STOMACH

Pericryptal fibroblast sheath in intestinal metaplasia and gastric carcinoma

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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.

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.¹ *H. pylori* infection is involved in the process of progression from normal gastric mucosa to superficial gastritis, chronic active gastritis, atrophic gastritis, and finally to intestinal metaplasia.¹ 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.^{2–6} Furthermore, we established Cdx2 transgenic mice expressing the transcription factor Cdx2 exclusively in the gastric epithelium.⁷ 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.⁸ 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,⁹ form the pericryptal fibroblast sheath (PCFS). PCFS consists of a network of fibroblast cells and extracellular matrix immediately subjacent to the crypt epithelial cells.^{8–10} Such pericryptal fibroblasts under the basement membrane envelop the glands of the intestine, are seen as elongated cells tightly appliquéd 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.¹¹ Colonic absorptive function depends not only on crypt luminal cells but also on the fibroblast cells of the surrounding pericryptal sheath.¹¹

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.^{8–9} Pericryptal fibroblasts have been shown to play a fundamental role in epithelial differentiation via epithelial-mesenchymal cell interactions during both fetal and adult life.^{12–13} 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.^{8–9} 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.⁸ 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.⁸

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.

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

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 $\times 400$.

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.⁷ The gastric mucosa of Cdx2 transgenic mice was completely changed to intestinal metaplastic mucosa.⁷

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 μ m, and stained with haematoxylin and eosin for histological evaluation.

Immunohistochemistry

Thick sections (3 μ m) 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% H_2O_2 . 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 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 $\times 100$ (A, C); $\times 400$ (B, D).

Figure 3 Normal human small (A, B) and large (C, D) intestinal mucosa. Immunohistochemical stain for α -smooth muscle actin. Pericryptal fibroblast sheath formed by pericryptal fibroblasts is closely embracing the epithelial cells of normal intestinal crypts. Magnification $\times 100$ (A, C); $\times 400$ (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 intestinal 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.⁷ 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.⁷ 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

Figure 4 Normal and intestinal metaplastic mucosa of the mouse stomach. Immunohistochemical stain for α -smooth muscle actin. Pericryptal fibroblast sheath (PCFS) formed by pericryptal fibroblasts is closely embracing the epithelial cells of the intestinal metaplastic mucosa (C, D) while PCFS is not seen in the normal gastric mucosa (A, B). Magnification $\times 100$ (A, C); $\times 400$ (B, D).

Figure 5 Normal and intestinal metaplastic mucosa of the human stomach. Immunohistochemical stain for α -smooth muscle actin. Pericryptal fibroblast sheath (PCFS) formed by pericryptal fibroblasts is closely embracing the epithelial cells of the intestinal metaplastic mucosa (C, D) while PCFS is not seen in the normal gastric mucosa (A, B). Magnification $\times 100$ (A, C); $\times 400$ (B, D).

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 7, B 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

Figure 6 Electron micrograph of intestinal metaplastic mucosa in the Cdx2 transgenic mouse. The fibroblast (arrows) around the epithelial cells of the crypt demonstrates the fusiform shape and broad contact with the epithelial basal lamina (arrowhead) which is typical of the pericryptal fibroblast. The basement membrane is indicated by the arrowhead (C).

mesenchyme. The glands of intestinal metaplastic mucosa induced by a single homeobox gene, Cdx2, in transgenic mouse stomach were surrounded by PCFS via the basement membrane while normal gastric glands were not. PCFS was also seen around the glands of human intestinal metaplastic mucosa. Cdx2 has been reported to induce expression of various types of intestine specific genes that have consensus 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.^{17, 18} Cdx2 haploinsufficiency results in

abnormal differentiation of midgut endoderm.¹⁹ The initial effect of heterozygous Cdx2 knockout mice, apparent in neonates, is seen as patches of forestomach epithelium present in the terminal ileum, caecum, or proximal colon.²⁰ 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 metaplastic epithelium was induced from gastric mucosa by expression of Cdx2,⁷ 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 fibroblasts 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.^{21, 22} Regional differentiation and morphogenesis of the gut epithelium requires tissue interactions involving instructive effects of mesenchyme and competence of the epithelium receiving them.²³ 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.^{24, 25} The chick embryonic stomach and the fetal rat colon endoderms are induced to achieve a small intestinal morphological and functional cytodifferentiation (induction of *sucrase-isomaltase* gene expression) under the influence of the small intestinal mesenchyme.^{25, 26} 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.²⁷ These effects of the mesenchyme on the epithelium indicate that mesenchyme is important in morphogenetic processes and in maintenance of the tissular integrity in the gut.

Laminin-1, an extracellular matrix component of the basement membrane, is known to stimulate intestinal cell differentiation.²⁸⁻³¹ The differentiating effect of laminin-1 coatings on Caco2-TC7 cells is accompanied by upregulation of Cdx2,³¹ 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 carcinoma by immunohistochemistry employing an antibody against

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 PCFS. Cdx2 staining for the adenocarcinoma lesion (D, H) is extremely weak compared with the intestinal metaplastic lesion (B, F). Magnification $\times 200$.

α -SMA.³² 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.³³ Nakayama *et al* reported the relationship between α -SMA positive stromal cells and gastric carcinoma.³⁴ Their α -SMA positive

stromal cells were high molecular weight caldesmon negative and different from PCFS which is positive for high molecular weight caldesmon.³⁴ 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

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 gastric carcinoma. 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.^{12 13 35} 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.³⁶ 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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REFERENCES

- Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;**52**:6735–40.
- Eda A, Osawa H, Yanaka I, et al. Expression of homeobox gene CDX2 precedes that of CDX1 during the progression of intestinal metaplasia. *J Gastroenterol* 2002;**37**:94–100.
- Satoh K, Mutoh H, Eda A, et al. Aberrant expression of CDX2 in the gastric mucosa with and without intestinal metaplasia: effect of eradication of *Helicobacter pylori*. *Helicobacter* 2002;**7**:192–8.
- Almeida R, Silva E, Santos-Silva F, et al. Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas. *J Pathol* 2003;**199**:36–40.
- Bai YQ, Yamamoto H, Akiyama Y, et al. Ectopic expression of homeodomain protein CDX2 in intestinal metaplasia and carcinomas of the stomach. *Cancer Lett* 2002;**176**:47–55.
- Mizoshita T, Inada K, Tsukamoto T, et al. 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. *Gastric Cancer* 2001;**4**:185–91.
- Mutoh H, Hakamata Y, Sato K, et al. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem Biophys Res Commun* 2002;**294**:470–9.
- Pascal RR, Kaye GI, Lane N. Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. I. Autoradiographic studies of normal rabbit colon. *Gastroenterology* 1968;**54**:835–51.
- Kaye GI, Lane N, Pascal RR. Colonic pericryptal fibroblast sheath: replication, migration, and cytodifferentiation of a mesenchymal cell system in adult tissue. II. Fine structural aspects of normal rabbit and human colon. *Gastroenterology* 1968;**54**:852–65.
- Desaki J, Fujiwara T, Komuro T. A cellular reticulum of fibroblast-like cells in the rat intestine: scanning and transmission electron microscopy. *Arch Histol Jpn* 1984;**47**:179–86.
- Naftalin RJ, Pedley KC. Regional crypt function in rat large intestine in relation to fluid absorption and growth of the pericryptal sheath. *J Physiol* 1999;**514**:211–27.
- Birchmeier C, Birchmeier W. Molecular aspects of mesenchymal-epithelial interactions. *Annu Rev Cell Biol* 1993;**9**:511–40.
- Kedinger M, Freund JN, Launary JF, et al. Cell interactions through basement membrane in intestinal development and differentiation. In: Sanderson IR, Walker WA, eds. *Development of the gastrointestinal tract*. London: BC Decker Inc, 1999:83–102.
- Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol Microbiol Scand* 1965;**64**:31–49.
- Schlemper RJ, Riddell RH, Kato Y, et al. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000;**47**:251–5.
- Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. *Gut* 2002;**51**:130–1.
- Silberg DG, Swain GB, Suh ER, et al. Cdx1 and Cdx2 expression during intestinal development. *Gastroenterology* 2000;**119**:961–71.
- 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.
- Chawengsaksophak K, James R, Hammond VE, et al. Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* 1997;**386**:84–7.
- Beck F, Chawengsaksophak K, Waring P, et al. Reprogramming of intestinal differentiation and intercalary regeneration in Cdx2 mutant mice. *Proc Natl Acad Sci U S A* 1999;**96**:7318–23.
- Simon-Assmann P, Kedinger M. Heterotypic cellular cooperation in gut morphogenesis and differentiation. *Semin Cell Biol* 1993;**4**:221–30.
- Simon-Assmann P, Kedinger M, De Arcangelis A, et al. Extracellular matrix components in intestinal development. *Experientia* 1995;**51**:883–900.
- Yasugi S. Regulation of pepsinogen gene expression in epithelial cells of vertebrate stomach during development. *Int J Dev Biol* 1994;**38**:273–9.
- Kedinger M, Simon PM, Grenier JF, et al. Role of epithelial-mesenchymal interactions in the ontogenesis of intestinal brush-border enzymes. *Dev Biol* 1981;**86**:339–47.
- Duluc I, Freund JN, Leberquien C, et al. Fetal endoderm primarily holds the temporal and positional information required for mammalian intestinal development. *J Cell Biol* 1994;**126**:211–21.
- Haffen KKM, Simon-Assmann P. *Cell-contact-dependent regulation of enterocyte differentiation*. New York: Raven Press, 1989.
- Fukuda K, Ishii Y, Saiga H, et al. Mesenchymal regulation of epithelial gene expression in developing avian stomach: 5'-flanking region of pepsinogen gene can mediate mesenchymal influence on its expression. *Development* 1994;**120**:3487–95.
- Hahn U, Stallmach A, Hahn EG, et al. Basement membrane components are potent promoters of rat intestinal epithelial cell differentiation in vitro. *Gastroenterology* 1990;**98**:322–35.
- Vachon PH, Beaulieu JF. Extracellular heterotrimeric laminin promotes differentiation in human enterocytes. *Am J Physiol* 1995;**268**:G857–67.
- Basson MD, Turowski G, Emenaker NJ. Regulation of human (Caco-2) intestinal epithelial cell differentiation by extracellular matrix proteins. *Exp Cell Res* 1996;**225**:301–5.
- Lorentz O, Duluc I, Arcangelis AD, et al. Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation. *J Cell Biol* 1997;**139**:1553–65.
- Yao T, Tsuneyoshi M. Significance of pericryptal fibroblasts in colorectal epithelial tumors: a special reference to the histologic features and growth patterns. *Hum Pathol* 1993;**24**:525–33.
- Li A, Hasui K, Yonezawa S, et al. Immunohistochemical analysis of pericryptal fibroblast sheath and proliferating epithelial cells in human colorectal adenomas and carcinomas with adenoma components. *Pathol Int* 1999;**49**:426–34.
- Nakayama H, Enzan H, Miyazaki E, et al. Alpha smooth muscle actin positive stromal cells in gastric carcinoma. *J Clin Pathol* 2002;**55**:741–4.
- Simo P, Simon-Assmann P, Arnold C, et al. Mesenchyme-mediated effect of dexamethasone on laminin in cocultures of embryonic gut epithelial cells and mesenchyme-derived cells. *J Cell Sci* 1992;**101**:161–71.
- Birchmeier C, Meyer D, Riethmacher D. Factors controlling growth, motility, and morphogenesis of normal and malignant epithelial cells. *Int Rev Cytol* 1995;**160**:221–66.