Isthmus Stem Cells Are the Origins of Metaplasia in the Gastric Corpus

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Isthmus Stem Cells Are the Origins of Metaplasia in the Gastric Corpus

The acquisition of genetic/epigenetic mutations in long-lived gastrointestinal stem cells leads to the development of cancer, as well as precancerous lesions such as metaplasia and dysplasia. In the proximal stomach corpus, this model of progression from stem cells has been supported by studies in mice and human beings, showing abundant proliferation in the isthmus and clonal expansion of mutated cells from the stem cell region. An alternative theory proposes that gastric metaplasia arises from mature differentiated chief cells. Despite reports of low levels of proliferation in chief cells in acute injury models, there is little evidence for reprogramming of chief cells into long-lived stem cells that continuously supply progeny over time. Critical flaws in the chief cell transdifferentiation theory include the definition of acute SPEM, the chief cell-damaging effect of chemical reagents, and the specificity of chief cell lineage tracing. In contrast, there is now strong evidence regarding the stem cell origins of gastric metaplasia that refutes the transdifferentiation theory. Here, we briefly review the history and definition of gastric metaplasia, and outline in detail the evidence that supports the stem cell origin of metaplasia. (Cell Mol Gastroenterol Hepatol 2017;4:89–94; http://dx.doi.org/10.1016/j.jcmgh.2017.02.009)

Metaplasia of the stomach gained increasing recognition when a link to gastric adenocarcinoma was noted and the Correa pathway was proposed. Although classic intestinal metaplasia (IM) with goblet cell differentiation initially received most of the attention, spasmolytic polypeptide-expressing metaplasia (SPEM) recently has attracted greater interest. SPEM was first characterized by "a marked expansion of an aberrant gastric mucous cell lineage that stained positive for spasmolytic polypeptide" in *Helicobacter felis*-infected mice (spasmolytic polypeptide was the original name given to trefoil factor family 2 [TFF2]).

*Helicobacter* species induce a variety of histopathologic changes in mice, including oxyntic atrophy (loss of corpus chief and parietal cells), surface mucous pit-cell hyperplasia, mucous metaplasia (MM), and pseudopyloric metaplasia (PM). In this process, chief cell disappearance precedes parietal cell loss and the development of SPEM. Both MM and PM are classified as SPEM that expresses neck cell markers TFF2, gastric mucin-6 (MUC6), and Griffonia simplicifolia leaf lectin II (GS2), but they need to be distinguished. SPEM-MM is characterized by large, foamy TFF2⁺ cells that secrete neutral and acid mucins and replace lost parietal and chief cells (Figure 1A). In addition to morphologic differences from normal mucous neck cells, SPEM-MM expresses unique markers (CD44 and Sox9) that are absent in normal neck cells. Thus, MM is clearly a form of metaplasia and not simply neck cell hyperplasia. In contrast, SPEM-PM occurs later and includes less differentiated cell types that resemble the pyloric antrum. Dysplasia emerges after SPEM-PM is established.

An entity similar to TFF2-expressing PM in mice was recognized in human gastric tissue in 1999 and the name SPEM formally was proposed to encompass TFF2-expressing metaplasia. SPEM development was linked to mucosal injury associated with parietal and chief cell loss, particularly in chronic *Helicobacter* species infection. Although initial studies pointed to SPEM as a preneoplastic lesion, knockout of the signature peptide, TFF2, in mice accelerated gastric inflammation and carcinogenesis, suggesting a possible role for TFF2 as a tumor suppressor.

Observations in Patients Indicate a Stem Cell Link

Analysis of resected gastric specimens showed the frequent co-existence of SPEM and IM in the same compound glands. This raised the question of whether SPEM originated from tissue resident stem cells or from another source. The stability and durability of IM and SPEM suggests that they are maintained by a self-renewing stem cell. Although there was an implicit assumption that metaplasia arises from epigenetic changes in multipotent gastric stem cells, more recent studies have shown that metaplastic gastric glands are clonal, maintained by multiple stem cells, and can form large patches that spread by glandular fission.
Thus, chronic inflammation leads to reconstruction and expansion of niche components with changes in the location of proliferation outside of the isthmus, which may cause the migration of isthmus stem/progenitor cells, rather than generation of new progenitors.

**Development of Short-Term Models Mimicking Gastric Metaplasia**

Although SPEM typically requires many months to develop, several acute chemically induced SPEM models have been described, including DMP-777, L-635, and high doses of tamoxifen. These models involve chemically induced injury with gastric atrophy and the development of SPEM-like lesions. Rodents treated with DMP-777 were reported to develop TFF2-expressing metaplasia after 7–10 days. Similarly, administration of high doses of tamoxifen caused rapid parietal and chief cell loss, and subsequently increased GS2⁺ cells near the base. The most rapid SPEM-like model involved treatment with L-635, which lead to TFF2-expressing metaplasia in 1 week, accompanied by massive inflammation.

In these models, a subset of metaplastic cells expressed chief cell markers and arose primarily in the lower third of the corpus glands. These correlated with other data suggesting a chief cell origin for these lesions. This led the researchers to formally propose that SPEM may be derived from mature chief cells through “transdifferentiation.”

To test this hypothesis, investigators performed lineage-tracing in Mist1-CreERT; Rosa26-LacZ mice to genetically mark Mist1⁺ chief cells and their progeny during SPEM development. After DMP-777 treatment, the number of TFF2-expressing cells in the midl gland region increased quickly, but most of these cells were not marked by Mist1-CreERT lineage, suggesting that they arose from neck progenitors or other cells that did not express Mist1. Indeed, there was actually a significant decrease in the number of Mist1-traced chief cells after DMP-777 treatment, suggesting that DMP-777 ablates not only parietal cells but also chief cells, as we confirmed recently. Nevertheless, a rare population of the remaining Mist1-traced chief cells expressed TFF2, indicating that a subset of Mist1⁺ chief cells (most often at the top of the chief cell zone) could contribute in a limited fashion to SPEM-like lesions.

The investigators also showed full Mist1-lineage tracing of SPEM in both L-635 and H. felis infection mouse models, and, more recently, the group confirmed our previous findings that expression of mutant KrasG12D in the Mist1 lineage leads to the development of SPEM, which they showed could be completely reversed by a mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) inhibitor. From these models, they concluded that the metaplasia originated primarily from chief cells.

**Weaknesses in the Conclusion That Metaplasia Originates From Chief Cells**

Although the conclusion of a chief cell origin is supported by expansion of double-positive cells (intrinsic factor (IF)⁺TFF2⁺) and Mist1 tracing, the transdifferentiation...
hypothesis remains flawed. First, very few actively cycling cells are detected at the gland base in chemical injury models; the majority of proliferating cells originate from the isthmus. The notion that chief cells interconvert into progenitor cells and migrate from the base toward the isthmus, against the flow from the main corpus stem cells, challenges numerous well-established paradigms. Although, a similar model was proposed in a study of Troy-CreERT knockin mice, the chief cell behavior they proposed was proven to be perturbed by Troy gene haploinsufficiency later.

Second, induction of metaplasia by the chemical agents is completely reversible within 2 weeks. Thus, these drugs do not cause permanent reprogramming at the stem cell level. Helicobacter species-induced metaplasia persists for >1 year and thus must be sustained by a long-lived stem cell. Thus, the nature of drug-induced metaplasia appears to be different from that of classic SPEM.

Third, DMP-777 damages chief cells and dramatically reduces the number of Mist1-traced chief cells, which accounts for the extension of metaplasia to the base of the gastric glands; this is likely the case with the very similar compound L-635. One of the earliest changes in the H felis mouse model is rapid chief cell disappearance, an event that precedes parietal cell loss. Indeed, the notion that part of the SPEM induction requires chief cell injury has been supported by a recent study showing that parietal cell ablation alone is insufficient to induce metaplasia. Therefore, the cellular changes observed at the gland base after drug treatment simply may reflect chief cell regeneration.

Indeed, although cells that express TFF2/GS2 and IF near the base often are categorized as SPEM, their histologic features are quite different from classic SPEM (either SPEMM-MM or SPEM-PM) identified in Helicobacter species–infected mice. The acute SPEM-like cells show a different appearance with limited expansion and less mucin. We conclude that the increase in TFF2þIFþ cells likely represents expansion and migration of progenitors that are known to be TFF2þIFþ from the neck region rather than true metaplasia. The definition of SPEM needs to be reconsidered carefully and more sharply limited, rather than loosely categorizing cells as metaplasia based simply on transient TFF2/gastric intrinsic factor (GIF) positivity.

Finally, the main problem with the transdifferentiation hypothesis is that it was based almost entirely on the assumption that Mist1 is a specific marker of chief cells and does not mark any additional stem/progenitor cells, which is not the case.

### Mist1 Is Expressed in Quiescent Stem Cells in the Isthmus

We showed that Mist1 is expressed not only in chief cells but also in quiescent, self-renewing stem cells within the corpus isthmus. There is an average of 1–2 isthmus Mist1þ cells per gland, much less than the number of Mist1þ chief cells (average 9–10/gland) at the glandular base (Figure 1B), which explains why isthmus expression previously was overlooked. We found abundant long-term lineage tracing from Mist1þ cells, but analyses of detailed time course and multicolor fluorescent reporter mice showed that Mist1þ-derived tracing arose from the isthmus, but not from the gland base. Consistent with this, ablation of >95% of Lgr5þ chief cells using Lgr5-DTR-EGFP mice that express diphtheria toxin receptor (DTR) and green fluorescent protein (GFP) in Lgr5þ cells did not reduce the numbers of long-term Mist1þ-derived tracing events (Figure 1A and Figure 1C–E).

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BrdU, bromodeoxyuridine; 5-FU, 5-fluorouracil.
contrast, when isthmus stem cells ablated with a well-defined 5-fluourouracil regimen, a Mist1-derived tracing events rarely were seen.  Thus, the long-term, lineage-tracing events in Mist1-CreERT mice derive from isthmus stem cells, rather than chief cells. Therefore, lineage tracing from Mist1þ cells cannot be used to support the concept of chief cell transdifferentiation. Long-term Mist1-traced lineage must be assumed to emanate from the isthmus stem cell.

KrasG12D expression in the corpus isthmus (Mist1þ) stem cell results in the development of mucous-producing metaplasia within 1 month. a Within 5–7 days after Kras activation of the Mist1þ cells, Ki67þ metaplastic foci were seen within the isthmus (Figure 1F). In contrast, few Ki67þ cells present at the base of the glands or associated with chief cells. At day 14, there was evidence for gland fission with Alcian blue mucous metaplasia, but only in the isthmus region (Figure 1F). These proliferating isthmal cell clusters then migrated toward the base and expanded laterally via gland fission (Figure 1F). Thus, temporal analysis showed a downward migration of the stem cell zone, consistent with our earlier findings in the CK19-Kras mice and Helicobacter-infected mouse models. b Moreover, depletion of Lgr5þ chief cells did not impair metaplasia development, consistent with earlier findings c showing that SPEM was not derived from Lgr5þ chief cells. Therefore, Mist1þ isthmus cells, rather than Mist1þ chief cells, are the clear origin of Kras-induced metaplasia.

Summary of the Evidence in Favor of a Stem Cell Origin of Metaplasia

The presence of Mist1þ isthmus stem cells accounts for the origin of metaplasia in the models discussed earlier (Table 1), as well as the results from other models. Our findings regarding Kras-induced metaplasia development c were confirmed by Choi et al., a who showed that Kras-induced SPEM subsequently gives rise to IM and even dysplasia. Kras-induced metaplasia stably persists over time, similar to H felis–induced metaplasia, suggesting reprogramming of long-lived stem cells rather than transient transdifferentiation. Indeed, when MEK inhibitors reverse the Kras-induced metaplasia, d normalization of gland structure occurs in a gland-by-gland fashion with upward regression of the isthmus lesion but without any proliferation in chief cells. This finding strongly argues that metaplasia indeed is generated by Kras-activated stem cells, with normal gland components renewed from normalized stem cells after MEK inhibition.

An occasional criticism of the argument for the isthmus origin of metaplasia is that Mist1þ corpus stem cells appear scarce and seem insufficient to account for all of the Kras-induced metaplasia, which replaces nearly the entire stomach within 1–2 months. e Indeed, in the initial report, Mist1þ isthmus stem cells were not well represented in the 5-um–thickness axial sections analyzed. f In fact, each gland actually contains 1–2 Mist1þ isthmus stem cells, which more clearly are shown in cross-sectional analysis (Figure 1B). Although Mist1þ chief cells are more abundant, Kras mutant chief cells rarely proliferate. In contrast, the majority of Kras-mutated Mist1þ isthmus cells become rapidly proliferative with expression of neck cell markers and Alcian blue mucins, suggesting a 50- to 100-fold greater proliferative potential in Mist1þ stem cells compared with chief cells (Figure 1F). In addition, the notion that the numbers of Mist1þ isthmus cells are insufficient ignores the fact that Krasþ glands are known to outcompete wild-type glands with lateral expansion in the neutral drift model (Figure 1F). A recent study with eR1-CreERT;LSL-KrasG12D mice has shown similar results, g with Alcian blue metaplasia also arising in the isthmus, supporting in our view an isthmal origin for metaplasia. Although an occasional gland base showed pepsinogen C (PGC) metaplasia in these mice, it should be noted that PGC also is expressed in neck cells or above (Figure 1C), thus it does not support chief cell origin.

Although our detailed analyses confirm that isthmus stem cells are the main source of SPEM in acute injury and Kras models, we cannot exclude the possibility that neck or chief cell precursors, residing immediately above mature chief cells, may contribute to short-term regeneration and metaplasia. The presence of chief cell precursors in this region initially was suggested by Karam and Leblond, h and indeed approximately 15% of normal neck/chief lineage cells express both GS2/GIF, and they appear to show occasional limited proliferation after injury (Figure 1E). If some of these cells express Mist1, they might contribute to some of the short-term GS2/GIF lesions.

Conclusions

Gastric metaplasia can be fully generated by the isthmus stem cell. There is as yet no evidence for clonal expansion or upward migration of mature zymogenic cells from the gland base, even after injury. The role of the Mist1þ isthmus stem cell remains to be fully elucidated in chemical injury models, but the definition of SPEM also needs further clarification. Although there may be some degree of plasticity for chief cells, they likely are unable to generate long-lived stem cells and the stable, clonal metaplasia found in patients and H felis–infected mice. Nevertheless, chief cells or their progenitors still may contribute in limited ways to early stages of gastric repair. More studies are needed to understand the factors involved in regulating the altered differentiation of the isthmus Mist1þ stem cell that leads to the production of long-term SPEM.

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Conflicts of interest
The authors disclose no conflicts.

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