Changes within the gastric mucosa.

III. Animal models of oxyntic atrophy and metaplasia

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Goldenring, James R., and Sachio Nomura. Animal models of oxyntic atrophy and metaplasia. Am J Physiol Gastrointest Liver Physiol 291: G999–G1004, 2006; doi:10.1152/ajpgi.00187.2006.—Gastric cancer in humans arises in the setting of oxyntic atrophy (parietal cell loss) and attendant hyperplastic and metaplastic lineage changes within the gastric mucosa. Helicobacter infection in mice and humans leads to spasmolytic polypeptide-expressing metaplasia (SPEM). In a number of mouse models, SPEM arises after oxyntic atrophy. In mice treated with the parietal cell toxic protonophore DMP-777, SPEM appears to arise from the transdifferentiation of chief cells. These results support the concept that intrinsic mucosal influences regulate and modulate the appearance of gastric metaplasia even in the absence of significant inflammation, whereas chronic inflammation is required for the further neoplastic transition.

Gastric adenocarcinoma; spasmolytic polypeptide-expressing metaplasia; trefoil factor 2; intestinal metaplasia

GASTRIC ADENOCARCINOMA remains a major cause of mortality worldwide. Deaths from gastric adenocarcinoma are the third largest cause of cancer-related mortality in the world. Early recognition and resection of gastric cancers remains the mainstay of gastric cancer therapy, and adjuvant treatments provide only minimal benefits. Although aggressive endoscopic screening procedures in Japan have led to earlier discovery and surgical removal of gastric cancers, little is known of the cellular etiology of gastric neoplasms. Moreover, this lack of knowledge of the sequence and progression of neoplastic events in gastric cancer is manifested in a general lack or inadequacy in screening methods for patient populations at risk for gastric cancer. Thus animal models of gastric carcinogenesis are critical for the elucidation of the precancerous process.

Gastric Cancer Pathogenesis in Humans

The pathway to gastric carcinogenesis is mediated through global changes in the lineages of the stomach. Studies over the past 15 years have demonstrated that the major primary cause of gastric cancer in humans is chronic infection with particular subclasses of the bacterium Helicobacter pylori (2). Indeed, the World Health Organization has designated H. pylori as a class I carcinogen. Two important factors contribute to the evolution of gastric cancer in the presence of chronic H. pylori infection: 1) the infection elicits a prominent inflammatory response throughout the gastric mucosa (2); and 2) chronic infection leads to a loss of glandular lineages in the gastric fundus, especially acid-secreting parietal cells and pepsin-secreting chief cells. Oxyntic atrophy, either focal or global, appears as a prerequisite for the development of gastric cancer. While the association of cancer with gastric atrophy and inflammation are now well accepted, the intervening cellular events that mediate the progression from atrophy to neoplasia remain controversial.

The loss of parietal cells leads to a number of attendant changes in cell lineages within the gastric mucosa. After oxyntic atrophy, patients may show varying levels of foveolar hyperplasia. This increase in surface cell numbers is likely a reactive response to increases in gastrin release secondary to hypochlorhydria. Oxyntic atrophy also leads to mucous cell metaplasia. Studies over the last decade have increasingly emphasized the association of precedent mucous cell metaplasias with the development of upper gastrointestinal cancers in the esophagus, pancreas, and stomach. The development of esophageal cancer is closely linked with Barrett’s epithelial metaplasia, and pancreatic adenocarcinoma arises from discrete mucous cell metaplasias (1, 3). While the association of intestinal-type cancers with chronic H. pylori infection and oxyntic atrophy is well accepted, the connections between discrete metaplasias and cancer are less clear (Fig. 1). Traditionally, most Western authorities have considered goblet cell intestinal metaplasia (IM) (Fig. 1) as the leading candidate for the origination of gastric cancer (5). 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 IM with dysplastic transformation (12). Indeed, IM is not the only possible metaplastic precursor of cancer. A number of investigators, especially in Asia, have focused attention on the presence of metaplastic glands in the fundus with a general phenotype similar to that of antral or pyloric glands (12). This phenotypic antralization of the fundus or pseudopyloric metaplasia is commonly associated with intestinal-type adenocarcinoma. We have described a similar metaplastic process as spasmolytic polypeptide-expressing metaplasia (SPEM) (22), which is characterized by the presence of trefoil factor 2 (TFF2; spasmolytic polypeptide)-immunoreactive cells in the gastric fundus with morphological characteristics similar to deep antral gland cells or Brunner’s gland cells (Fig. 1). SPEM was association with >90% of resected gastric cancers in three studies in the United States, Japan, and Iceland (11, 22). In addition, similar findings were recently reported for patients from Korea, and the expression of TFF2 correlated with metastasis (7). In all of these studies, SPEM was present as often or more often in association with cancer than goblet cell IM. Although TFF2 immunoreactivity was less prominent.
in advanced cancers, in the Iceland study (11), TFF2 immunoactivity was observed in >50% of early gastric cancers. All of these studies in humans demonstrate the importance of SPEM and IM as putative preneoplastic metaplasias in humans.

**Mouse Models of Oxyntic Atrophy and Metaplasia**

Over the past decade, a number of mouse models have been devised, which 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 (Table 1). Critical to these studies is the analysis of gastric mucosal lineages using a number of histological and immunohistochemical markers. Thus, as an introductory preamble, it is important to note that direct analogies between human pathological criteria and those in the mouse must be approached with caution. There is no place of greater confusion than the use of the term IM. In human pathology, IM primarily refers to the aberrant presence of goblet cells in the gastric mucosa as defined by staining with Alcian blue. Although this is a very reliable marker in humans, its use in mice is more problematic. The Alcian blue stain reflects the presence of specific classes of sugar residues on mucins, and these can vary radically among species. Thus, it is important to note that the deep glandular cells of the mouse antrum, which are also immunoreactive for both TFF2 and mucin Muc6, are also Alcian blue positive (Fig. 2). Thus, classification of metaplastic cells as “IM” by Alcian blue in the mouse stomach in the absence of goblet cell morphology is problematic. Indeed, deep antral gland cells and SPEM are also periodic acid-Schiff (PAS) positive with a more pink staining reaction, in contrast with the deep carmine staining in surface cells. Thus, in the mouse, specific immunostaining for mucin subtypes and TFFs provides a more accurate reflection of the phenotype of various fundic metaplasias (Table 2). In this discourse, we will only use the term IM to refer to the presence of goblet cells identified by morphological criteria or immunostained with MUC2 or TFF3 antibodies.

**Table 1. Animal models of oxyntic atrophy and gastric metaplasia**

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Example</th>
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<tbody>
<tr>
<td>Chronic infection</td>
<td>H. felis infection</td>
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<tr>
<td></td>
<td>H. pylori infection</td>
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<tr>
<td>Transgenic and knockout mouse manipulations</td>
<td>KLF4 knockout mouse</td>
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<tr>
<td></td>
<td>H/K-CDT×2 mouse</td>
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<td></td>
<td>Insulin-gastrin mouse</td>
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<td></td>
<td>H/K-Cholera toxin mouse</td>
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<td>H/K-Diphtheria toxin mouse</td>
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<tr>
<td></td>
<td>H/K-thymidine kinase mouse</td>
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<tr>
<td>Acute oxyntic atrophy</td>
<td>DMP-777 treatment</td>
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KLF4, Kruppel-like factor 4; H/K, H⁺-K⁺-ATPase.
Alterations in transcription factors regulating developmental patterns can lead to oxyntic atrophy. Thus, Kruppel-like factor 4 (KLF4)-deficient mice develop oxyntic atrophy and SPEM throughout the fundus (15). As in *H. felis* mice, the antrum is spared of changes. KLF4-deficient mice develop extensive TFF2-expressing metaplasia throughout the gastric fundus. Of interest, these mice do not appear to develop any significant inflammatory response in the mucosa, and no dysplastic changes have been reported to date.

While Cdx2, an intestinal transcription factor that is expressed throughout the small and large intestines, is not expressed in the normal stomach, forced expression of Cdx2 in the stomach using a short H⁺-K⁺-ATPase promoter leads to intestinalization of the gastric fundus (18, 23). H/K-Cdx2 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-Cdx2 mice. Thus, although previous studies using *Helicobacter* infection in mice have not observed goblet cell IM, the establishment of IM in the stomach in mice does represent a preneoplastic scenario with analogy to humans.

Interestingly, several models have reported oxyntic atrophy after the long-term induction of acid hypersecretion. While all three of these models have led to the loss of parietal cells at >6 mo of age, the atrophic phenotypes are dissimilar. The expression in parietal cells of a point mutant of H⁺-K⁺-ATPase, which is incompetent for endocytosis, leads to eventual atrophic gastritis with cystic changes (6). The phenotype in these mice appears to be primarily due to foveolar hyperplasia, although no formal systematic 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 (24). These older animals develop SPEM and gastritis cystica profunda. Furthermore, infection with *H. felis* leads to the accelerated development of SPEM and dysplastic cystic changes. Most recently, Samuelson and colleagues (17) have studied the phenotype of transgenic mice expressing cholera toxin targeted to parietal cells. These mice show a progression of oxyntic atrophy after 6 mo of age with initial mucous neck cell hyperplasia followed later by development of fully replaced SPEM-like glands. It is unclear whether this phenotype reflects a progressive expansion of mucous neck cells or mucous neck cell hyperplasia combined with eventual SPEM development. Interestingly, 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 anti-parietal cell antibodies. Interestingly, human patients with pernicious anemia associated with anti-parietal cell antibodies

### Table 2. Characteristics identifying gastric metaplasias

<table>
<thead>
<tr>
<th>Markers</th>
<th>IM</th>
<th>SPEM</th>
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<tbody>
<tr>
<td>Morphology</td>
<td>Intestinal goblet cell</td>
<td>Deep antral gland cell</td>
</tr>
<tr>
<td>Mucin</td>
<td>MUC2</td>
<td>MUC6</td>
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<td>Trefoil factor</td>
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IM, intestinal metaplasia; SPEM, spasmolytic polypeptide-expressing metaplasia.
do not appear to have as prominent SPEM as those with *H. pylori* infection-associated adenocarcinoma (our unpublished results). All of these three models demonstrate the complexity of phenotypes that may develop after parietal cell loss.

**Direct genetic parietal cell ablation.** Finally, two models of direct genetic parietal cell ablation have been reported in mice. H/K-Diphtheria toxin mice demonstrate parietal cell-specific expression of toxin, leading to the rapid demise of parietal cells as they begin to express the proton pump (16). This genetic ablation model leads to an expansion of preparietal cells in the midportion of gastric glands. At ages >1 yr, 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 (4). These mice demonstrate parietal cell-specific expression of thymidine kinase, but treatment with ganciclovir results in a complete loss of the glandular fundic mucosa, likely due to the transit of toxic adducts through the extensive system of gap junctions among mucosal cells. Thus, this model could not address issues of reactive metaplasia.

**Induction of SPEM After Acute Oxyntic Atrophy**

The orally active, cell-permeant neutrophil elastase inhibitor DMP-777 has allowed the examination of SPEM induction after acute oxyntic atrophy in the absence of significant inflammatory infiltrate. Mice or rats treated with high doses of oral DMP-777 (>200 mg·kg⁻¹·day⁻¹) demonstrate a rapid loss of parietal cells with 3 days of administration (10, 19). The acute oxyntic atrophy is followed immediately with prominent foveolar hyperplasia, and, after 7–10 days of oxyntic atrophy, SPEM then develops in the fundus. These investigations have demonstrated that the induction of gastric metaplasia is a direct result of the loss of parietal cells. These results are compatible with the loss of prodifferentiative growth factors secreted by parietal cells, which could include the EGF receptor ligands transforming growth factor-α, amphiregulin, and heparin-binding EGF as well as sonic hedgehog. In this model of acute oxyntic atrophy, gastrin seems to be the major driving force for foveolar hyperplasia, because gastric knockout mice do not develop surface cell hyperplasia in response to DMP-777 treatment (19). Nevertheless, the absence of gastrin appears to promote the development of SPEM, with the rapid induction of metaplasia after only 1 day of DMP-777 treatment. More recent studies have demonstrated that the reduction in EGF receptor signaling in wave-2 mice carrying a hypomorphic parietal cell-specific knockout of the EGF receptor tyrosine kinase mice (4). These mice demonstrate parietal cell-specific expression of thymidine kinase, but treatment with ganciclovir results in a complete loss of the glandular fundic mucosa, likely due to the transit of toxic adducts through the extensive system of gap junctions among mucosal cells. Thus, this model could not address issues of reactive metaplasia.

**From Metaplasia to Dysplasia: the Role for Bone Marrow-Derived Cells**

As noted above, we have observed that in the absence of chronic inflammation, dysplasia does not develop from gastric metaplasia. Recent investigations have demonstrated that bone marrow-derived cells (BMDCs) contribute to the metaplasia (SPEM) observed in *H. felis*-infected mice (13). These studies demonstrated through multiple methods that BMDCs differentiate into SPEM in *H. felis*-infected mice (13). Furthermore, metaplasia derived from BMDCs contributed prominently in the progression to gastric dysplasia. Importantly, in models without chronic inflammation, such as DMP-777 treatment, no BMDC engraftment was observed. Thus, engraftment of bone BMDCs as SPEM absolutely required inflammation. The presence of BMDCs in SPEM provides the first evidence that gastric epithelial dysplasia can derive from cells of bone marrow.
In contrast with many previous studies, these investigations demonstrate that marrow-derived cells can both engraft and proliferate, establishing a predominance of BMDC-derived metaplasia within the mucosa.

Our present results favor the presence of a two-step model for gastric preneoplasia in the presence of chronic infection with Helicobacter sp. (Fig. 3). First, infection causes inflammation, and the combined presence of Helicobacter-derived factors with inflammatory mediators leads to the loss of parietal cells. The loss of parietal cells then leads to the emergence of SPEM, likely through a normal repair protocol. However, in a second step, with continued chronic infection, BMDCs enter the field containing metaplasia and adopt the metaplastic phenotype. The greater proliferative rate in SPEM associated with H. felis infection compared with that in SPEM observed in mice treated with DMP-777 could accrue from dysregulation of cell cycle control in metaplastic cells derived from bone marrow precursors. This model would suggest that engraftment of BMDCs contributes to the dysplastic transition. Alternatively, elevated proliferative rates may reflect a response to the ongoing inflammatory milieu.

In any case, these bone marrow transplantation studies demonstrate a clear connection between SPEM and the progression to dysplasia in mice. Whether similar engraftment can be observed in mice with goblet cell IM, as in the mice overexpressing Cdx2 in the gastric fundus, remains to be determined. Certainly the present studies suggest that a combination of inflammation and metaplasia must coexist to observe neoplastic progression.

Implications of Mouse Studies for the Pathogenesis of Human Gastric Prenecroplasia

All of these studies indicate that oxyntic atrophy leads to the development of SPEM, likely through the transdifferentiation of chief cells. The results of mouse model studies further support the role of gastric mucous cell metaplasias as precursors of gastric cancer. The analogy between gastric metaplasias observed in humans and mice is incomplete. The origin of IM in humans remains elusive, in part because goblet cell IM is not observed in mouse models of Helicobacter infection (9). Thus IM could arise separately from SPEM or could represent a further differentiation of a metaplastic lineage from SPEM. One is therefore left with a series of hypothetical constructs for the development of cancer from precedent metaplasias in humans. Either of the observed metaplasias, SPEM or IM, could be paracancerous, whereas the other is truly preneoplastic. Alternatively, as noted above, IM could evolve from SPEM, either as a precancerous transition or a paracancerous transition. There presently is no evidence for the evolution of SPEM from IM, because antralization and SPEM appear to develop earlier in the process of oxyntic atrophy. Evidence in Asian literature suggests that gastric cancers can be classified into gastric-type or intestinal-type tumors based on differentiated morphologies (12). Thus, it is possible that each metaplasia gives rise to a distinct type of cancer, e.g., goblet cell IM could evolve into intestinal-type cancers, whereas SPEM evolves into gastric-type cancers. One should also note that there is even a possibility that both SPEM and IM are paracancerous, reflecting a proneoplastic mucosal milieu without
direct responsibility for the ultimate neoplastic transformation. Still, investigations in mice, at least, suggest that SPEM can lead to neoplasia. From a practical point of view, these studies indicate that the development of biomarkers for metaplastic processes holds great promise for the identification of those at risk for the development of gastric cancer. Further studies are needed to define with greater clarity the key regulators of the metaplastic process.

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