Evidence for Repatterning of the Gastric Fundic Epithelium Associated With Ménétrier's Disease and TGFα Overexpression

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Background & Aims: **Increase of intramucosal transforming growth factor α (TGFα) levels in the gastric fundus leads to oxyntic atrophy and massive foveolar hyperplasia in both metallothionein (MT)-TGFα mice and patients with Ménétrier's disease. We have evaluated the hypothesis that increased levels of TGFα in the fundus induces an antral pattern of cell differentiation in fundic glands by studying Pdx1, a transcription factor whose expression normally is confined to the gastric antrum.** ***Methods:*** **Induction of Pdx1 expression was evaluated in Pdx1$^{lacZ/+}$/MT-TGFα bigenic mice treated with zinc. The distribution of Pdx1 in MT-TGFα mice and Ménétrier's disease patients was evaluated with anti-Pdx1 antibodies. Transcript levels were evaluated by quantitative polymerase chain reaction in mouse and human tissues and AGS cells.** ***Results:*** **In Pdx1$^{lacZ/+}$ mice, Pdx1 was expressed in antral mucosal cells including gastrin cells and TFF2-expressing deep glandular mucous cells. Zinc treatment for 2 to 8 weeks in Pdx1$^{lacZ/+}$/MT-TGFα transgenic mice resulted in expression of Pdx1 throughout the fundus. No ectopic fundic Pdx1 expression was observed in either *H felis*–infected or DMP777-treated mice. In MT-TGFα mice, 8 weeks of zinc treatment elicited nuclear Pdx1 staining throughout the fundic mucosa. TGFα treatment in AGS cells led to increases in Pdx1 and gastrin messenger RNA expression. Fundic sections from Ménétrier's disease patients showed nuclear Pdx1 staining throughout the fundic glands. Treatment of a Ménétrier's disease patient with an anti–epidermal growth factor receptor monoclonal antibody reduced fundic expression of both Pdx1 and gastrin.** ***Conclusions:*** **Overexpression of TGFα in MT-TGFα mice and Ménétrier's disease patients elicits ectopic expression in the fundus of Pdx1, consistent with the phenotype of antralization.**

Oxyntic atrophy, the loss of gastric parietal cells, is the major phenotypic pathology associated with chronic changes in the stomach.[1,2] Oxyntic atrophy is a prerequisite for the development of gastric metaplasias and gastric neoplasia.[2–4] Although the importance of atrophic gastritis in gastric pathophysiology is clear, the range of differing causes for this process is more obscure. Chronic infection with *Helicobacter pylori* leads to loss of glandular lineages in the fundus and the development of various gastric metaplasias and foveolar hyperplasia.[2] Investigations in a number of animal models have recapitulated the onset of oxyntic atrophy after *Helicobacter* sp. infection.[5–7] Nevertheless, the exact influences that lead to changes in the gastric mucosa after *Helicobacter* infection remain obscure.

Studies over the past decade have shown that overexpression of transforming growth factor α (TGFα) leads to morphologic antralization of the gastric fundus. Metallothionein (MT)-TGFα mice develop massive foveolar hyperplasia in the gastric fundus, along with tortuous glands and cystic glandular dilatations.[8–11] The antral glands largely are unaffected. In MT-TGFα mice, the overexpression of TGFα leads to an overproduction of surface mucous cells at the expense of glandular lineages including chief and parietal cells.[9–12] In addition, we have observed that MT-TGFα mice display TFF2-expressing lineages in their deep fundic glands that are similar phenotypically to the deep gland cells of the antrum.[8] This phenotype is consistent with both oxyntic atrophy and antralization of the fundus.

The phenotype in the MT-TGFα mice recapitulates the histologic phenotype in patients with Ménétrier's disease. Patients with Ménétrier's disease show massive

Abbreviations used in this paper: EGF, epidermal growth factor; GAPDH, glyceraldehyde triphosphate dehydrogenase; MT, metallothionein; PCR, polymerase chain reaction; siRNA, short interfering RNA; TGF, transforming growth factor; X-gal, 4-chloro-5-bromo-3-indolyl-β-D-galactopyranoside.

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foveolar hyperplasia in the gastric fundus, usually without coincident disease in the antrum.[13] Immunostaining for TGFα shows prominent up-regulation of TGFα production throughout the foveolar cells of the gastric fundus in Ménétrier's disease patients.[14] Indeed, the recognition of the ligand-induced overstimulation of the epidermal growth factor (EGF) receptor in the stomach as the driving force for the disease has led to the successful treatment of Ménétrier's disease patients with Erbitux, a blocking monoclonal antibody that blocks ligand binding to the EGF receptor.[15] Although these studies establish the importance of TGFα and EGF receptor–mediated signaling in the pathogenesis of Ménétrier's disease, less is known of the mechanisms underlying the phenotypic changes in cell lineages within the gastric fundus in these patients. A Ménétrier's disease phenotype can be observed with severe lymphocytic gastritis, likely associated with *H pylori* infection.[13] However, up-regulation of TGFα expression has not been reported with *Helicobacter* infection.

Pdx1 is a helix-turn-helix homeobox transcription factor that plays a critical role in foregut development. Pdx1 is expressed highly in the developing pancreas, antrum, and duodenum.[16] Previous investigations have documented the expression of Pdx1 in islet β-cells, duodenal Brunner's glands, and endocrine cells of the adult antrum.[17] Mice with targeted deletion of the Pdx1 gene show pancreatic islet agenesis and loss of mucosal cells in the gastric antrum and duodenum along with morphologic alteration of the pylorus and rostral duodenum.[18,19] All of these studies have stressed a role for Pdx1 in the proper genesis of foregut endocrine cells. Previous studies have shown that overexpression of TGFα in MT-TGFα mice causes a chronic pancreatitis with expansion of Pdx1-positive metaplastic epithelia from Pdx1-expressing precursors.[20] We have investigated the changes in Pdx1 expression attendant with the alterations in fundic gastric lineages associated with overexpression of TGFα in both MT-TGFα mice and patients with Ménétrier's disease. The results indicate that oxyntic atrophy and foveolar hyperplasia in both MT-TGFα mice and Ménétrier's disease patients is associated with the ectopic expression of Pdx1 in the fundus.

Materials and Methods

Materials

Monoclonal murine immunoglobulin M anti-TFF2 antibodies were a gift from Professor Nicholas Wright (Cancer UK, London, UK).[21] DMP777 was a gift from DuPont Pharmaceuticals (Wilmington, DE). Guinea pig anti-Pdx1 antibodies were generated by Strategic Biosolutions (Newark, DE) against a GST-Pdx1 fusion protein.

Animals

MT-TGFα, $Pdx1^{lacZ/+}$, and $Pdx1^{lacZ/+}$/MT-TGFα bigenic mice were propagated and offspring were genotyped as described previously.[20] For induction of TGFα expression, single transgenic or bigenic mice received 25 mmol/L $ZnSO_4$ in drinking water beginning at 4 weeks of age for 2, 8, or 20 weeks. For DMP777 treatment, $Pdx1^{lacZ/+}$ mice were treated with DMP777 (350 mg/kg/day per gavage) for 7 or 14 days. For *H felis* infection, male $Pdx1^{lacZ/+}$ mice were inoculated per gavage with *H felis* at 8 weeks of age, as described previously.[5]

Fixation and β-Galactosidase Histochemistry

Animal tissues were fixed by intracardial perfusion with 4% paraformaldehyde, .5% glutaraldehyde in .1 mol/L Sorensen's phosphate buffer (pH 7.4) with 2 mmol/L $MgCl_2$, 5 mmol/L ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, and 30% (wt/vol) sucrose, and their stomachs were excised. The stomachs were opened along the greater curvature and washed in 3 changes of Sorensen's buffer containing .01% (wt/vol) sodium deoxycholate and .02% (wt/vol) Nonidet P-40. For the whole-mount staining, the stomachs were incubated for 4 hours at 37°C with 4-chloro-5-bromo-3-indolyl-β-D-galactopyranoside (X-gal) in a solution containing 2 mmol/L $MgCl_2$, 5 mmol/L ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, .01% (wt/vol) sodium deoxycholate, .02% (wt/vol) Nonidet P-40, 5 mmol/L $K_3Fe(CN)_6$, and 5 mmol/L $K_4Fe(CN)_6$-$6H_2O$ in .1 mol/L Sorensen's phosphate buffer. The X-gal was prepared as a 4% stock solution in dimethylformamide and was added to the mixture just before use. For microscopic sections, the stomachs were embedded into OCT compound (Sakura Finetek U.S.A., Torrance, CA) and frozen on dry ice immediately after perfusion fixation. The frozen blocks were cut into 8- to 10-μm thick sections, washed in 3 changes of Sorensen's buffer containing .01% sodium deoxycholate and .02% Nonidet P-40, and incubated for 4 hours at 37°C in a X-gal solution as described earlier. After the reaction, the sections were washed, counterstained with Nuclear fast red (DAKO, Carpinteria, CA), dehydrated, and mounted with Cytoseal XYL (Richard-Allan, Kalamazoo, MI).

Immunohistochemistry

For immunostaining of $Pdx1^{lacZ/+}$ mouse stomach, the frozen sections were cut in the same way as for X-gal histochemistry. Double staining with X-gal reactions was performed for the sections washed in 3 changes of Sorensen's buffer containing .01% sodium deoxycholate and .02% Nonidet P-40, and in 3 changes of Sorensen's buffer after the reaction with X-gal. For immunostaining for MT-TGFα single-transgenic mice, the mouse tissues were fixed by cardiac perfusion of 4% paraformaldehyde in .01 mol/L Sorensen's phosphate buffer for 10 minutes and postfixation for 2 hours.