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Oxyntic atrophy, metaplasia and gastric cancer

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Abstract

The process of gastric carcinogenesis involves the loss of parietal cells (oxyntic atrophy) and subsequent replacement of the normal gastric lineages with metaplastic lineages. In humans, two metaplastic lineages develop as sequelae of chronic *Helicobacter pylori* infection: intestinal metaplasia and Spasmolytic Polypeptide-expressing Metaplasia (SPEM). Mouse models of both chronic *Helicobacter* infection and acute pharmacological oxyntic atrophy have led to the recognition that SPEM arises from transdifferentiation of mature chief cells. The presence of inflammation promotes the expansion of SPEM in mice. Furthermore, studies in Mongolian gerbils as well as increasing evidence from human studies indicates that SPEM likely represents a precursor for development of intestinal metaplasia. These findings indicate that loss of parietal cells, augmented by chronic inflammation, leads to a cascade of metaplastic events. Identification of specific biomarkers for SPEM and intestinal metaplasia hold promise for providing both early detection of pre-neoplasia as well as information on prognostic outcome following curative resection.

Keywords

Gastric adenocarcinoma; metaplasia; SPEM; intestinal metaplasia

I. Organization of the normal gastric oxyntic mucosa

The acid secreting or oxyntic mucosa is made up of a complex set of differentiated lineages that are specialized for initiation of digestion and protection of the epithelial lining. These include the acid-secreting parietal cells, zymogen secreting chief cells, two types of mucous secreting cells (surface mucous cells and mucous neck cells) and multiple types of endocrine cells that secrete histamine (enterochromaffin-like cells), somatostatin and other local hormones. All of these lineages appear to differentiate from a progenitor cell population that is located in the upper neck region of glands. These progenitor cells give rise to a set of second order progenitor cells that can then give rise to specific sets of differentiated cell lineages (1–5). Pre-surface cells give rise to surface mucous cells that secrete Muc5AC and Trefoil Factor 1 (TFF1) and migrate towards the luminal surface. Pre-parietal cells give rise

to acid secreting parietal cells, which migrate towards the bases of fundic glands. Mucous neck cells, which secrete Muc6 and TFF2, differentiate from pre-neck cells and also migrate towards the bases of fundic glands. As mucous neck cells reach the lower portions of the fundic glands, they undergo a further differentiation into zymogen-secreting chief cells (3, 6). Endocrine cells are also assumed to differentiate from progenitors located in the neck region, but there are no data to determine precisely the pathways of endocrine cell differentiation.

Given the variety of differentiated lineages that populate the gastric fundic mucosa, the phenotypic architecture of the gland requires the assembly of lineages with varying lifetimes migrating either towards the lumen or towards the base. Thus, surface cells migrate towards the lumen and have a short lifetime of 4 to 7 days. Thus, pathological conditions that induce foveolar cell hyperplasia such as Ménétrier's disease can be reversed rapidly once the drive for foveolar hyperplasia is relieved with treatment using an antibody against the EGF receptor. In contrast, the lineages in the gland regions of the fundus are relatively long-lived with lifetimes of 50–150 days according to various estimates. This combination of short-lived lineages moving towards the lumen and long-lived lineages migrating towards the bases results in the fundic gland phenotype with displaying the presence of the progenitor cell region in the upper third of the glands.

The development of pre-neoplastic metaplasia in the stomach

Gastric adenocarcinoma remains the second leading cause of cancer-related death worldwide (7). While gastric cancer rates have been declining in the United States throughout the last century, recent studies indicate that gastric cancer may be rising again, especially in younger populations. Over 20 years ago Correa delineated the association of intestinal metaplasia with the development of intestinal-type gastric cancer (8). While the initiating event in gastric pre-neoplasia in humans is chronic infection with the gram-negative bacterium, *Helicobacter pylori* (9), the details of how metaplasia is initiated and how it can progress towards cancer have remained elusive until recently. Chronic *H. pylori* infection initiates both parietal cell loss (or oxyntic atrophy) and prominent inflammation. Loss of parietal cells appears to represent a key fulcrum in the process of preneoplasia due to the role the acid secreting cells in as the coordinate source of a number of critical growth factors including amphiregulin, TGF- α , HB-EGF, and Shh (10–13). Loss of parietal cell-derived signaling molecules disrupts the proper differentiation of other lineages most notably the zymogen-secreting chief cells (14, 15). The chronic *Helicobacter* infection also elicits prominent inflammation throughout the mucosa. Thus, the combination of oxyntic atrophy along with prominent inflammation is a prerequisite for progression of metaplasia to gastric adenocarcinoma (16).

Oxyntic atrophy triggers a series of changes in the cells lining the gastric mucosa. In humans, two types of metaplasia arise in the milieu of oxyntic atrophy and inflammation: intestinal metaplasia and spasmolytic polypeptide expressing metaplasia (SPEM). Both intestinal metaplasia and SPEM have been associated with the progression to intestinal type gastric cancer (8, 17–25). Thus, oxyntic atrophy in association with prominent inflammation incites the induction of gastric lineages that predispose to the development of gastric cancer.

Nevertheless, the factors mediating progression from oxyntic atrophy to gastric cancer remain unclear.

Intestinal metaplasia, characterized by the presence of intestinal goblet cells in the stomach (26), represents one of the clearest examples of metaplasia, since intestinal goblet cells are not present in the normal stomach. Correa proposed that intestinal metaplasia represented the critical pre-neoplastic metaplasia leading to intestinal type cancer (8). The goblet cells in intestinal metaplasia express appropriate intestinal markers including Muc2 and Trefoil factor 3 (TFF3) as well as other intestinal brush border markers including sucrase-isomaltase, villin, Muc13 and fatty acid binding protein (27, 28). More recent investigations have focused increased interest on a second possible pre-neoplastic metaplasia, SPEM. The mucous metaplastic lineages in SPEM display morphological characteristics of deep antral gland cells or Brunner's glands and express Muc6 and Trefoil Factor 2 (TFF2) (23). Despite the similarity between SPEM and the deep antral glands, no gastrin cells are observed in SPEM glands. Recent studies have suggested that both SPEM and intestinal metaplasia may be pre-neoplastic metaplasias. In three separate studies, SPEM was associated with 90% of resected gastric cancers (23, 25, 29). TFF2 was also expressed in greater than 50% of early gastric cancers from Iceland (30). Nevertheless, the relationship between SPEM and intestinal metaplasia has been elusive.

Parietal cell loss leading to metaplasia mice

While discrete steps leading to metaplasia have been difficult to discern in humans, investigations over the past decade have made considerable progress in understanding the intramucosal changes associated with the induction of metaplasia in rodents. Chronic infection of mice with *H. pylori* or with a different, but related, subspecies of *Helicobacter felis* (*H. felis*) results in loss of parietal cells and inflammation throughout the mucosa (31–33). *H. felis*-infected C57BL/6 mice develop SPEM after 4 to 6 months of infection, demonstrating that oxyntic atrophy and inflammation can also lead to SPEM in mice. After 12 months of infection, the mice progress to gastritis cystica profunda, which is considered a dysplastic process in the fundic mucosa (31). Importantly, however, the *Helicobacter*-infected mice never develop intestinal metaplasia, suggesting that SPEM is the critical pre-neoplastic metaplasia in mice.

To distinguish between the roles of oxyntic atrophy and inflammation in the development of SPEM, we have utilized administration of DMP-777 to examine the role of acute oxyntic atrophy. DMP-777 is a cell permeant neutrophil elastase inhibitor that ablates parietal cells without inciting an inflammatory response. Loss of parietal cells is due to the secondary activity of DMP-777 as a parietal cell-specific protonophore. DMP-777 partitions into the acid secretory canalicular membranes of parietal cells, and then when the cells secrete, DMP-777 provides an open pathway for protons to flow back into parietal cells, causing their rapid death. Pretreatment of mice with omeprazole prior to DMP-777 administration abrogates parietal cell toxicity (34). Thus, after 3 days of DMP-777 administration, mice develop oxyntic atrophy. However, no significant inflammatory infiltrate is observed, presumably because of the action of DMP-777 as a potent cell permeant neutrophil elastase inhibitor. Despite the lack of inflammation, the oxyntic atrophy gives rise to SPEM after 10

to 14 days of DMP-777 administration (35). Thus, SPEM develops as a direct result of the loss of parietal cells. However, it is critical to note that DMP-777-induced SPEM never progresses to dysplasia even after a year of administration (36). These findings demonstrate the crucial role of inflammation in the progression to gastric neoplasia. Immunodeficient mice infected with *H. felis* do not develop oxyntic atrophy and thus do not develop SPEM or progress to dysplasia (37). Therefore, in the case of acute pharmacological ablation of parietal cells, parietal cell loss is sufficient for the development of SPEM.

What factors regulate the induction of metaplasia?

While oxyntic atrophy appears to be an absolute pre-requisite for the development of SPEM, the identification of clear mediators of induction have remained obscure. Indeed, investigations over the past several years have identified a number of alterations that promote SPEM induction, but little information points to manipulations that inhibit SPEM induction. Parietal cells produce a number of paracrine factors that affect the growth and differentiation of the other gastric lineages. In particular, parietal cells are a major source of the EGF receptor ligands, TGF α , amphiregulin and HB-EGF. To examine the role of the EGF signaling pathway in the development of SPEM, the *waved-2* mouse, which has a hypomorphic mutation EGF receptor that reduces tyrosine kinase activity. Accelerated development of SPEM was observed in the *waved-2* mice treated with DMP-777 (38). While these studies suggested that loss of EGF receptor signaling could promote metaplasia, investigations of specific EGF receptor ligands have shown differential effects. DMP-777-induced SPEM in TGF α -deficient mice developed similarly to that seen in wild type mice (39). In contrast, loss of amphiregulin led to accelerated SPEM development (39). Indeed, the effect of amphiregulin on DMP-777 induction were more prominent than those in *wave-2* mice. Interestingly, in further studies we have observed that amphiregulin deficient mice develop SPEM spontaneously after 8 months of age and go on to develop dysplastic changes after one year of age (40). These results suggest that loss of amphiregulin secretion by parietal cells may be an important trigger for the induction of SPEM in the setting of acute oxyntic atrophy.

The gastric mucosa also contains a number of endocrine cell lineages, which coordinate both cell secretory function and lineage growth and differentiation. Gastrin secreting G cells and histamine secreting enterochromaffin-like (ECL) cells (41–47) are primarily responsible for the regulation of parietal cell acid secretion (42). However, gastrin is also a primary driver of general fundic mucosal proliferation and has a critical direct role in promoting the differentiation of surface mucous cells (48). Histamine secreted by the ECL cells also plays an important role in the differentiation of the gastric lineages (49). Additionally, somatostatin cells coordinate inhibitory influences both on acid secretion and hormonal secretion (50). Thus, parietal cells, G cells, somatostatin cells and ECL cells have established a number of complex feedback loops to regulate the gastric mucosal milieu. Alteration or interruption of this balance of intramucosal factors affects the maturation of normal gastric lineages as well as the development of SPEM. Since oxyntic atrophy leads to elevated levels of gastrin, we initially anticipated that gastrin would be a prominent driver of metaplasia. In fact, we found that elevated gastrin was not associated with SPEM. Rather, gastrin deficient mice showed a prominent potentiation of SPEM development following

DMP-777 administration (48). Gastrin deficient mice develop SPEM after only a single oral dose of DMP-777, while it takes 10 days of DMP-777 administration for the development of SPEM in wild type mice (35). Although not as prominent as the gastrin knockout mice, histidine-decarboxylase (HDC) deficient mice (which lack histamine) also have an accelerated development of SPEM upon administration of DMP-777 (49). Thus loss of specific paracrine and hormonal regulators can lead to an imbalance of feed back controls and this may promote the formation of metaplasia, perhaps as a perceived response to injury.

Cellular origin of metaplasia

Although the association of oxyntic atrophy with the emergence of SPEM in mice is clear, the cellular origin of SPEM has remained elusive. The morphological characteristics of SPEM seemed similar to a pre-zymogenic cell in that the mouse SPEM cells expressed both TFF2 and intrinsic factor (39, 48, 51). Indeed, the most reliable signature of SPEM in mice appears to be the emergence of cells at the bases of glands expressing both intrinsic factor and TFF2. One important difference between pre-zymogenic cells and SPEM cells is that in the pre-zymogenic cells, mucous components and zymogenic components appear to be present in the same granules (6), whereas in SPEM they are in distinct granule populations (48). As noted above, as the post-mitotic mucous neck cells migrate towards the base of the gland, they alter their expression profiles as well as their morphology to redifferentiate into chief cells (3, 6). Both mucous neck cells and chief cells have conventionally been thought of as post-mitotic. Nevertheless, following treatment with DMP-777, we observed scattered Ki-67 positive cells in SPEM at the bases of fundic glands in wild type mice as well as in gastrin and amphiregulin knockout mice (39, 48, 51). In the normal gastric mucosa, no dividing cells are observed at the bases of fundic glands. Therefore, these findings indicated that cells at the bases of fundic glands that were contributing to the development of SPEM were re-entering the cell cycle.

All evidence suggested that the emergence of this zone of proliferation in SPEM cells at the bases of fundic glands was independent of the normal progenitor zone located near the gland lumen. Indeed, in gastrin deficient mice, the rapid emergence of SPEM after only a single dose of DMP-777 suggested that there was insufficient time for cells to migrate out of the normal proliferative zone to the gland bases where SPEM cells were emerging. Gene microarray studies of emerging SPEM lineages isolated by laser capture microdissection from gastrin knockout mice treated with DMP-777 for 1 or 3 days suggested that the SPEM cells were upregulating a large cohort of genes involved in G1/S transition, including multiple MCM proteins involved in chromatin unwinding (51). In addition to these changes in gene expression, we also demonstrated that some SPEM cells expressed both TFF2 and Mist1, a mature chief cell differentiation specific marker (51). Even though in general we say a decrease in Mist1 expression following DMP-777 treatment, because of the rapid time course of SPEM induction in gastrin knockout mice, we were actually able to “capture” the transition of cells between chief cells and SPEM. All of these studies have now led to the novel hypothesis that SPEM is derived from transdifferentiation of mature chief cells into SPEM. Central to this process is the transition of through cells that can express markers of both cell lineages, e.g TFF2 and intrinsic factor. Lineage ampping studies are presently

under way to provide direct evidence for the pathway of transdifferentiation of chief cells into SPEM.

The relationship of SPEM to intestinal metaplasia. The origin of intestinal metaplasia has been harder to examine because *Helicobacter* infection in mice does not result in intestinal metaplasia. However, *H. pylori* infection of other species, such as Mongolian gerbils, does induce intestinal metaplasia and eventually dysplasia and cancer (53, 54). (55–57). Recent studies by Nomura and colleagues have now addressed the relationship of SPEM and intestinal metaplasia through a detailed examination of the histological progression of oxyntic atrophy to metaplasias in the Mongolian gerbil (57). After only 3 weeks of infection, the gerbils developed SPEM in the presence of oxyntic atrophy. While extension and expansion of SPEM was observed over the following weeks, intestinal metaplasia arose later at 24 and 39 weeks of infection. Importantly, the sites of intestinal metaplasia were surrounded by the mucosa with pre-existing SPEM. Moreover, single gland units were present containing both intestinal metaplasia and SPEM lineages (57). Similar developmental patterns of intestinal metaplasia have also been observed in humans (58). In the gerbils, cells were observed in the transition between SPEM and intestinal metaplasia that expressed markers of both lineages (e.g. Muc2 and TFF2). In another recent investigation, Nam and colleagues have also observed the development of intestinal metaplasia and dysplasia in older amphiregulin knockout mice. As with the gerbils, Muc2 expressing intestinal metaplasia developed within glands also expressing SPEM lineages and hybrid cells expressing both Muc2 and TFF2 were observed in transition zones within glands and dysplastic regions (40). Importantly, in both of these rodent studies, intestinal metaplasia was defined by expression of specific markers such as Muc2 and TFF3 rather than staining with Alcian blue histochemistry. This is important because, in contradistinction with humans, antral cells in rodents are often positive for Alcian blue staining (59). All of these results increasingly suggest that SPEM is the first metaplasia arising after induced oxyntic atrophy, and then, especially in humans, with subsequent on-going chronic inflammation intestinal metaplasia arises as a second metaplastic transition. Such an evolution of metaplastic lineages has previously been observed by Wright and colleagues in the context of mucosal restitution in inflammatory bowel disease (60).

An understanding of metaplasia and the development of biomarkers for pre-neoplasia

Given the clear association of metaplasias in the stomach with increased risk for gastric cancer, the identification of effective biomarkers of metaplasia and metaplastic progression to dysplasia could provide screening methods for patients at risk for gastric cancer. Recent studies in both mice and humans have begun to identify a number of biomarkers that have implications for cancer outcome. Recent gene microarray analyses of SPEM in mice identified the secreted WAP domain protein, HE4 (WFDC2) as a putative biomarker of metaplasia (51). While there was no HE4 expression in normal mouse gastric lineages, SPEM lineages from both DMP-777 treated and *H. felis* infected mice showed strong expression of HE-4. Similarly, HE4 was expressed in all samples of SPEM and intestinal metaplastic lineages in humans. Additionally, strong HE4 expression was maintained in the majority of intestinal-type gastric cancers, making it a promising biomarker candidate.

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Figure 1.

Comparison of normal fundic gastric glands and metaplastic SPEM glands in mice. The diagram at the top depicts alterations in gland lineages between normal and SPEM glands with emergence of SPEM and foveolar hyperplasia following loss of parietal cells. Panels below show immunostaining patterns for normal and SPEM-containing glands. TFF2 (red) expressing mucous neck cells redifferentiate into intrinsic factor (green) expressing chief cell at the base of normal glands. However, TFF2 expression is expanded to the base of SPEM glands and appears within cells that show dual staining for intrinsic factor. Mist1 (green) is a differentiated chief cell marker in normal gastric glands. However, Mist1 is also expressed in some TFF2-expressing SPEM cells, suggesting transdifferentiation of chief cells. Although proliferation as detected by BrdU (red) is normally only in the progenitor zone of the upper normal gland, TFF2-expressing SPEM cells show clear proliferating cells also expressing intrinsic factor (IF). Previous studies have led to the identification of promising biomarkers of SPEM such as HE4. HE4 is not detected in normal chief cells or any normal fundic cells, but HE4 staining is strongly observed in SPEM.

Figure 2.

Current model for the origin and progression of gastric metaplasias in humans. Studies have demonstrated that loss of parietal cells results in chief cell transdifferentiation and the SPEM emergence. In the presence of chronic inflammation from *H. pylori* infection, SPEM evolves into intestinal metaplasia then progresses on to gastric cancer. Establishment of biomarkers of these metaplastic lineages is a priority. Recent studies in mice identified HE4 as a SPEM biomarker. In humans, HE4 is not expressed in the cells of the normal fundus. However, HE4 is detected in both SPEM and intestinal metaplasia, supporting the hypothesis of SPEM progression to intestinal metaplasia.