Chapter 13
Insights into the Development of Preneoplastic Metaplasia: Spasmolytic Polypeptide-Expressing Metaplasia and Oxyntic Atrophy

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Introduction

Deaths from gastric adenocarcinoma are the third largest cause of cancer-related mortality in the world (Pisani et al. 1999). Early recognition and resection of gastric cancers remains the mainstay of gastric cancer therapy, and adjuvant treatment regimens provide only minimal benefits beyond surgery (Wainess et al. 2003). Although aggressive endoscopic screening procedures in Asian countries, such as Japan and South Korea, have led to earlier discovery and surgical removal of gastric cancers (Rembacken et al. 2001), little is known of the cellular etiology of gastric neoplasms. Although the concept of preneoplasia does spur screening endoscopy, there is a general lack of knowledge of the sequence of events leading first to metaplasia and later to preneoplastic lesions and gastric cancer. This uncertainty in the analysis of precancerous events hobbles efforts to develop effective screening methods for patient populations at risk for gastric cancer. Thus, detailed analyses of the pathways to carcinogenesis in humans and in animal models of gastric carcinogenesis are critical for elucidation of the precancerous process.

Human Gastric Cancer Pathogenesis

Inherent to a broader understanding of gastric cancer pathogenesis is the critical concept that cancer arises in a field of altered mucosal lineages, either focally or globally. Studies over the past 15 years have demonstrated that the major proximate cause of gastric cancer in humans is chronic infection with particular strains of the bacterium *Helicobacter pylori* (Blaser and Parsonnet 1991, 1994; Peura 1997). The World Health Organization has designated *H. pylori* as a class I carcinogen (Peura 1997). Two important factors contribute to the evolution of gastric cancer in the presence of chronic *H. pylori* infection: first, the infection elicits a prominent inflammatory response throughout the gastric mucosa (Blaser 1992; Blaser and Parsonnet 1994); second, chronic infection leads to loss of glandular lineages in the gastric fundus, especially acid-secreting parietal cells and pepsin-secreting chief

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cells (Ormand et al. 1991). The overall picture of the stomach mucosa in patients with chronic *Helicobacter* infection is one of dynamic mucosal lineage changes and prominent inflammatory response. Oxyntic atrophy (the loss of parietal cells) either focal or global is a prerequisite for the development of gastric cancer (El-Zimaity et al. 2002). Whereas an association of human gastric cancer with gastric atrophy and inflammation are now well accepted, the intervening cellular events that mediate the progression from atrophy to neoplasia remain controversial.

Studies over the last decade have increasingly emphasized the association of mucous cell metaplasias as precursors for upper gastrointestinal cancers in the esophagus, pancreas, and stomach. Development of esophageal adenocarcinoma is closely linked with Barrett's epithelial metaplasia and pancreatic adenocarcinoma arises from discrete mucous cell metaplasias (Biankin et al. 2003; Cameron et al. 1985, 1995). In the case of pancreatic adenocarcinoma, sequential preneoplastic PANIN lesions evolve from mucous cell metaplasia of pancreatic acinar cells (Biankin et al. 2003; Means et al. 2005). Whereas the association of intestinal-type cancers with chronic *H. pylori* infection and oxyntic atrophy is well accepted (Figure 13.1A), the connections between discrete metaplasias and cancer are less clear. Most Western authorities have considered goblet cell intestinal metaplasia (Figure 13.1A) as the leading candidate for origination of gastric cancer (Correa 1988; Filipe et al. 1994). Goblet cells are not found in the normal stomach, so the presence of cells with goblet cell morphology represents a clear metaplastic process with intestinal phenotype cells. Nevertheless, little evidence exists linking directly intestinal metaplastic cells with dysplastic transformation (Brito et al. 1995; Hattori 1986; Hattori and Fujita 1979; Takizawa and Koike 1998). Interestingly, recent investigations have found that intestinal metaplasias uniformly express the transcription factor Pdx1, which is expressed in the antrum and duodenum (distal foregut) (Leys et al. 2006). Thus, although many have thought of the presence of goblet cells in the stomach as a colonic-type metaplasia, it now seems more likely that intestinal metaplasia in the stomach actually represents a duodenal metaplasia, mimicking cells from the contiguous gut segment.

A number of recent studies have revealed that intestinal metaplasia is not the only possible metaplastic precursor of cancer. A number of investigators, especially in Asia, have reported on metaplastic glands in the fundus with a general phenotype similar to that of the antral or pyloric glands (Hattori 1986; Hattori and Fujita 1979; Hattori et al. 1982; Xia et al. 2000). This antralization of the fundus, also known as pseudopyloric metaplasia, is frequently associated with intestinal-type adenocarcinoma (Figure 13.1A). We have described a similar metaplastic process as spasmolytic polypeptide-expressing metaplasia or SPEM (Schmidt et al. 1999), which is characterized by the presence of trefoil factor 2 (TFF2 or spasmolytic polypeptide) immunoreactive cells in the gastric fundus with morphologic characteristics similar to those of deep antral gland cells or Brunner's gland cells. We have observed SPEM in association with greater than 90% of resected gastric cancers in three studies in the United States, Japan, and Iceland (Halldorsdottir et al. 2003; Schmidt et al. 1999; Yamaguchi et al. 2001). SPEM is observed as TFF2 immunostaining cells emerging from the bases of fundic glands, often associated

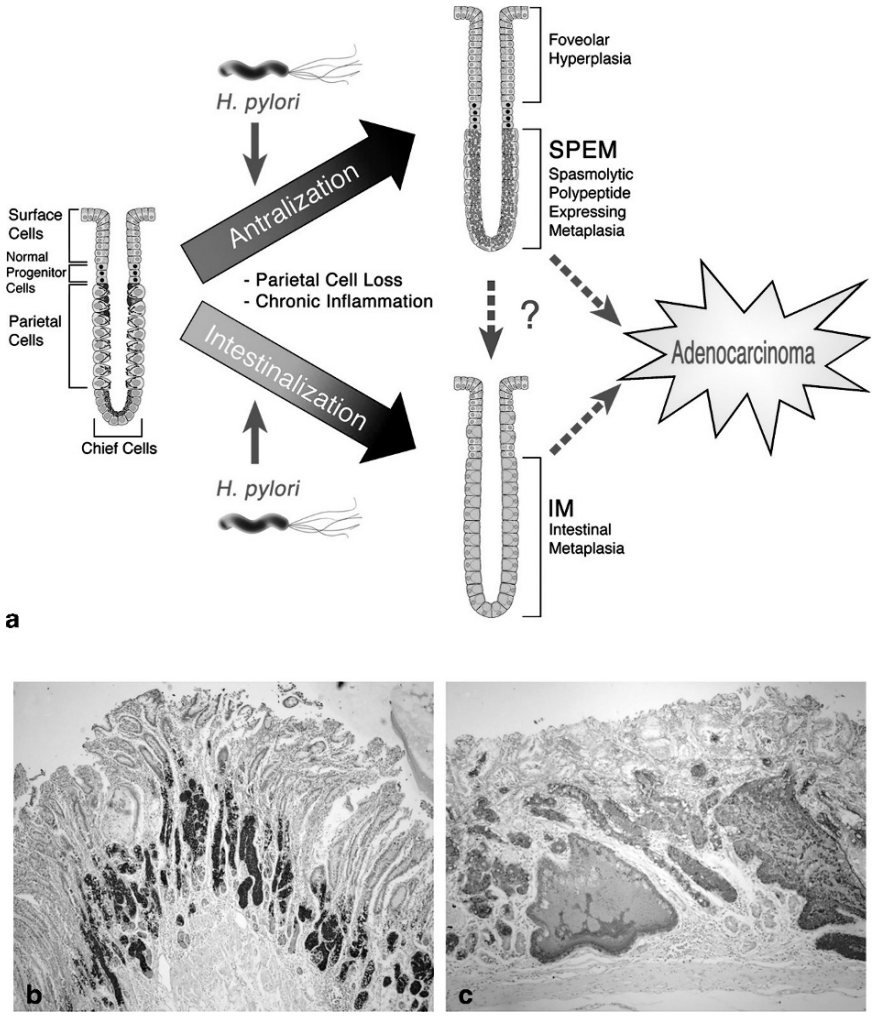

Fig. 13.1 Gastric metaplasia in humans. **(A)** Scheme for the development of gastric metaplasias in humans and their relationship to gastric cancer. (Adapted from Goldenring and Nomura 2006.) **(B, C)** Trefoil factor 2 (TFF2) staining in specimens from patients resected for gastric cancer. **(B)** Spasmolytic polypeptide-expressing metaplasia (SPEM) stained with TFF2 antibodies arises from the base of the glands, and foveolar hyperplasia is observed luminal to the SPEM cells. **(C)** TFF2 staining shows strong labeling of gastritis cystica profunda, a preneoplastic pathology, surrounded by further SPEM glands

with foveolar hyperplasia in the same glands (Figure 13.1B). TFF2 immunostaining is also observed in gastritis cystica profunda, which is associated with the development of intramucosal dysplasia (Figure 13.1C). In addition, similar findings were recently reported for patients from Japan, where expression of TFF2 correlated with metastasis (Dhar et al. 2003). In all of these studies, SPEM was present as

often or more often in association with cancer than goblet cell intestinal metaplasia. Whereas TFF2 immunoreactivity was less prominent in advanced cancers, in the Iceland study, TFF2 immunoreactivity was observed in greater than 50% of early gastric cancers (Halldorsdottir et al. 2003). It is important to note at this point that TFF2 is expressed in the normal fundic mucosa and the expression that they are referring to here is an overexpression in an aberrant cell lineage. All of these investigations have suggested that SPEM and intestinal metaplasia share equal importance as putative preneoplastic lesions in the stomach.

Several pathologic processes lead to oxyntic atrophy in humans. First, antibodies generated against parietal cell proteins, especially the H/K-ATPase, in patients with autoimmune gastritis lead to profound oxyntic atrophy (Marshall et al. 2005). Autoimmune gastritis is associated with carcinoid tumors in the stomach, but is less associated with gastric adenocarcinoma (Borch et al. 1985). Second, chronic infection with *H. pylori* leads to loss of parietal cells and other attendant changes in the gastric mucosa (Peura 1997). Chronic *H. pylori* infection represents the most prominent worldwide cause for gastric adenocarcinoma. Third, in Ménétrier's disease, patients demonstrate loss of parietal cells along with massive foveolar hyperplasia often leading to loss of serum proteins through the leaky gastric mucosa (Coffey et al. 1987; Wolfsen et al. 1993). Although Ménétrier's disease has been linked with gastric cancer in the past, it seems likely that this association is more appropriately assigned to the lymphocytic gastritis subtype which is associated with concurrent *H. pylori* infection. In patients without *H. pylori* infection and significant inflammatory infiltrate, the pathology seems to stem from vast overproduction of transforming growth factor (TGF)α in the gastric mucosa (Dempsey et al. 1992). Administration of antibodies, which block binding to the epidermal growth factor (EGF) receptor, can radically ameliorate the disease and reverse oxyntic atrophy (Burdick et al. 2000). Importantly, although the former two pathways to oxyntic atrophy implicate degenerative influences on parietal cells, recent investigations suggest that the influence of TGFα leads to the induction of a true antral phenotype with expression of both antral mucosal lineage related genes, such as *Pdx1*, and the presence of gastrin cells in the affected fundic mucosa (Nomura et al. 2005).

Mouse Models of Oxyntic Atrophy and Metaplasia

Over the past decade, a number of mouse models have been devised that have led to insights into the ramifications of oxyntic atrophy. These studies can be divided into three general categories: 1. studies of chronic *Helicobacter* sp. infection, 2. studies of genetic manipulations that lead to oxyntic atrophy, and 3. models of toxicity against parietal cells. Critical to these studies is the analysis of gastric mucosal lineages using a number of histologic and immunohistochemical markers. As we have noted previously, classification of mouse lineages is best accomplished through specific immunostains. Intestinal metaplasia in humans is defined by goblet cells stained with Alcian blue. Human intestinal metaplasia also stains with

markers of intestinal goblet cells, including TFF3 and Muc2. Only a few Alcian blue–staining mucous neck cells are present in the normal human stomach. However, in mice, essentially all of the deep gland cells of the antrum are stained with Alcian blue (Goldenring and Nomura 2006). These deep antral cells express TFF2 and MUC6, rather than TFF3 and MUC2 as in goblet cells. Thus, assignment of pathologies as "intestinal metaplasia" in mice should only be made when TFF3 or MUC2 staining have been established. Similarly, SPEM assignment should require staining with TFF2 or MUC6. Nevertheless, TFF2 and MUC6 are also present in normal fundic mucous neck cells in both humans and rodents. Thus, assignment of lineages as SPEM requires immunostaining as well as morphologic correlation. More recently, we have also identified novel markers, such as HE-4, which are not present in the normal stomach, but are present in both SPEM and intestinal metaplasia (Nozaki et al. 2008). Thus, a battery of immunostains can be utilized to assign more precisely the origins of metaplasia. Caution is also recommended in the use of periodic acid–Schiff (PAS) to assign surface cell lineages. SPEM is usually also PAS positive, although the color intensity is usually less in SPEM and, indeed, the contrast between deep carmine staining of surface cells versus a more pink staining of SPEM can be useful in rapid histologic analysis of foveolar hyperplasia and SPEM.

Oxyntic Atrophy and Metaplasia After Chronic **Helicobacter felis** *Infection*

C57BL/6 mice infected with *Helicobacter* sp., particularly *H. felis*, demonstrate profound loss of parietal cells and the replacement of fundic mucosa with a mucous cell metaplasia, which is highly proliferative and expresses TFF2 (Figure 13.2A) (Fox et al. 1996, 2003; Wang et al. 1998). These mice demonstrate prominent SPEM by 6 months of infection. SPEM is also observed in other models of *Helicobacter* infection using *H. pylori* strains in both mice and gerbils (Fox et al. 1996, 2003; Kirchner et al. 2001; Wang et al. 1998). Importantly, in these *Helicobacter* infection models, SPEM represents the only observed metaplasia and no goblet cell intestinal metaplasia is present (Figure 13.2A). Also importantly, inflammation is critical to the development of atrophy and metaplasia. Thus, immunodeficient mice do not develop atrophy and metaplasia after *H. felis* infection (Fox et al. 1993). Similarly, TNFα-deficient mice do not develop SPEM after chronic *H. pylori* infection (Oshima et al. 2005).

Just as in humans, a number of studies have indicated that, in *Helicobacter*-infected mice, SPEM can progress to dysplasia and intramucosal cancer. This process is accelerated in infected insulin-gastrin mice, where gastritis cystica profunda and dysplasia develop along a more rapid time course (Wang et al. 2000). The importance of SPEM as a precursor of gastric dysplasia was recently highlighted in studies on the engraftment of bone marrow cells in the stomachs of *H. felis*–infected C57BL/6 mice (Houghton et al. 2004). The questions of bone marrow engraftment

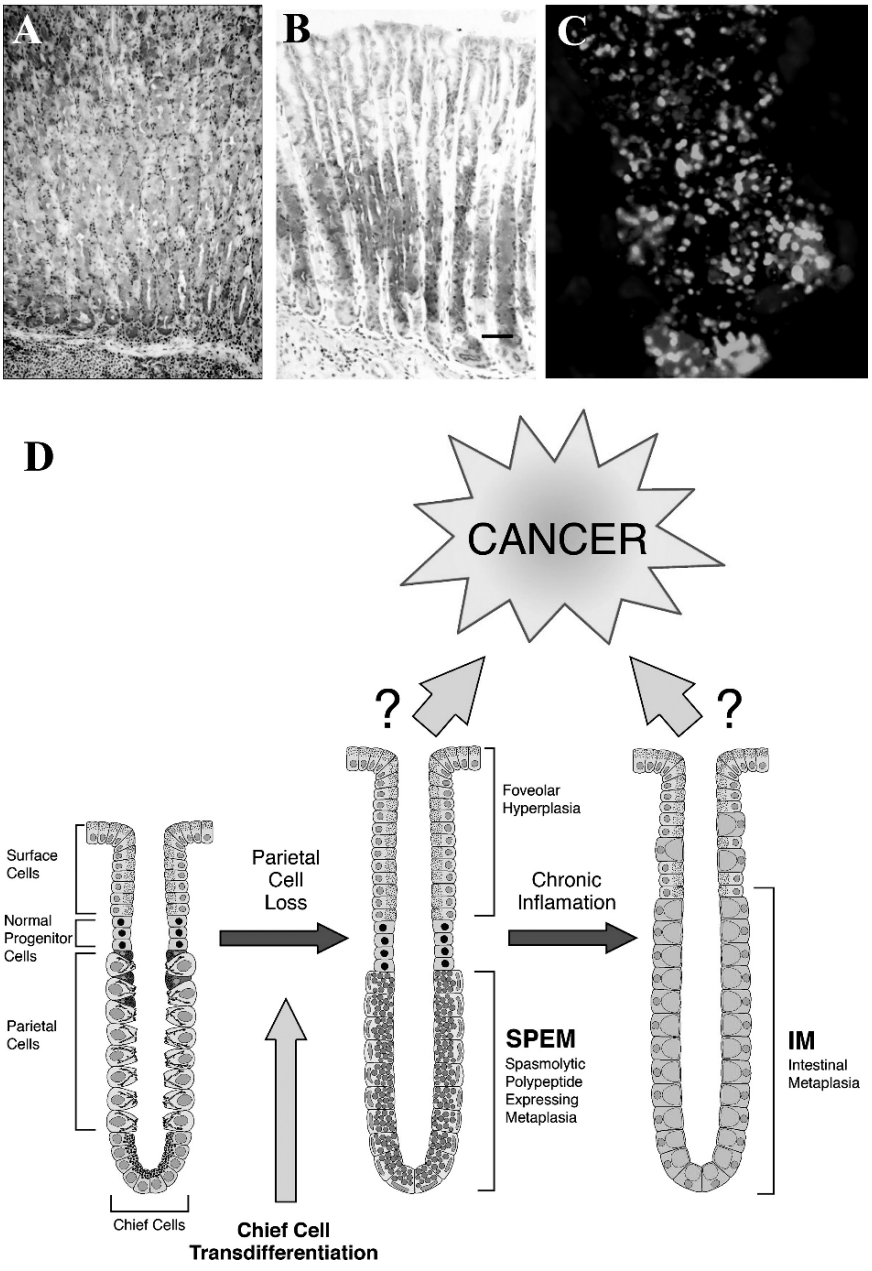

Fig. 13.2 Spasmolytic polypeptide-expressing metaplasia (SPEM) in mice. **(A)** Trefoil factor 2 (TFF2) staining reveals near complete replacement of fundic glands with SPEM in a C57BL/6 mouse infected with *Helicobacter felis* for 9 months. **(B)** TFF2 staining shows prominent SPEM in a mouse treated with DMP-777 for 14 days. **(C)** Dual immunofluorescence staining for intrinsic factor (green) and TFF2 (red) demonstrates dual-staining SPEM cells at the bases of glands from a mouse treated with DMP-777 for 14 days. Note that the intrinsic factor and TFF2 are present in separate granule populations in the SPEM cells. **(D)** A unified hypothesis for the origin of gastric metaplasias. Our studies suggest that SPEM arises from transdifferentiation of chief cells after parietal cell loss. In addition, we hypothesize that, in the presence of chronic inflammation in humans, intestinal metaplasia emerges from further differentiation of SPEM. (Adapted from Goldenring and Nomura 2006.) (*See Color Plates*)

are addressed in detail elsewhere in this volume. However, it is important to note that these studies demonstrated that bone marrow–derived cells engrafted into the SPEM cell lineage in the fundus. Moreover, over time, markers for bone marrow–derived cell origin also were found in gastritis cystica profunda and dysplasia, suggesting that dysplastic pathologies develop from SPEM. Whether similar engraftment can be observed in mice with goblet cell intestinal metaplasia is unclear because mice infected with *Helicobacter* sp. do not develop intestinal metaplasia. Nevertheless, the bone marrow transplantation studies demonstrate a clear connection between SPEM and the progression to dysplasia in mice, and implicate the SPEM lineage in the origin of gastric neoplasia in mice.

Oxyntic Atrophy, Hyperplasia, and Metaplasia After Genetic Manipulation

A number of mouse models of transgenic and targeted deletion have led to oxyntic atrophy phenotypes. Most of these manipulations develop either hyperplastic or metaplastic phenotypes. It should be noted that not all of these changes may truly represent metaplasias, because the mucosal phenotypes are often established during development because of a change in global lineage patterning. Several models are illustrative of mucosal changes associated with oxyntic atrophy. The metallothionein (MT)-TGFα mice demonstrate foveolar hyperplasia and oxyntic atrophy similar to that seen in patients with Ménétrier's disease (Dempsey et al. 1992; Sharp et al. 1995). These mice, as in Ménétrier's disease patients, demonstrate upregulation in the gastric fundus of the distal foregut transcription factor Pdx-1 (Nomura et al. 2005). The presence of neoplasia in these mice is controversial. Although the mice do appear to have marked changes in their mucosa, most of the pathologies are more consistent with benign cystic changes than dysplasia. Indeed, although Ménétrier's disease is considered preneoplastic in humans, it now seems probable that patients without inflammatory infiltrates are likely not precancerous and experience disease based on overexpression of TGFα. More recently, others have studied the effects of the knockout of the H_2-histamine receptor in mice and also found a Ménétrier's disease–like phenotype (Ogawa et al. 2003). H_2-receptor knockout mice develop profound oxyntic atrophy and foveolar hyperplasia. Interestingly, these mice also show prominent elevations in gastric TGFα levels and demonstrate some the serum albumin losses seen in a subset of Ménétrier's disease patients. It is not clear whether these mice develop truly dysplastic changes in the gastric mucosa.

Recent investigations have noted that KLF4-deficient mice develop oxyntic atrophy and SPEM phenotype throughout the fundus (Katz et al. 2005). As in the *H. felis* mice, the antrum is spared of changes. KLF4-deficient mice develop extensive TFF2-expressing metaplasia throughout the gastric fundus. It is notable that these mice do not seem to develop any significant inflammatory response in the mucosa and no dysplastic changes have been reported to date.

Cdx-2 is an intestinal transcription factor that is expressed throughout the small and large intestines. Although Cdx-2 is not expressed in the normal stomach, forced

expressing of Cdx-2 in the stomach using a short H/K-ATPase promoter leads to intestinalization of the gastric fundus (Mutoh et al. 2002; Silberg et al. 2002). H/K-Cdx-2 mice demonstrate profound oxyntic atrophy with expression of intestinal goblet cells throughout the fundus of the stomach. Recent investigations have noted that dysplasia develops in the stomachs of older H/K-Cdx-2 mice. Thus, although *Helicobacter*-infected mice do not develop intestinal goblet cell metaplasia, the presence of intestinal metaplasia in the stomach in mice does represent a potentially preneoplastic scenario with analogy to humans. The metaplasia profile after *Helicobacter* infection seems to be species dependent, because *H. pylori*–infected Mongolian gerbils do develop intestinal metaplasia. Importantly, recently we have noted that goblet cell intestinal metaplasia in gerbils develops from SPEM (Yoshizawa et al. 2007).

Somewhat paradoxically, a number of models have reported oxyntic atrophy after long-term induction of acid hypersecretion. Although all three of these models lead to the loss of parietal cells at greater than six months of age, the atrophic phenotypes are different. Expression in parietal cells of a point mutant of the H/K-ATPase, which cannot be endocytosed efficiently after delivery to the apical lumen, leads to eventual atrophic gastritis with cystic changes in older animals (Courtois-Coutry et al. 1997). The phenotype in these mice seems to be primarily attributable to foveolar hyperplasia although no formal analysis of metaplasias has been performed. Insulin-gastrin transgenic mice demonstrate elevated serum gastrin levels with acid hypersecretion early in life followed by oxyntic atrophy in older animals (Wang et al. 2000). These older animals develop SPEM and gastritis cystica profunda. Furthermore, infection with *H. felis* leads to accelerated development of SPEM and dysplastic cystic changes. Most recently, Samuelson and colleagues have studied the phenotype of transgenic mice with targeted expression of cholera toxin in parietal cells(Lopez-Diaz et al. 2006). These mice show a progression of oxyntic atrophy after 6 months of age with initial mucous neck cell hyperplasia followed later by development of fundic glands fully replaced with cells showing a SPEM-like morphology. This phenotype could reflect progressive expansion of mucous neck cells (mucous neck cell hyperplasia) combined with eventual SPEM development. Notably, the full manifestation of this phenotype correlates with the detection of anti-H/K-ATPase antibodies. At present it is not clear whether other models of acid hypersecretion followed by oxyntic atrophy also might accrue from antiparietal cell antibodies. Interestingly, human patients with pernicious anemia associated with antiparietal cell antibodies do not exhibit SPEM as an associated metaplasia (our unpublished results).

Two models of direct genetic parietal cell ablation have been reported in mice. H/K-diphtheria toxin mice demonstrate parietal cell–specific expression of tetanus toxin, leading to the rapid demise of parietal cells as they begin to express the proton pump (Li et al. 1996). This genetic ablation model leads to an expansion of preparietal cells in the midportion of gastric glands. At ages older than 1 year, these mice develop cystic changes and alterations consistent with dysplasia. No analysis of metaplastic lineages was performed in these mice, so it is presently unclear

whether dysplastic lesions may arise from SPEM or some other metaplastic process. One other genetic ablation model has been reported in H/K-thymidine kinase mice (Canfield et al. 1996). These mice demonstrated parietal cell–specific expression of thymidine kinase, but treatment with ganciclovir resulted in complete loss of the glandular fundic mucosa likely because of the exchange of toxic adducts through the extensive system of gap junctions among the mucosal cells. Thus, this model could not address issues of reactive metaplasia. Nevertheless, it is notable that after cessation of ganciclovir treatment, animals could reassemble the normal pattern of mucosal lineages in the reconstituted fundic mucosa.

Insights into the Origin of Spasmolytic Polypeptide-Expressing Metaplasia After Acute Oxyntic Atrophy

Although the above discussion has detailed a number of scenarios leading to the observation of SPEM, they have not provided particular insights into the origin of metaplasia. One difficulty with these models has been that induction of SPEM was a chronic process. Thus, we have turned to a model of acute oxyntic atrophy to provide information on the emergence of SPEM after the loss of parietal cells. The orally active, cell permeant neutrophil elastase inhibitor, DMP-777, at high doses (>200 mg/kg/day) induces acute oxyntic atrophy in all mammals tested without inducing a significant inflammatory infiltrate. This has allowed investigations of the influences of parietal cell loss in the absence of the intramucosal inflammation seen in *Helicobacter* infection models. In mice or rats, administration of high doses of oral DMP-777 leads to a rapid loss of parietal cells within 3 days of treatment (Goldenring et al. 2000; Nomura et al. 2005). The acute loss of parietal cells leads within 1 day of treatment to rapid increases in gastrin levels and prominent foveolar hyperplasia. After this initial reactive surface mucous cell hyperplasia, SPEM then develops in the gastric fundus between 7 to 10 days of treatment (Figure 13.2B). The entire process is the result of parietal cell loss caused by the action of DMP-777 as a parietal cell secretory membrane protonophore. Parietal cell necrosis presumably follows acid reflux back into the cell through protonophore channels in the apical membrane. Indeed, pretreatment of animals with the proton pump inhibitor omeprazole blocks the loss of parietal cells after DMP-777 treatment (Ogawa et al. 2006a).

This acute model of oxyntic atrophy has proven amenable to detailed study, providing important knowledge into the mucosal responses to parietal cell loss. In this model, gastrin is the major driving force for foveolar hyperplasia, because gastrin knockout mice do not develop surface cell hyperplasia in response to DMP-777 treatment (Nomura et al. 2004). These results confirmed previous results in other rodent models (Konda et al. 1999). Nevertheless, the absence of gastrin seems to promote the development of SPEM, with rapid induction of metaplasia after only 1 day of DMP-777 treatment. Gastrin-deficient mice develop SPEM after only one dose of DMP-777, compared with the 7–10 days required in wild-type C57BL/6

(Nomura et al. 2004). This rapid development of SPEM in gastrin-deficient mice is too rapid for metaplasia to arise from the normal progenitor zone located in the neck region. Although we had previously suggested that SPEM might develop from cryptic progenitor cells located at the bases of fundic glands, more recent studies suggest that SPEM develops through transdifferentiation of chief cells. Indeed, we have found that the presence of cells at the base of glands expressing both intrinsic factor (a chief cell marker in mice) and TFF2 in separate granules is the best reflection of SPEM induction (Figure 13.2C). Gastrin-deficient mice treated with DMP-777 have a rapid increase in these dual-expressing cells after only 1 day of treatment. Electron micrographs of SPEM cells demonstrate separate populations of granules with characteristics of either zymogen or mucous granules (Nozaki et al. 2008). In addition to dual-expressing cells, we also observed the presence of BrdU labeling S phase cells at the bases of fundic glands, distinct from the normal progenitor zone located near the lumen (Goldenring et al. 2000; Nomura et al. 2004). Although the proliferative rate observed in DMP-777–treated mice is considerably lower than that in SPEM in *H. felis*–infected mice, the rapid induction basally located proliferating cells suggest that some transdifferentiating cells can reenter the cell cycle. Thus, SPEM cells may eventually become self-renewing or be influenced by inflammatory regulators toward metaplastic expansion or dysplastic transformation.

The reduction in EGF-receptor signaling in *waved-2* mice carrying a hypomorphic mutation, which reduces EGF-receptor tyrosine kinase activity, also causes acceleration of SPEM development after DMP-777 treatment (Ogawa et al. 2006b). More recent investigations have demonstrated that loss of amphiregulin signaling seems to account for most of the effects observed in *waved-2* mice (Nam et al. 2007). In these studies, specific loss of amphiregulin accelerated the development of SPEM similar to the findings observed in *waved-2* mice. However, TGFα-deficient mice developed SPEM along a time course similar to that observed in wild-type mice. Thus, specific EGF-receptor ligands seem to have differing influences on mucosal lineage differentiation. In addition, it is notable that the amphiregulin-deficient mice seemed to have altered somatostatin dynamics (Nam et al. 2007). These studies demonstrate how alteration of an important intramucosal factor may have widespread effects on the dynamic regulation of the mucosal milieu.

It is clear that intrinsic paracrine and endocrine regulators modulate the emergence of metaplasia after the loss of parietal cells. Although the DMP-777 treatment model allows rapid induction of metaplasia, it should be noted that even after prolonged administration of drug for up to a year, no dysplastic lesions are ever observed in mice or rats despite the profound oxyntic atrophy and SPEM. These results seem to accrue from the absence of significant inflammatory infiltrate in DMP-777–treated animals. The lack of infiltrate likely is a result from the major action of this drug as a cell-permeant inhibitor of neutrophil elastase. It is also notable that no bone marrow–derived cell engraftment was observed in DMP-777–treated mice, even though they showed extensive SPEM (Houghton et al. 2004). Thus, SPEM can develop solely in response to the loss of parietal cells, especially in the absence of inflammatory infiltrate. Moreover, these investigations point out

that the presence of chronic inflammation is a requirement for development of dysplasia from metaplasia.

Toward a Unified Hypothesis for the Origin of Gastric Metaplasias

Studies analyzing the phenotypes of the growing number of genetic mouse models in induced-atrophy scenarios in mice have demonstrated how complicated the influences are within the gastric mucosa. Chronic overexpression or knockout of key regulators may lead to a watershed of alterations in a number of cytokines and growth factors that normally regulate the dynamics of mucosal homeostasis. Thus, a loss of gastrin could lead to decreases in sonic hedgehog expression, which could in turn lead to alterations in growth factor expression. Similarly, loss of an EGF-receptor ligand such as amphiregulin may lead to augmentation of the influence of other EGF-receptor ligands such as HB-EGF. Thus, emergence and persistence of metaplastic lesions are likely regulated by the balance of an array of intramucosal factors. Alterations in the balance of these factors likely lead to a number of aberrant mucosal lineage phenotypes, from foveolar hyperplasia and SPEM to dysplasia.

Studies in rodents have demonstrated that SPEM develops in the setting of oxyntic atrophy from activation of cryptic progenitor cells at the bases of gastric glands distinct from the normal progenitor cells in the gland neck (Goldenring et al. 2000; Wang et al. 1998; Yamaguchi et al. 2002). As noted above, studies in gastrin- and amphiregulin-deficient mice support the origin of SPEM from transdifferentiation of chief cells (Figure 13.2D). The origin of intestinal metaplasia remains elusive (Hattori and Fujita, 1979), in part because goblet cell intestinal metaplasia is not observed in mouse models of *Helicobacter* infection (Fox et al. 1996). Thus, intestinal metaplasia could arise separately from SPEM or could represent a further differentiation of a metaplastic lineage from SPEM (Figure 13.2D). The evolution of one mucous cell metaplasia from another has been observed in the setting of injury and repair associated with Crohn's disease (Wright et al. 1990). No investigation has sought to evaluate systematically the relative contribution of discrete metaplasias to the development of gastric dysplasia and gastric cancer in humans. One is therefore left with a series of hypothetical constructs for the development of cancer from precedent metaplasias. Either of the observed metaplasias, SPEM or intestinal metaplasia, could be paracancerous, whereas the other is truly preneoplastic. Alternatively, as noted above, intestinal metaplasia could evolve from SPEM, either as a precancerous transition or a paracancerous transition. There presently is no evidence for the evolution of SPEM from intestinal metaplasia, because antralization and SPEM seem to develop earlier in the process of oxyntic atrophy (Takizawa and Koike 1998). Finally, it is possible that each metaplasia gives rise to a distinct type of cancer; for example, intestinal metaplasia could evolve into intestinal-type cancers while SPEM evolves into gastric-type cancers.

One should also note that there is even a possibility that both SPEM and intestinal metaplasia are paracancerous, but investigations in mice, at least, suggest that SPEM can lead to cancer (Wang et al. 1998, 2000).

The predominance of studies in mice now indicates that SPEM is the proximate precursor of dysplasia and cancer. But as noted above, there is no evidence for intestinal metaplasia in most models of murine gastric cancer after *Helicobacter* infection. Thus, it remains uncertain how SPEM is related to intestinal metaplasia. Recent studies in mongolian gerbils, where SPEM precedes the development of intestinal metaplasia, have indicated that intestinal metaplasia develops from SPEM glands (Yoshizawa et al. 2007). Indeed, we have observed a number of examples of intestinal metaplasia emanating from basal SPEM in human resection specimens. These studies now lead to a unified hypothesis: Loss of parietal cells during the initial stages of infection with *H. pylori* leads to the induction of SPEM through transdifferentiation of chief cells. In the course of chronic and sustained infection, the SPEM lineage may undergo further differentiation into intestinal metaplasia (Figure 13.2D). It remains to be determined whether either or both of these metaplasias can progress to dysplasia or neoplasia. Given the results in mice, intestinal metaplasia may reflect a further benign attempt by the mucosa to increase repair in the face of chronic infection and inflammation. Further studies of human metaplastic lineages are required to determine relationships of individual metaplasias to observed neoplastic progression.

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