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Lrig1+ gastric isthmal progenitor cells restore normal gastric lineage cells during damage recovery in adult mouse stomach

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Abstract

Objective—Lrig1 is a marker of proliferative and quiescent stem cells in the skin and intestine. We examined whether Lrig1-expressing cells are long-lived gastric progenitors in gastric glands in the mouse stomach. We also investigated how the Lrig1-expressing progenitor cells contribute to the regeneration of normal gastric mucosa by lineage commitment to parietal cells after acute gastric injury in mice.

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Design—We performed lineage labelling using *Lrig1-CreERT2/+;R26R-YFP/+* (Lrig1/YFP) or *R26R-LacZ/+* (Lrig1/LacZ) mice to examine whether the Lrig1-YFP-marked cells are gastric progenitor cells. We studied whether Lrig1-YFP-marked cells give rise to normal gastric lineage cells in damaged mucosa using Lrig1/YFP mice after treatment with DMP-777 to induce acute injury. We also studied Lrig1-*CreERT2/CreERT2* (Lrig1 knockout) mice to examine whether the Lrig1 protein is required for regeneration of gastric corpus mucosa after acute injury.

Results—Lrig1-YFP-marked cells give rise to gastric lineage epithelial cells both in the gastric corpus and antrum, in contrast to published results that Lgr5 only marks progenitor cells within the gastric antrum. Lrig1-YFP-marked cells contribute to replacement of damaged oxyntic glands during the recovery phase after acute oxyntic atrophy in the gastric corpus. Lrig1 null mice recovered normally from acute gastric mucosal injury indicating that Lrig1 protein is not required for lineage differentiation. Lrig1+ isthmal progenitor cells did not contribute to transdifferentiating chief cell lineages after acute oxyntic atrophy.

Conclusions—Lrig1 marks gastric corpus epithelial progenitor cells capable of repopulating the damaged oxyntic mucosa by differentiating into normal gastric lineage cells in mouse stomach.

INTRODUCTION

The stomach is geographically demarcated into the corpus and antrum, which are distinguished by two distinct glands, oxyntic glands and antral glands. In the corpus glands, proliferative progenitor cells are located in the isthmal region near the gland lumen and give rise to short-lived surface mucous cell lineages that migrate toward the gland lumen and long-lived parietal, enteroendocrine and chief cell lineages that migrate toward the base.¹ Previous investigations have shown that acute or chronic gastric injury after non-steroidal anti-inflammatory drug administration or *Helicobacter pylori* infection in the corpus results in parietal cell loss and development of metaplasia.²⁻⁴ Severe gastric damage can in part be repaired by metaplastic lineages,^{5,6} and damaged oxyntic mucosa can also be repaired by committed gastric epithelial stem/progenitor cell differentiation into mature gastric epithelial cells.⁷ However, it is largely unknown which stem/progenitor cells are responsible for the repair of damaged gastric oxyntic mucosa after injury. Unlike other GI tract organs where stem cells reside at the base of crypts or glands, gastric epithelial stem cells in the mouse oxyntic glands are uniquely located in the neck region of oxyntic glands, designated the isthmus region, and the stem cell progeny migrate bi-directionally between the gastric lumen and the base of gland.^{7,8}

The stem cells in the isthmus of oxyntic glands give rise to committed stem cells such as surface cell progenitors or common progenitors (pregland) that later differentiate into parietal cells, mucus neck cells and chief cells.¹⁹⁻¹² Several groups have reported markers for gastric stem/progenitor cells that generate oxyntic gland lineages and/or antral gland lineage cells. TFF2 transcript-expressing cells represent a common gland progenitor (or pregland cells).¹² Sox2-expressing cells give rise to all gastric lineages including endocrine cells in the corpus and antrum.¹³ Recently, rare Mist1 transcript-expressing cells have been reported that can give rise to all corpus lineages.¹⁴ Lgr5-expressing cells represent long-lived progenitor cells in the gastric antrum, but they do not contribute to corpus gland cell lineage differentiation.¹⁵ Most recently, Matsuo *et al*¹⁶ have used an enhancer region of the

Runx1 promoter (eR1) to mark endogenous stem cells in both the corpus and antrum of the stomach.

Leucine-rich repeats and immunoglobulin-like domains 1 (Lrig1) is a pan-ErbB negative regulator that marks epithelial stem cells in the skin, small intestine and colon,¹⁷⁻¹⁹ as well as the interstitial cells of Cajal in the intestine and colon.²⁰ Lrig1 functions as a negative regulator of ErbB, Met and Ret receptor signalling²¹ and facilitates Egfr degradation.²² Consistent with this, Lrig1 null mice have heightened levels of Egfr and phosphorylated Egfr within the small intestine,^{17,18} and these mice develop spontaneous duodenal tumours, providing evidence that Lrig1 acts as a tumour suppressor in vivo. Proper function of the oxyntic mucosa of the stomach is regulated by the ErbB family ligands and receptors.²³ However, the control of this signalling to promote proper growth and cellular differentiation in both homeostasis and injury is incompletely understood. Intriguingly, short-term lineage tracing studies suggested that Lrig1+ progenitor cells may give rise to daughter cells within the oxyntic and antral mucosa.¹⁷ However, these studies did not evaluate the ability for Lrig1+ cells to act formally as stem cells in homeostasis or in injury states. Given the lack of markers for progenitor cells involved in damage recovery in the oxyntic mucosa, as well as the evidence for significant ErbB regulation in those glands, we sought to determine whether Lrig1-expressing cells are long-lived gastric stem/progenitor cells in the mouse stomach. We also examined how Lrig1-expressing cells contribute to the regeneration of normal gastric mucosa in the corpus by lineage commitment to parietal cells after acute gastric injury in mice.

RESULTS

Lrig1 marks long lived-gastric epithelial progenitor cells in mouse stomach

Previously, we have reported that Lrig1 lineage-labelled cells are detected in gastric epithelium.¹⁷ In this study, we performed short- and long-term lineage tracing to investigate whether Lrig1 messenger RNA (mRNA) expression marks gastric epithelial stem/progenitor cells and whether the Lrig1+ cells are capable of self-renewal. We crossed Lrig1-Cre^{ERT2} mice with either R26R-YFP or R26R-LacZ mice, and a single 2 mg dose of tamoxifen was administered to mice by intraperitoneal (IP) injection. Lrig1/YFP mice were sacrificed 1–40 days after tamoxifen injection, while Lrig1/LacZ mice were examined 1 year after induction of lineage labelling. At 1 day after tamoxifen treatment, we observed YFP+ cells both in the corpus and the antrum (figure 1A, arrows). At 3–40 days after tamoxifen treatment, the YFP+ cells were present in the proliferative isthmus zone in the corpus mucosa (figure 1B), as well as in surface cells and parietal cells in the oxyntic glands. We continued to detect β -gal+ cells at 1 year after tamoxifen treatment, and β -gal staining was observed along the entire length of oxyntic glands. In the antrum, at 3 days after tamoxifen treatment, the YFP+ cells were localised to the base region of antral glands where the antral gland progenitor cells are located (figure 1B). At 10 and 40 days, the YFP+ cells were observed extending throughout the entire antral glands, and we observed β -gal-labelled antral glands even 1 year after tamoxifen treatment. Thus, Lrig1-YFP+ cells in the corpus divide more slowly than in the antrum, but the Lrig1-YFP+ cells both in the corpus and the antrum show characteristics of long-lived stem/progenitor cells.

To characterise what types of gastric epithelial lineages were derived from Lrig1+ cells in the oxyntic glands, we coimmunostained with antibodies against gastric lineage markers as well as YFP or β -gal. YFP+ cells in the gland isthmus were positive for Ki-67 at 10 and 40 days after tamoxifen treatment, and β -gal+ cells were positive for Ki-67 at 1 year after tamoxifen treatment (figure 1C, white arrows), indicating that the Lrig1-YFP/ β -gal marked cells are capable of long-term self-renewal. The YFP or β -gal+ cells also colocalised with gastric epithelial lineage cells, such as H/K-ATPase-positive parietal cells (figure 1C, yellow arrows) and GS-II lectin-positive mucus neck cells (figure 1C, red arrows), as well as *Ulex europaeus* agglutinin I (UEAI)-positive surface cells, intrinsic factor (IF)-positive chief cells, chromogranin A-positive endocrine cells, antral gastrin cells and doublecortin-like kinase 1 (Dclk1)-expressing tuft cells at 10 days after tamoxifen induction (figure 2A). These results demonstrate that Lrig1-YFP-marked cells in the gastric corpus and antrum self-renew under normal conditions and have the capacity to differentiate into all of the gastric cell lineages.

To assess whether these Lrig1-YFP-marked cells are present at birth and can give rise to adult lineages, we lineage traced Lrig1/LacZ mice from postnatal day 1 and examined adult stomach tissue for the presence of β -gal staining. We found multiple glands throughout the stomach that were β -gal labelled (figure 2B), suggesting they arose from Lrig1+ stem cells present at birth and that these cells continue to support gland formation throughout development to adult oxyntic glands.

Lrig1-YFP-marked cells reconstitute gastric epithelial mucosa after acute oxyntic atrophy

We used DMP-777, which is a parietal cell-specific protonophore, to induce reversible acute oxyntic atrophy. Fourteen days after induction of YFP expression in Lrig1/YFP mice, we administered DMP-777 for 3 days and analysed the mouse stomachs 7 days after DMP-777 withdrawal to examine whether the Lrig1-YFP-marked progeny gives rise to differentiated gastric epithelial lineage cells, especially parietal cells, after oxyntic atrophy (figure 3A). As expected, DMP-777 caused significant loss of parietal cells compared with untreated mice (figure 4A). This atrophy was reversed over the course of 1 week (figures 3B and 4A). The total number of glands with YFP+ cells increased significantly following DMP-777 treatment (figure 3C). Additionally, we observed a further significant increase in YFP+ lineage-traced glands during the recovery phase (figure 3C and E), suggesting that the Lrig1-YFP-marked progenitor cells are dividing in response to atrophy in the recovery phase. To examine progenitor behaviour further, we evaluated the YFP+ cells in the oxyntic mucosa of untreated, DMP-777-treated and recovery mice for expression of the proliferative marker, Ki-67. In untreated mice, the YFP+ cells rarely expressed Ki-67 (figure 3D). However, in recovery, the number of cells coexpressing YFP and Ki-67 significantly increased compared with untreated mice (figure 3D and F). In addition, the marked YFP+ cells coexpress H/K-ATPase, GS-II lectin or IF in the recovery phase (figure 4A, B), suggesting that the Lrig1-YFP-marked cells can give rise to all of the gland lineages during repair.

Lrig1-YFP-marked cells differentiate into parietal cells during recovery phase of acute oxyntic atrophy

To confirm that Lrig1-YFP-marked cells participate in regeneration of the entire gland, we performed lineage tracing in Lrig1/YFP mice by administering tamoxifen after 3 days of

DMP-777 treatment to trace the Lrig1-YFP-marked cell progeny in the recovery phase after acute oxyntic atrophy. Then, we sacrificed the Lrig1/YFP mice on the next day or 5 days after tamoxifen treatment (figure 5C). We still observed parietal cell loss on the first day after 3 days of DMP-777 treatment, and the remaining parietal cells were scattered through the middle and base portion of the oxyntic glands.

Previous studies have shown that CD44 variant 9 (CD44v9) is absent from the normal stomach, but basolateral staining for CD44v9 is upregulated in metaplastic cells.²⁴²⁵ Basolateral CD44v9 was detected in the earliest stages of initiation of transdifferentiation of chief cells at only 1 day following the induction of acute parietal cell loss with DMP-777 (figure 6). In addition, we observed further upregulation of CD44v9 expression after 3 days of DMP-777 treatment (figure 6). Scattered proliferating cells were also present in the base of the glands where the CD44v9-expressing chief cells in the early stages of transdifferentiation were located (figure 5A). However, we only rarely observed YFP+ cells throughout the corpus area in the Lrig1/YFP mouse stomachs after 3 days of DMP-777 treatment.

Five days after withdrawal of DMP-777, the corpus mucosa was repopulated with many parietal cells and GS-II lectin+ mucus neck cells were normally located in the neck area of the glands (figure 5B). After 5 days of recovery from acute oxyntic atrophy, YFP+ cells were increased about 30-fold compared with day 1 (figure 5D). Groups of YFP+ cells were co-positive for either Ki-67 (figure 5B, yellow arrow) or the parietal cell marker, H/K-ATPase (figure 5B, white arrows). Importantly, about 65% of YFP+ cells coexpressed H/K-ATPase, indicating that the Lrig1-YFP-marked stem/progenitor cells rapidly differentiate into parietal cells during the recovery phase to restore the damaged oxyntic glands with normal gastric lineage cells. Additionally, CD44v9+ chief cells still were observed at the base portion of the glands, but they were negative for YFP, indicating that Lrig1-Cre was not expressed in chief cells transdifferentiating into spasmolytic polypeptide-expressing metaplasia (SPEM) cells.

Lrig1 protein is not required for regeneration of gastric corpus mucosa after oxyntic atrophy

We next addressed whether the Lrig1 protein plays a direct role during recovery after acute parietal cell loss by DMP-777 treatment. We treated Lrig1 heterozygous (Lrig1 Het) or Lrig1 null (Lrig1 knockout (KO)) mice with DMP-777 for 3 days. DMP-777 treatment led to parietal cell loss and foveolar hyperplasia in the gastric corpus mucosa in both Lrig1 Het and KO mice (R1 in figure 7A). Lrig1 Het or KO mouse stomachs showed that surface cells (UEAI) and mucus neck cells (GS-II lectin) were still present after DMP-777 treatment, but H/K-ATPase staining parietal cells were lost (figure 7A). Also, we observed cells staining for both CD44v9 and GS-II, consistent with induction of transdifferentiation in chief cells. We next examined the Lrig1 Het or KO mouse stomachs after withdrawal of DMP-777 for 5 days. H&E staining revealed that the damaged gastric mucosa had recovered reflected by normal parietal cell numbers in both Lrig1 Het and KO mice (figure 7A). The number and cellular morphology of the parietal cells following recovery in Lrig1 KO stomachs were similar to Lrig1 Het stomachs (figure 7A and B).

As Lrig1 functions as a negative regulator of ErbB signalling, we next evaluated the oxyntic mucosa in both wild-type and Lrig1 KO mice for total levels of Egfr or Her2 and downstream modulators. We first examined Lrig1 protein expression in normal gastric mucosa. The Lrig1 protein was detected both in the corpus and antrum (figure 8A), and some of Lrig1 protein-expressing cells costained for Ki-67 (figure 8A, arrows). We also confirmed that Lrig1 mRNA expression by in situ hybridisation remained in the isthmal progenitor cell region both in the corpus and the antrum, as well as in the base region of the oxyntic glands in the corpus (online supplementary figure S1). In the corpus, most of Lrig1 protein-expressing cells were costained for H/K-ATPase, a parietal cell marker, but not for ghrelin, Dclk1, IF or GS-II lectin (figure 8B). We detected loss of Lrig1 protein in Lrig1 KO mice, as expected (online supplementary figure S2). Overall, our results suggest that Lrig1 protein does not exert a major influence on regeneration of gastric corpus mucosa after acute oxyntic atrophy.

DISCUSSION

We have identified that the pan-ErbB-negative regulator Lrig1 marks isthmal progenitor cells in the gastric corpus and antrum, which give rise to differentiated daughter cells in homeostasis and in recovery after short-term injury. Our findings support two important conclusions: First, our data show that Lrig1-YFP⁺ cells represent bona fide progenitor cells in the corpus. Lrig1-YFP-marked cells give rise to all lineages within the oxyntic epithelium during homeostasis. Second, previous studies have reported that Lrig1 expression is restricted to intestinal epithelial stem/progenitor cell populations.¹⁷¹⁹ In contrast, our data also show that Lrig1 mRNA and protein mark both progenitor cells and differentiated parietal cells, implying roles for Lrig1 in both stem and differentiated epithelial compartments.

Lrig1-YFP- or Lrig1-LacZ-marked progenitor cells are long-lived and play critical roles in gastric biology

While Lrig1 mRNA and protein were detected by in situ hybridisation and immunostaining, respectively, the Lrig1/YFP mouse marked only isthmal progenitors in the corpus. Our ability to evaluate the lineage mapping of Lrig1-YFP-marked or Lrig1-LacZ-marked isthmal progenitors more specifically stems from the fortuitous lack of lineage marking of gastric parietal cells (see figure 1A). While the reason for this lack of parietal cell lineage marking is not clear, the specific marking of isthmal cells has allowed us to use the Lrig1/YFP mouse to evaluate dynamics of lineage differentiation from these putative progenitor cells. Thus, our lineage tracing data show that Lrig1-YFP-marked isthmal cells contribute to tissue maintenance in the oxyntic and antral glands, even up to 1 year after genetic marking with YFP. Our data provide evidence that stem/progenitor cells in the oxyntic mucosa persist for at least 1 year and can still actively give rise to daughter cells. The rate of turn-over or the overlap of these long-lived cells with other suggested markers of corpus stem cells, like Sox2- or Tff2-transcriptional unit-expressing cells, is unknown. In future studies, it will be important to examine these cells in the context of these markers, both in homeostasis and during oxyntic injury.

A notable finding was the expression of Lrig1 protein in both stem/progenitor cells and parietal cells, implying possible roles for Lrig1 protein in both cell types. Lrig1 is a pan-ErbB-negative regulator. Parietal cells express high levels of Egfr and Tgfa,²³²⁶ and these previous studies have suggested that Tgfa may act as a tonic autocrine suppressor of parietal cell acid secretion. It will be interesting to investigate if Lrig1 has a role in such autocrine regulation, since activated Egfr can also increase ligand production.²⁷ Interestingly, the Lrig1 KO mouse did not display any other tissue defects during homeostasis or injury recovery. One possible interpretation is that another ErbB-negative regulator or potentially another Lrig family member is acting in a compensatory role both in homeostasis and in injury in null animals. Further studies are needed to evaluate a functional role for another ErbB-negative regulator in these null animals. In the future, it will also be important to perform these studies using inducible Lrig1 KO mice, as this approach will allow for more temporal resolution examining how loss of Lrig1 affects both gastric stem and parietal cells, which may shed light on the role of Lrig1 protein in the stomach.

Lrig1+ isthmal progenitor cells in injury and neoplasia

In human gastric cancer, preneoplastic metaplasias, such as SPEM and intestinal metaplasia, are considered as an origin of gastric adenocarcinoma, and the preneoplastic metaplasias are initiated by oxyntic atrophy (parietal cell loss) and foveolar hyperplasia after chronic *H. pylori* infection.²⁸ Previous studies have reported that Lrig1 is associated with many types of cancer development. Differential expression of Lrig1 has been correlated with decreased prognosis in ovarian, pituitary and squamous cell adenocarcinomas and non-small cell lung cancer,²⁹⁻³² and Lrig1 modulates EMT behaviour in breast cancer.³³ Furthermore, the mutation rate of Lrig1 was detected as 3% in gastric cancer and loss of Lrig1 led to gastric metaplasia in the proximal duodenum.³⁴³⁵

We and others have previously demonstrated that SPEM originates from transdifferentiation of chief cells.²⁴³⁶ However, a recent investigation has suggested that isthmal progenitor cells may be responsible for the development of metaplastic lineages rather than chief cells.¹⁴ In contradistinction to that report, the results presented here indicate that Lrig1-YFP-marked isthmal progenitor cells do not give rise to metaplastic lineages marked by CD44v9 expression. These findings are consistent with the results of Matsuo *et al.*,¹⁶ who demonstrated that isthmal progenitors gave rise to foveolar hyperplasia, but not to SPEM. The data therefore indicate that the origin of metaplastic lineages from transdifferentiation of chief cells is distinct from the function of isthmal Lrig1-YFP-marked cells. It remains to be determined whether Lrig1-expressing stem cells in the stomach are identical to the eR1-expressing stem cells identified by Matsuo *et al.*¹⁶

In summary, our studies have identified long-lived Lrig1-expressing progenitor cells in the isthmus of the gastric corpus mucosa glands that give rise to all gastric lineages and can respond to acute injury. Further studies will be required to examine the role of Lrig1-expressing progenitor cells in reparative events following chronic injury.

METHODS

Mouse treatments

The generation of *Lrig1-CreERT2*^{+/+}, *Lrig1-CreERT2/CreERT2* (*Lrig1*^{tm1.(cre/ERT)Rjc}) mice has been described previously.¹⁷ To trace the lineages of *Lrig1*-expressing cells, *Lrig1-CreERT2*^{+/+} mice were bred with *R26R-YFP*^{+/+} (*Lrig1*/YFP) or *R26R-LacZ*^{+/+} (*Lrig1*/LacZ) mice. For adult studies, 2 mg of tamoxifen (Sigma) in corn oil, one dose, was administered to 8-week-old male/ female *Lrig1*/YFP mice by IP injection and sacrificed at the time points indicated in the results. For postnatal studies, 30 mg/kg of tamoxifen (Sigma) in corn oil, one dose, was administered to postnatal day 0 (p0) *Lrig1*/LacZ mice by IP injection and sacrificed at the time points indicated in the results. Preparation and treatment of mice with DMP-777 have been described previously.³⁷ DMP-777 was administered to 8-week-old *Lrig1*/YFP mice orally as a gavage treatment (350 mg/kg/day) once daily for 3 days beginning 14 days after tamoxifen treatment and sacrificed on the next day or 7 days after DMP-777 administration. DMP-777 was also administered to *Lrig1-CreERT2/CreERT2* (*Lrig1* KO) mice for 3 days and sacrificed on the next day or 5 days after DMP-777 administration. For the reversal study, DMP-777 was administered to 8-week-old *Lrig1*/YFP mice orally as a gavage treatment once daily for 3 days, and the 2 mg of tamoxifen was administered on the third day of DMP-777 administration and sacrificed 1 day or 5 days after tamoxifen treatment. The care, maintenance and treatment of mice used in this study followed protocols approved by the Institutional Animal Care and Use Committees of Vanderbilt University and the University of Oregon.

Tissue preparation and antibody staining

Tissue preparation and subsequent staining of frozen and formalin-fixed, paraffin-embedded (FFPE) tissues were performed as previously described.¹⁷ For FFPE tissues, sections were deparaffinised in HistoClear (Electron Microscopy Services) and rehydrated in a series of ethanol washes; then antigen retrieval was performed using target retrieval solution, pH 6 (Dako), using a pressure cooker. After incubating paraffin sections in serum-free protein block solution (Dako) at room temperature for 1.5 hours, primary antibodies diluted in antibody diluent with background reducing components (Dako) were incubated at 4°C overnight. The primary antibodies used for immunostaining are listed in the online supplementary table 1. Primary antibodies were detected with Alexa-fluor (Invitrogen) secondary antibodies or Dako Envision+ System-HRP DAB (Dako North America). 4',6-Diamidino-2-phenylindole (DAPI) was used to detect nuclei in immunofluorescence images. All fluorescence images were acquired using a Zeiss Axio Imager M2, equipped with a SPOT Explorer camera and using SPOT Basic software. Image overlay and preparation were performed in Adobe Photoshop.

RNA scope of *Lrig1*

In situ hybridisation for *Lrig1* was performed on 4% paraformaldehyde-fixed, paraffin-embedded normal mouse stomach tissue sections using the RNAscope Fluorescent Multiplex Kit according to the manufacturer's instructions (Advanced Cell Diagnostics). Negative control probe supplied by Advanced Cell Diagnostics was also hybridised as a

negative control. Nuclei were counterstained with DAPI, and surface cells were counterstained with fluorescein isothiocyanate-conjugated UEAI.

Statistics

For quantitation of YFP+ cells, three to eight representative images were taken from a section of each mouse stomach of biological replicates at 20× magnification. We defined a zone of corpus in the proximal mouse stomach based on the distribution of oxyntic glands, about 4–5 mm of corpus zone from the first gland of the proximal stomach, and considered the entire area of the corpus zone to analyse the YFP-expressing cells. The captured images were analysed using manually counted data. The average numbers of the YFP-expressing cells per gland in each representative image were used to obtain an average value of the YFP-expressing cells per gland for each mouse. The average values from each mouse were then compared by Mann-Whitney U test for dual comparisons or for multiple biological replicate groups by Kruskal-Wallis test with post hoc comparison of significant means with Dunn's test ($p < 0.05$ for significance).

To quantify the number of parietal cell number in the corpus of *Lrig1*-Het or *Lrig1*-KO mouse stomachs at 1 or 5 days after DMP-777 treatment for 3 days, H&E-stained stomach tissue sections from three mice per each group were imaged on the Leica SCN400 Slide Scanner. Three representative images in the corpus were taken from the scanned image of each mouse stomach of biological groups at 16× magnification, and parietal cells were manually counted in the entire mucosal area. Parietal cells per field were analysed between 1- and 5-day recovery groups by Mann-Whitney U test ($p < 0.05$ for significance).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Significance of this study

What is already known on this subject?

- ▶ Lrig1 marks proliferative and quiescent stem cells in the skin and intestine.
- ▶ Damaged oxyntic mucosa can be repaired by committed gastric epithelial progenitor cell differentiation.
- ▶ Lgr5-expressing cells do not contribute to corpus gland cell lineage differentiation in the stomach.

What are the new findings?

- ▶ Lrig1-YFP expression in mice marks long-lived gastric progenitor cells.
- ▶ Lrig1-YFP-expressing isthmal cells contribute to gastric corpus epithelial mucosa regeneration after acute oxyntic injury.
- ▶ Lrig1 protein is not required for regeneration of gastric mucosa after oxyntic atrophy.

How might it impact on clinical practice in the foreseeable future?

- ▶ Identification of discrete stem cell markers in the antrum and corpus of the stomach may allow future development of targeted therapies to promote more rapid healing in the gastric mucosa.

Figure 1.

Lineage tracing of Lrig1-expressing cells. (A) Immunostaining for YFP (green) and Ki-67 (red) at 1 day following a single intraperitoneal injection of 2 mg of tamoxifen in 8-week-old Lrig1/YFP (Lrig1-Cre^{ERT2/+};R26R^{YFP/+}) mouse lineage-labelled stomachs. Arrows indicate the lineage-labelled cells in the isthmus region in the corpus at 1 day after tamoxifen injection. (B) Immunostaining for YFP at 3, 10, 40 days and 1 year following tamoxifen treatment in Lrig1/YFP (Lrig1-Cre^{ERT2/+};R26R^{YFP/+}) or Lrig1/LacZ (Lrig1-Cre^{ERT2/+};R26R^{LacZ/+}) lineage-labelled stomachs. (C) The YFP/LacZ+ glands in the corpus of Lrig1/YFP (Lrig1-Cre^{ERT2/+};R26R^{YFP/+}) or Lrig1/LacZ (Lrig1-Cre^{ERT2/+};R26R^{LacZ/+})

mice were coimmunostained with antibodies against Ki-67 (proliferating cells, white arrows), H/K-ATPase (parietal cells, yellow arrows) and GS-II lectin (neck cells, red arrows). 4',6-Diamidino-2-phenylindole was used for nuclear staining. Boxes indicate regions enlarged. Scale bars 100 μ m.

Figure 2.

Lrig1 lineage labelling in the gastric corpus. (A) Lrig1/YFP lineage traced corpus sections of adult mouse stomachs at 10 days after tamoxifen injection displaying YFP and differentiation marker costaining cells. Panels (a) and (b) display YFP and *Ulex europaeus* agglutinin I (UEAI; red, arrow) or intrinsic factor (IF; blue, arrow) co-positive cells, respectively. Panel (c) displays a YFP and chromogranin A (CGA; red, arrow) co-positive cell. Panel (d) displays a YFP and gastrin (red, arrow). Panel (e) displays a YFP and doublecortin-like kinase 1 (red, arrow) co-positive cell. Scale bars represent 100 μm . (B) Representative low-power view of Lrig1/LacZ lineage-labelled stomach at 1 year following a single intraperitoneal injection of 2 mg tamoxifen at postnatal day 0. Blue indicates lineage-labelled glands in both the corpus and antrum. (a) Higher power view of transition zone labelling. (b, c) Higher power view of corpus labelling. Scale bars represent 500 μm in low power and 50 μm in high power.

Figure 3.

Lineage tracing of Lrig1-expressing cells during and after acute oxyntic atrophy. (A) DMP treatment schematic. At 10 days after tamoxifen (TAM) treatment, Lrig1/YFP mice were examined after 3 days of DMP-777 treatment or 1 week after cessation of DMP-777. (B) H&E-stained stomachs from untreated mice (10 days after tamoxifen), mice treated with DMP-777 for 3 days or mice 1 week after DMP-777 treatment (Recovery). (C) Quantitation of YFP+ glands. D, DMP-777 treated; R, Recovery (1 week after DMP-777 treatment); U, untreated. Quantitation was performed per analysed image (n=3, *p<0.05). (D) The YFP+ glands in the corpus were coimmunostained with antibodies against Ki-67 (proliferating cells). 4',6-Diamidino-2-phenylindole was used for nuclear staining. Boxes indicate regions

enlarged. Scale bars 100 μ m. (E, F) Quantitation of YFP⁺ cells or dual YFP⁺/Ki-67⁺ cells per gland in U, D or R mouse stomachs (n=3, *p<0.05). Error bars indicate SD.

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Figure 4.

Lrig1-expressing cells give rise to parietal, mucous neck and chief cells after DMP-777 treatment. (A) In untreated lineage-traced Lrig1/ YFP mice (left panel), co-positive YFP (green) and H/K-ATPase (red) are occasionally observed; however, YFP does not colocalise with GS-II lectin (blue). Immediately following DMP-777 treatment (middle panel), fewer H/K-ATPase-expressing cells are observed. In the recovery phase after DMP-777 treatment (right panel), more H/K-ATPase-expressing cells are observed, and the YFP+ lineage-traced cells also expressed both H/K-ATPase and GS-II lectin. (B) In untreated lineage-traced

Lrig1/YFP mice (left panel), costaining YFP (green) and intrinsic factor (IF, red) were occasionally observed; however, YFP did not colocalise with IF immediately following DMP-777 treatment (middle panel). In the recovery phase after DMP-777 treatment (right panel), YFP+ lineage-traced cells also expressed IF. Images are from the same tissue generated for figure 3. All scale bars represent 100 μ m.

Figure 5. Lrig1+ cells actively give rise to all cell lineages of the corpus in response to acute DMP-induced injury. (A) DMP treatment schematic. Lrig1/YFP mice were treated with tamoxifen at the end of 3 days of DMP-777 treatment, and the mice were examined on the next or 5 days after TAM injection. R1, recovery for 1 day; R5, recovery for 5 days (1 day or 5 days after TAM injection). (B, C) The YFP+ glands in the corpus of Lrig1/YFP mice were coimmunostained with antibodies against H/K-ATPase (parietal cells), GS-II lectin (neck cells), Ki-67 (proliferating cells) and CD44v9 (metaplastic cells). 4',6-Diamidino-2-phenylindole was used for nuclear staining. Boxes indicate regions enlarged. Note that CD44v9 staining at the lumen surface represents non-specific staining of surface gastric mucin. Scale bars 100 μ m. (D) Quantitation of YFP+ cells per 20 $\times$ field during recovery. (E)

Quantitation of YFP+ or YFP+H/K-ATPase+ (YFP+ parietal cells) cells per 20× field at 5 days after tamoxifen injection (n=3). Error bars indicate SD.

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Figure 6.

Staining of transdifferentiation-initiating chief cells with CD44v9 antibody in DMP-777-treated mouse stomachs. Immunostaining for intrinsic factor (IF, a chief cell marker, green), CD44v9 (red) and GS-II lectin (a mucus neck cell or metaplastic cell marker, blue) in untreated mouse stomach or mouse stomachs treated with DMP-777 for 1 day or 3 days. Metaplastic cells are co-positive for IF and GS-II lectin. CD44v9 is not observed in untreated normal mouse stomach corpus, but it is observed in transdifferentiating chief cells, which are co-positive for GS-II lectin, after DMP-777 treatment for 1 day and 3 days (arrows).

Figure 7.

Examination Lrig1 in stomach of Lrig1 knockout (KO) mice compared with wild-type mice. (A) Lrig1 Het (Lrig1-^{CreERT2/+}) or Lrig1 KO (Lrig1-^{CreERT2/CreERT2}) mice were treated with DMP-777 for 3 days and sacrificed on the next day or after 5 days of treatment.

Immunohistochemistry for gastric lineage markers, *Ulex europaeus* agglutinin I (UEAI, surface cells), GS-II lectin (neck cells) and H/K-ATPase (parietal cells), as well as a metaplasia-specific marker, CD44v9. (B) Quantitation of parietal cells per field in Lrig1 Het and Lrig1 KO mice 1 day (R1) and 5 days (R5) after cessation of DMP-777 treatment. Both Het and KO mice recovered their parietal cell mass after 5 days of recovery (*p=0.05 compared with respective R1 animals).

Figure 8.

Lrig1 protein expression is expressed by both parietal cells and proliferative cells in the gastric corpus. (A) Lrig1 expression (green) colocalises with the proliferative marker, Ki-67 (red), in both the corpus (upper panel) and antrum (lower panel). Arrowheads indicate Lrig1 and Ki-67 double-positive cells. (B) Lrig1+ expression in the corpus colocalises with the parietal cell marker, H/K-ATPase (top left, arrowheads), but it does not colocalise with ghrelin, doublecortin-like kinase-1 (Dclk1), gastric intrinsic factor (IF) or GS-II lectin (all shown in red). Scale bars in A and B represent 50µm.