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REVIEW

The Origins of Gastric Cancer From Gastric Stem Cells:
Lessons From Mouse ModelsYoku Hayakawa,¹ James G. Fox,² and Timothy C. Wang³

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SUMMARY

In this perspective, we briefly summarize recent advances in our understanding of gastric stem cells and their link to gastric cancer. Although the discussion is focused largely on mouse experimental models and the gastric mucosa, we correlate experimental observations with human studies as well as findings from other cancer fields, most of which have pointed to stem cells as the origin of cancer. We also comment on the role of alterations in the stem cell niche as fundamental to the development of gastric cancer. Although other gastric cell types may possess some degree of cellular plasticity and could contribute to cancer development, accumulating evidence indicates that stem cells are the major cellular origin of most cancers, including gastric cancer.

The cellular origin of digestive cancers has been a long-standing question in the cancer field. Mouse models have identified long-lived stem cells in most organ systems, including the luminal gastrointestinal tract, and numerous studies have pointed to tissue resident stem cells as the main cellular origin of cancer. During gastric carcinogenesis, chronic inflammation induces genetic and epigenetic alterations in long-lived stem cells, along with expansion of stem cell niches, eventually leading to invasive cancer. The gastric corpus and antrum have distinct stem cells and stem cell niches, suggesting differential regulation of cancer initiation at the 2 sites. In this short review, we discuss recent experimental models and human studies, which provide important insights into the pathogenesis of gastric cancer. (*Cell Mol Gastroenterol Hepatol* 2017;3:331–338; <http://dx.doi.org/10.1016/j.jcmgh.2017.01.013>)

Keywords: Gastric Cancer; Stem Cell; Stem Cell Niche; Lgr5; Mist1; CCK2R.

Considerable data exist regarding gastric pre-neoplasia and early histopathologic changes that can progress to gastric cancer. Gastric cancer was linked strongly to chronic inflammation of the stomach, later recognized as being caused by *Helicobacter pylori* infection. However, gastric cancer takes many years to develop. Pathologic studies by Correa¹ have shown that intestinal-type gastric cancer develops through a series of

histopathologic stages that include chronic gastritis, atrophic gastritis, intestinal metaplasia (IM), dysplasia, and cancer. However, although there has been intense interest in the past in altered cellular differentiation or metaplasia, it is highly likely that gastric cancer, similar to other cancers, arises from aberrant stem cells through a process of field cancerization, the clonal expansion from multipotent stem cells that possess gene mutations,^{2–8} and thus a deep understanding of gastric carcinogenesis will require detailed elucidation of the role of gastric stem cells.

In organs such as the luminal gastrointestinal tract, which turn over rapidly and continuously, multipotent stem cells reside at the top of the self-renewal hierarchy and govern organ homeostasis.^{9–12} Gastrointestinal stem cells give rise to committed progenitor cells of the epithelial lineages, including proliferative transit-amplifying cells, which then give rise to fully differentiated epithelial lineages that are mostly nonproliferative. Because cancers arise only after the acquisition of multiple mutagenic events, long-lived cells would be the only cells capable of serving as reservoirs of mutated stem cells.^{4,13,14} Stem cells appear to be the ideal cellular targets for the accumulation of genetic alterations, given their fundamental properties of longevity and self-renewal.^{2,15} Mutations have been shown to be accumulated in stem cells,¹⁶ whereas mutations that occur in differentiated, postmitotic epithelial cells would not be propagated to progeny, and in general differentiated cells would not survive for the many decades required to achieve the mutational threshold for malignant transformation.¹⁴ In addition, studies in mice have suggested that mutations that occur in gastrointestinal stem cells more often lead to cancer,^{10,14,17–19} although cancer can arise when mutations are targeted to some long-lived differentiated cells, such as tuft cells.²⁰ Indeed, although there has been some debate as to whether the primary determinant of cancer risk at a particular organ site is the actual number of stem cells vs other extrinsic factors/environmental risk, there has been

Abbreviations used in this paper: CCK2R, cholecystokinin receptor 2; IM, intestinal metaplasia; SPEM, spasmolytic polypeptide-expressing metaplasia.

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little debate regarding the stem cell origins of cancer.^{21,22} Short-lived progenitors are able to interconvert into stem-like cells after tissue damage,^{10,23,24} but it remains unclear whether such interconverted cells can develop into cancer. In organs such as pancreas and liver that do not frequently divide in the normal state, cancers may arise from more differentiated acinar and hepatocyte compartments, but recent evidence has suggested the existence within these compartments of specific facultative reserve stem-like cells, which preferentially may contribute to regeneration and give rise to cancer.²⁵⁻³⁰ In any case, although it may be possible for non-stem cells to contribute to gastric regeneration and cancer, chronic inflammation associated with *H pylori* infection typically induces a regenerative and reparative response orchestrated by tissue resident stem cells,³¹ which are activated and expand as part of an injury response, predisposing to the acquisition of genetic and epigenetic alterations (Figure 1).

Nevertheless, exactly how cancer is initiated remains unclear. Traditionally, cancer initiation was thought to commence after mutation in an oncogene or tumor-suppressor gene, such as *APC* in the colon, and this may be true in a subset of colon cancers.³² However, in gastric cancer, The Cancer Genome Atlas (TCGA) and other studies have shown 4 distinct molecular subtypes, but have not defined a clear dominant mutational pathway.^{33,34} The different molecular subtypes could in theory represent different cellular origins, as has been postulated for colorectal cancer and other cancers,^{35,36} but this notion has not yet been supported experimentally. *TP53* is the most mutated gene found in approximately half of all gastric cancer patients, and a *TP53* mutation sometimes is observed even in early lesions.^{33,34,37,38} However, the role of *TP53* mutations in cancer initiation has not been fully elucidated.

Furthermore, more than 10% of gastric cancer cases have very few gene mutations, suggesting that genetic events may not be required for cancer initiation and progression.^{33,34,37} It is possible that in many tumors, epigenetic changes induced by chronic *Helicobacter* infection^{39,40} or other factors⁴¹ may play more important roles in triggering cancer initiation. There is strong evidence from analysis of human resection specimens that these mutations are established within stem cells in the gastric glands, and that clonal alterations can spread by means of gland fission.⁵⁻⁸

Mutations present in gastric dysplasia or cancer also can be found in adjacent IM, suggesting a shared clonal origin.⁵⁻⁸ Fifty years ago, researchers assumed that gastric dysplasia and cancer arose directly from IM cells. However, it now has become evident that IM is not highly proliferative and likely represents terminally differentiated, postmitotic cells⁴² (Figure 1). Indeed, in Barrett's esophagus, which likely originates from migrated gastric cardia glands,^{38,43-46} goblet cell differentiation is associated with reduced Notch signaling and reduced proliferation,^{46,47} and clinically a high goblet cell count is associated with a reduced risk of cancer.^{48,49} Greater focus has been given recently to another form of metaplasia called spasmolytic polypeptide-expressing metaplasia (SPEM), which appears more proliferative and shows a stronger association with gastric cancer.⁵⁰⁻⁵² SPEM usually is located closer to the stem cell zone, likely precedes IM, and probably represents a precursor of IM.⁴² However, evidence again suggests that SPEM does not give rise directly to dysplasia and cancer,^{42,53} but rather represents a fairly stable differentiated lesion that may in fact inhibit tumor progression^{54,55} (Figure 1). The cardinal marker for SPEM, the peptide Trefoil factor 2 (TFF2), is anti-inflammatory and tumor suppressive, and knockout of the *Tff2* gene leads to more rapid gastric cancer

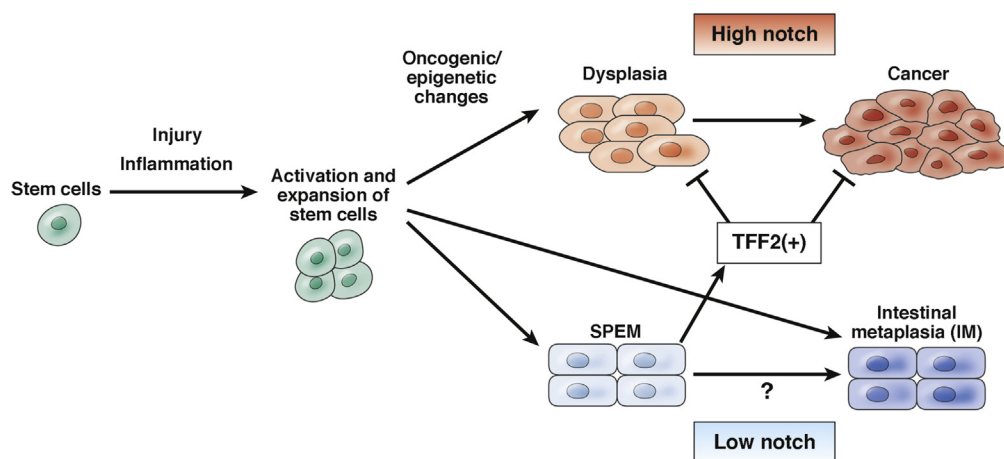


Figure 1. Model of stem cell-derived gastric carcinogenesis. During gastric carcinogenesis, long-lived stem cells and their niche are activated and expanded in response to tissue injury and inflammation. Activated stem cells give rise to metaplasia and dysplasia after accumulation of genetic and epigenetic changes. During Barrett's esophagus development, dysplasia can progress to cancer with high Notch expression, while metaplasia appears to be postmitotic and a distinct lineage with low Notch expression.⁴⁶ Given that Barrett's esophagus may originate from gastric cardia glands, this may be the case in gastric metaplasia/dysplasia development. Indeed, notch signaling has been shown to increase proliferation and decrease differentiation, and thus clearly regulate mucous cell phenotypes in the stomach.⁹⁹⁻¹⁰² Aberrant notch activation leads to hyperplasia and dysplasia both in the corpus and antrum.^{68,101,102} SPEM cells express TFF2, which inhibits cancer progression.^{54,55}

progression.^{54,55} At a population level, SPEM and IM almost certainly are risk factors for gastric cancer,^{38,50,52} given their association with *H pylori* infection and chronic atrophic gastritis, but this is not sufficient to assume that they are direct cellular precursors of cancer.³⁸ Indeed, the presence of SPEM and IM likely are risk factors for cancer because they reflect early changes to the pool of undifferentiated gastric stem cells that are the origins of cancer. Nevertheless, in our opinion, metaplasia is a distinct lineage from cancer, and greater attention should be given to the undifferentiated stem cells that are the direct source of cancer (Figure 1).

Gastric antral stem cells were the first epithelial stem cells characterized in the stomach. Earlier studies have suggested that stem cells reside at the isthmus in the antral glands, a region above the base where the glands narrow, with clear evidence of bidirectional migration from the stem cell zone.⁵⁶ Leucine-rich repeat-containing G-protein coupled receptor 5 (*Lgr5*) is a reported antral stem cell marker, and similar to the findings in the intestine, *Lgr5* was expressed at the base of the glands.¹² Although the location of *Lgr5*⁺ antral cells was not entirely consistent with the location of stem cells from earlier studies, *Lgr5*⁺ cells did overlap somewhat with the antral proliferative zone in the lower isthmus, and lineage-tracing studies with *Lgr5*-CreERT mice consistently showed tracing events of the antral glands. Recently, more rapidly cycling, long-lived stem cells were identified that express the gastrin receptor cholecystokinin receptor 2 (CCK2R).⁵⁷ These *Lgr5*-negative CCK2R⁺ antral stem cells are highly proliferative, divide on average once a day, and often are present as single

cells near the +4 position (Figure 2). Several other stem cell markers such as Sox2 or eR1 have been reported,^{58,59} but the possible overlap between these markers needs to be elucidated further.

In animal models, both *Lgr5*⁺ stem cells and CCK2R⁺ stem cells are able to interconvert with each other, and can give rise to cancer.^{12,60,61} Importantly, the expression of CCK2R in antral +4 stem cells potentially explains the gastrin-mediated effects on cancer progression in mouse models.⁶² In mice, increased levels of amidated gastrin promote proximal corpus carcinogenesis but inhibit antral cancer development, whereas knockout of gastrin promotes antral cancer development.^{41,63-65} In the antrum, amidated gastrin suppresses CCK2R⁺ stem cell activity⁵⁷; on the other hand, CCK2R-expressing stem cells in the cardia and progenitors in the corpus are activated by amidated gastrin⁴⁵ (Figure 2). Therefore, CCK2R modulates proximal and distal gastric stem cells in distinct ways, and thus cancer risk.⁶² In recent decades, there has been a dramatic epidemiologic shift in the location of gastric cancer, with a decrease in antral cancer and an increase in proximal corpus/cardia cancer.³⁸ These trends in theory could be related in part to increased levels of amidated gastrins, which might be facilitated by the frequent use of acid-suppressing drugs.

In contrast to the antral glands, the corpus glands are functionally and morphologically distinct, with very different stem cell populations (Figure 2). Earlier studies that included electron microscopy suggested the presence of undifferentiated, granule-free stem cells within the

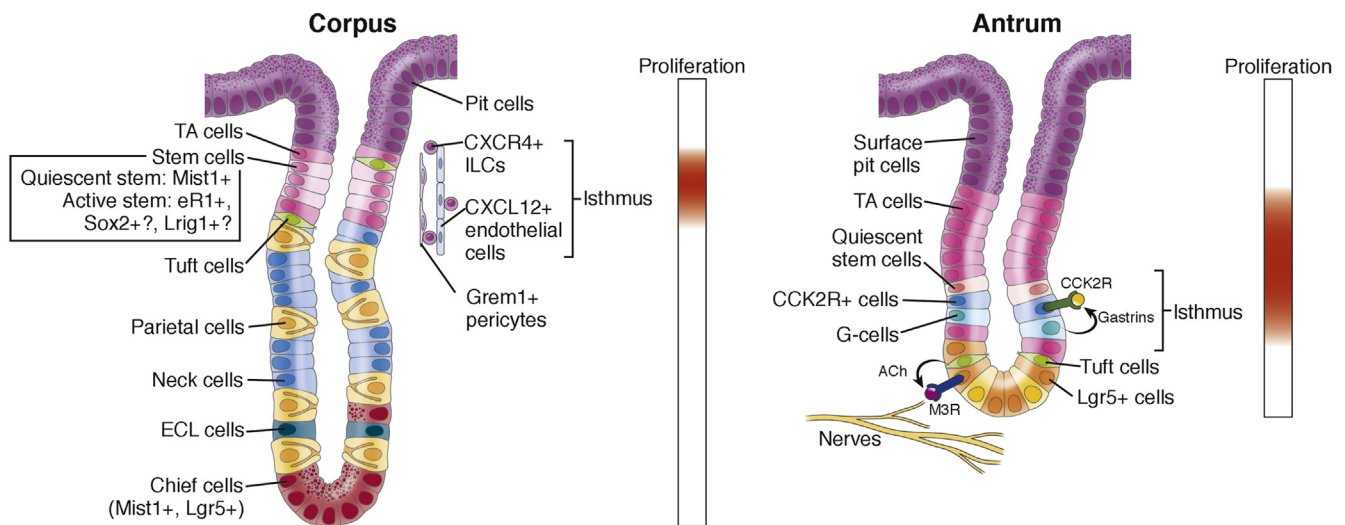


Figure 2. Gastric corpus and antrum stem cells and their niche. Stem cells are thought to reside within proliferative isthmus (upper part of the corpus glands and lower part of the antral glands). In the corpus glands, the isthmus is located between abundant transit-amplifying (TA) cells. There is a slow-cycling stem cell that expresses *Mist1* and a rapidly cycling stem cell that may express eR1, Sox2, or Lrig1, and they can differentiate into various cell types such as pit, parietal, neck, enterochromaffin-like (ECL), and tuft cells. C-X-C motif chemokine ligand 12 (CXCL12)⁺ endothelial cells, C-X-C motif chemokine receptor type 4 (CXCR4)⁺ innate lymphoid cells (ILCs), and Grem1⁺ pericytes appear to compose the corpus stem cell niche. Chief cells at the base express *Mist1* and *Lgr5*, but they normally are postmitotic and do not divide. In the antrum, several rapidly cycling stem cells are reported just below the major TA cell zone, including *Lgr5*⁺ cells at the base and CCK2R⁺ cells at +4. *Lgr5*⁺ cells can be activated by acetylcholine (ACh)-producing nerves and tuft cells through muscarinic acetylcholine receptor subtype 3 (M3R). Gastrins secreted from antral G cells can regulate CCK2R⁺ stem cell function in a paracrine manner. Slow-cycling stem cells in the antrum have not been identified yet.

corpus isthmus with bidirectional migration of progenitor and daughter cells.⁶⁶ *Lgr1* and *Sox2* may be expressed in a subset of corpus stem or progenitor cells,^{59,67} but to date these markers have not been linked directly to the granule-free stem cell. We recently found that *Mist1*, a marker of gastric chief cells, is expressed significantly within the corpus granule-free stem cell population.⁶⁸ Although *Mist1* protein expression is difficult to confirm in these isthmus stem cells, endogenous *Mist1* messenger RNA readily is detectable. *Mist1*⁺ isthmus cells are single, relatively slow-cycling stem cells, dividing on average every 5 days, and can serve as a cellular origin of gastric corpus cancer. Given that *Mist1* is expressed abundantly in 9–10 mature chief cells per gland, but present in only 1–2 *Mist1*⁺ isthmus stem cells per gland, *Mist1* is clearly not a specific stem cell marker. However, most stem cell markers are not specific for stem cells and label a heterogeneous cell population, and classically purification and characterization of stem cell populations have been achieved by flow cytometry using 4–5 markers, rather than relying on a single marker.

Importantly, lineage tracing in *Mist1*-CreERT mice clearly indicates that glands are traced from rare *Mist1*⁺ isthmus stem cells, not basal *Mist1*⁺ chief cells. This can be shown by ablating chief cells using *Lgr5*-DTR-GFP mice, which express diphtheria toxin receptor (DTR) and green fluorescent protein (GFP) in *Lgr5*⁺ cells,⁶⁹ and by ablating isthmus stem cells using treatment with 5-fluorouracil.⁷⁰ Lineage tracing in *Mist1*-CreERT mice is not reduced by chief cell ablation, but is inhibited by isthmus cell ablation.⁶⁸ It is perhaps not widely appreciated that although *Lgr5*-CreERT recombines in only a small subset of chief cells, *Lgr5* (and GFP) are expressed broadly at the base of oxyntic glands as shown in *Lgr5*-DTR-GFP mice,⁷⁰ (Figure 2). Therefore, ablation of *Lgr5*⁺ chief cells eliminated Cre-mediated recombination of chief cells by *Mist1*-CreERT, but did not reduce oxyntic gland tracing.

Recent studies have suggested a tissue stem cell model that comprises both rapidly cycling (active) and relatively slow-cycling (quiescent) stem cells, which can interconvert.⁷¹ Although *Lgr5*⁺ stem cells are reported to divide symmetrically and expand clonally through the neutral-drift model,^{72,73} our discovery of *Mist1*⁺ relatively quiescent gastric stem cells seems more consistent with the classic paradigm of rare master stem cells that maintain the glands.^{74–76} Earlier label-retaining studies in the intestine suggested a model of solitary +4-type stem cells that divide asymmetrically in the normal state, with symmetric division (leading to crypt fission) occurring only when there is a need to expand the stem cell population, such as in regenerative and carcinogenic states.^{75,76} Recent studies have suggested that such +4-type stem cells are present within the intestinal crypts, and may express several markers.^{77–79} In the gastric antrum, CCK2R⁺ cells are rapidly cycling stem cells, and thus there also may exist an additional adjacent, relatively slow-cycling antral stem cell. Similarly, in the corpus, it is probably the case that in addition to the slow-cycling *Mist1*⁺ isthmus stem cell, there also are rapidly cycling,

non-*Mist1* stem cells within the corpus glands (Figure 2). Recently, eR1⁺ cells have been described in the gastric isthmus, and these might represent active corpus stem cell,⁵⁸ although eR1 does not mark a solitary cell. Further characterization of the heterogeneous isthmus stem cell population is required.

Mist1⁺ corpus stem cells can serve as an origin of gastric cancer, including both intestinal and diffuse types. Diffuse-type gastric cancer was generated by knockout of the *Cdh1* gene in *Mist1*⁺ isthmus cells, in combination with *Helicobacter* species infection, whereas intestinal-type cancers were generated by knockout of *Apc* along with *Kras* mutation in *Mist1*⁺ cells.⁶⁸ Induction of a mutant *Kras* gene in *Mist1*⁺ corpus stem cells leads to the rapid development of SPEM, and the finding of SPEM in *Mist1*-CreERT;LoxP-Stop-LoxP (LSL)-*Kras* mice was confirmed by another group.⁸⁰

The stem cell niche is critical for the maintenance and control of tissue stem cells, maintaining their quiescence and promoting self-renewal, but stem cell niches can be altered and expanded after injury and inflammation.^{68,81–84} An activated niche can facilitate the development of cancer derived from stem cells, and we and others have defined important cellular components of the gastric stem cell niche, including myofibroblasts, nerves, endothelial cells, innate lymphoid cells, pericytes, tuft cells, and hormone-producing cells^{20,45,57,68,85–88} (Figure 2). Targeting these niche cells may be potentially useful in the inhibition of stem cell expansion and cancer development.

Gastric cancer develops after many years of chronic *H pylori* infection, in a chronically inflamed stomach characterized by atrophic glands and expanded stroma. Thus, alterations in specified niches precede the activation of gastric stem cells and the initiation of cancer. Although a number of mouse models of gastric cancer now have been described (please see Hayakawa et al⁸⁹ for a more complete summary), the *Helicobacter* infection model and the interleukin 1 β overexpression mouse model⁹⁰ have been used commonly to model inflammation-dependent gastric carcinogenesis. Given that *Rag2*^{-/-} immunodeficient mice have minimal *Helicobacter*-associated pathology,^{91,92} one could argue that *H pylori* induces cancer indirectly through recruitment of immune cells and induction of high levels of proinflammatory cytokines and oxidative stress.^{93–95} In particular, activation and recruitment of immature CD11b⁺Gr1⁺ myeloid cells,⁹⁰ along with cancer-associated fibroblasts,⁸⁷ are early events associated with malignant progression in the stomach.

Although *H pylori* can have direct effects on gastric epithelial cells, questions remain as to whether *H pylori* interacts directly with gastric stem cells to promote neoplasia. Given that the relative abundance of *H pylori* in the stomach markedly decreases with the development of atrophy and metaplasia, any direct effects of *H pylori* more likely occur during early preneoplasia rather than as a late event in carcinogenesis. Indeed, *Lgr5*⁺ antral stem cells can be activated by *H pylori* colonization at early histopathologic stages.³¹ An additional role for *H pylori* may be to induce gastric dysbiosis or bacterial overgrowth at later stages.

Chronic infection with *H pylori* and subsequent gastric atrophy lead to an increased gastric pH level, resulting in expansion of the microflora of the stomachs.^{96–98} This bacterial overgrowth, which can sustain a high level of chronic inflammation and oxidative stress, is associated significantly with late-stage progression to gastric cancer because the development of dysplasia in monoassociated *H pylori*-infected *Ins-Gas* mice was delayed significantly by housing the mice in otherwise germ-free conditions.^{97,98}

Finally, relatively novel contributors to the evolving cancer niche are neurons and their close cousins, tuft cells (Figure 2). Gastric stem cells are supported strongly by cholinergic signaling emanating in part from the vagus nerve acting through the muscarinic-3 receptor, and thus vagotomy markedly suppresses gastric cancer development in a variety of mouse models.⁸⁵ More recently, we have shown that both *Dclk1*⁺ tuft cells and nerves are important sources of acetylcholine within the gastric mucosa, and the cholinergic stimulation induces nerve growth factor expression, leading to an expansion of enteric nerves in the stomach, thus promoting gastric carcinogenesis.⁸⁶ Blockade of nerve growth factor signaling inhibits gastric tumor development, thus establishing the acetylcholine–nerve growth factor axis as a key component of the gastric cancer stem cell niche.⁸⁶ Comprehensive understanding of gastric stem cells and their niche is indeed a key to open up a new road for cancer therapy, and thus still needs to be intensely elucidated further.

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Conflicts of interest

The authors disclose no conflicts.

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