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Sox2 expression is maintained while gastric phenotype is completely lost in Cdx2-induced intestinal metaplastic mucosa ☆

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ABSTRACT

Sox2 is closely related to the gastric phenotype. Sox2 plays a pivotal role in gastric epithelial differentiation in the adult. Sox2 expression is reduced in *Helicobacter pylori*-associated intestinal metaplastic change of the gastric epithelium. The gastric mucosa is replaced by intestinal metaplastic mucosa in the stomach of caudal type homeobox 2 (Cdx2)-transgenic mice. The aim of this study was to use Cdx2-transgenic mice to investigate: (i) Sox2 expression in the intestinal metaplastic mucosa of the Cdx2-transgenic mouse stomach; and (ii) the relationship between Sox2 and Cdx2. Quantitative real-time PCR was performed to determine *Sox2*, *Cdx2*, *Muc5Ac*, and *alkaline phosphatase* mRNA expression levels and single- or double-label immunohistochemistry was used to evaluate the localization of Sox2, Cdx2, gastric mucin and alkaline phosphatase activity. We determined that *Sox2* mRNA in the intestinal metaplastic mucosa of the Cdx2-transgenic mouse stomach was expressed 3.5-fold compared to the normal mouse stomach. Immunohistochemical analysis showed that the same cells in the intestinal metaplastic mucosa expressed both Cdx2 and Sox2. Gastric mucin was not expressed while alkaline phosphatase activity was recognized in the intestinal metaplastic mucosa in spite of the Sox2 expression. Cdx2 increased the transcriptional activity of the Sox2 gene, and Sox2 increased the transcriptional activity of the Muc5Ac gene, which was reduced by cotransfecion of Cdx2 together with Sox2 in the human gastric carcinoma cell line AGS. In conclusion, Sox2 expression is maintained while gastric phenotype is completely lost in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach.

1. Introduction

Sox gene family plays important roles in the regulation of organ development and cell type specification (Wegner, 1999; Bowles et al., 2000; Schepers et al., 2002; Boiani and Scholer, 2005). Among the *Sox* gene family, *Sox2* gene is expressed in the stomach (Tsukamoto et al., 2006). Sox2 is expressed in the foregut-derived organs such as pharynx, oesophagus and stomach, but not in the hindgut-derived organs such as intestine, where the intestine-specific homeobox gene caudal type homeobox 1 (Cdx1) and Cdx2 are present and play an important role in cell differentiation (Ishii et al., 1998; Kamachi et al., 2000; Que et al., 2007). Since expression of Sox2 has been shown to be confined to the stomach and undetectable from the duodenum though to the rectum, it could be a key molecule for gastric differentiation in the gastrointestinal tract of mammals (Yasugi, 2000; Tsukamoto et al., 2004). Moreover, *in vitro* analyses show that Sox2 up-regulates stomach-specific expressions of *pepsinogen A* (Tani et al., 2007) and *Muc5AC* (Li et al., 2004) genes. These studies suggest that *Sox2* is one of the candidate genes related with the differentiation in the stomach.

Intestinal metaplasia of the human stomach has been extensively studied as a possible premalignant condition (Morson, 1955; Stemmermann and Hayashi, 1968; Correa, 1992; You et al., 1993). *Cdx2* has been shown to be aberrantly expressed in intestinal metaplasia. *Cdx2* is a mammalian member of the caudal-related homeobox gene family (Mallo et al., 1997). In the adult mouse and in humans, expression is strictly confined to the gut, from the duodenum to the rectum. The normal stomach does not express the transcription factor Cdx2. We and others have reported the presence of Cdx2 in the intestinal metaplastic mucosa of *H. pylori*-infected human stomach (Silberg et al., 1997; Mizoshita et al., 2001; Eda et al., 2002; Satoh et al., 2002). In addition, we have previously generated Cdx2-transgenic mice that developed intestinal metaplastic mucosa in the stomach (Mutoh et al., 2002; Mutoh et al., 2005).

☆ Author contributions: Hiroyuki Mutoh and Miho Sashikawa contributed equally to this work. Hiroyuki Mutoh and Miho Sashikawa conceived the ideas, planned and designed experimental strategies, analysed the data and prepared the manuscript. Hiroyuki Mutoh and Miho Sashikawa performed the experiments. Hiroyuki Mutoh, Miho Sashikawa and Kentaro Sugano contributed to data analysis.

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H. pylori infection is known to be one of the major virulence factors interfering with the differentiation of the gastric epithelium by causing chronic inflammation (Marshall and Warren, 1984). *H. pylori* infection is associated with reduced expression of Sox2 in the gastric epithelium. The loss of Sox2 and the aberrant expression of Cdx2 occur in the intestinal metaplastic mucosa (Tsukamoto et al., 2004; Tsukamoto et al., 2005). The expression patterns of Sox2 and Cdx2 are inversely related, and down-regulation of Sox2 could thus be an important mechanism in intestinal metaplasia, in addition to the ectopic expression of Cdx2 (Tsukamoto et al., 2004). Sox2 and Cdx2 appear to play opposing roles, with Sox2 being essential for gastric epithelial differentiation, and Cdx2 being required for conversion to an intestinal metaplastic phenotype. However, the relationship between Sox2 down-regulation and Cdx2 up-regulation in the intestinal metaplastic mucosa has not yet been clarified.

In the present study, Cdx2-transgenic mouse, which is a model mouse of intestinal metaplasia of the stomach, was used to examine the relationship between Sox2 and Cdx2 in the intestinal metaplastic mucosa.

2. Materials and methods

2.1. Cdx2-transgenic mice

Cdx2-transgenic mice, with stomach-specific expression of Cdx2 under the control of the rat H^+/K^+-ATPase β-subunit (*Atp4b*) gene promoter, were used (Mutoh et al., 2002). The gastric mucosa of these mice is completely changed to intestinal metaplastic mucosa (Mutoh et al., 2002; Mutoh et al., 2005). Mice had free access to standard food and drinking water and were maintained on a 12 h light/12 h dark cycle. All experiments in this study were performed in accordance with the Jichi Medical University Guide for Laboratory Animals.

2.2. RNA isolation and qRT-PCR (quantitative real-time PCR)

Total RNA was extracted from the gastric mucosa (normal mice), the intestinal mucosa (normal mice), and the intestinal metaplastic mucosa (Cdx2-transgenic mice) using the guanidinium isothiocyanate/phenol method (Isogen; Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed at 37 °C for 1 h in a final volume of 20 μl of reverse transcription buffer (50 mM Tris–HCl pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM spermidine and 10 mM dithiothreitol (DTT)) containing reverse transcriptase (ReverTraAce; Toyobo, Osaka, Japan), 200 pmol random primers and 1 mM dNTPs (Sigma, St. Louis, MO). The cDNA (100 ng) was then used in each real-time PCR to determine the expression levels for each specific gene. The PCR was performed ready-to-use Assay-on-Demand gene expression products (Applied Biosystems, Foster City, CA): *Cdx2* Mm00432449_ml; *Sox2* Mm03053810_s1; *Alp* Mm00475847_g1; *Muc5Ac* Mm01276735_m1. Each Assay-on-Demand gene expression product contains target-specific primers and probes and a TaqMan Gene Expression Master Mix containing AmpErase uracil-N-glycosylase (Applied Biosystems) to prevent re-amplification of carryover PCR products. PCR amplification and fluorescence data collection were performed with an ABI PRISM 7900 HT sequence detection system (Applied Biosystems), using the following conditions: 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of amplification (95 °C for 15 s, 60 °C for 1 min). All PCRs were performed in 96-well plates using a final volume of 20 μl and each gene was studied in triplicate. To normalise RNA transcript abundance for each gene, the housekeeping gene β-actin (Pre-Developed TaqMan Assay Reagents, Applied Biosystems) was used to calculate the ΔC_T [C_T (target)/C_T (actin)]. The C_T values for β-actin for the normal mouse gastric tissues, the normal mouse intestinal tissues and Cdx2-transgenic mouse gastric tissues fell in a close range with no specific pattern of spatial or temporal variation detected (data not shown). A relative quantification approach was used in the present study to describe the change in expression of the target gene in a test sample relative to a calibrator sample (reference). The relative RNA transcript abundance value was calculated as follows: first, the ΔC_T for the normal and Cdx2-transgenic mouse stomach tissues was calculated; and secondly, differences between the normal and Cdx2-transgenic mouse stomach tissues were calculated as $\Delta\Delta C_T$ [ΔC_T (target)/ΔC_T (reference)]. The normal mouse stomach was used as the reference for *Sox2* and *Muc5Ac* expression, and Cdx2-transgenic mouse stomach was used as the reference for *Cdx2* and *Alp* expression. Finally, the fold difference (relative abundance) was calculated using the formula $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001) and was plotted as the mean ($n=6$).

2.3. Immunohistochemistry

Murine tissues sectioned at a thickness of 3 μm were used for immunohistochemistry as described previously (Mutoh et al., 2002; Mutoh et al., 2005). Primary antisera were diluted in PBS and incubated overnight at 4 °C. The next day, slides were washed in PBS and incubated with $Cy3^-$ or Alexa-conjugated secondary antibody (for immunofluorescence) or HRP (horseradish peroxidase)-labelled secondary antibody at 37 °C for 30 min. Colour development was carried out by incubating the sections with DAB (3,3-diaminobenzidine tetrahydrochloride; Wako Pure Chemical Industries, Osaka, Japan) as the chromogenic substrate. Finally, the sections were lightly counterstained with haematoxylin, mounted and viewed under a light microscope.

Our panel of primary antisera included the anti-Cdx2 antibody (dilution of 1:100, rabbit IgG; Epitomics, Burlingame, CA), the anti-Sox2 antibody (dilution of 1:200, goat IgG; Neuromics, Edina, MN) and the anti-gastric mucin antibody (dilution of 1:50, mouse IgG; Novocastra, Newcastle upon Tyne, UK).

2.4. Luciferase assays

To construct the luciferase reporter vector pGL4.10[luc2]-Sox2 and pGL4.10[luc2]-Muc5Ac, 1550 bp (−1403 to +147) fragment that is located at the 5′ end of the mouse Sox2 coding sequence and 2106 bp (−2068 to +38) flagment that is located at the 5′ end of the mouse Muc5Ac coding sequence were amplified by PCR with specific primers (Sox2-fw: ggctttgtttgactccgtgt, Sox2-rv: gggctcc aaacttctctcct; Muc5Ac-fw: acccagagcagtcctgagaa, Muc5Ac-rv: acccagaatgggaccaactt) from 500 ng of mouse genomic DNA isolated from mouse tails according to standard phenol–chloroform extraction procedures. The amplified fragments of the Sox2 and Muc5Ac promoter were directly cloned into the TA cloning vector pCRII (Invitrogen) to yield the plasmid pCRII/Sox2 promoter and pCRII/Muc5Ac promoter. pCRII/Sox2 promoter construct was digested with XhoI and HindIII, and the resulting fragments were subcloned into the XhoI and HindIII restriction sites of the pGL4.10[luc2] vector (Promega, Madison, WI) and confirmed by sequence analysis. pCRII/Muc5Ac promoter construct was digested with SacI and HindIII, and the resulting fragments were subcloned into the SacI and HindIII restriction sites of the pGL4.10[luc2] vector (Promega, Madison, WI) and confirmed by sequence analysis.

The human gastric cancer cell line AGS was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1 mM L-glutamine, 10% foetal bovine serum, and 1% antibiotic solution. AGS cells were seeded at 2×10^5 cells/well in 24-well dishes (Nunc, Rochester, NY), 18–24 h before transfection. Transient transfections were performed using Lipofectamine 2000

(Invitrogen) according to the manufacturer's instructions. 100 ng of Sox2 promoter reporter plasmid with 800 ng of Cdx2 expression vector (pRC/CMV–Cdx2) or empty vector (pRC/CMV) were added to each plate, together with 50 ng of the Renilla luciferase control reporter plasmid (pGL4.70[hRluc]; Promega) as a control for the transfection efficiency. 100 ng of Muc5Ac promoter reporter plasmid with 800 ng of Sox2 expression vector (pRC/CMV-Sox2), Cdx2 expression vector (pRC/CMV–Cdx2), pRC/CMV-Sox2+pRC/CMV-Cdx2, or empty vector (pRC/CMV) were added to each plate, together with 50 ng of the Renilla luciferase control reporter plasmid (pGL4.70[hRluc]; Promega) as a control for the transfection efficiency. At 24 h after the transfection, the cells were lysed in lysis buffer (Promega) and the firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) in a luminometer, according to the manufacturer's instructions. The relative firefly luciferase activities were calculated by normalising the transfection efficiency according to the Renilla luciferase activities produced by the internal control plasmid pGL4.70[hRluc]. Three separate experiments were carried out in triplicate.

2.5. Statistical analysis

Data in bar graphs represent means ± SD. Statistical analysis was performed by Student's *t*-test. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control values. $P < 0.05$ was considered to be statistically significant.

3. Results

In the Cdx2-transgenic mice we generated previously, all of the epithelial cells in the gastric corpus expressed Cdx2 and were replaced by intestinal metaplastic cells (Mutoh et al., 2002; Mutoh et al., 2005). First, we examined Cdx2 expression in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach using qRT-PCR (Fig. 1A). The *Cdx2* mRNA level in Cdx2-transgenic mouse stomach was increased by 2800-fold compared with the normal mouse stomach (Fig. 1A). As *Sox2* expression is lost in the intestinal metaplasia of the human stomach (Tsukamoto et al., 2004), we next examined *Sox2* expression in the Cdx2-induced intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach. *Sox2* gene expression was measured by qRT-PCR (Fig. 1B). The *Sox2* mRNA level in the Cdx2-transgenic mouse stomach was increased by 3.5-fold compared with the normal mouse stomach (Fig. 1B).

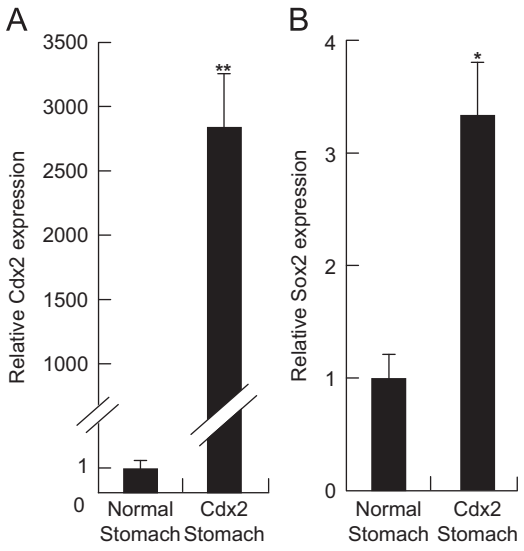

Fig. 1. qRT-PCR analysis of *Cdx2* and *Sox2* expression. (A) *Cdx2* gene expression characterised by qRT-PCR. *Cdx2* mRNA levels in Cdx2-transgenic mouse stomach were increased by 2800 fold compared with normal mouse stomach. (B) *Sox2* gene expression characterised by qRT-PCR. *Sox2* mRNA levels in Cdx2-transgenic mouse stomach were increased by 3.5-fold compared with normal mouse stomach. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control values.

Furthermore, we investigated Sox2 and Cdx2 expression in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach using immunohistochemistry. Sox2 was expressed in both the intestinal metaplastic mucosa of Cdx2-transgenic mouse gastric corpus and the stratified epithelium of Cdx2-transgenic mouse fore-stomach (Fig. 2B and D). Cdx2 was expressed in the intestinal metaplastic mucosa of Cdx2-transgenic mouse gastric corpus, but not in the stratified epithelium of Cdx2-transgenic mouse fore-stomach (Fig. 2A and C). The Sox2 expression in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach indicates that Sox2 reduction might not be necessary for the induction of intestinal metaplastic mucosa. In the normal mouse stomach, Cdx2 was expressed in neither corpus nor fore-stomach (Fig. 2E) while Sox2 was expressed in both corpus and fore-stomach (Fig. 2F).

Next, we examined whether the same cells expressed both Sox2 and Cdx2 by double immunostaining. Almost all of the epithelial cells of intestinal metaplastic mucosa in Cdx2-transgenic mouse stomach expressed both Sox2 and Cdx2 in the same cells (Fig. 3A, B, and C). Alkaline phosphatase (ALP) is expressed in the normal intestinal absorptive enterocytes. ALP activity was recognized in the intestinal metaplastic epithelium that expressed both Sox2 and Cdx2 (Fig. 3D). Next, we examined ALP expression in the normal mouse stomach, the normal mouse intestine and the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach using qRT-PCR (Fig. 3E). The *ALP* mRNA level in Cdx2-transgenic mouse stomach was increased by 26-fold compared with normal mouse stomach (Fig. 3E). The expression level of *ALP* in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach was 2-fold compared with the normal mouse intestine (Fig. 3E).

Furthermore, we examined the effects of the transfection of the Cdx2 expression plasmid on the transcriptional activities of the Sox2 promoter luciferase construct containing the region between −1403 and +147 (relative to the Sox2 transcription start site). Cotransfection with the Cdx2 expression plasmid increased the transcriptional activities of the Sox2 reporter gene in AGS cells (Fig. 3F).

Sox2 plays a pivotal role in gastric epithelial differentiation. Next, we examined the gastric mucin-positive foveolar cells in both normal and Cdx2-transgenic mouse stomach (Figs. 4A and B). The normal gastric foveolar cells were positive for gastric mucin (Fig. 4A). On the other hand, the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach had lost the gastric mucin-positive foveolar cells (Fig. 4B). Using adjacent sections, we examined the relationship between Sox2 expression and gastric mucin expression in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach (Fig. 4B–F). Although Sox2 was diffusely expressed in the intestinal metaplastic mucosa (Fig. 4C and E), gastric mucin was not recognized (Fig. 4B and D). The positive cells in the mesenchyme (Fig. 4B and D) are plasma cells because antibody for gastric mucin is mouse monoclonal antibody. We examined *Muc5Ac* expression in normal mouse stomach, Cdx2-transgenic mouse stomach and normal mouse intestine (Fig. 4F). *Muc5Ac* was expressed in the normal mouse stomach (Fig. 4F), but was neither expressed in the Cdx2-transgenic mouse stomach nor in the normal mouse intestine (Fig. 4F). This indicates that the gastric epithelial cells of Cdx2-transgenic mouse cannot produce Muc5Ac although they express Sox2.

Furthermore, we examined the effects of the transfection of the Cdx2 and Sox2 expression plasmids on the transcriptional activities of the Muc5Ac promoter luciferase construct containing the region between −2068 and +38 (relative to the Muc5Ac translation start site). Cotransfection with Sox2 expression plasmid increased and

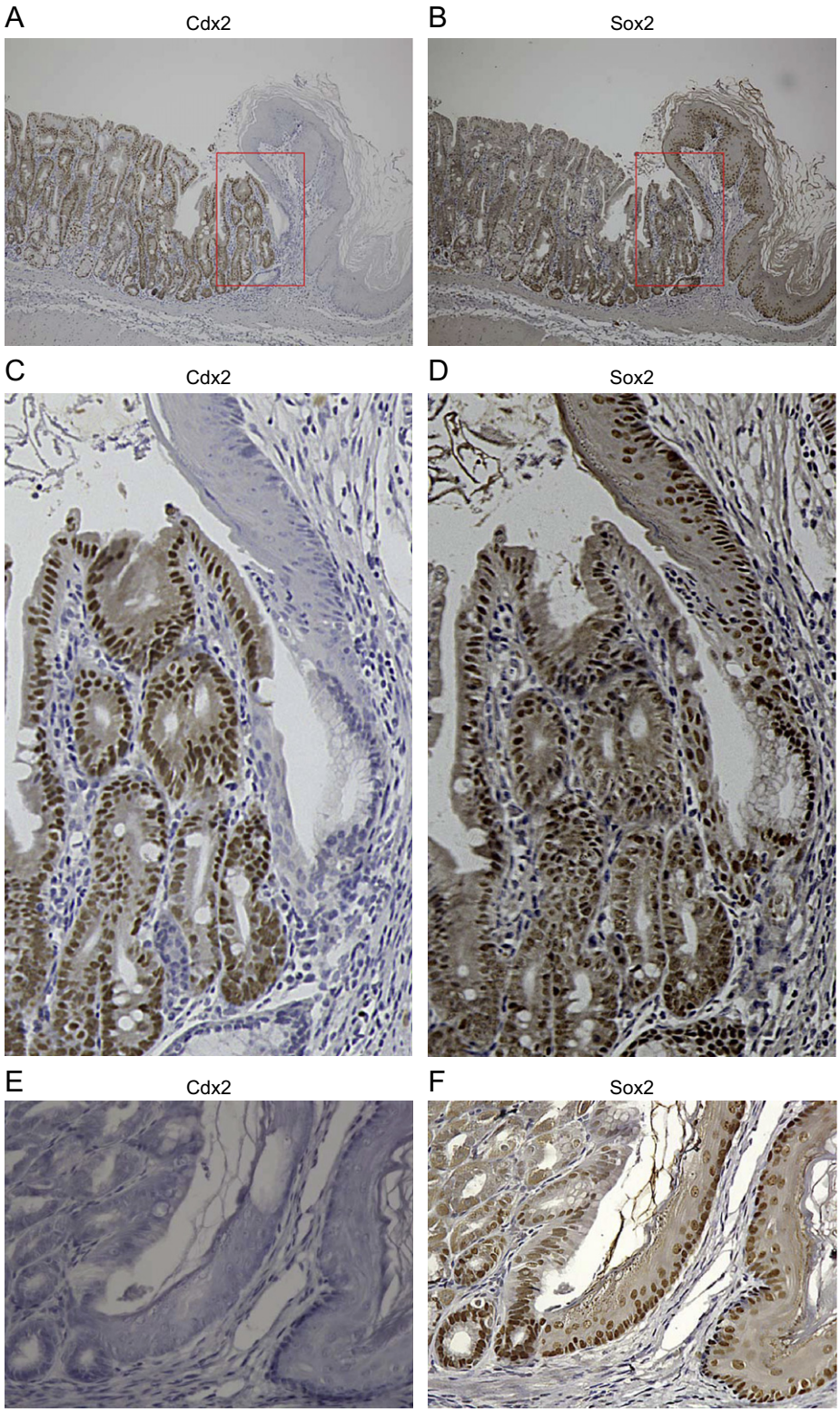

Fig. 2. Immunohistochemical staining for Cdx2 ((A), (C), and (E)) and Sox2 ((B), (D), and (F)) in Cdx2-transgenic mouse stomach ((A)–(D)) and normal mouse stomach ((E) and (F)) using adjacent sections, respectively. All of the epithelial cells in the gastric corpus expressed Cdx2 while the epithelial cells in the stratified epithelium of fore-stomach did not express Cdx2 ((A) and (C): Cdx2-transgenic mouse stomach). The area surrounded by square in panel (A) is magnified in panel (C). All of the epithelial cells in the gastric corpus and all of the epithelial cells in the stratified epithelium of fore-stomach expressed Sox2 ((B) and (D): Cdx2-transgenic mouse stomach). The area surrounded by square in panel (B) is magnified in panel (D). In the normal mouse stomach, Cdx2 was expressed in neither corpus nor fore-stomach (E) while Sox2 was expressed in both corpus and fore-stomach (F). Original magnification $\times 100$ ((A) and (B)) and $\times 200$ ((C)–(F)).

cotransfection with Cdx2 expression plasmid decreased the transcriptional activities of the Muc5Ac reporter gene (Fig. 4G). Transcriptional activities increased by the cotransfection with Sox2 expression plasmid were reduced by cotransfecion of Cdx2 expression plasmid together with Sox2 expression plasmid in AGS cells (Fig. 4G).

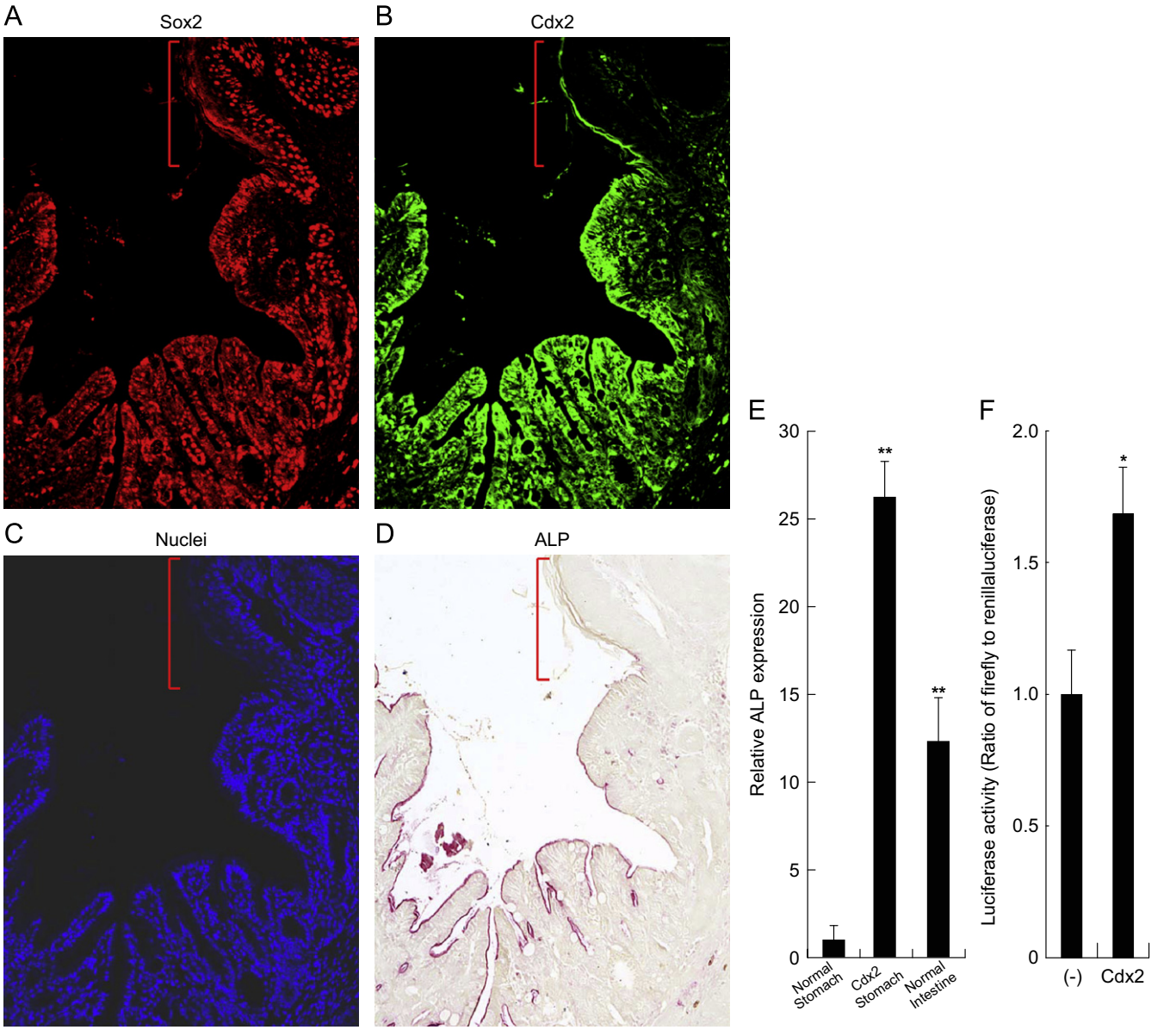

Fig. 3. Immunohistochemical staining for Sox2, Cdx2, and ALP ((A)–(D); same section); qRT-PCR analysis of *ALP* expression (E); Sox2 promoter reporter gene activity (F). Panels (A) and (B) show fluorescence immunohistochemical staining for Sox2 and Cdx2 in Cdx2-transgenic mouse stomach. Panel (C) shows nuclear staining (DAPI). Panel (D) shows ALP activity. Square bracket indicates the stratified epithelium of fore-stomach. Panel (E) shows *ALP* gene expression characterised by qRT-PCR. Sox2 promoter reporter gene activity was increased by the tranfection of Cdx2 expression vector (pRC/CMV–Cdx2) compared to empty vector (pRC/CMV) (F). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control values.

4. Discussion

This study has confirmed that both Sox2 and Cdx2 are expressed in the intestinal metaplastic mucosa of the Cdx2-transgenic mouse stomach. The present results suggest that Cdx2, which is usually induced by *H. pylori* infection, does not prevent the expression of Sox2 that plays a pivotal role for the normal gastric differentiation.

4.1. Sox2 and Cdx2 in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach

To analyze the shift from a gastric to an intestinal phenotype, one should focus on the gastric transcription factor Sox2 and the intestinal transcription factor Cdx2 (Bowles et al., 2000). In the human digestive tract, Sox2 expression is found in the rostral gut epithelium from the pharynx to the stomach epithelium including fundic and pyloric mucosae but is not in intestine (Ishii et al., 1998). On the other hand, Cdx2 is expressed in the duodenum to rectum, but not in the stomach. In intestinal metaplasia, Sox2 transcripts begin to decrease and gradually disappear as intestinal metaplasia progresses (Tsukamoto et al., 2004). The expression patterns of Sox2 and Cdx2 appear inversely related and down-regulation of Sox2 could be an important mechanism in intestinal metaplasia, in addition to ectopic expression of Cdx2 at the transcriptional and translational levels. It seems possible either that Sox2 may negatively regulate Cdx2 expression or vice versa. However, the present results indicate that Cdx2 did not negatively regulate Sox2 expression, indicating that the loss of Sox2 in intestinal metaplastic mucosa is independent of Cdx2 expression. *H. pylori* infection itself might reduce the Sox2 expression in human intestinal metaplasia. Furthermore, the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach, which completely lost the gastric phenotype, clarified that Cdx2 expression is sufficient and the loss of Sox2

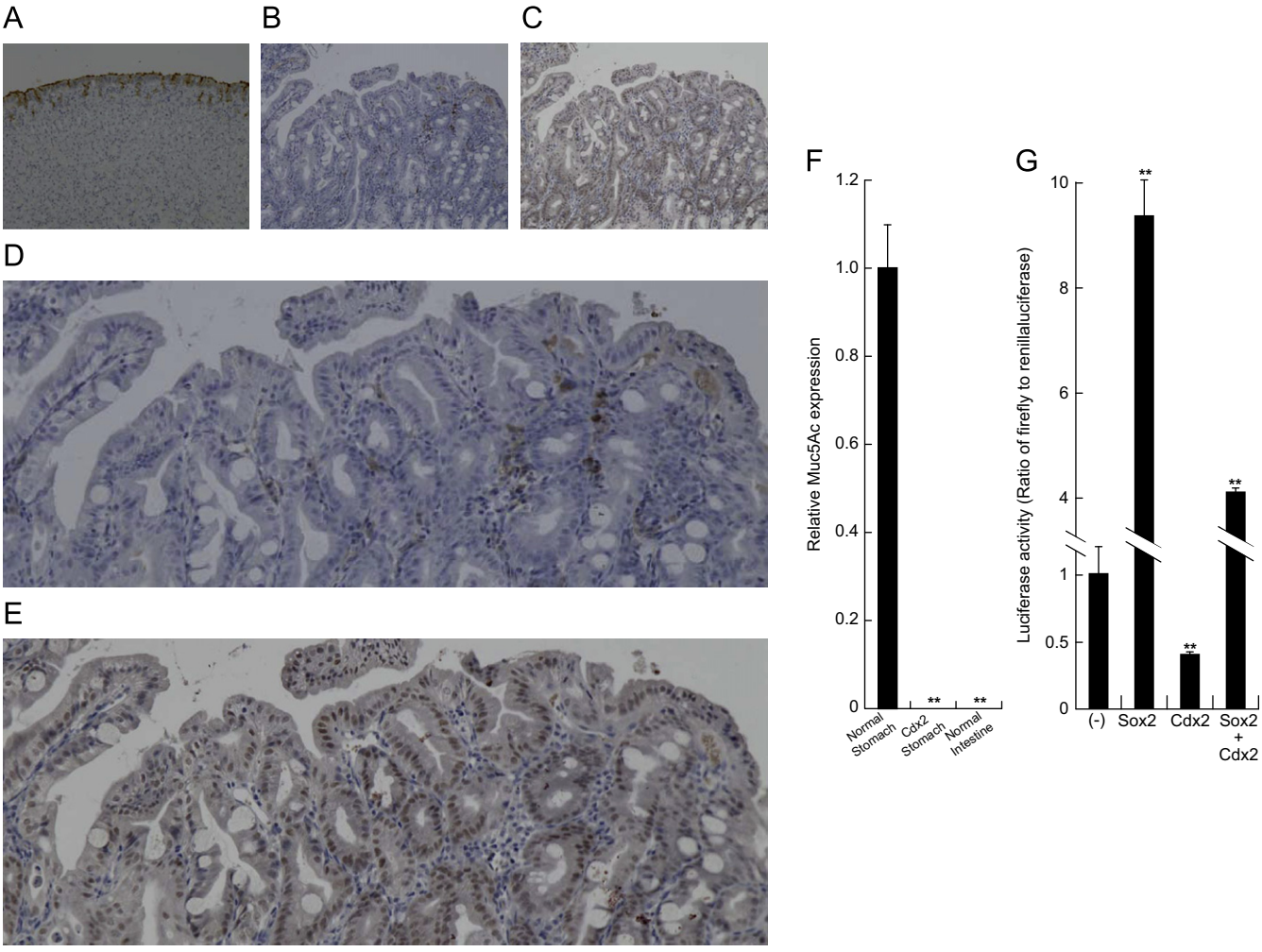

Fig. 4. Immunohistochemical staining for gastric mucin ((A), (B), and (D)) and Sox2 ((C) and (E)) in normal mouse stomach (A) and Cdx2-transgenic mouse stomach ((B)–(E); adjacent sections). All of the foveolar cells in the normal mouse stomach expressed gastric mucin (A) while the surface cells in the Cdx2-transgenic mouse stomach did not express gastric mucin ((B) and (D)). The positive cells in the mesenchyme ((B) and (D)) are plasma cells because antibody for gastric mucin is mouse monoclonal antibody. All of the epithelial cells in the Cdx2-transgenic mouse stomach expressed Sox2 ((C) and (E)). Original magnification × 100 ((A)–(C)), × 200 ((D) and (E)). Panel (F) shows *Muc5Ac* gene expression characterised by qRT-PCR. Muc5Ac promoter reporter gene activity was increased by the tranfection of Sox2 expression vector and decreased by the transfection of Cdx2 expression vector (G). Muc5Ac promoter reporter gene activity upregulated by the tranfection of Sox2 expression vector was reduced by cotransfection of Cdx2 expression vector together with Sox2 expression vector (G). $^{**}P < 0.01$ versus control values.

expression is not necessary for the development of intestinal metaplasia. Aberrant Cdx2 expression and loss of Sox2 may independently occur during the generation of intestinal metaplasia in *H. pylori*-infected human intestinal metaplastic mucosa.

Like the relationship of Cdx2 and Muc2, Sox2 stimulates the expression of gastric differentiation markers including Muc5Ac (Fig. 5). It remains to be elucidated whether gastric and intestinal gene transcription is regulated independently or in a coordinated fashion. The present results indicate that over-expressed Sox2 cannot induce Muc5AC in the Cdx2-expressing gastric mucosa. These results indicate that *Muc5Ac* gene transcription is regulated in a coordinated fashion by Cdx2 and Sox2, not independently by Sox2 alone (Fig. 5).

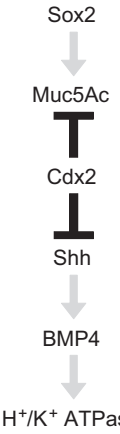

Fig. 5. Scheme for the generation of intestinal metaplasia. Muc5Ac expression was reduced by Cdx2 expression while Cdx2 expression did not reduce Sox2 expression.

4.2. *Shh is related to the differentiation of gastric phenotype, but not Sox2, in Cdx2-transgenic mouse stomach*

Sonic hedgehog (Shh) plays a role in maintaining stem cell populations in the stomach. In humans and mice, *Shh* mRNA and Shh protein are expressed in the normal gastric fundus. The gastric mucosa of Shh-null mice shows epithelial hyperplasia and alkaline phosphatase expression, a sign of intestinal-type differentiation (Ramalho-Santos et al., 2000). Shh expression is lost in intestinal metaplasia of the stomach and increased in fundic gland ectopies,

such as gastric metaplasia of the oesophagus and Meckel's diverticulum (van den Brink et al., 2001; van den Brink et al., 2002). *H. pylori* infection is associated with reduced expression of Shh in the fundic glands. The loss of Shh and the aberrant expression of Cdx2 are recognized in the human intestinal metaplastic mucosa (Shiotani et al., 2005). *H. pylori* eradication is associated with an increase in Shh expression and a decrease in Cdx2 expression in the gastric mucosa (Shiotani et al., 2005). Shh and Cdx2 appear to play opposing roles, with Shh being essential for fundic gland differentiation, and Cdx2 being required for conversion to an intestinal metaplastic phenotype. In a Mongolian gerbil model of *H. pylori* infection, it has been shown that *H. pylori* leads to down-regulation of Shh expression (Suzuki et al., 2005). We have recently reported that Shh was completely lost in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach (Fig. 5; Mutoh et al., 2010). However, in the present study, Sox2 was not reduced in the intestinal metaplastic mucosa of Cdx2-transgenic mouse stomach (Fig. 5). These results indicate that loss of gastric phenotype in the intestinal metaplastic mucosa could be related to the loss of Shh rather than Sox2 expression.

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I hereby declare no conflicts of interest exist.

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