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

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

| Citation          | Hayakawa, Yoku et al. “Isthmus Stem Cells Are the Origins of Metaplasia in the Gastric Corpus.” Cellular and Molecular Gastroenterology and Hepatology 4, 1 (July 2017): 89–94 © 2017 The Authors |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| As Published      | http://dx.doi.org/10.1016/J.JCMGH.2017.02.009                                                                                                                                                        |
| Publisher         | Elsevier BV                                                                                                                                                                                         |
| Version           | Final published version                                                                                                                                                                             |
| Citable link      | http://hdl.handle.net/1721.1/117671                                                                                                                                                                 |
| Terms of Use      | Creative Commons Attribution-NonCommercial-NoDerivs License                                                                                                                                           |
| Detailed Terms    | http://creativecommons.org/licenses/by-nc-nd/4.0/                                                                                                                                                     |
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.
**A** MM | PM

**B** [Images of cellular and molecular structures]

**C** Mist1/Lgr5

**D** [Images showing cellular and molecular structures with labels]

**E** Normal

**F** TFF2^IF^ pre-chief cell

**G** DMP-777 L-635 TAM Lgr5-DTR

**H** Acute injury (Mostly due to loss of chief cells)

**I** MUC6

**J** Ki67

**K** Clonal expansion
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 mid 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.9

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.2 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 SPEM-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.6 We conclude that the increase in TFF2+/IF+ cells likely represents expansion and migration 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 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.6

**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 isthmus 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.6 Consistent with this, ablation 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).5 In

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

| Evidence for chief cells | Evidence for Mist1+ isthmus stem cells |
|--------------------------|----------------------------------------|
| **Proliferation**        | None in normal state, and minimal (<5%) in injury | Normally slow proliferation, and marked expansion in injury |
| BrdU labeling            | No uptake in normal state, with rare labeling after injury (eg, 5-FU), but no upward migration | Labeled cells migrate bidirectionally from the isthmus upward toward the pits and downward to the base |
| Lineage tracing          | Troy-derived long-term tracing in haploinsufficient Troy knockin models, but not in Troy BAC models | Mist1-CreERT labels the isthmus stem cell as well as chief cells, and tracing arises from the isthmus stem cell |
|                          | 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 | 5-FU-induced stem cell ablation eliminates Mist1 tracing |
|                          | No evidence that chief cells expand clonally | Confetti mice show clonal expansion from the isthmus |
|                          | **DMP-777** | **Mist1+** isthmus stem cell–derived tracing is not altered after DMP-777 |
|                          | TFF2+/IF+ cells present in lower third of glands after treatment, but morphology of these SPEM cells is atypical and expansions are transient (<2 wk) | Human gastric metaplasia is stable and clonal with a field effect, indicating stem cell origins |
|                          | DMP-777 reduces Mist1-traced chief cell number | |
|                          | **Mutant Kras** | **Mucous-producing proliferating GS2/MUC6+ SEMP that is Alcian blue+ is present early only in the isthmus | |
|                          | Proliferating GS2+ IF+ cells at the base after 1 month | Gland fission is present early only in the isthmus |
|                          | eR1-CreERT with Kras mutation leads to occasional GS2+ metaplasia near the gland base | Lgr5-DTR–mediated ablation of chief cells does not reduce metaplasia, whereas suppression of the isthmus stem cell (with 5-FU) blocks metaplasia |
|                          | Most chief cells do not proliferate even after Kras induction at early time points, and are replaced rapidly by the migration of isthmus-derived SEMP cluster | Metaplasia in eR1-CreERT/LSL-Kras mice starts primarily in the isthmus |

BrdU, bromodeoxyuridine; 5-FU, 5-fluorouracil.
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+ 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 Mist1+ stem cell that leads to the production of long-term SPEM.

YOKU HAYAKAWA, MD, PhD
Department of Gastroenterology
Graduate school of Medicine
University of Tokyo
Tokyo, Japan

JAMES G. FOX, DVM, PhD
Division of Comparative Medicine
Massachusetts Institute of Technology
Cambridge, Massachusetts

TIMOTHY C. WANG, MD
Division of Digestive and Liver Diseases
Department of Medicine, Irving Cancer Research Center
Columbia University Medical Center
New York, New York
References

1. Wang TC, Goldenring JR, Dangler C, et al. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection. Gastroenterology 1998;114:675–689.

2. Fox JG, Rogers AB, Whary MT, et al. Accelerated progression of gastritis to dysplasia in the pyloric antrum of TFF2 -/- C57BL6 x Sv129 Helicobacter pylori-infected mice. Am J Pathol 2007;171:1520–1528.

3. Gutierrez-Gonzalez L, Graham TA, Rodriguez-Justo M, et al. The clonal origins of dysplasia from intestinal metaplasia in the human stomach. Gastroenterology 2011;140:1251–1260 e1–6.

4. Huh WJ, Khurana SS, Geahlen JH, et al. Tamoxifen induces rapid, reversible atrophy, and metaplasia in mouse stomach. Gastroenterology 2012;142:21–24 e7.

5. Nam KT, Lee HJ, Sousa JF, et al. Mature chief cells are cryptic progenitors for metaplasia in the stomach. Gastroenterology 2010;139:2028–2037.e9.

6. Hayakawa Y, Ariyama H, Stancikova J, et al. Mist1 expressing gastric stem cells maintain the normal and neoplastic gastric epithelium and are supported by a peri-vascular stem cell niche. Cancer Cell 2015;28:800–814.

7. Choi E, Hendley AM, Bailey JM, et al. Expression of activated Ras in gastric chief cells of mice leads to the full spectrum of metaplastic lineage transitions. Gastroenterology 2016;150:918–930.

8. Stange DE, Koo BK, Huch M, et al. Differentiated troy(+) chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. Cell 2013;155:357–368.

9. Nam KT, O’Neal RL, Coffey RJ, et al. Spasmolytic polypeptide-expressing metaplasia (SPEM) in the gastric oxyntic mucosa does not arise from Lgr5-expressing cells. Gut 2012;61:1678–1685.

10. Matsuo J, Kimura S, Yamamura A, et al. Identification of stem cells in the epithelium of the stomach corpus and antrum of mice. Gastroenterology 2017;152:218–231.

11. Karam SM, Leblond CP. Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. Anat Rec 1993;236:259–279.

Conflicts of interest
The authors disclose no conflicts.

Funding
The authors are supported by the National Institute of Health grant U54CA126853, R01CA093405, R01CA120979, R01DK052778, R35CA210088 (T.C.W.), T32OD010978, P30ES002109, and P01CA28842 (J.G.F.), the Clyde Wu Family Foundation (T.C.W.), the Project for Cancer Research And Therapeutic Evolution (P-CREATE) from the Japan Agency of Medical Research and Development, AMED, the Grant-in-Aid for Research Activity Start-up from Japan Society for the Promotion of Science, the Kobayashi Foundation for Cancer Research, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, and the Tokyo Society of Medical Sciences (Y.H.).

Most current article
© 2017 The Authors. Published by Elsevier Inc. on behalf of the AGA Institute. This is an open access article under the CC BY-NC-ND license [http://creativecommons.org/licenses/by-nc-nd/4.0/].

http://dx.doi.org/10.1016/j.jcmgh.2017.02.009