**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)

Point Counterpoint

FROM THE 2016 AGA
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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 pylorogenic 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 midgland 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 _Kras^G12D_ 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

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**Figure 1. (See previous page).** (A) Left: Histopathology of SPEM-MM. H&E staining of 9-month-old _H pylori_ SS-1 strain–infected TFF2 knockout mice (top and middle) and Alcian blue staining of 4-month-old _H pylori_ PMSS-1 strain–infected wild-type mice (bottom). Right: Histopathology of SPEM-PM. H&E (top and middle) and Alcian blue (bottom) staining of 6-month-old PMSS-1–infected wild-type mice. (B) Mist1^+^ isthmus stem cells are present in almost every gland. Cross-section (top) and axial section (bottom) of Mist1-CreERT; R26-TdT homozygous mouse corpus 1 day after tamoxifen induction. Bottom panel is stained with GS2 (green). Mist1 (red) is expressed abundantly in the basal chief cell region, but also consistently in isthmus stem cells. Cross-sectional analysis showed that an average of 1–2 Mist1^+^ isthmus stem cells are present in each corpus gland, but the number of Mist1^+^ chief cells is much greater than of Mist1^+^ stem cells. (C) Left: Lgr5 marks chief cells. Gland base of Mist1-CreERT; R26-TdT; Lgr5-DTR-EGFP mouse corpus 1 day after tamoxifen. Lgr5 expression (green) is restricted at the base and overlaps with the majority of Mist1^+^ chief cells (red). Right: GS2 (green) and PGC (red) staining in wild-type mice. PGC is expressed in both chief and neck cells. (D) Schema of expression pattern in mouse corpus glands of Mist1, Lgr5, IF, PGC, TFF2/GS2, and Kid7. Neck cell markers such as TFF2 or GS2 are found between Mist1^+^ chief cells and Mist1^+^ isthmus cells. Proliferation (Kid7^+^) is found solely within the isthmus region in normal state. Evidence suggests the presence of chief cell precursors (pre-chief cells) in the lower neck/top of the chief cell region, which express both chief and neck cell markers. (E) GS2 (green) and Kid7 (red) staining in wild-type (left) and Lgr5-DTR (right) mice 3 days after treatment with diphtheria toxin. Lgr5–diphtheria toxin treatment efficiently ablates chief cells, and there is a marked expansion of proliferation at the isthmus after chief cell ablation. PGC and GS2-expressing neck cells are increased and show hyperplasia. Because of the absence of basal chief cells, isthmus and neck cells migrate rapidly downward to the base, and therefore GS2^+^ pre-chief cells can be localized at the base. These cells occasionally proliferate (Kid7^+^) and expand, reflecting their neck progenitor function, to compensate and regenerate ablated chief cells. Other acute injury models including DMP-777, L-635, and high dose of tamoxifen (TAM), cause a rapid loss of parietal cells, as well as a reduction in chief cells, therefore the increase of basal TFF2^+^ cells in these models is the result of a downward shift of TFF2^+^ neck/pre-chief cells that can proliferate to regenerate chief cells. During this process, immature IF^+^TFF2^+^ cells are produced from upper isthmus stem cells and chief cells, not from dedifferentiation of ablated mature chief cells. Loss of parietal cells alone is not sufficient to induce such IF^+^TFF2^+^ cell hyperplasia, therefore acute SPEM lesions in injury models likely are caused by loss of damaged chief cells. (F) Diagram of changes in the gastric glands with _Kras^G12D_ activation in the Mist1^+^ cells. Mutant _Kras_ expression is induced in both Mist1^+^ isthmus stem cells and chief cells, but only isthmus stem cells can give rise to metaplasia, and they migrate toward the base in a manner that can be reversed with a MEK inhibitor. _Kras^-mutated Mist1^+^ isthmus stem cells are proliferating rapidly. Bottom: MUC6 (left) staining and Kid7 (middle) staining with Alcian blue at 7 days after tamoxifen induction. Because of rapid turnover, chief cells are depleted at the base and MUC6^+^ neck cells occupy the basal region at this point, but importantly these MUC6^+^ cells do not show SPEM-like morphology, mucins, or proliferation. Bottom right: Alcian blue staining at 14 days after tamoxifen induction. Metaplastic Mist1^+^ isthmus cell cluster expand toward the base as well as laterally through gland fission, eventually replacing the entire stomach with SPEM cells.
hypothesis remains flawed. First, very few actively cyclingcells are detected at the gland base in chemical injury models;the majority of proliferating cells originate from the isthmus. The
total that chief cells interconvert into progenitor cellsmigrate from the base toward the isthmus, against theflow from the main corpus stem cells, challenges numerouswell-established paradigms. Although, a similar model wasproposed in a study of Troy-CreERT knockin mice, the chiefcell behavior they proposed was proven to be perturbed byTroy gene haploinsufficiency later.

Second, induction of metaplasia by the chemical agents iscompletely reversible within 2 weeks. Thus, these drugs do notcause 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 dramaticallyreduces the number of Mist1-trae chief cells, whichaccounts for the extension of metaplasia to the base of thegastric gands, this is likely the case with the very similarcompound L-635. One of the earliest changes in the H felis mouse model is rapid chief cell disappearance, an event thatprecedes parietal cell loss. Indeed, the notion that part ofthe SPEM induction requires chief cell injury has been-supported by a recent study showing that parietal cellablation alone is insufficient to induce metaplasia. Therefore, the cellular changes observed at the gland base afterdrug 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 SPEM-MM or SPEM-PM) identified in Helicobacter species–infectedmice. The acute SPEM-like cells show a different appearancewith limited expansion and less mucin. We conclude thatthe increase in TFF2+IF+ cells likely represents expansion andmigration of progenitors that are known to be TFF2+IF+ 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 transdifferentiationhypothesis is that it was based almost entirely on theassumption that Mist1 is a specific marker of chief cells anddoes not mark any additional stem/progenitor cells, whichis 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 Mist1+ cells per gland, much less than the number of Mist1+ chief cells (average 9–10/gland) at the glandular base (Figure 1B), which explains why ismus expression previously was overlooked. We found abundant long-term lineage tracing from Mist1+ cells, but analyses of detailed time course and multicolor fluorescent reporter mice showed that Mist1-derived tracing arose from the isthmus, but not from the gland base. Consistent with this, ablution of >95% of Lgr5+ chief cells using Lgr5-DTR-EGFP mice that express diphtheria toxin receptor (DTR) and green fluorescent protein (GFP) in Lgr5+ cells did not reduce the numbers of long-term Mist1-derived tracing events (Figure 1A and Figure 1C–E).

### Table 1. Evidence for Cellular Origins of Metaplasia

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<th>Evidence for chief cells</th>
<th>Evidence for Mist1+ isthmus stem cells</th>
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<tr>
<td>Proliferation</td>
<td>None in normal state, and minimal (&lt;5%) in injury</td>
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<td>BrdU labeling</td>
<td>No uptake in normal state, with rare labeling after injury</td>
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<td>Lineage tracing</td>
<td>Troy-derived long-term tracing in haploinsufficient Troy knockin models, but not in Troy BAC models</td>
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<td>Chief cells express Lgr5, but no tracing from Lgr5+ chief cells in normal and injury states; ablation of Lgr5+ chief cells does not decrease Mist1-derived tracing</td>
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<td>No evidence that chief cells expand clonally</td>
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<td>DMP-777</td>
<td>TFF2+IF+ cells present in lower third of glands after treatment, but morphology of these SPEM cells is atypical and changes are transient (&lt;2 wk)</td>
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<td>DMP-777 reduces Mist1-traced chief cell number</td>
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<td>Mutant Kras</td>
<td>Proliferating GS2+IF+ cells at the base after 1 month; eR1-CreERT with Kras mutation leads to occasional GS2+ metaplasia near the gland base</td>
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<td>Most chief cells do not proliferate even after Kras induction at early time points, and are replaced rapidly by the migration of isthmus-derived SPEM cluster</td>
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BrdU, bromodeoxyuridine; 5-FU, 5-fluorouracil.
contrast, when isthmus stem cells ablated with a well-defined 5-fluorouracil regimen,\textsuperscript{6} Mist1-derived tracing events rarely were seen.\textsuperscript{7} Thus, the long-term, lineage-tracing events in Mist1-CreERT mice derive from isthmus stem cells, rather than chief cells. Therefore, lineage tracing from Mist1\textsuperscript{+} 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.

\textit{Kras}\textsuperscript{G12D} expression in the corpus isthmus (Mist1\textsuperscript{+}) stem cell results in the development of mucus-producing metaplasia within 1 month.\textsuperscript{6} Within 5–7 days after \textit{Kras} activation of the Mist1\textsuperscript{+} cells, Ki67\textsuperscript{+} metaplastic foci were seen within the isthmus (Figure 1F). In contrast, few Ki67\textsuperscript{+} 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\textsuperscript{+} 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.\textsuperscript{5} Moreover, depletion of Lgr5\textsuperscript{+} chief cells did not impair metaplasia development, consistent with earlier findings\textsuperscript{6} showing that SPEM was not derived from Lgr5\textsuperscript{+} chief cells. Therefore, Mist1\textsuperscript{+} isthmus cells, rather than Mist1\textsuperscript{+} chief cells, are the clear origin of \textit{Kras}-induced metaplasia.

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

The presence of Mist1\textsuperscript{+} 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 \textit{Kras}-induced metaplasia development\textsuperscript{6} were confirmed by Choi et al.,\textsuperscript{7} who showed that \textit{Kras}-induced SPEM subsequently gives rise to IM and even dysplasia. \textit{Kras}-induced metaplasia stably persists over time, similar to \textit{H felis}-induced metaplasia, suggesting reprogramming of long-lived stem cells rather than transient transdifferentiation. Indeed, when MEK inhibitors reverse the \textit{Kras}-induced metaplasia,\textsuperscript{9} 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 \textit{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 Mist1\textsuperscript{+} corpus stem cells appear scarce and seem insufficient to account for all of the \textit{Kras}-induced metaplasia, which replaces nearly the entire stomach within 1–2 months.\textsuperscript{6} Indeed, in the initial report, Mist1\textsuperscript{+} isthmus stem cells were not well represented in the 5-um–thickness axial sections analyzed.\textsuperscript{6} In fact, each gland actually contains 1–2 Mist1\textsuperscript{+} isthmus stem cells, which more clearly are shown in cross-sectional analysis (Figure 1B). Although Mist1\textsuperscript{+} chief cells are more abundant, \textit{Kras} mutant chief cells rarely proliferate. In contrast, the majority of \textit{Kras}-mutated Mist1\textsuperscript{+} isthmus cells become rapidly proliferative with expression of neck cell markers and Alcian blue\textsuperscript{+} mucins, suggesting a 50- to 100-fold greater proliferative potential in Mist1\textsuperscript{+} stem cells compared with chief cells (Figure 1F). In addition, the notion that the numbers of Mist1\textsuperscript{+} isthmus cells are insufficient ignores the fact that \textit{Kras}\textsuperscript{+} glands are known to overcompete wild-type glands with lateral expansion in the neutral drift model (Figure 1F). A recent study with eR1-CreERT;LSL-Kras\textsuperscript{G12D} mice has shown similar results,\textsuperscript{10} with Alcian blue\textsuperscript{+} metaplasia also arising in the isthmus, supporting in our view an isthmal origin for metaplasia. Although an occasional gland base showed pepsinogen C (PGC)\textsuperscript{+} 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 \textit{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,\textsuperscript{11} 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 Mist1\textsuperscript{+} 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 \textit{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 Mist1\textsuperscript{+} 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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