TFF2 mRNA Transcript Expression Marks a Gland Progenitor Cell of the Gastric Oxyntic Mucosa

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BACKGROUND & AIMS: Gastric stem cells are located in the isthmus of the gastric glands and give rise to epithelial progenitors that undergo bipolar migration and differentiation into pit and oxyntic lineages. Although gastric mucus neck cells located below the isthmus express trefoil factor family 2 (TFF2) protein, TFF2 messenger RNA transcripts are concentrated in cells above the neck region in normal corpus mucosa, suggesting that TFF2 transcription is a marker of gastric progenitor cells. **METHODS:** Using a BAC strategy, we generated a transgenic mouse with a tamoxifen-inducible Cre under the control of the TFF2 promoter (TFF2-BAC-Cre^{ERT2}) and analyzed the lineage derivation from TFF2 mRNA transcript–expressing (TTE) cells. **RESULTS:** TTE cells were localized to the isthmus, above and distinct from TFF2 protein-expressing mucus neck cells. Lineage tracing revealed that these cells migrated toward the bottom of the gland within 20 days, giving rise to parietal, mucous neck, and chief cells, but not to enterochromaffin-like-cell. Surface mucus cells were not derived from TTE cells and the progeny of the TTE lineage did not survive beyond 200 days. TTE cells were localized in the isthmus adjacent to doublecortin CaM kinase-like–1$^+$ putative progenitor cells. Induction of spasmolytic polypeptide-expressing metaplasia with DMP-777–induced acute parietal cell loss revealed that this metaplastic phenotype might arise in part through transdifferentiation of chief cells as opposed to expansion of mucus neck or progenitor cells. **CONCLUSIONS: TFF2 transcript–expressing cells are progenitors for mucus neck, parietal and zymogenic, but not for pit or enterochromaffin-like cell lineages in the oxyntic gastric mucosa.**

Keywords: Trefoil Factor Family; Stem Cell; Oxyntic Lineage; Parietal Cell.

Tissue stem cells are difficult to identify morphologically and not easily distinguished from other epithelial cells. They are characterized by multipotentiality and the ability to self-renew. Tissue stem or progenitor cells reside within a "niche," an area providing an optimal microenvironment for normal differentiation.[1] Stem cells are present in small numbers and are thought to remain largely quiescent or undergo division at a very slow rate, such that they are negative for proliferation markers. Instead, proliferation markers such as Ki67, proliferating cell nuclear antigen, or 5-bromo-2-deoxyuridine label transit-amplifying progenitor cells, immediate descendents of stem cells are located adjacent to the stem cells. Transit-amplifying progenitor cells divide quickly and are responsible for the bulk of cell division, but seem to have a limited lifespan and are replaced periodically by descendents of the true stem cell. However, recent data suggest that some classes of intestinal stem cells may be actively dividing with a higher cellular turnover rate than previously thought.[2]

In the gastric oxyntic glands, the proliferative zone encompassing the putative gastric stem cell is localized to the isthmus of the glands. From the isthmus, cells migrate bidirectionally, upward to differentiate into gastric surface mucus cells that coat the gastric pits and downward to give rise to gastric parietal and zymogenic cells.[3] Analysis of genetic mosaic mice[4,5] and of somatic mitochondrial mutations in humans[6] suggest that most gastric units are monoclonal, with all epithelial cells derived from a single stem cell.[7] Although the gastric stem cell has been the subject of investigation for several decades, it has not yet been identified except perhaps through studies using tritiated thymidine labeling, electron microscopic autoradiography, and chemical mutagenesis.[8–12] These studies have helped to define pre-pit, pre-neck, and pre-parietal cells, which derive from a more undifferentiated granule-free cell, potential stem cell of the gastric epithelium. As defined originally by electron microscopy, these rare cells are located in the center of the isthmus, are generally stationary, and divide rarely to give rise to progenitor cells.[8–11] Thus, in these early studies, zymogenic or chief cells were derived from neck cells and parietal cells developed from a separate progenitor, although this model was more recently modified to suggest some overlap in chief and parietal cell origins in humans.[3]

A well-validated stem cell marker, LGR5, shows lineage labeling in some antral gastric glands and might mark the antral stem or progenitor cells.[13,14] In the oxyntic mucosa, similar lineage tracing has not been achieved, although a potential candidate, doublecortin-like kinase-1 (Dclk1), was identified

Abbreviations used in this paper: Dclk1, doublecortin-like kinase-1; IF, intrinsic factor; IHC, immunohistochemistry; mRNA, messenger RNA; TFF2, trefoil factor family 2; TTE, TFF2 transcript–expressing; SPEM, spasmolytic polypeptide-expressing metaplastic lineage.

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from sequencing of laser capture microdissected mouse gastric and small intestinal epithelial progenitors.[15] However, to date, direct evidence for the presence of long-lived progenitors has been limited, although one study involving chemical mutagenesis of random epithelial cells showed the presence of clones containing only a single mature cell type indicating the presence of committed progenitors in the gastric epithelium.[12]

Trefoil factor family 2 (TFF2), also known as spasmolytic polypeptide, is a small peptide that is coexpressed with mucin 6 in discrete cell populations. There are 3 known members of the TFF family (TFF1, -2, and -3), which all contain 3-looped structural motifs resembling a 3-leaf trefoil. Under normal conditions, TFF2 is expressed and secreted by gastric mucous neck cells and deep antral gland mucous cells, and data from knockout mice indicate that it plays an important role in gastric cytoprotection and repair.[16] Trefoils are strongly induced after epithelial damage and facilitate both short-term (restitution) and long-term (glandular re-epithelialization) repair processes by stimulating cell migration,[17] inhibiting apoptosis,[18] and reducing antigen access to the healing epithelium.[19] In addition, they are regulated by proinflammatory and anti-inflammatory cytokine expression,[20,21] and have been postulated to participate in the mucosal immune response by modulating immunocyte migration, possibly through the CXCR4 receptor.[22] Although a number of potential biological roles for TFF2 have been reported, a precise understanding of the function of TFF2 has remained elusive. There appears to be a relationship between TFF2 gene expression and expansion of the proliferative zone of the mucosa, raising the possibility that TFF2 may be involved in regulating epithelial proliferation or differentiation in response to injury. In addition, loss of parietal cells induces development of a TFF2-expressing metaplastic lineage (spasmolytic polypeptide-expressing metaplastic lineage [SPEM]) in both mice and humans.[23]

Although TFF2 protein expression is confined to mucous neck cells, in situ hybridization studies have demonstrated that TFF2 messenger RNA (mRNA) expression is above the neck region in the gastric isthmus,[24] raising the possibility that TFF2 mRNA transcript expression is a marker of gastric stem or progenitor cells. Using a tamoxifen-inducible Cre recombinase under the control of the TFF2 promoter, we have identified a population of TFF2 transcript–expressing (TTE) cells as specific progenitors for parietal, mucous neck, and zymogenic cell lineages, but not for pit or enterochromaffin-like cell lineages in the oxyntic gastric mucosa. Thus, although previous studies suggested that corpus mucosa stem cells gave rise to 3 second-order transit amplifying cell populations (pre-pit, pre-neck, and pre-parietal), these results are consistent with a model that the TTE cell represents a gland lineage progenitor cell population that then gives rise to both mucous neck and parietal cells.

Methods

For BAC recombineering, a CreERT2FrtNeoFrt cassette was ligated into the p451 plasmid upstream of the ATG and 40 bp downstream with a 198-bp replacement of exon 1. BAC DNA was microinjected in the pronucleus of fertilized CBA × C57BL/6J oocytes. After back-crossing (F6) to C57BL/6, 2 of 3 founders were mated with B6.129S4-*Gt(ROSA)26Sor*^{tm1Sor}/J, referred to as Rosa26R-lacZ, or B6; 129-*Gt(ROSA)26Sor*^{tm2Sho}/J, referred to as Rosa26R-GFP (Jackson Laboratories, Bar Harbor, ME) and histological analysis was performed. For detailed methods see Supplementary information.

Results

TFF2 Promoter-Dependent Cre Expression in the Stomach, Lung, Kidney, and Duodenum

Lineage tracing, using inducible Cre recombinase, has become a powerful strategy to analyze the progeny of stem cells. Using a recombineering approach, we generated 3 positive founder lines of transgenic mice with a tamoxifen-inducible Cre^{ERT2} driven by a BAC-TFF2 promoter (Figure 1*A*). The resulting transgenic TFF2-Cre^{ERT2} mice exhibited TFF2 and Cre expression in the gastric corpus as analyzed by reverse transcription polymerase chain reaction in the corpus and antrum (Figure 1*B*) and immunohistochemistry (IHC) (Figure 1*C*), generally located above the known localization of TFF2 protein expression, as also demonstrated by in situ hybridization and protein staining for TFF2 (Figure 1*E*). The transgenic mice showed no Cre expression in liver, spleen, muscle, and pancreas (data not shown). Taken together, TFF2-Cre^{ERT2} mice showed a pattern of Cre expression that was consistent with endogenous TFF2 mRNA expression, located above the TFF2 protein expression.

After backcrossing to a C57BL/6J background, we crossed the TFF2-Cre^{ERT2} transgenic mice to Rosa26R-lacZ or Rosa26R-GFP reporter mice. Injection of tamoxifen activates the Cre^{ERT2} enzyme in TTE cells, and Cre-mediated excision of the floxed STOP cassette in the Rosa26 reporter then irreversibly marks TTE cells and any subsequent progeny of these cells, facilitating lineage tracing (Figure 1*A*).

To visualize the location of TTE cells in the gastric corpus glands, we analyzed 2- to 3-month-old mice 48 hours after tamoxifen induction. Single cells with GFP or LacZ signals only rarely appeared in the isthmus region in gastric glands in the corpus (Figure 2*A* and *B*). Mostly, several cells (5–10) in the isthmus were labeled. Expression of LacZ or GFP was not observed in noninduced mice (Figure 2*B*), excluding any leakiness of the Cre expression. A single dose of tamoxifen activated Cre expression in up to 10% of the gastric glands, and dosing over 3 consecutive days increased this to approximately 30% of glands (data not shown). Tamoxifen treatment itself did not induce any cellular changes in the stomach. The absence of uniform expression in all glands, which has been observed previously in lineage tracking experiments in the gut, has been attributed to issues regarding administration of tamoxifen or an incomplete or mosaic expression of the Cre^{ERT2} transgene. In the corpus, all of the Cre-GFP− or Cre-LacZ−labeled cells occurred at positions in the isthmus or upper neck region, similar to that observed in the past for