 7A, E). PCFS was absent in all 10 Cdx2 transgenic mouse and 10 human intestinal-type adenocarcinomas. Both human and murine gastric carcinomas were classified as intestinal-type according to the criteria of Lauren14 and as category 5.2 according to the five categories of the Vienna classification.15 16 We examined expression of Cdx2 in intestinal metaplasia and adenocarcinoma. Cdx2 staining for the adenocarcinoma lesion (fig 7D, H) was extremely weak compared with the intestinal metaplastic lesion (fig 7B and F). The decrease in Cdx2 may explain, in part, the cause of the disappearance of PCFS in the gastric adenocarcinoma. There was no difference in immunoreactivities for Cdx2 and α-SMA in intestinal metaplastic mucosa between 12 and 50 week old Cdx2 transgenic mice (data not shown).

**DISCUSSION**

Our results demonstrate that intestinal metaplasia is not merely epithelial metaplasia but also affects the underlying
Cdx2 expression is only activated distal to the gastric- 
H pylori differentiated from the gastric type to the intestinal type by 
epithelium as well as the subjacent mesenchyme is trans-
human intestinal metaplastic mucosa indicates that the 
mainly caused by H pylori epithelium. Furthermore, as human intestinal metaplasia is 
through expression of transcription factor Cdx2 in the 
mucosa, the epithelium as well as the mesenchyme changes 
study is the first to show that in the intestinal metaplastic 
generated by us. To the best of our knowledge, the present 
was demonstrated with Cdx2 transgenic mice previously 
role not only in differentiating intestinal metaplastic mucosal 
sequences for Cdx2 directly to bind. That Cdx2 has a pivotal 
various types of intestine specific genes that have consensus 
mucosa. Cdx2 has been reported to induce expression of 
also seen around the glands of human intestinal metaplastic 
membrane while normal gastric glands were not. PCFS was 
induced by a single homeobox gene, Cdx2, in transgenic 
mouse stomach were surrounded by PCFS via the basement 
mesenchyme. The glands of intestinal metaplastic mucosa 
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sequences for Cdx2 directly to bind. That Cdx2 has a pivotal 
role not only in differentiating intestinal metaplastic mucosal 
epithelial cells but also in inducing PCFS in the mesenchyme 
was demonstrated with Cdx2 transgenic mice previously 
generated by us. To the best of our knowledge, the present 
study is the first to show that in the intestinal metaplastic 
mucosa, the epithelium as well as the mesenchyme changes 
from a gastric-type mucosa to an intestinal-type mucosa 
through expression of transcription factor Cdx2 in the 
epithelium. Furthermore, as human intestinal metaplasia is 
mainly caused by H pylori infection, generation of the PCFS in 
human intestinal metaplastic mucosa indicates that the 
epithelium as well as the subjacent mesenchyme is trans-
differentiated from the gastric type to the intestinal type by 
H pylori infection.

The stomach and intestine are derived from the primitive 
undifferentiated gut tube formed during gastrulation but 
Cdx2 expression is only activated distal to the gastric-
duodenal junction. Cdx2 haploinsufficiency results in 
abnormal differentiation of midgut endoderm. The initial 
effect of heterozygous Cdx2 knockout mice, apparent in 
neonates, is seen as patches of forestomach epithelium 
present in the terminal ileum, cecum, or proximal colon. Thus in Cdx2 deficiency, cells which would normally 
differentiate into caecum and proximal colon follow a default 
pathway and form the stratified squamous epithelium 
characteristic of forestomach. In contrast, intestinal meta-
plastic epithelium was induced from gastric mucosa by 
expression of Cdx2, indicating that Cdx2 may be a key 
regulator for intestinal epithelial cell fate determination and 
differentiation. The intestinal metaplastic mucosa of Cdx2 
transgenic mouse stomach also generated PCFS in the 
mesenchyme indirectly through expression of Cdx2 in the 
epithelium. The crypts of intestinal metaplastic mucosa in the 
human stomach were also embraced by pericryptal fibro-
blasts via the basement membrane. These results suggest that 
Cdx2 may have a master function in the coordinate process 
leading to both epithelial and mesenchymal differentiation 
during continuous renewal of the intestinal mucosa.

The embryonic gut of vertebrates consists of endodermal 
epithelium and surrounding mesenchyme. The involvement 
of epithelial-mesenchymal cell interactions in the control of 
cell differentiation during intestinal ontogeny and during 
continuous cell renewal in the mature organ has been 
demonstrated. Regional differentiation and morpho-
genesis of the gut epithelium requires tissue interactions 
involving instructive effects of mesenchyme and competence 
of the epithelium receiving them. The importance of the 
mesenchyme has been shown by in vivo models in which 
hybrid recombinants composed of fetal or adult mesenchyme 
associated with epithelial cells are grafted. The chick 
embryonic stomach and the fetal rat colon endoderms are 
duced to achieve a small intestinal morphological and 
functional cytodifferentiation (induction of sucrase-isomaltase 
gene expression) under the influence of the small intestinal 
mesenchyme. In the avian embryonic muscular stomach 
epithelium (proventriculus), expression of embryonic chick 
pepsinogen gene, which is specific to developing glandular 
stomach epithelium, is regulated by the instructive influences 
of the chick glandular stomach mesenchyme. These effects 
of the mesenchyme on the epithelium indicate that 
mesenchyme is important in morphogenetic processes and 
in maintenance of the tissue integrity in the gut. 
Laminin-1, an extracellular matrix component of the 
basement membrane, is known to stimulate intestinal cell 
differentiation. The differentiating effect of laminin-1 
coatings on Caco2-TC7 cells is accompanied by upregulation 
of Cdx2, suggesting that Cdx2 plays a key role in the cascade 
of events involved in extracellular matrix mediated intestinal 
cell differentiation. These data indicate that intestinal 
mesenchyme influences intestinal epithelial differentiation. 
Conversely, the present results showed that Cdx2 induced 
intestinal epithelium affected PCFS formation in the 
mesenchyme. PCFS in intestinal metaplasia of the stomach 
was formed simply by expressing Cdx2 in gastric mucosal 
epithelial cells. The results indicate that expression of Cdx2 in 
epithelial cells directly plays a pivotal role in generating PCFS 
around Cdx2 expressing glands. It is therefore possible that 
the intestinal metaplastic mucosa of Cdx2 transgenic mouse 
stomach results from direct interaction of Cdx2 protein with 
the promoters of enterocytic differentiation markers as well 
as from indirect effects of the mesenchyme. Taken together, 
these observations indicate that reciprocal interactions 
between epithelium and mesenchyme might be important 
for the development and differentiation of intestinal mucosa, 
including both epithelium and mesenchyme.

PCFS has been investigated in colorectal cancer by 
imunohistochemistry employing an antibody against
The amount of PCFS was reduced significantly in colorectal carcinoma. A significant reverse correlation was seen between the degree of colorectal neoplasia progression and the quantity of neoplastic glands with PCFS. Nakayama et al reported the relationship between α-SMA positive stromal cells and gastric carcinoma. Their α-SMA positive stromal cells were high molecular weight caldesmon negative and different from PCFS which is positive for high molecular weight caldesmon. The relationship between PCFS and gastric carcinoma has not been investigated to date. In the present study, we have shown that PCFS also disappeared in the gastric carcinoma of both humans and mice, similar to 

**Figure 7** Relationship between Cdx2 and α-smooth muscle actin (α-SMA) positive pericryptal fibroblast sheath (PCFS) in vivo. Gastric intestinal metaplasia of Cdx2 transgenic mice (A, B) and humans (E, F). Gastric adenocarcinoma of Cdx2 transgenic mice (C, D) and humans (G, H). Immunohistochemical staining for α-SMA (A, C, E, G) and Cdx2 (B, D, F, H). Cdx2 expressing intestinal metaplastic epithelial cells are surrounded by α-SMA positive pericryptal fibroblasts in the intestinal metaplastic mucosa (A, B, E, F) while PCFS is not recognised in the adenocarcinoma (C, G). α-SMA positive cells in gastric carcinoma tissue is not histologically PCSF. Cdx2 staining for the adenocarcinoma lesion (D, H) is extremely weak compared with the intestinal metaplastic lesion (B, F). Magnification ×200.
the colonic carcinoma. Although intestinal-type gastric carcinoma is thought to develop from gastric epithelium that has undergone intestinal metaplasia, intestinal-type gastric carcinoma cells were not surrounded by PCFS, which is different from the intestinal metaplastic mucosa around the gastrointestinal mucosa. The presence of mesenchymal cells or mesenchyme derived extracellular matrix molecules has been shown to play a fundamental role in epithelial cell proliferation and differentiation via epithelial-mesenchymal cell interactions.\textsuperscript{22, 23} Mesenchymal cells express important growth factors whose receptors are found on the epithelial cell surface which is exemplified by expression of hepatocyte growth factor/scatter factor and of keratinocyte growth factor which regulate epithelial cell behaviour.\textsuperscript{24} These instructive actions of the mesenchyme on gastrointestinal tract epithelial cells indicate that the generation and disappearance of PCFS might be closely related to the development of intestinal-type gastric carcinoma.

In conclusion, ectopic expression of Cdx2 in gastric mucosal epithelial cells induced not only intestinal epithelial cell differentiation but also PCFS generation in the mesenchyme, indicating that Cdx2 may be important for intestinal mucosal differentiation involving both the epithelium and mesenchyme. In addition, PCFS in the mesenchyme may be related to the development of gastric carcinoma.

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