

Research Paper

Intestinal Metaplasia with a High Salt Diet Induces Epithelial Proliferation and Alters Cell Composition in the Gastric Mucosa of Mice

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KEY WORDS

Cdx2, transgenic mice, intestinal metaplasia, high salt, gastric cancer, gastric mucosa

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ABSTRACT

Intestinal metaplasia of the gastric mucosa is an important component in the pathway to adenocarcinoma. The mechanisms that induce the progression from intestinal metaplasia to cancer have not been elucidated. High dietary salt has been known as one of the risk factors for gastric cancer development in humans. Therefore, we investigated the role of high salt diet on gastric epithelial cell proliferation and differentiation, using our mouse model that ectopically expressed Cdx2 homeodomain transcription factor and induced an intestinal metaplastic phenotype in the gastric epithelia. Sixty Cdx2 transgenic and sixty age-matched wild-type littermates were studied. Fifty-percent Cdx2 transgenic and wild type mice were administered a high-salt diet and the other fifty-percent was fed a standard diet starting at 12 weeks after birth. At 10, 20 and 40 weeks after initiation of the diets, histopathological changes were determined by Hematoxylin and Eosin, alcian blue, and periodic acid-Schiff (PAS) staining. Cell types and cell kinetics were assessed by immunohistochemistry. At 52 weeks, significant alterations in pathology were observed in the Cdx2 transgenic mice fed a high-salt diet, including elongation of gastric pits, reduction of the glandular zone in the gastric corpus, and deepening of glands in the antrum. In the Cdx2 transgenic mice fed a high salt diet, the parietal and chief cells were significantly decreased in the gastric corpus. A significant increase in cell proliferation and apoptosis in the corpus and antrum were observed in Cdx2 transgenic mice fed a high-salt diet as compared to wild-type littermates. Taken together, these data implicate that intestinal metaplasia in concert with a high-salt diet induces epithelial proliferation, apoptosis, and alters cellular types in the gastric mucosa of mice. Alteration in the composition of the gastric epithelium may play a role in influencing the microenvironment to engender susceptibility to carcinogens.

INTRODUCTION

In the progression to gastric adenocarcinoma, a common malignancy worldwide, a generally accepted sequence is that chronic gastritis leads to atrophic gastritis, followed by intestinal metaplasia, dysplasia, and finally carcinoma.¹ Intestinal metaplasia is a common pathological condition in the human stomach, which is characterized by the transdifferentiation of gastric epithelial cells to an intestinal phenotype, accompanied by the expression of intestine-specific genes, including MUC2, sucrase-isomaltase, and carbonic anhydrase I. As an intermediate step in this series, intestinal metaplasia is generally considered to be a precancerous condition, especially for the intestinal-type of gastric cancer.^{2,3} This notion is mainly based upon epidemiological studies, however, the mechanisms that induce the progression of intestinal metaplasia to gastric cancer have not been elucidated.

In most cases intestinal metaplasia follows the appearance of atrophic gastritis. By using various histological and immunohistochemical techniques, intestinal metaplasia can be classified into complete and incomplete types.⁴ Complete type, or type I, resembles the small intestine and contains mature absorptive cells, Paneth cells, and goblet cells secreting sialomucins. Types II and III, are incomplete metaplasia, and contain gastric and intestinal epithelia with goblet cells secreting sialomucins and/or sulfomucins. Type I intestinal metaplasia is more common in benign conditions, whereas types II and III, particularly those with marked secretion of sulfomucins, show a significant association with carcinoma, and may represent a variant with a higher malignant potential.⁵ Thus far, environmental factors, including *Helicobacter pylori* infection,^{6,7} high salt diet^{8,9} and nitrites¹⁰ have been implicated in the initiation of the process leading to intestinal metaplasia. However, the molecular mechanisms underlying the initiation of intestinal metaplasia remain unclear.

CDX2, a member of the caudal-related homeobox gene family, induces cellular differentiation characterized by multilayered structures with microvilli and intestinal specific

gene expression in IEC6 cells, an undifferentiated rat intestinal epithelial cell line.¹¹ In addition, Cdx2 is able to inhibit cellular growth in human colon cancer cell lines.^{12,13} Cdx1 and Cdx2 are commonly detected in human intestinal metaplasia, although they are not expressed in the normal stomach.^{14,15} A causal link between Cdx2 and the development of intestinal metaplasia has been revealed by ectopic expression of Cdx2 in gastric epithelia of mice.^{16,17} Furthermore, gastric polyps developed in 100 wk-old transgenic mice expressing Cdx2 in the parietal cell lineage.¹⁸ In that study, APC and p53 mutations were detected, suggesting that long-term intestinal metaplasia can progress to gastric adenocarcinoma.

High dietary salt has been established as one of the risk factors for gastric adenocarcinoma development in humans and experimental animals. In case controlled studies, high salt intake has been shown to have a significant positive association with the development of gastric cancer.¹⁹⁻²¹ The role of salt consumption has also been implicated in experimental animal models in which animals fed high-salt diets developed gastric mucosal changes. The combination of high salt and *Helicobacter pylori* causes a significant increase in proliferation in the proximal corpus and antrum with atrophy of parietal cells, indicating that elevated salt intake may potentiate *H. pylori* associated carcinogenesis by inducing proliferation, pit cell hyperplasia, and glandular atrophy.²² Swiss/ICR mice fed salted rice diets developed glandular atrophy.²³ In addition, a concentrated salt diet caused excessive cell proliferation in the gastric mucosa in rodent models.²⁴ A recent study showed that outbred Mongolian Gerbils fed a 2.5% salt diet for 56 weeks developed atrophic gastritis and intestinal metaplasia.²⁵ However, the role of high salt diets in combination with intestinal metaplasia has not been studied.

To understand the molecular mechanisms that underlie intestinal metaplasia, our group successfully established Cdx2-transgene mice in which ectopic expression of Cdx2 alone in the gastric mucosa produced type III intestinal metaplasia, characterized by the presence of sulfomucins.¹⁶ In the current study, we have investigated the modulation of intestinal metaplasia by a high-salt diet in Cdx2 transgenic mice.

MATERIALS AND METHODS

Animals. Transgenic Cdx2 and control CD1 mice, as described previously, were used in this study.¹⁶ Pathogen-free mice were bred and housed in polycarbonate cages under a 12hr light/12hr dark cycle, fed experimental diets including ordinary food: containing 0.75% w/w sodium chloride, or high-salt food: containing 8% w/w sodium chloride (Purina 5001-2, TestDiet, Richmond, IN), and given water ad libitum. All experiments were approved by the committee on Research and Animal Care at the University of Pennsylvania.

Cdx2 transgenic and wild-type littermates were age matched and randomly distributed into four groups comprising: (1) wild-type mice with a standard diet; (2) wild-type mice fed a high salt diet; (3) Cdx2 transgenic mice fed a standard diet; and (4) Cdx2 transgenic mice fed a high salt diet. The mice (wild-type and transgenic) in the high salt diet groups began this diet at 12 weeks after birth. At the time of sacrifice, weeks 22, 32 or 52 after birth, ten mice in each group were weighed and injected with 1ml of bromodeoxyuridine (BrdU) (Zymed, San Francisco, CA) per 100 g body weight intraperitoneally 1 hr before being euthanized. Stomachs were excised, cut along the greater and lesser curvatures into halves. One half of the stomach was immediately flattened in a cassette and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) (pH 7.4). Twenty-four hours after fixation, the stomachs were cut into 3-5 small strips extending from body to the antrum and arranged in a cassette to maintain accurate orientation of the gastric mucosa.

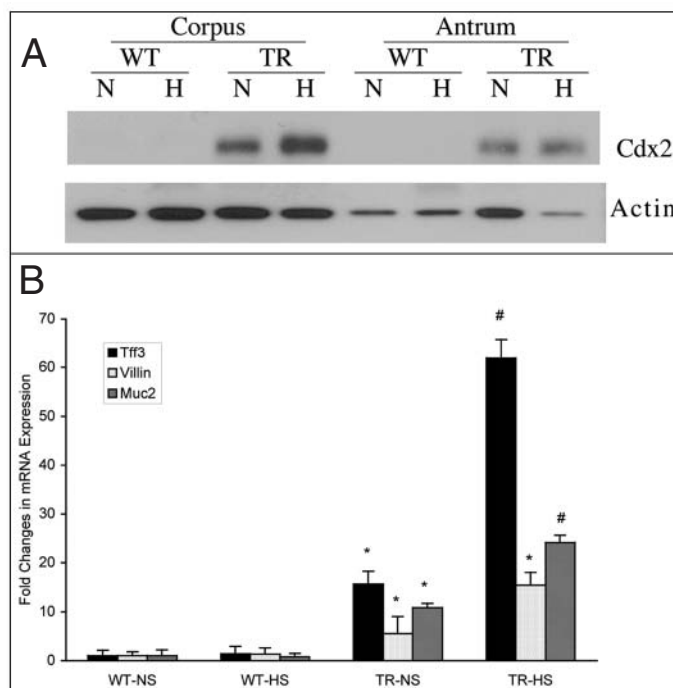


Figure 1. (A) Representative western blot for Cdx2 using protein isolated from the gastric corpus and antrum of the mice in four individual groups. WT, wild-type littermates; TR, transgenic mice; N, normal diet; H, high-salt diet. (B) Intestine specific gene expression by Real-time PCR analysis. Tff3, villin, and Muc2 mRNA expression levels was determined with RNA isolated from the glandular stomach of the wild-type littermates and transgenic mice fed either standard salt or high-salt diet. Data was normalized to GAPDH and expressed as fold induction relative to that observed in wild-type littermates fed a standard diet (arbitrarily set at a value of 1). WT-NS: wild-type fed a standard diet; WT-HS: wild-type fed a high-salt diet; TR-NS: transgenic mice fed a standard diet; and TR-HS: transgenic mice fed a high-salt diet. Each bar represents the mean \pm SD from three animals. #, $p < 0.001$ versus transgenic mice fed a standard diet; *, $p < 0.05$ versus wild-type mice fed a standard diet.

The tissue was placed in PBS, processed in xylene and embedded in paraffin for sectioning. The other half was used for extraction of total RNA and protein.

RNA isolation and quantitative reverse-transcription polymerase chain reaction. The surface of the glandular stomach of the mice was gently scraped with a spatula to obtain the gastric mucosa. Total RNA was then extracted using the RNeasy Midi Kit (Qiagen, Valencia, CA) from collected cells from the mucosa. Two micrograms of total RNA was reverse-transcribed into cDNA by the Superscript First-strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Quantification of mRNA expression for intestine-specific genes, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels, was performed on an ABI 7000 Sequence Detector (Foster City, CA) using the following pairs of primers. Mouse Tff3: forward 5'-TCTGTCA-CATCGGAGCAGTGT-3', reverse 5'-TCTCCTGCAGAGGTTGAAGC-3'; Muc2 forward 5'-CATCCCTCAAACCAGACCAG-3', reverse 5'-CCCACAGGACCCAAAACAGTA-3'; and villin forward 5'-CCTGGAGCAGCTGTAAACAA-3', reverse 5'-TGAAGTCTTCGGTGGACAGGT-3'. GAPDH: forward 5'-ACATGTTCCAGTATGACTCCACTCA-3', reverse 5'-GGTCTCGCTCCTGGAAGAT-3'.

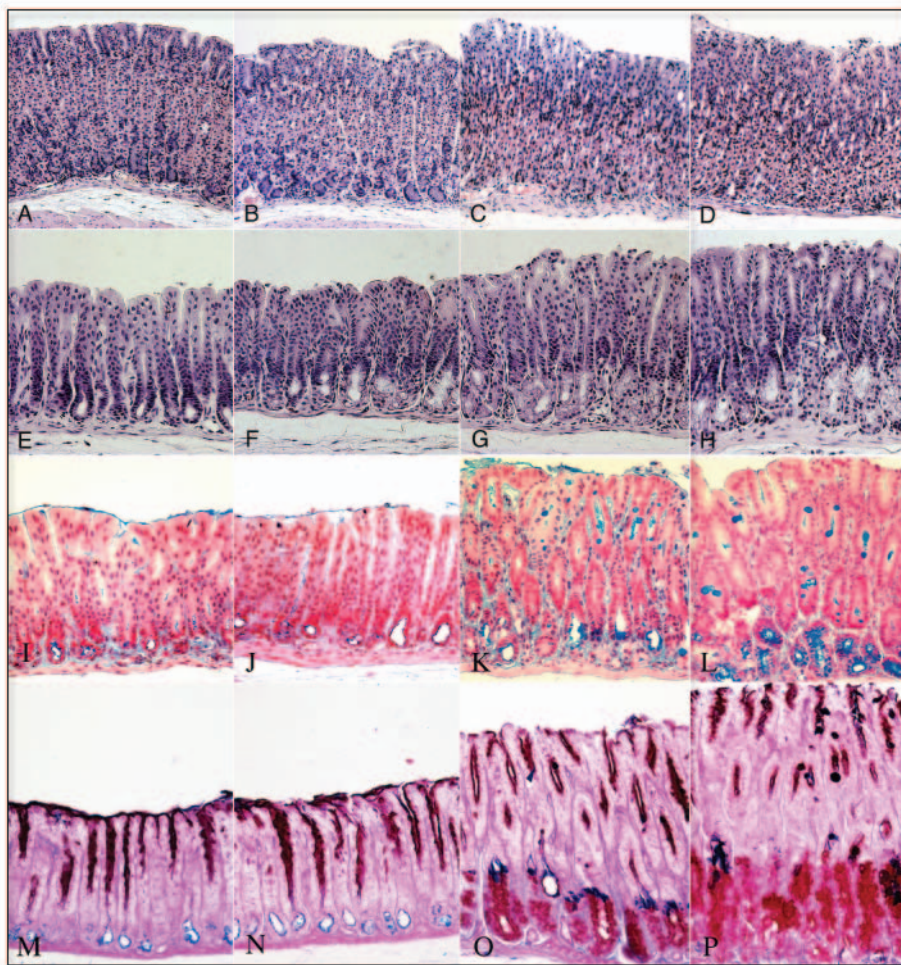


Figure 2. Photomicrographs of HE(A-E), Alcian-Blue, and PAS staining showing histological features of gastric tissue from transgenic mice and wild-type littermates at 52 weeks after birth. In the gastric corpus, the Cdx2 transgenic mice (C, standard diet group, and D, high-salt diet group) had elongation of the gastric pits and reduction of the glandular zone compared with wild-type littermates (A, standard diet group, and B, high-salt diet group). In the antrum, the thickness of gastric mucosa was increased in the Cdx2 transgenic mice fed a standard diet (G) and especially those in the high salt diet group (H). Alcian blue stained goblet cells were well maintained in the Cdx2 transgenic mice (K and L). PAS staining was less in the surface and pit cells and greater in the glandular cells in the Cdx2 transgenic mice (O and P) than wild-type littermates (M and N). Original magnification, $\times 100$ (A-D); $\times 200$ (E-P).

Protein isolation and Western blot. Protein was isolated from the gastric mucosa scraped from the corpus and antrum, by homogenization in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate. After centrifugation, the protein concentration was measured by the Bio-Rad protein assay (Bio-Rad laboratories, Inc.). The protein lysate was mixed with an equal volume of sample buffer and denatured at 95°C for 10 min. Equal amounts of proteins (30 μ g/lane) were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred on to PVDF Immobilon-P Transfer Membrane (Millipore, Billerica, MA) by electroblotting. The blot was incubated in block solution (5% nonfat milk in 1x PBS with 0.25% Tween-20) at 4°C overnight, followed by a 1 hr incubation at room temperature with a polyclonal rabbit anti-mouse Cdx2 antibody CNL¹⁶ at 1:2500 dilution in blocking solution. After washing, the blot was incubated with a donkey anti-rabbit horseradish peroxidase IgG secondary antibody (1:5000; Amersham, Buckinghamshire, England) and developed using an ECL Plus kit (Amersham, Buckinghamshire, England) according to manufacturer's instructions.

Histological evaluation. The paraffin embedded stomach strips were sectioned at 5- μ m. The gastric tissues were examined in an orientation to

view the squamocolumnar junction toward the gastroduodenal junction. The sections were stained in a standard fashion with hematoxylin-eosin (H and E), alcian blue for goblet cell, or periodic acid-Schiff (PAS) for neutral mucins. The glandular mucosa of the corpus and the antrum were examined histologically for epithelial changes and inflammation.

Immunohistochemistry. Immunohistochemical staining for BrdU (Boehringer Mannheim, Germany) and activated caspase-3 (R and D Systems Inc. Minneapolis, MN) incorporation was performed to determine the epithelial proliferation and apoptosis status, respectively. Antibodies for H⁺/K⁺-adenosine triphosphatase (ATPase) (Medical and Biological Labs, Co., Inc. Japan), pepsinogen C (Biodesign, Saco, ME), Chromogranin A (Diasonin, San Diego, CA), and trefoil factor family 1 (Tff1) (generous gift from Dr. Andrew Giraud) were employed to identify cell lineages in the gastric mucosa. After deparaffinization in xylene and graded ethanol series, the 5- μ m tissue sections were immersed in 10 mM citric acid buffer at pH 6.0 and boiled in a microwave oven for 6 minutes. Endogenous peroxidase activity in the tissue sections was blocked by immersing the slides in 3% hydrogen peroxide for 15 min at room temperature. The slides were then incubated in Avidin D, Biotin blocking reagent (Vector Labs, Burlingame, CA) and additional Protein Block Agent (Coulter-Immunotech, Miami, FL) to block non-specific binding sites. Primary antibodies were used in the following concentrations: BrdU and activated caspase-3, 1:750; H⁺/K⁺-ATPase, 1:2500; pepsinogen C, 1: 200; Chromogranin A, 1:3000, and Tff1, 1:1000, incubated overnight at 4°C. As a negative control, slides were incubated with the same concentration of normal immunoglobulin (IgG) as used for the primary antibody. After overnight incubation, biotinylated goat anti-mouse or anti-rabbit IgG at a dilution of 1:200 and horseradish peroxidase-conjugated streptavidin were applied to the sections for 30 min at 37°C. The immunostaining was visualized by applying 3'3'-diaminobenzidine and counterstained with hematoxylin.

Quantification of BrdU, activated caspase-3, H⁺/K⁺-ATPase, pepsinogen C, Chromogranin A staining. In a blinded fashion to the observer, positively stained cells were counted in 10–15 contiguous gastric units for each section, and expressed as the number of positive cells per gastric unit. Epithelial proliferation and apoptosis were quantified based on BrdU incorporation and caspase-3 staining, respectively in the middle corpus mucosa and the antrum. Quantification of the parietal and chief cells in the corpus mucosa was based on H⁺/K⁺-ATPase and pepsinogen C staining, respectively. Endocrine cells were quantified by the number of Chromogranin A positive cells. Only glands in the appropriate orientation were counted.

Statistical analysis. A two-tailed student's t test for unpaired data was used for the statistical analysis to determine the differences between the groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Cdx2 and intestine specific gene expression. To access Cdx2 expression level in the glandular stomach of the transgenic mice during the long-term experiment, western blotting was performed on proteins isolated from the

gastric mucosa in the corpus and the antrum separately at 52 weeks of age. As previously determined, almost every glandular epithelial cell expressed Cdx2 in the stomach.¹⁶ Furthermore, Cdx2 expression was not only maintained in both the corpus and antrum until 52 weeks after birth, but was upregulated by the addition of a high-salt diet (Fig. 1A).

We next examined expression of a panel of intestinal specific genes including Tff3, Muc2, and villin by quantitative RT-PCR using RNA from different individual groups. These markers have been useful in the identification of metaplastic tissue. As shown in Figure 1B, Tff3, Muc2, and villin expressions were induced in the stomachs of the transgenic mice ($p < 0.05$). In addition, Tff3, villin, and Muc2 expressions were further induced in the Cdx2 transgenic mice fed a high salt diet compared with Cdx2 transgenic mice fed standard diet by 4, 2.8 and 2.2 fold, respectively. The result suggested that a high-salt diet induced further progress of the intestinal metaplasia in the Cdx2 transgenic mice.

High salt diet induced histological changes in the Cdx2 transgenic mice.

Changes in the cellular types were detectable at 32 weeks of age (data not shown), gradually increased in the animals with the longer treatment, and significantly different by 52 weeks. At 52 weeks of age, the Cdx2 transgenic mice had elongation of the gastric pits and reduction of the parietal cell zone in the gastric corpus. These findings were more profound in the Cdx2 transgenic mice fed a high-salt diet (Fig. 2C and D). In the antrum, the Cdx2 transgenic mice continued to exhibit intestinal metaplasia (Fig. 2K and L) and the thickness of the gastric mucosa was increased (Fig. 2G), particularly in the high-salt diet groups (Fig. 2H), compared with the wild-type littermates (Fig. 2E and F). However, this increase was not caused by deepening of the pits, but by an increase in glands that secrete neutral mucins as identified by periodic acid-Schiff (PAS) staining (Fig. 2O and P) as compared to wild type littermates (Fig. 2M and N). In the wild type littermates, no significant changes were observed in the animals on either a standard or a high salt diet. No inflammatory lesions were observed in any of the groups.

Identification of the cell lineages. To further examine the changes in cellular types, a number of cell-specific markers were used. H⁺/K⁺-ATPase and pepsinogen C, markers for parietal and chief cells respectively, were significantly decreased in the gastric corpus of the Cdx2 transgenic mice at 52 weeks of age (Fig. 3C and D, and G and H; Table 1). Among the Cdx2 transgenic mice, a significant decrease (Fig. 3D; Table 1 $p < 0.001$) in parietal cells was associated with a high-salt diet, whereas no significant changes in parietal and chief cells were observed in the wild-type littermates fed either a standard or a high-salt diet (Fig. 3A, B, E, and F).

We next examined expression of mucin epitopes and related trefoil factor family (Tff) peptides to clarify cell-lineage differentiation. Tff1, one

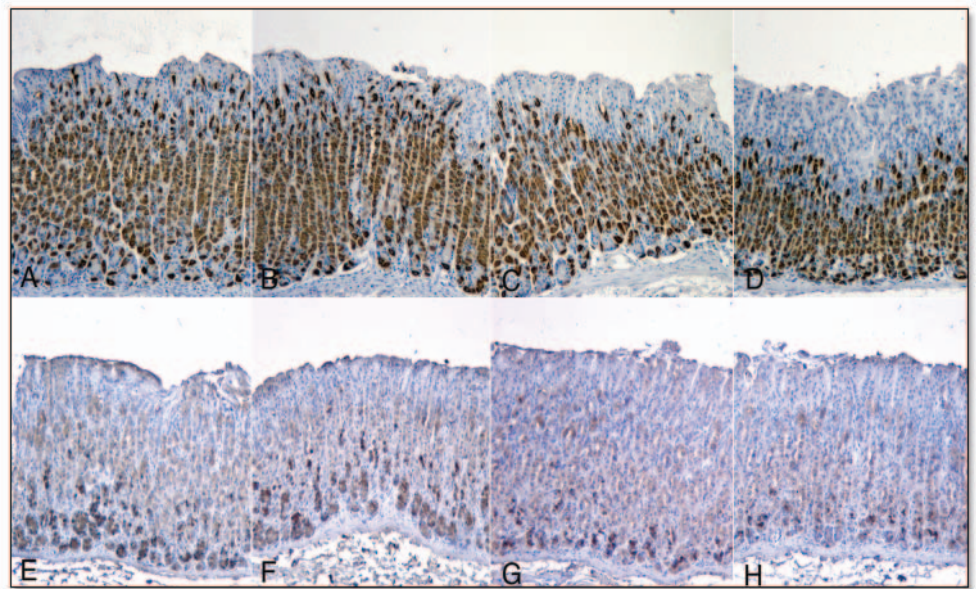


Figure 3. Immunohistochemical staining of hydrate potassium ATPase (H⁺/K⁺-ATPase, A–D) and Pepsinogen C staining (E–H) in the gastric corpus at 52 weeks. (A and E), wild-type littermates fed a standard diet; (B and F), wild-type littermates fed a high-salt diet; (C and G), transgenic mice fed a standard diet; and (D and H), transgenic mice fed a high-salt diet. Parietal cells and chief cells were decreased in the transgenic mice in either standard diet or high-salt diet groups. Original magnification, $\times 100$.

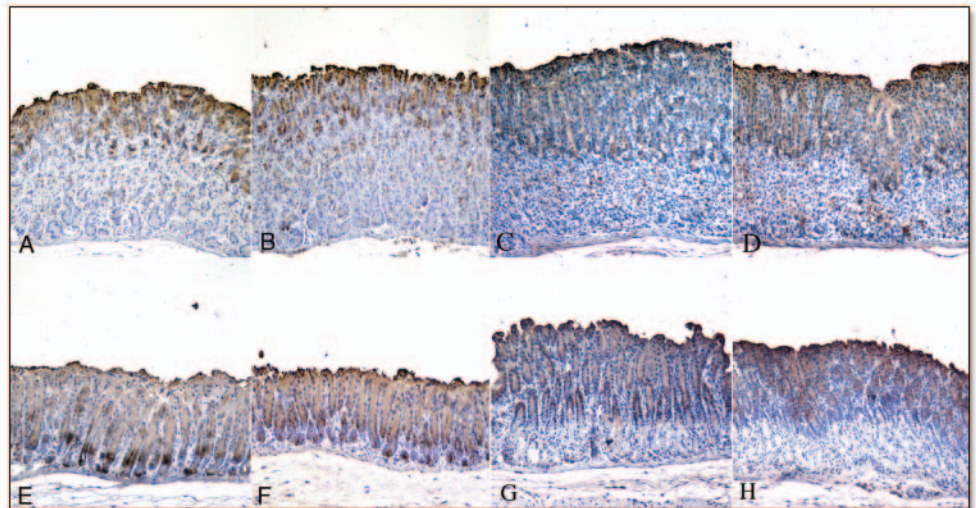


Figure 4. Immunolocalization of Tff1 in the gastric corpus (A–D) and the antrum (E–H). Detection of Tff1 on surface and pit cells was increased in the corpus of the Cdx2 transgenic mice littermates at 52 weeks after birth on either standard diet (C) or high-salt diet (D), compared with age-matched wild-type littermates fed standard diet (A) and high-salt diet (B). In contrast, in the antrum, Tff1 positive cells were unchanged among the four groups, while glandular cells were increased in Cdx2 transgenic mouse (G), especially on a high-salt diet (H). Original magnification, $\times 100$.

of the trefoil factor family is normally expressed in the gastric surface epithelium (Fig. 4A and B). Tff1 has been associated with the carcinogenesis process in the stomach.^{26–28} In the Cdx2 transgenic mice, the distribution of cells expressing Tff1 was expanded and spread to a deeper glandular area of the corpus mucosa compared to wild-type littermates (Fig. 4C and D). However, in the antral mucosa of the Cdx2 transgenic mice, Tff1 was relatively decreased (Fig. 4G), especially in those animals on the high-salt diet (Fig. 4H), compared to wild-type littermates (Fig. 4E and F). There were no apparent changes in Tff1 expression in either diet group of the wild-type

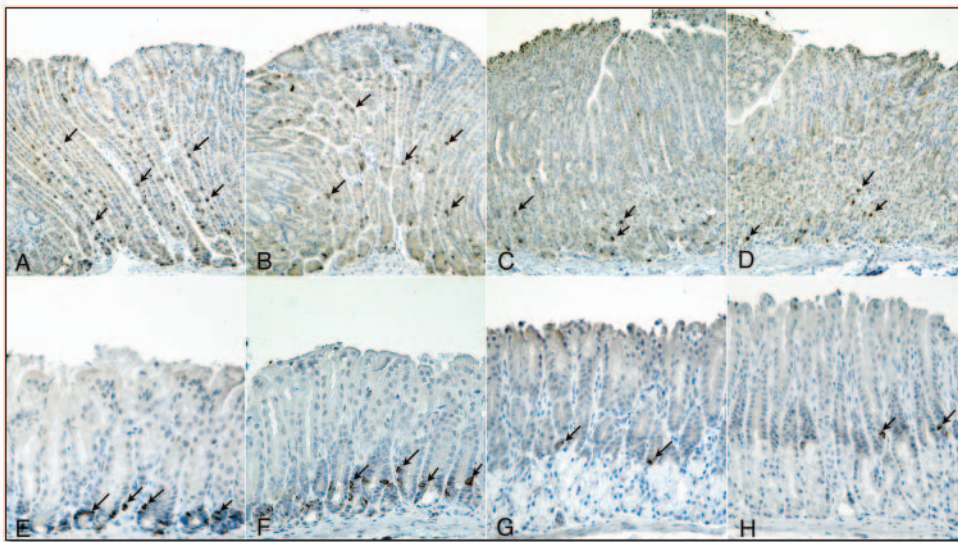


Figure 5. Immunohistochemical staining of Chromogranin A in both gastric corpus (A–D) and antrum (E–H) showing Chromogranin A was reduced in Cdx2 transgenic mice littermates at 52 weeks after birth (shown in arrow). (A and E), wild-type littermates fed a standard diet; (B and F), wild-type littermates fed a high salt diet; (C and G), transgenic mice fed a standard diet; and (D and H), Cdx2 transgenic mice fed a high-salt diet. Original magnification, x 100 (A–D), x 200 (E–H).

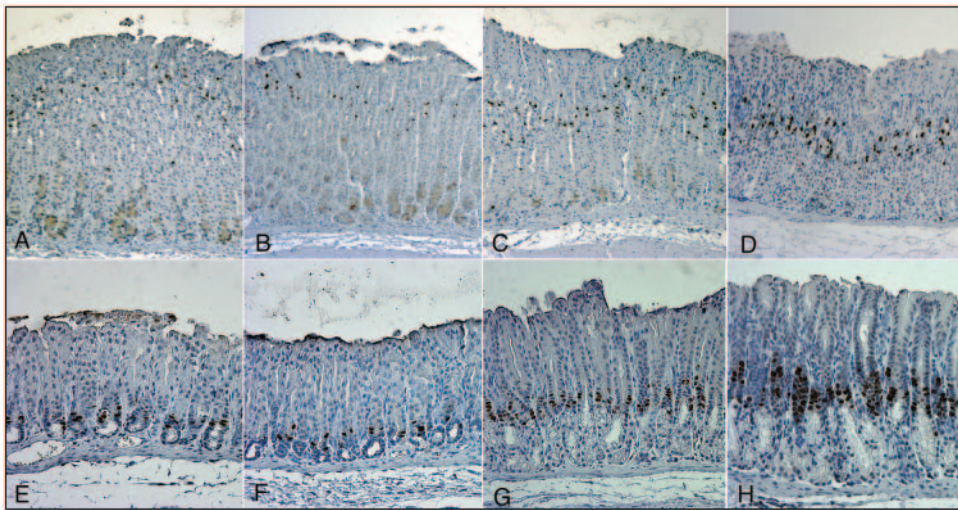


Figure 6. Immunohistochemical detection of BrdU in the gastric corpus (upper panel) and the antral mucosa (lower panel) at 52 weeks. In the wild type littermates, immunopositive cells are located in a narrow band at the junction of the glandular zone and the overlying gastric pit in the mouse fed a standard diet (A and E) and a high salt diet (B and F). In the Cdx2 transgenic mice, the number of BrdU-positive cells were increased in the corpus (C) and the antrum (G) of the mice in a standard diet and further expanded to the base of the corpus (D) and surface of the antrum (H) in the high salt diet group. Original magnification, x 100 (A–D); x 200 (E–H).

littermates. This result suggests that a high salt diet in combination with intestinal metaplasia further reduced cells producing gastric type mucins in the antrum.

Endocrine cells of the gastric mucosa play an important role in cellular composition and stomach function. Therefore, we next examined Chromogranin A expression, which is a marker for endocrine cells. In wild-type littermates, Chromogranin A staining was diffused in the gastric mucosa. By contrast, strong staining in Chromogranin A positive cells was detected rarely in the Cdx2 transgenic mice fed both a standard diet and a high salt diet and these were limited to the lower part of glands in both the

corpus and antral mucosa ($P < 0.05$) (Fig. 5 and Table 1). This observation suggests that the endocrine cell lineage might be altered in the Cdx2 transgenic mice.

Analysis of cell kinetics. The changes in cellular composition observed by histology and immunohistochemistry suggested alterations in proliferation or programmed cell death. Therefore, BrdU incorporation was evaluated to determine the status of proliferation. At 52 weeks of age, the number of BrdU-positive cells in both the corpus and the antrum was significantly increased ($P < 0.05$) in the Cdx2 transgenic mice compared with wild-type littermates (Fig. 6 and Table 1). Moreover, the proliferative zone was expanded towards the base in the corpus and towards the surface in the antrum compared with the wild-type littermates in which a limited number of BrdU-positive cells were present in a narrow band at the junction of the glandular zone and the overlying gastric pit. Among the Cdx2 transgenic mice fed a high-salt diet there was a significant increase ($p < 0.05$) in BrdU labeling as compared to those fed a standard diet. However, in the wild type littermates, no significant changes were observed between the standard and high salt diet groups, indicating that the high-salt diet only affected the proliferation of the gastric mucosa in the Cdx2 transgenic mice. To evaluate the apoptosis in the gastric mucosa, activated caspase-3 labeling was examined. Caspase-3 positive cells were mainly seen in the surface area with a high turnover rate, with no significant difference in the location and the number among the four groups. However, the number of caspase-3 positive cells in the lower zone of the gastric mucosa was increased in the Cdx2 transgenic mice fed a high-salt diet compared with the other groups ($p < 0.05$) (Fig. 7 and Table 1). In the wild-type littermates, no significant difference in caspase-3 expression in the lower zone was observed in the groups with either a high or a standard salt diet. These data reveal that a high-salt diet promotes both proliferation and apoptosis in the gastric epithelial cells in the Cdx2 transgenic mice.

DISCUSSION

Intestinal metaplasia, one of the most commonly occurring pathological processes in the stomach, has been studied primarily in a descriptive fashion. Although it is generally believed to be a precancerous condition based upon epidemiological studies, a functional link between intestinal metaplasia and carcinogenesis remains elusive. Following the recognition that Cdx2 and Cdx1 play a critical part in regulating intestinal differentiation and proliferation, our group and other investigators studied the expression of Cdx1 and Cdx2 in human intestinal metaplasia as a potential mediator of the process.^{13,14} A functional link was established by our group between Cdx2 expression

and intestinal transdifferentiation in the gastric epithelia.¹⁶ In that study, we generated a mouse model that ectopically expressed Cdx2 and exhibited an intestinal phenotype in the gastric epithelia. However, no discernible neoplastic phenotype was observed in this model by 18 months with continual observation. To further explore the role of intestinal metaplasia in carcinogenesis, in the current study, we examined a carcinogenic factor, namely high-salt diet.

One significant finding was that gastric epithelial proliferation was significantly increased in the Cdx2 transgenic mice compared with wild type littermates. Moreover, the addition of a high-salt diet accentuated the increase in proliferation in transgenic mice, but not in wild type littermates. An elevated apoptosis index was observed in Cdx2 transgenic mice fed a high-salt diet, but not in transgenic mice with a standard diet or wild type littermates in either the standard or high-salt diet groups. Thus, intestinal metaplasia alone fosters a background through which mutagens may exert their effect, where a high salt diet may stimulate turn over of intestinal metaplastic epithelial cells. These observations are consistent with the cellular content analysis discussed below. The role of dietary salt in the development of gastric cancer has been debated in numerous studies. Previous study reported that a high-salt diet alone induced elongation of gastric pits and increased epithelial proliferation in C57BL/6mice fed a high-salt diet.²² By contrast, we did not observe any significant changes on gastric cellular types or cell turnovers in age-matched wild-type littermates correlate with a high-salt in the present study. It is clear from previous mouse work that C57BL/6 is the most susceptible strain for carcinogens such as *H. pylori*. In the current study, the Cdx2 transgenic mice were bred in a CD-1 background, suggesting that genetic background might play a role on susceptibility to causative agents.

Another interesting observation in the present study was the alteration of the gastric mucosal structure. In the transgenic mice with a standard diet, we found that the glandular length was shortened and foveolae were deepened in the gastric corpus, while antral glands were elongated which is a common pathological change in hyperplasia.^{29,30} In addition, a significant decrease of parietal cells is a key step toward gastric adenocarcinoma, which has been suggested in

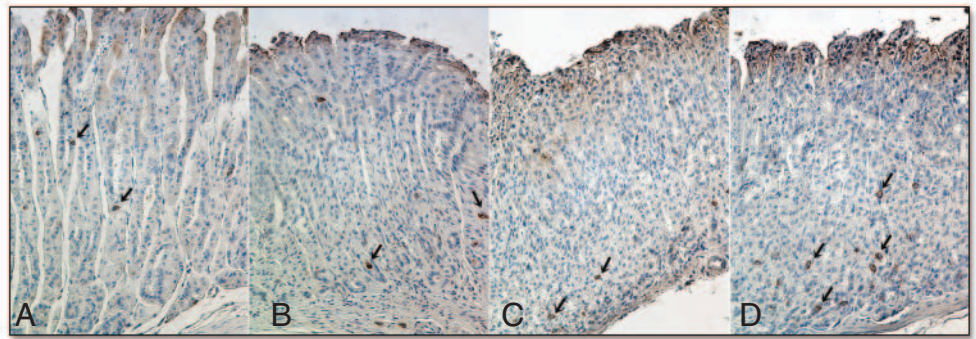


Figure 7. Apoptotic cells detected by activated caspase-3 staining (black arrow) in the gastric corpus at 52 weeks. The Cdx2 transgenic mice fed a high-salt diet (D) had an increase in apoptotic cells in the gastric corpus mucosa especially in the glandular part compared with the other three groups (A, wild-type fed a standard diet; B, wild-type fed a high salt diet; and C, transgenic mouse fed a standard diet). Original magnification. x 100.

previous studies.^{22,31} Therefore, similar structural characteristics observed in this study suggest that a synergistic effects of a high salt diet and intestinal metaplasia may facilitate the conversion of the gastric mucosa to a hyperplastic state, although the entire thickness of gastric mucosa was not increased. Thus, we speculate that these pathological alterations may change the microenvironment in the stomach, which is beneficial to other carcinogenic factors, such as *H. pylori* infection or the transformation of nitrites to nitrates.

In conclusion, intestinal metaplasia with a high-salt diet induces epithelial proliferation, apoptosis, and alters the cellular composition of the gastric mucosa in mice. Altered cellular types may change the microenvironment in the stomach that may be more susceptible to other carcinogens. Acceleration of cell turnover increases the probability of gene mutations. We speculate that these changes provide a platform towards oncogenesis.

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.
- Yuasa Y. Control of gut differentiation and intestinal-type gastric carcinogenesis. *Nat Rev Cancer* 2003; 3:592-600.
- Leung WK, Sung JJ. Review article: Intestinal metaplasia and gastric carcinogenesis. *Aliment Pharmacol Ther* 2002; 16:1209-16.
- Filipe MI. Mucins in the human gastrointestinal epithelium: A review. *Invest Cell Pathol* 1979; 2:195-216.
- Ito H, Hiraiwa N, Sawada-Kasugai M, Akamatsu S, Tachikawa T, Kasai Y, Akiyama S, Ito K, Takagi H, Kannagi R. Altered mRNA expression of specific molecular species of fucosyl- and sialyl-transferases in human colorectal cancer tissues. *Int J Cancer* 1997; 71:556-64.

Table 1 Effect of Cdx2/IM and high-salt diet on proliferation, apoptosis, and cellular type in the gastric mucosa*

		Corpus				Antrum			
		Wild-type		Transgenic		Wild-type		Transgenic	
		NS	HS	NS	HS	NS	HS	NS	HS
Proliferation	BrdU	1.3 ± 0.8	1.6 ± 0.9	2.3 ± 1.2*	3.8 ± 1.5**	2.7 ± 1.2	2.9 ± 0.9	5.6 ± 1.8*	11.1 ± 4.7**
Apoptosis	Caspase3	0.4 ± 0.7	0.3 ± 0.5	0.3 ± 0.5	1.2 ± 1.1**				
Cell types	Parietal	31.9 ± 2.1	31.1 ± 2.6	26.0 ± 2.4*	15.8 ± 1.5**				
	Chief	12.8 ± 3.9	13.3 ± 2.8	4.6 ± 1.2*	3.8 ± 1.5*				
	Goblet	0	0	0	0	0	0	1.5 ± 0.7*	2.8 ± 1.5**
	Endocrine	2.9 ± 1.7	3.1 ± 1.9	1.5 ± 1.0*	1.3 ± 0.9*	2.4 ± 1.3	1.8 ± 0.8	0.5 ± 0.2*	0.3 ± 0.4*

*Data were expressed as mean ± SD of four or five animals. #Significant effect associated with high-salt diet, p < 0.05. *Significant effect associated with transgene/IM, p < 0.05. IM, intestinal metaplasia; NS, normal diet; HS, high-salt diet.

6. Forman D. *Helicobacter pylori*: The gastric cancer problem. *Gut* 1998; 43:S33-4.
7. Asaka M, Takeda H, Sugiyama T, Kato M. What role does *Helicobacter pylori* play in gastric cancer? *Gastroenterology* 1997; 113:S56-60.
8. De Stefani E, Correa P, Boffetta P, Deneo-Pellegrini H, Ronco AL, Mendilaharsu M. Dietary patterns and risk of gastric cancer: A case-control study in Uruguay. *Gastric Cancer* 2004; 7:211-20.
9. Tsugane S, Sasazuki S, Kobayashi M, Sasaki S. Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. *Br J Cancer* 2004; 90:128-34.
10. Shiotani A, Iishi H, Uedo N, Kumamoto M, Nakae Y, Ishiguro S, Tatsuta M, Graham DY. Hypoacidity combined with high gastric juice nitrite induced by *Helicobacter pylori* infection is associated with gastric cancer. *Aliment Pharmacol Ther* 2004; 20:48-53.
11. Suh E, Traber PG. An intestine-specific homeobox gene regulates proliferation and differentiation. *Mol Cell Biol* 1996; 16:619-25.
12. Mallo GV, Soubeyran P, Lissitzky JC, Andre F, Farnarier C, Marvaldi J, Dagorn JC, Iovanna JL. Expression of the *Cdx1* and *Cdx2* homeotic genes leads to reduced malignancy in colon cancer-derived cells. *J Biol Chem* 1998; 273:14030-6.
13. Lorentz O, Cadoret A, Duluc I, Capeau J, Gespach C, Cherqui G, Freund JN. Downregulation of the colon tumour-suppressor homeobox gene *Cdx-2* by oncogenic ras. *Oncogene* 1999; 18:87-92.
14. 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.
15. Bai YQ, Yamamoto H, Akiyama Y, Tanaka H, Takizawa T, Koike M, Kenji Yagi O, Saitoh K, Takeshita K, Iwai T, Yuasa Y. Ectopic expression of homeodomain protein CDX2 in intestinal metaplasia and carcinomas of the stomach. *Cancer Lett* 2002; 176:47-55.
16. Silberg DG, Sullivan J, Kang E, Swain GP, Moffett J, Sund NJ, Sackett SD, Kaestner KH. *Cdx2* ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology* 2002; 122:689-96.
17. Mutoh H, Hakamata Y, Sato K, Eda A, Yanaka I, Honda S, Osawa H, Kaneko Y, Sugano K. Conversion of gastric mucosa to intestinal metaplasia in *Cdx2*-expressing transgenic mice. *Biochem Biophys Res Commun* 2002; 294:470-9.
18. Mutoh H, Sakurai S, Satoh K, Tamada K, Kita H, Osawa H, Tomiyama T, Sato Y, Yamamoto H, Isoda N, Yoshida T, Ido K, Sugano K. Development of gastric carcinoma from intestinal metaplasia in *Cdx2* transgenic mice. *Cancer Res* 2004; 64:7740-7.
19. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: A comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res* 1985; 76:705-16.
20. Tuyns AJ, Kaaks R, Haelterman M, Riboli E. Diet and gastric cancer. A case-control study in Belgium. *Int J Cancer* 1992; 51:1-6.
21. You WC, Blot WJ, Chang YS, Ershow AG, Yang ZT, An Q, Henderson B, Xu GW, Fraumeni Jr JF, Wang TG. Diet and high risk of stomach cancer in Shandong. *China Cancer Res* 1988; 48:3518-23.
22. Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances *Helicobacter pylori* colonization in C57BL/6 mice. *Cancer Res* 1999; 59:4823-8.
23. Kodama M, Kodama T, Suzuki H, Kondo K. Effect of rice and salty rice diets on the structure of mouse stomach. *Nutr Cancer* 1984; 6:135-47.
24. Charnley G, Tannenbaum SR. Flow cytometric analysis of the effect of sodium chloride on gastric cancer risk in the rat. *Cancer Res* 1985; 45:5608-16.
25. Bergin IL, Sheppard BJ, Fox JG. *Helicobacter pylori* infection and high dietary salt independently induce atrophic gastritis and intestinal metaplasia in commercially available outbred Mongolian gerbils. *Dig Dis Sci* 2003; 48:475-85.
26. May FE, Westley BR. Trefoil proteins: Their role in normal and malignant cells. *J Pathol* 1997; 183:4-7.
27. Kim BW, Kim KM, Lee BI, Maeng LS, Choi H, Cho SH, Chae HS, Kim JK, Choi KY, Chung IS. Expression of trefoil peptides in the subtypes of intestinal metaplasia. *Peptides* 2004; 25:779-83.
28. Leung WK, Yu J, Chan FK, To KF, Chan MW, Ebert MP, Ng EK, Chung SC, Malfertheiner P, Sung JJ. Expression of trefoil peptides (TFF1, TFF2, and TFF3) in gastric carcinomas, intestinal metaplasia, and nonneoplastic gastric tissues. *J Pathol* 2002; 97:582-8.
29. Xiao F, Furuta T, Takashima M, Shirai N, Hanai H. Involvement of cyclooxygenase-2 in hyperplastic gastritis induced by *Helicobacter pylori* infection in C57BL/6 mice. *Aliment Pharmacol Ther* 2001; 15:875-86.
30. Oshima H, Oshima M, Inaba K, Taketo MM. Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. *EMBO J* 2004; 23:1669-78.
31. Brembeck FH, Schreiber FS, Deramandt TB, Craig L, Rhoades B, Swain G, Grippo P, Stoffers DA, Silberg DG, Rustgi AK. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. *Cancer Res* 2003; 63:2005-9.