Mature Chief Cells Are Cryptic Progenitors for Metaplasia in the Stomach

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BACKGROUND & AIMS: Gastric cancer evolves in the setting of a pathologic mucosal milieu characterized by both loss of acid-secreting parietal cells and mucous cell metaplasias. Indeed, mucous cell metaplasia is considered the critical preneoplastic lesion for gastric cancer. Previous investigations have shown that infection of mice with Helicobacter felis or induction of acute parietal cell loss with the drug DMP-777 leads to the emergence of a type of metaplasia designated spasmolytic polypeptide-expressing metaplasia (SPEM). We have hypothesized that SPEM arises from proliferating cells in gland bases, either from a cryptic progenitor cell or by transdifferentiation of mature chief cells. METHODS: Taking advantage of the chief cell–restricted expression of Mist1-Cre-ERT2, we used lineage mapping to examine whether SPEM lineages were derived from chief cells in 3 independent models of induction by DMP-777 treatment, L-635 treatment, or H felis infection. RESULTS: Treatment of mice with L-635 for 3 days led to rapid parietal cell loss, induction of a prominent inflammatory infiltrate, and emergence of SPEM. In all 3 models, SPEM developed, at least in part, from transdifferentiation of chief cells. We further found that acute parietal cell loss in the setting of inflammation (L-635 treatment) led to more rapid induction and expansion of SPEM derived from transdifferentiation of chief cells. CONCLUSIONS: These studies provide direct evidence by lineage tracing that SPEM evolves from differentiated chief cells. Thus, mature gastric chief cells have the ability to act as cryptic progenitors and reacquire proliferative capacity within the context of mucosal injury and inflammation.

Keywords: SPEM; Chief Cell; Transdifferentiation; Metaplasia.

In the normal gastric fundic mucosa, cell lineages differentiate from progenitor cells located in the neck regions of glands through the initial differentiation of 3 types of second-order progenitor cells: presurface, parietal, and preneck cells.1 Of particular relevance to the present discussion, preneck cells differentiate into mucous neck cells as they migrate toward the base of the glands and then redifferentiate at the bottoms of glands into zymogen-secreting chief cells.2 Intestinal-type gastric cancer predominantly develops in the setting of parietal cell loss (oxyntic atrophy) and mucous cell metaplasia.3 Although loss of parietal cells from the gastric epithelium appears to lead to mucous cell metaplasia, the origin of these metaplastic lineages remains obscure. Two types of mucous cell metaplasia develop in the stomach of human beings: spasmolytic polypeptide-expressing metaplasia (SPEM), a metaplasia in the gastric fundus resembling deep antral gland cells, expresses Trefoil Factor 2 (TFF2; also known as spasmolytic polypeptide) and MUC6.4 Intestinal metaplasia develops in both the fundus and antrum and resembles intestinal goblet cells with expression of both TFF3 and MUC2.5,6 Recent investigations suggest that intestinal metaplasia in the fundus likely develops from precedent SPEM.7,8 However, in mouse models of either Helicobacter infection or acute oxyntic atrophy, only SPEM is observed.9,10 C57BL/6 mice infected with Helicobacter felis for more than 9 months develop SPEM and progress to dysplasia by 1 year of infection,10 indicating a direct link between SPEM and gastric neoplasia.11

Although previous studies have indicated that SPEM in mice is the precursor for dysplasia,10,11 the origin of SPEM has remained unclear. To understand better the factors that lead to the emergence of SPEM, we have studied the induction of metaplasia after the acute destruction of parietal cells by treatment with DMP-777, a parietal cell–specific protonophore that partitions into the apical acid secretory membranes of parietal cells, leading to acute death after acid secretion.9 Importantly, because DMP-777 is also a potent neutrophil elastase inhibitor, we observed no significant inflammatory response in reaction to this acute parietal cell loss. Still,
loss of parietal cells led to the emergence at the bases of fundic glands of SPEM after 10 days of DMP-777 treatment.\textsuperscript{12} Observation of SPEM was preceded by an apparent loss of normal chief cells, which express the basic Helix-Loop-Helix (bHLH) transcription factor Mist1 and secrete pepsinogen and intrinsic factor.\textsuperscript{12,13} Although the normal proliferative zone for the gastric fundus is located toward the lumen in fundic gastric glands, in regions of emerging SPEM, we observed scattered proliferating mucosal cells at the bases of gastric glands.\textsuperscript{12,14} In evaluating the SPEM in gastrin-deficient mice and other models, we determined that the most reliable reflection of the emergence of SPEM was the presence at the bases of gastric glands of cells that co-expressed both TFF2 and intrinsic factor.\textsuperscript{12,15} We therefore hypothesized that SPEM cells are derived from transdifferentiation of mature chief cells.

To address this hypothesis, we performed lineage mapping studies using Mist1<sup>CreER<sup>+</sup></sup>/Rosa26R<sup>LacZ</sup> mice, which express bacterial β-galactosidase after tamoxifen-induced activation of Cre recombinase. The β-galactosidase is expressed exclusively in mature chief cells because tamoxifen-responsive Cre is knocked into the chief cell-specific Mist1 locus. In 3 different models of SPEM induction, SPEM cells predominantly were derived from mature (ie, Mist1-expressing) chief cells. Importantly, in models of SPEM that also induced inflammatory infiltrates, we observed a substantial expansion of the chief cell-derived, proliferative SPEM lineage. These results show that a key gastric metaplastic mucous cell lineage derives in large part from transdifferentiation of mature chief cells. Because similar scenarios for mucous cell metaplasia are linked to gastric carcinogenesis in human beings,\textsuperscript{5} our results may have major implications for our understanding of the origins of human gastric neoplasms.

### Materials and Methods

#### Mice

Eight- to 10-week-old mice were used for all studies. Generation of Mist1<sup>CreER<sup>+</sup></sup> and Rosa26R<sup>LacZ</sup> mice has been described previously.\textsuperscript{16} Mist1<sup>CreER<sup>+</sup></sup> mice were generated by standard embryonic stem cell targeting in which the complete Mist1 coding region was replaced with the Cre<sup>ERT2</sup> coding region. Cre recombinase was activated in Mist1<sup>CreER<sup>+</sup></sup>/Rosa26R<sup>LacZ</sup> mice by intraperitoneal injection of tamoxifen (1 mg/0.1 mL corn oil) for 3 doses every other day. \textit{H felis} infection was performed as previously described.\textsuperscript{17} \textit{H felis} was grown from freezer stock on trypticase soy agar plates containing 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ), at 37°C with 5% CO<sub>2</sub>, and inoculated into \textit{Brucella} broth containing 5% heat-inactivated fetal bovine serum and 10 mg/mL vancomycin. After 24 hours, cells were harvested by centrifugation. Mice were fasted overnight and orogastrically inoculated 3 times (2-day interval) with 0.5 mL of an \textit{H felis} cell suspension of 1 × 10<sup>6</sup> colony-forming units/mL using feeding needles.

#### β-Galactosidase Analysis

Assessment of β-galactosidase activity was used to examine the efficiency of recombination at the Rosa26R<sup>LacZ</sup> reporter allele after induction of Cre activity. Stomach whole-mounts were prepared, fixed, and exposed to 5-bromo-4-chloro-3-indoly-β-D-galactopyranoside (X-gal) substrate using a method previously reported.\textsuperscript{19} The stomachs were opened along the greater curvature and washed with ice-cold fixative containing 1% formaldehyde, 0.2% glutaraldehyde, and 0.02% Nonidet P-40 (NP-40) in phosphate-buffered saline (PBS) (Ca<sup>2+</sup> and Mg<sup>2+</sup> free). The stomachs were immediately incubated for 2 hours in a 20-fold volume of ice-cold fixative at 4°C on a rolling platform. The fixative was removed and the tissues were washed twice in PBS for 20 minutes at room temperature (20°C) on a rolling platform. The β-galactosidase substrate (5 mmol/L K<sub>4</sub>Fe(CN)<sub>6</sub>, 5 mmol/L K<sub>3</sub>Fe(CN)<sub>6</sub> × 3 H<sub>2</sub>O, 2 mmol/L MgCl<sub>2</sub>, 0.02% NP-40, 0.1% sodium deoxycholate, and 1 mg/mL X-gal in PBS) then was added and the tissues were incubated in the dark overnight at room temperature. The substrate was removed and the tissues were washed twice in PBS for 20 minutes at room temperature on a rolling platform. The tissues then were fixed overnight in a 20-fold volume of 4% paraformaldehyde in PBS at 4°C in the dark on a rolling platform. The paraformaldehyde was removed and the tissues were washed twice in PBS for 20 minutes at room temperature on a rolling platform. The stained tissues were transferred to tissue cassettes and paraffin blocks prepared using standard methods. Tissue sections were prepared and counterstained with neutral red.

#### Immunohistochemistry

We performed immunohistochemistry according to the manufacturer’s recommendations, typically using a modified citric acid unmasking protocol followed by

### Drugs

Preparation and treatment of the DMP-777 have been described previously.\textsuperscript{18} L-635 was a gift of Merck & Co, Inc (Rahway, NJ). Mice were administered L-635 orally as a gavage (350 mg/kg) once daily for 3 days.

#### \textit{H felis} Challenge

\textit{H felis} infection was performed as previously described.\textsuperscript{17} \textit{H felis} was grown from freezer stock on trypticase soy agar plates containing 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ), at 37°C with 5% CO<sub>2</sub>, and inoculated into \textit{Brucella} broth containing 5% heat-inactivated fetal bovine serum and 10 mg/mL vancomycin. After 24 hours, cells were harvested by centrifugation. Mice were fasted overnight and orogastrically inoculated 3 times (2-day interval) with 0.5 mL of an \textit{H felis} cell suspension of 1 × 10<sup>6</sup> colony-forming units/mL using feeding needles.
standard detection with 3,3-diaminobenzidine or alkaline phosphatase using a kit from Vector Laboratories (Burlingame, CA). Samples were counterstained with hematoxylin. In some cases, secondary antibodies were conjugated to AlexaFluor 488 (Invitrogen, Camarillo, CA) or Cy-3 and nuclei were counterstained with 4,6-diamidino-2-phenylindole. We used the following primary antibodies: mouse anti-TFF2 (1:100; a gift from Dr Nicholas Wright, Cancer UK, London, England), mouse anti-H+/K+-adenosine triphosphatase (ATPase) (1:2000; a gift from Dr Adam Smolka, Medical University of South Carolina, Charleston, SC), rabbit anti-intrinsic factor (1:1000; a gift from Dr David Alpers, Washington University, St. Louis, MO), rabbit anti-β-galactosidase (1:200; Abcam, Cambridge, MA), rat anti-CD45 (1:1000; BD Biosciences, San Jose, CA), goat anti-CD3-ε (1:1000; Biotechnology, Santa Cruz, CA), rat anti-CD45R (1:200; BD Biosciences), rat anti-F4/80 (1:500; Invitrogen), rat anti-MCA771G (1:100; Abcam, Cambridge, MA), rabbit anti-phospho-STAT 1 (1:50; Cell Signaling, Danvers, MA), rabbit anti-phospho-STAT 3 (1:50; Cell Signaling), rabbit anti-STAT 6 (1:100; Abcam), and rabbit anti-Mini Chromosome Maintenance Protein (MCM) 2 (1:100; Abcam).

**Quantitation**

X-gal–positive area and MCM2-positive cells were analyzed using an Ariol SL-50 automated slide scanner (Genetix, San Jose, CA) as described previously.14

**Statistics**

The data were analyzed with the JMP software package (version 4.0; SAS Institute, Cary, NC). X-gal-positive areas and MCM2-positive cell numbers were compared with analysis of variance followed by post hoc analysis of significant means by the Dunnett test. For all comparisons, P values less than .05 were considered statistically significant.

**RNA Extraction and Real-Time Reverse-Transcription Polymerase Chain Reaction**

Total RNA was isolated from the gastric fundus of untreated C57BL/6 mice and mice treated with L-635 for 3 days, treated with DMP-777 for 14 days, or infected with H. felis for 9 months (3 animals in each group) using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The RNA (1 μg) was treated with RQ1 RNase-free DNase (Promega, Madison, WI) and then reverse-transcribed using the Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA).

Equal amounts of each complementary DNA were analyzed for the expression of tumor necrosis factor-α, interleukin (IL)-1β, IL-4, IL-10, and interferon-γ by real-time polymerase chain reaction (PCR) using specific primers (200 nmol/L) and the EXPRESS SYBR GreenER quantitative PCR SuperMix (Invitrogen, Carlsbad, CA) in an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). The cycling conditions were as indicated by the SYBR Green supermix manufacturer’s protocol. Each sample was measured in triplicate. The primer sequences were as follows: tumor necrosis factor-α (forward: 5'-CTGTGAAGGGATGAGTGTT-3' and reverse: 5'-GGTCACTGTCCCGAGATCTT-3'); IL-1β (forward: 5'-CGTGGACCTCCAGGATGAG-3' and reverse: 5'-ATGCGAAGCTCACAACACG-3'); IL-4 (forward: 5'-TCACAGCAGGAAGAACAC-3' and reverse: 5'-CTGCAACTCCATGAGAAC-3'); IL-10 (forward: 5'-CAAAAGGACAGCTGGACAAC-3' and reverse: 5'-TCATTTCCGATAAGGCTTG-3'); interferon-γ (forward: 5'-GCAAGCCAGCACGCTCATTGAA-3' and reverse: 5'-CGCTTCTGTCGTTGCTAGA-3'). Cycle threshold was converted to relative expression according to the 2^-ΔΔCT cycle threshold method, using TATA–box-binding protein as an endogenous control. For each relative expression analysis, the mean value of the normalized cycle thresholds of all normal mouse samples was taken as reference.

Statistical significance (P < .05) of the differences in the expression levels was determined using an unpaired t test with Welch’s correction.

**Results**

**Lineage Mapping of SPEM in DMP-777–Treated Mice, a Model of Parietal Cell Loss Without Inflammation**

We performed lineage tracing of chief cells using Mist1CreER1/2/Rosa26RLOX mice, which express Cre-ER22 from the Mist1 locus in mature chief cells.13 The mice (n = 8) were first treated with tamoxifen for 3 days to induce Rosa26RLOX recombination and β-galactosidase expression in mature chief cells, and then recovered drug-free for 10 days before administration of DMP-777 for 14 days to elicit SPEM. In mice that received tamoxifen, we observed strong β-galactosidase expression in chief cells of the gastric fundus (Figure 1A), Brunner’s gland cells (Figure 1B), and pancreatic acinar cells (Figure 1C), but no staining was observed in the gastric antrum (Figure 1D). In animals that were not treated with DMP-777 (n = 4), we observed complete separation of TFF2 immunostaining mucous neck cells and X-gal staining in gastric fundus cells (Figure 1E). Although a previous study has indicated that some prezygogenic cells in the transition zone between mucous neck cells and chief cells transiently express markers of both lineages,20 we did not observe any cells that stained for both TFF2 and X-gal.

These results suggest that, after the 10-day period after tamoxifen treatment, all of the β-galactosidase–expressing cells in the fundus were mature chief cells. As previously reported,18 DMP-777 induced both a marked loss of parietal cells and prominent emergence of SPEM stained with antibodies against TFF2 (Figure 1F).
observed basal glandular cells labeling with antibodies against TFF2, which concomitantly showed β-galactosidase enzymatic activity (Figure 1F), indicating that these SPEM cells were derived from mature, Mist1-expressing chief cells. These cells also showed immunoreactivity for β-galactosidase protein using both immunohistochemistry and immunofluorescence detection (Supplementary Figures 1 and 2). No β-galactosidase staining was detected in Rosa26RlacZ mice (n = 8) treated with DMP-777 (n = 2), in Mist1CreER+/Rosa26RlacZ mice without tamoxifen induction treated with DMP-777 (n = 2), or in wild-type mice treated with DMP-777 (n = 2) (Supplementary Figures 3–5). We also observed an expansion of TFF2-expressing cells in the midgland region in mice treated with DMP-777. Those cells did not show expression of β-galactosidase, suggesting that they may arise from mucous neck cells. Therefore, the phenotype of SPEM glands in DMP-777-treated mice was composed of 2 groups of TFF2-expressing mucous cells: one unequivocally derived from transdifferentiation of chief cells and another likely derived from mucous neck cells, which normally express TFF2, likely through arrest of forward differentiation into chief cells.

A Novel Model of Acute Parietal Cell Loss With Inflammation

As noted earlier, DMP-777 abrogates development of inflammation, presumably by its inhibition of neutrophil elastase, blocking the inflammatory response early in the innate immune phases. However, in human beings, metaplasia usually develops in the setting of severe inflammation. We therefore examined the effects of a structurally related β-lactam compound, Merck L-635 (L-635), which retains potent parietal cell protonophore activity, but does not have any significant activity against neutrophil elastase. We treated C57BL/6 mice with 3 daily doses of L-635 (n = 4) and examined the stomachs. Compared with the normal gastric mucosa (Figure 2A), and as seen with DMP-777 treatment, L-635 caused prominent parietal cell loss (Figure 2B and C). However, in contrast with DMP-777 treatment, we also observed a prominent submucosal and intramucosal inflammatory infiltrate (Figure 2B). Although DMP-777 treatment for only 3 days does not elicit any metaplasia, L-635 over the same interval caused a marked mucous cell metaplasia that dominated the fundic mucosa and was strongly
positive for TFF2 (Figure 2D). As previously reported for SPEM induced by DMP-777, the L-635–induced metaplasia showed dual expression of both TFF2 and intrinsic factor (Figure 2E). L-635–induced metaplastic cells also stained for Ki-67, indicating the adoption of proliferative capacity in the metaplastic cells (Figure 2F). It is important to note that although 3 doses of DMP-777 does elicit parietal cell loss in the fundic mucosa, we do not observe induction of SPEM until 10–14 days of drug treatment.18 These results indicated that a combination of parietal cell loss and inflammation could potentiate the development of SPEM.

Given the apparent effects of inflammation on the development of SPEM, we compared the inflammatory infiltrates observed in L-635–treated mice with *H felis*–infected mice. Both models showed prominent intramucosal and submucosal lymphocytic infiltrates comprising both B and T cells (Supplementary Figure 6). Infiltrates in both models also contained both neutrophils and macrophages (Supplementary Figure 7). To evaluate the

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**Figure 2.** Characteristics of stomachs from C57BL/6 mice treated with L-635. (A) Cell lineage markers in normal mouse fundus. First panel: H&E staining; second panel: periodic acid–Schiff (PAS) staining; third panel: H⁺/K⁺-ATPase staining for parietal cells; and fourth panel: dual-immunofluorescence staining for both intrinsic factor and TFF2. A few dual-stained cells were located at the junction between the mucous neck cell and chief cell zones. Green, intrinsic factor for chief cell; red, TFF2; blue, 4,6-diamidino-2-phenylindole (DAPI). (B–F) Extensive SPEM accompanying inflammation induced by L-635. (B) H&E staining of gastric mucosa from a mouse treated with L-635 showing metaplasia with parietal cell loss and a prominent submucosal and intramucosal inflammatory infiltrate. Right panel: higher magnification view. (C) PAS staining showing prominent mucous cell metaplasia. (D) H⁺/K⁺-ATPase staining showing marked loss of parietal cells. (E) Immunostaining for TFF2 (red) in L-635–treated mice showing a marked TFF2-staining mucous cell metaplasia that dominated the fundic mucosa. (E) Dual-immunofluorescence staining for both intrinsic factor and TFF2 showing cells with dual expression of both TFF2 and intrinsic factor at the bases of fundic glands. Green, intrinsic factor; red, TFF2; blue, DAPI. (F) L-635–induced metaplastic cells also were strongly stained for Ki-67, indicating the adoption of proliferative capacity in the metaplastic cells. Green, intrinsic factor; red, Ki-67. Bar, 50 μm.
functional impact of these mixed immune cell infiltrates, we studied the expression of cytokines by quantitative PCR. Supplementary Figure 8 shows that, similar to previous reports, chronic H. felis infection elicited significant increases in the expression of IL-1β, tumor necrosis factor-α, and IL-4. In contrast, acute L-635 treatment elicited significant increases in IL-1β as well as IL-10. DMP-777 treatment does not cause a significant infiltrate, and we did not observe any significant increases in any of the cytokines tested. In concert with these quantitative PCR studies, we also stained sections of L-635–treated and H. felis–infected mouse stomachs for activated phosphorylated-STAT proteins as downstream indicators of cytokine activity (Supplementary Figure 9). In H. felis–infected mice, the nuclei of epithelial cells were stained extensively at the bases of fundic glands with antibodies against phospho-STAT3, and phospho-STAT1 staining was observed in the nuclei of scattered cells in the upper portions of the glands. Nevertheless, no phospho-STAT staining was observed in L-635–treated mice, perhaps reflecting the acute nature of the inflammation.

**Lineage Mapping of SPEM in Mouse Models of Parietal Cell Loss and Inflammation**

To evaluate the origin of metaplasia in L-635–treated mice, we treated the Mist1CreER+/Rosa26RLacZ mice with tamoxifen to induce β-galactosidase activity in all mature chief cells. Ten days later, we treated those mice with 3 doses of L-635 (n = 6) and evaluated X-gal staining (Figure 3). Compared with both untreated animals (n = 4) and DMP-777–treated mice (n = 8), L-635 treatment (n = 6) caused a significant expansion of the number of X-gal–stained cells (Figure 3C). These cells also showed β-galactosidase protein immunoreactivity (Supplementary Figures 1C, D and 2). TFF2 expression was observed in all of the X-gal–stained cells in mice treated with L-635 (Figure 3E and F). These results suggested that L-635 induced transdifferentiation of chief cells into SPEM and that the attendant inflammation induced rapid expansion of that metaplasia.

**Lineage Mapping of SPEM in 6-Month-Old H. felis–Infected Mice**

Previous studies have shown that infection of C57BL/6 mice with H. felis leads to development of SPEM in the presence of a prominent inflammatory infiltrate.10,12,21,22 We therefore sought to determine whether SPEM elicited by H. felis infection also was derived from chief cell transdifferentiation. Mist1CreER+/Rosa26RLacZ mice were treated with tamoxifen and then 10 days later were infected with H. felis (n = 6). Mice were killed 6
months after challenge and evaluated for expression of β-galactosidase and TFF2. The *H. felis*-infected mice showed prominent X-gal staining lineages in the fundic mucosa, comprising more than half the height of the glands (Figure 4A–C). The X-gal-positive cells were stained prominently with TFF2 antibodies throughout the length of the glands (Figure 4D and E and Supplementary Figure 2), indicating that SPEM elicited by *H. felis* infection was derived from mature chief cells. Dual immunostaining for TFF2 and β-galactosidase protein also was observed throughout the SPEM cells (Supplementary Figure 2). These data indicate that SPEM induced and sustained in the setting of chronic inflammation is derived predominantly from chief cells.

**Proliferative Cell Lineage Analysis in SPEM Induced by DMP-777 and Acute and Chronic Inflammation**

To confirm that the attendant inflammation induced rapid expansion of metaplasia, we analyzed the proliferative cell lineages in the 3 different SPEM models. We evaluated MCM2 staining of proliferative cells in SPEM induced by DMP-777 (no inflammation), L-635 (acute inflammation), and *H. felis* infection (chronic inflammation) (Figure 5). In untreated mice (*n* = 4), MCM2 stained only proliferating normal progenitor cells in the upper gland neck, although no proliferating cells were observed among the X-gal–staining chief cells (Figure 5A and E). In DMP-777–treated mice (*n* = 8), MCM2-expressing proliferative cells were also X-gal positive at the bases of fundic glands (Figure 5B and E). In mice treated with L-635 (*n* = 6) and in *H. felis*–infected mice (*n* = 6), MCM2 staining was observed in X-gal–staining cells along the entire gland length (Figure 5C and D). The number of MCM2-labeled proliferative cells in L-635–treated animals and *H. felis*–infected mice was significantly greater than in DMP-777–treated mice (Figure 5E). Similar results were observed for assessment of proliferating cells with either bromodeoxyuridine or Ki-67 staining (data not shown). Thus, metaplastic cells derived from transdifferentiating chief cells in L-635–treated mice and *H. felis*–infected mice acquire substantial proliferative capacity.

**Discussion**

In human beings, metaplastic cell lineages are acknowledged as critical precursors for the development of intestinal-type gastric cancer. Although human beings show both intestinal metaplasia and SPEM as preneoplastic lesions, in mice infected with *Helicobacter* species only SPEM develops and progresses toward dysplasia. In both human beings and mice, the cellular origin of metaplastic lineages has remained elusive. Previous concepts of preneoplasia have focused on derivation of metaplasia from aberrant differentiation of pro-

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**Figure 4.** Lineage mapping of SPEM in 6-month-old *H. felis*–infected Mist1CreER/+/Rosa26RLacZ mice. Sections of fundic mucosa stained with (A–C) X-gal or dual stained for (D and E) X-gal and TFF2 (brown). Chronic *H. felis* infection caused an expansion of the number of X-gal–stained cells. (A) Note the lymphoid follicle and inflammatory cell infiltration in the mucosa. (B) Mucosa adjacent to section in panel A. (C) Higher magnification view of panel B. (D) Dual immunostaining for TFF2 in X-gal–stained sections. Brown (3,3-diaminobenzidine) = TFF2. TFF2 expression was observed throughout the X-gal–stained cells in *H. felis*–infected mice. (E) Higher magnification view of panel D. Bar, 50 μm.
profesional progenitor cells. The current results have defined a new paradigm for the induction of preneoplastic metaplasia in the stomach. An initial loss of parietal cells is required for induction of metaplasia. Although parietal cells are best known for their secretion of acid into the gastric lumen, they also secrete a number of important mucosal growth factors including transforming growth factor-α, amphiregulin, Heparin-binding EGF-like growth factor (HB-EGF), and Sonic hedgehog (Shh).24–27 The loss of these important factors may induce the transdifferentiation of mature chief cells into SPEM. Our results suggest that chief cells represent a repository of potential progenitor cells at the bases of fundic glands. We show here that modification of the gland milieu with loss of parietal cells alone (as in the case of DMP-777 treatment) is sufficient to activate transdifferentiation of chief cells. Nevertheless, the additional presence of inflammation in L-635–treated and H. felis–infected mice leads to expansion of SPEM and conversion from a relatively indolent metaplasia into an expanded proliferative metaplasia. These effects appear to be further amplified and sustained in SPEM observed

Figure 5. Proliferative cell lineage analysis in SPEM induced by DMP-777, L-635, and H. felis infection in Mist1CreER1/Rosa26RLacZ mice. Proliferating cells in mice were visualized with antibodies against MCM2. (A) MCM2 stained only proliferating normal progenitor cells in the upper fundic gland neck, whereas no proliferating cells were observed in the X-gal-staining chief cells (n = 4). (B) In DMP-777–treated mice (n = 8), we observed MCM2 expression in X-gal-staining cells at the bases of fundic glands. Right panel: higher magnification view. (C) In mice treated with L-635 (n = 6), MCM2 staining was observed in X-gal-staining cells along the entire gland length. Right 2 panels: higher magnification views. (D) In H. felis–infected mice (n = 6), MCM2-staining nuclei were present in X-gal–staining cells throughout the length of the glands. SM, submucosa; LF, lymphoid follicle. Right panel: higher magnification view. (E) MCM2-positive proliferative cells were quantitated (± standard error of the mean). The number of proliferating chief cell-derived, X-gal–positive cells in L-635–treated animals and H. felis–infected mice was significantly greater than in DMP-777–treated mice. **P < .01. Bar, 50 μm.
in the setting of chronic inflammation after 6 months of *H. felis* infection. The relationship of chief cells to intestinal metaplasia cannot be addressed in mice. However, recent investigations in both *H. pylori*-infected Mongolian gerbils and in human beings indicates that intestinal metaplasia may arise from pre-existing SPEM. Thus, the transdifferentiation of chief cells into SPEM represents the critical proximate step in the development of metaplasia and gastric carcinogenesis in the setting of oxyntic atrophy.

Previous studies have shown an extremely rapid induction of SPEM in gastrin-deficient mice treated with DMP-777. Microdissection of the SPEM lineages forming in gastrin-deficient mice suggested that parietal cell loss leads to both alteration in differentiation transcripts and re-entry into proliferation. In the present investigations, a key hallmark of the transdifferentiation process for chief cells appears to be the recrudescence of proliferative capacity that is augmented in the presence of inflammation. The present studies as well as previous investigations have not noted any patterns of apoptosis in chief cell populations after loss of parietal cells. These results along with the rapid progression of SPEM in gastrin-deficient mice treated with DMP-777 and L.635–treated mice suggest that transdifferentiation represents a pervasive phenomenon affecting a broad population of chief cells. Recent studies in the intestine have highlighted a role for discrete stem cell populations expressing Lgr5 in renewal of the normal mucosa and promotion of carcinogenesis. Further investigations have documented the presence of progenitor cells expressing Lgr5 at the bases of antral glands, but few Lgr5-positive cells were present in the fundic mucosa, and those were located only along the lesser curvature. In addition, we have performed a number of studies with laser capture microdissection of SPEM from both mouse models and human tissue and have not observed Lgr5 expression in metaplasia. In contrast, our results reported here indicate a novel mechanism for the generation of neoplastic metaplasia in stomach. Although recent studies have suggested that neoplasias are fueled from rare abnormal progenitors (cancer stem cells), presumably derived from normal progenitor cells, the present investigations show that mature, terminally differentiated cells, can transdifferentiate into proliferating metaplastic mucous cells. Whether specific subpopulations of transdifferentiating chief cells respond to inflammatory influences remains to be determined.

Although the present studies do not address the further question of how metaplasia progresses to dysplasia, these findings on the origin of metaplastic lineages have important implications for a general understanding of gastric carcinogenesis. Importantly, mature chief cells gave rise to metaplasia induced by infection with *Helicobacter*, which is considered the major etiologic factor for preneoplastic metaplasia and a recognized carcinogen in human beings. Indeed, the persistence of metaplastic lineages derived from mature chief cells 6 months after Cre-mediated induction of β-galactosidase expression suggests that *H. felis* promotes the persistence of self-renewing metaplastic cells. Because SPEM is associated directly with the development of dysplasia in mice and human beings, these findings suggest that gastric neoplasias in human beings ultimately may develop from metaplasias that arise from transdifferentiation of chief cells. Recent investigations also have indicated that transdifferentiation of pancreatic acinar cells may lead to ductular adenocarcinoma. Thus, in contrast with the concept of a cancer stem cell derived from normal progenitor cells, we now implicate here an alternative origin of neoplastic cells: an array of mature cells may have the potential to transdifferentiate, adopting metaplastic characteristics and becoming proliferative in the presence of inflammatory mediators. Because these metaplastic cells do not arise from professional progenitors, but rather have re-entered the cell cycle from a formerly postmitotic state, they may be predisposed to aberrant growth and acquisition of mutations. In any case, our current studies suggest that metaplasia itself is not neoplastic without inflammatory influences. Further study will be required to better understand the nature of the metaplastic cells and to define the particular inflammatory regulators that promote neoplastic transformation of metaplastic cells.

In summary, using lineage mapping with Mist1CreER/+ mice, we have shown in 3 separate mouse models of oxyntic atrophy that resulting SPEM originates in part or predominantly from transdifferentiation of chief cells. The results further suggest that metaplasias derived from chief cells undergo expansion in the presence of inflammatory infiltrates. These findings indicate that preneoplastic metaplastic lineages in the gastric fundic mucosa can arise from differentiated cell lineages, rather than professional progenitor populations.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at **www.gastrojournal.org**, and at doi: 10.1053/j.gastro.2010.09.005.

**References**


Supplementary Figure 1. Expression of β-galactosidase in Mist1\textsuperscript{CreER\textsuperscript{+}\textsuperscript//-}Rosa26RLacZ mice treated with DMP-777 and L-635. To identify expression of β-galactosidase protein in Mist-Cre\textsuperscript{ER\textsuperscript{+}\textsuperscript//-}Rosa26RLacZ mice after treatment with DMP-777 and L-635, we immunostained gastric tissues with antibodies against β-galactosidase (Vector Red staining). (A) In DMP-777–treated mice, we observed β-galactosidase expression at the bases of fundic glands. (B) Higher magnification view of panel A. (C) As seen with X-gal staining, β-galactosidase expression was observed in L-635–treated mice along the entire gland length. (D) Higher magnification view of panel C. Bar, 50 μm.
Supplementary Figure 2. Dual immunostaining for TFF2 (red) and bacterial β-galactosidase (green) in mouse models of metaplasia. Mist1CreER+/Rosa26RlacZ mice were treated with 3 doses of tamoxifen to induce β-galactosidase expression in mature chief cells and after a 10-day, drug-free interval, metaplasia was induced with 3 different protocols: 14 days of DMP-777, 3 days of L-635, or 6 months' infection with H. felis. Frozen sections of gastric fundus were dual immunostained for TFF2 (red) and bacterial β-galactosidase (green). A dual staining overlay is shown at the right. Bar, 50 μm.
Supplementary Figure 3. No expression of β-galactosidase in Rosa26RLacZ mice treated with DMP-777 and L-635 without or with tamoxifen treatment. To confirm whether endogenous β-galactosidase activity was induced in Rosa26RLacZ mice after treatment with DMP-777 (n = 2) and L-635 (n = 2), we evaluated the β-galactosidase activity by incubation of tissue from mice that either never received tamoxifen (n = 2) or were treated with 3 doses of tamoxifen (n = 2). β-galactosidase activity was visualized by incubation of stomachs with X-gal followed by paraffin-embedding and sectioning. No β-galactosidase expression was observed in the entire stomach including fundic glands after treatment with either (A and B) DMP-777 or (C and D) L-635. Bar, 50 μm.

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Supplementary Figure 4. No expression of β-galactosidase in Mist1<sup>CreER<sup>+/</sup></sup>/Rosa26R<sup>LacZ</sup> mice treated with DMP-777 and L-635 without tamoxifen treatment. To confirm whether endogenous β-galactosidase activity was induced in Mist1<sup>CreER<sup>+/</sup></sup>/Rosa26R<sup>LacZ</sup> mice after treatment with DMP-777 (n = 2) and L-635 (n = 2), we evaluated the β-galactosidase activity by incubation of tissue from mice that never received tamoxifen. β-galactosidase activity was visualized by incubation of stomachs with X-gal followed by paraffin-embedding and sectioning. No β-galactosidase expression was observed in the entire stomach including fundic glands after treatment with either (A) DMP-777 or (B) L-635. Bar, 50 μm.
Supplementary Figure 5. Induction of SPEM in mice does not induce spurious β-galactosidase immunostaining. Mice were maintained either without treatment (normal) or were treated with 14 days of DMP-777 (n = 2), 3 days of L-635 (n = 2), or 6 months’ infection with *H. felis* (n = 2). Frozen sections of gastric fundus were dual immunostained for TFF2 (red) and bacterial β-galactosidase (green). A dual staining overlay of TFF2 and 4,6-diamidino-2-phenylindole (DAPI) is shown at the right. No immunostaining for β-galactosidase was observed in gastric sections with any treatment protocol. Bar, 50 μm.
Supplementary Figure 6. Characterization of infiltrating inflammatory cells in SPEM models with inflammation using lymphocyte cell-surface antigen markers restricted to the T- and B-cell lineage. In sections of mouse stomachs from L-635–treated or H. felis–infected mice, general lymphocyte staining of both B and T cells was determined with CD45 immunostaining (a general immune cell marker). T-cell–specific staining was performed for CD3-ε, and B-cell–specific staining was performed with antibodies against CD45R. Both T-cell and B-cell staining was observed in both SPEM models with inflammation in the mucosa as well as in the submucosa. Bar, 50 μm.
Supplementary Figure 7. Characterization of infiltrating inflammatory cells in SPEM models using macrophage and neutrophil markers. In sections of mouse stomachs from L-635–treated or H. felis–infected mice, immunostaining was performed for F4/80, a macrophage-restricted cell surface glycoprotein antigen, and MCA771G, which recognizes a polymorphic antigen expressed by polymorphonuclear cells, but is absent on resident tissue macrophages. Both neutrophils and macrophages were observed within infiltrating inflammatory cells in both models. Bar, 50 μm.
Supplementary Figure 8. Expression of cytokines in the gastric mucosa of L-635–treated and H. felis–infected mice. Transcript levels for tumor necrosis factor (TNF)-α, IL-1β, IL-4, IL-10, and interferon-γ were determined by real-time PCR in fundic RNA samples isolated from untreated wild-type (WT), DMP-777–treated, L-635–treated, and H. felis–infected mice (n = 3 for all groups). Results are expressed as a ratio of the mean expression in wild-type mice (±standard error of the mean). *P < .05 vs untreated wild-type mice.
Supplementary Figure 9. The expression of cytokine receptor-associated transcription factor STATs. Sections of mouse stomachs from L-635–treated or *H. felis*–infected mice were stained with antibodies against phospho-STAT1, phospho-STAT3, or phospho-STAT6. Although phospho-STAT1 and phospho-STAT3 were not activated in L-635–induced inflammatory cells, we observed activated STAT1 in both the fundus and antrum and phospho-STAT3 in the fundus in the *H. felis*–infected mouse model. No phospho-STAT6 staining was observed in either model (data not shown). Bar, 50 μm.