LECTURE

Gastric stem cells: an update

Irvin M. Modlin, Mark Kidd, Kevin D. Lye and Wright NA[1]

Gastrointestinal Surgical Pathobiology Research Group, Department of Surgery, Yale University School of Medicine, New Haven, CT, USA, [1]Histopathology Unit, Cancer Research UK, Lincoln's Inn Fields, London, UK

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Abstract. Cells of the gastric mucosa undergo constant renewal, the rate depending on the health of the tissue (inflammation, ulceration, carcinogenesis). While much attention has been focused on the mechanism of mucosal damage and the pathogenesis of ulceration, there has recently been the recognition that elucidation of the nature of the gastric stem cell lineages as well as the regulators of phenotype expression of this system may yield considerable biological information as well as open the door to the identification of areas of therapeutic relevance. Chimeric and X-inactivation studies in mice and humans demonstrate that each region of the gastric mucosa is morphologically diverse (antrum is different to the fundus), with its own repertoire of cell types and glandular structures. The current evidence suggests that a single stem cell in every gastric gland indirectly gives rise to a clone of all differentiated cells, by production of committed progenitor cells. It is also this multipotential cell that produces new crypts by crypt fission, repairs entire crypts when damaged, and gives rise to the ulcer-associated cell lineage and gastric carcinomas. It is likely that this stem cell occupies a niche in the isthmus composed of mesenchymal cells and extracellular matrix factors, which regulates the function of the cell via mesenchymal-epithelial cross talk. The molecular events (IGF-signaling) that regulate the development of the gastric gland in the mice have begun to be understood. Ultimately, the identification of these pathways will play an important role in identifying new molecular targets for the treatment of gastric disease. (Keio J Med 52 (2): 134–137, June 2003)

Key words: crypt, gastric, growth factor, proliferation, stem cell

Introduction

While much attention has been focused in the past on the mechanisms of mucosal damage and the pathogenesis of ulceration and carcinogenesis, there has however more recently been recognition that the elucidation of the gastric multipotent cell niche may yield considerable biological information as well as open the door to the identification of areas of therapeutic relevance. In this respect, the question of the nature of the stem cell lineage as well as the identification of the regulators of phenotype expression are important areas that require delineation and elucidation.

Murine gastric stem cells

Mouse gastric glandular epithelium is composed of tubular invaginations termed gastric units (Fig. 1).[1] In the acid-secreting portion of the stomach, each unit contains three predominant cell lineages: pit, parietal and zymogenic (chief). The multipotent stem cell and its first incarnation, the undifferentiated granule-free progenitor cell (committed precursor cell), reside in the isthmus of the unit. Within the gastric glands, migration of cell precursors is bi-directional from the neck/isthmus region to form the gastric epithelium. Thus, the pre-pit precursor differentiates into mucus-secreting pit cells as it moves up the isthmus, while the preneck cell precursor differentiates successively into pepsinogen-producing neck cells and then zymogenic (chief) cells as it migrates downwards.[2] In contrast, acid-producing parietal cells differentiate within the isthmus from pre-parietal cell precursors which then migrate upwards or downwards. This precursor cell population – gastric epithelial progenitor cells (which include the multipotent stem cell) make up approximately 3% of the

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Reprint requests to: Dr. Irvin M. Modlin, Department of Surgery, Yale University School of Medicine, 333 Cedar Street, PO Box 208062, New Haven, CT 06520-8062, USA

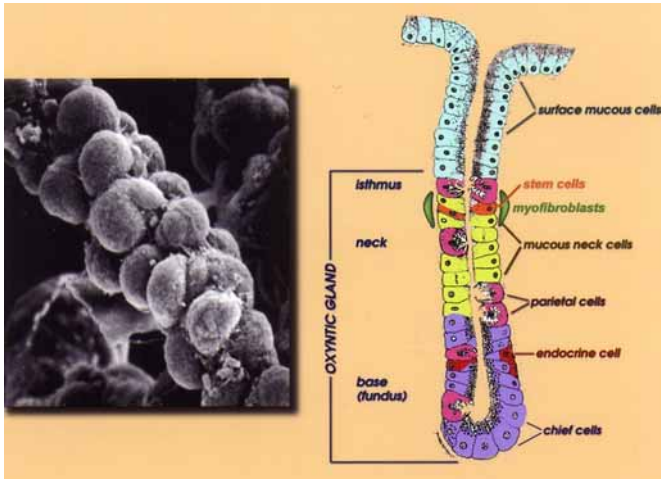

Fig. 1 An electron micrograph of an isolated gastric gland (*left*) and a depiction of the gland unit (*right*). The pit and the luminal surface are lined by surface mucous cells. The isthmus contains stem and progenitor cells and is enclosed by a sheath of myofibroblastic cells. Mucous neck cells are found in the neck, while chief and endocrine cells are present in the base of the gland. In contrast, parietal cells are scattered throughout the gland.

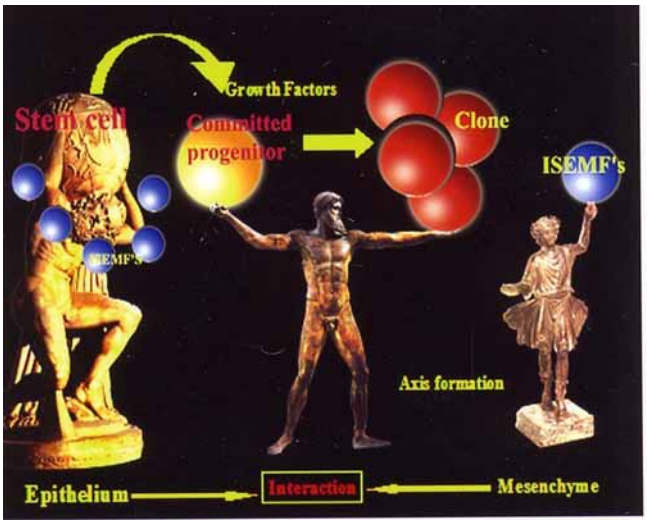

Fig. 2 A cartoon depicting the interrelationships between stem, committed progenitor cells and ISEMFs in the gastric mucosa. This is a bi-directional cross-talk that allows the epithelium (and its environment) to affect the fate of stem cells via growth factors and the mesenchyme.

gastric epithelium in adult mice. It is considered that the antral gastric mucosa, including the endocrine cells, derive from a common stem cell.[1]

Experiments in XX/XY and CH3 ↔ BALB/c chimeric mice have identified the presence of homotypic gastric glands derived from each of the parent strains.[3,4] This indicates that gastric glands in the mouse are clonally derived. In addition, an examination of the chromosomal complement of the gastrin-producing endocrine cell in the XX/XY chimeras demonstrated that these were or were not Y-positive, depending on whether the gland developed from a Y-containing clone or not. This indicates that at least the gastrin-secreting endocrine cell of the antrum is derived from a common stem cell.[3]

Experiments in X-inactivation mosaic mice expressing the *lacZ* reporter gene investigating the clonality of gastric glands in the fundic and pyloric regions of the developing mice demonstrated that while most glands are initially polyclonal with three or four stem cells per gland, they become monoclonal during the first six weeks of murine life.[5] This potentially occurs through gland fission or when division (repeated mitoses) of one of the stem cells overrides all other stem cells in the gland. A population of approximately 5–10% of mixed, polyclonal glands, however, persists into adulthood. They are potentially derived from glands with reduced fission rates. The significance of these multi-stem cell-containing glands is, however, unknown.

Stem cells within the gastric glands are thought to reside within a niche or group of cells and extracellular substrates, which provide an optimal microenvironment for stem cells to give rise to their differentiated progeny (Fig. 2).[1] Two populations of cell types may play important roles in this process. Gastric glands are enclosed in a protective fenestrated sheath of intestinal sub-epithelial myofibroblasts (ISEMFs). These cells exist as a syncytial organ which extends throughout the lamina propria and merges with the pericytes of the blood vessels. ISEMFs are closely linked to the gastric epithelium and are believed to play an important role in epithelial–mesenchymal interactions. They secrete hepatocyte growth factor (HGF), transforming growth factor-β (TGF β), and keratinocyte growth factor (KGF); the receptors for these growth factors are present on the epithelial cells. Secretion of these growth factors by ISEMFs is thought to play an important role in the regulation of epithelial cell differentiation.[6] In contradistinction, epithelial cells secrete platelet-derived growth factor-α (PDGF-α) which acts by paracrine signaling via its mesenchymal receptor PDGFR-α to regulate essential epithelial–mesenchymal interactions during development.[7] Parietal cells have also been identified to potentially regulate cell determination in gastric units by expression of insulin-like growth factors binding protein–2.[8] Gastric epithelial cells are therefore able to regulate the activity of ISEMFs.

A second myofibroblast population are the interstitial cells of Cajal (ICC). These are located close to mural neurons and act as pacemakers for gastrointestinal smooth muscle activity, propagate electrical events, and modulate neurotransmission.[9] Their role in regu-

lating stem cells is unknown but appears likely given the importance of neural pathways (*e.g.*, PACAP and neuroendocrine ECL cell proliferation) in the gastric mucosa.[10] An increasing number of genes and growth factors expressed by intestinal mesenchymal cells and epithelial cells have, however, also been identified that regulate development, proliferation, and differentiation in adulthood. These include members of the fibroblast growth factor (FGF) family, epidermal growth factor (EGF) family, TGF-β, insulin-like growth factors (IGF)-1 and -2, HGF/scatter factor, sonic and Indian hedgehog, and PDGF-α. Of these, it is clear that the Wnt/β-catenin signaling pathway and downstream molecules such as APC, Tcf-4, Fkh-6, Cdx-1 and Cdx-2 are vital for normal gastrointestinal function, as mutations of these at any stage appear to induce tumorigenesis. It has been postulated that these pathways may play an important role in stem cell biology.[1]

While gastric multipotent stem cells have not been isolated, the genetic analysis of an enriched mouse gastric epithelial progenitor cell population from the fundus has facilitated the understanding of some of the molecular pathways that regulate this precursor cell proliferation and differentiation in the murine stomach.

Transgenic mice with mutant diptheria toxin A fragment-ablation of parietal cells have an increased number of gastric epithelial cell progenitors (20% compared to 3% in normal animals). This is increased in transgenic embryonic animals (day 18) to ~90%. Gene-chip analysis of the epithelial progenitor cell populations derived from intact stomachs from different transgenic mice and normal mice have been performed.[11] The results demonstrate the prominence of growth factor (particularly IGF) response pathways and regulators of protein turnover pathways in these cells. Transcripts encoding products for mRNA processing and cytoplasmic localization were also present in a substantial proportion of genes as were homologues of genes required for axis formation during oogenesis. This indicates the importance of growth factor signaling and the ability to communicate specifically with different cell types in the epithelial progenitor stem cell niche for the development of different gastric cell types.[11]

Gastric mucosa cells can be derived from cell types other than the gastric epithelial cell progenitor. The adult hematopoietic bone marrow stem cell has a great deal of plasticity and can differentiate into a number of different cell types including gastric epithelia. Thus, bone marrow-derived epithelial cells in the gastrointestinal tract have been demonstrated in the mouse.[12] In this study, which examined the long-term repopulation of hematopoietic stem cells (HSC) in lethally irradiated hosts, female mice received a single labeled male-derived HSC. Five long-term survivors were sacrificed after 11 months and homing and differentiation of the HSC cell was examined. Donor-derived epithelial cells (cytokeratin-positive) were identified in the gastric pits in the stomach and constituted $0.3 \pm 0.2\%$ of cells. They had differentiated into columnar epithelial cells, appeared to be randomly scattered, and did not appear to be fully functional since they did not seem to proliferate. This demonstrates that an exogenously applied multipotent stem cell of a very different organ system can contribute to an established, organized glandular system like the gastric mucosa, even in the adult animal.

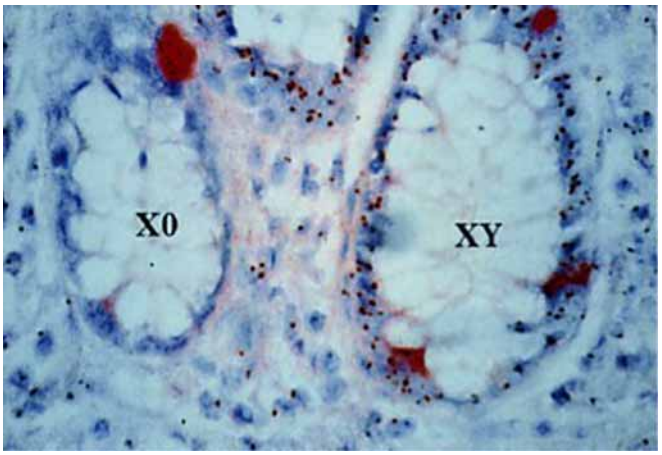

Fig. 3 Monoclonal origin of crypts in an X0/XY mosaic individual. Staining using in situ hybridization for a Y-chromosome-specific probe demonstrates an X0 crypt (*left*) and an XY crypt (*right*). (Courtesy of M. Novelli)

Human gastric stem cells

Glandular clonality studies in human gastric mucosa using x-chromosome-linked inactivation (the phosphoglycerate kinase and human androgen receptor – (HUMARA) genes were used to distinguish the two different x-chromosomes) demonstrated that while pyloric glands appear homotypic for either of the loci and are therefore monoclonal, approximately 50% of the fundic glands were heterotypic and thus polyclonal (Fig. 3).[13] This suggests a more complex situation than in chimeric mice and indicates both regional and species-specific differences in the development of the gastric mucosa. Unlike the mouse, however, no technique exists to isolate human gastric stem cells (or epithelial progenitor cell populations), and little is known about the molecular pathways that regulate stem cell proliferation and differentiation in the human stomach.

Ulceration in the stomach is, however, known to induce a novel cell lineage derived from the gastric stem cell (ulcer associated cell lineage – UACL).[14] This cell lineage grows from the bases of existing crypts, ramifies to form a new gland, and ultimately emerges

onto the mucosal surface. Cells produce neutral mucin, show a unique lectin-binding profile and immunophenotype, and secrete abundant immunoreactive EGF (urogastrone). It would appear that the production of EGF/urogastrone by the precursor cell stimulates cell proliferation, regeneration, and ultimately ulcer healing under these conditions.

In an analogous fashion to the mouse, human multipotent hematopoeitic stem cells can also contribute cells to the gastric mucosa. Thus, an *in situ* hybridization study with Y-chromosome–specific probes combined with immunohistochemical staining of gastric biopsies was able to demonstrate the presence of mucosal cells (derived from the donor) in the gastric cardia of female patients who had undergone sex-mismatched peripheral blood stem cell transplantation.[15] This suggests the future potential therapeutic possibility of transdifferentiation in gastric diseases.

Conclusion

Cells of the gastric mucosa undergo constant renewal, the rate of which varies depending on the demand dictated by the health of the tissue (inflammation, ulceration, carcinogenesis). Each region of the gastric mucosa appears to be morphologically diverse (antral *versus* fundic), with its own repertoire of cell types and glandular structures. The current evidence suggests that a single stem cell in every gastric gland indirectly gives rise to a clone of differentiated cells, by production of committed progenitor cells. It is also this cell (the multipotential stem cell) that produces new crypts by crypt fission, will repair entire crypts when damaged, and give rise to the UACL and gastric carcinomas. It is most likely that this stem cell occupies a niche in the isthmus composed of mesenchymal cells and extracellular matrix factors. This environment regulates the function of the epithelial stem cell via mesenchymal–epithelial cross talk. The molecular events (IGF-signaling) that regulate the development of the gastric gland, at least in the mouse, are beginning to be understood, but it remains to be determined whether such information will be directly pertinent to the human situation.

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