

Gastric epithelial stem cells in development, homeostasis and regeneration

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Summary statement: The tissue-resident gastric stem cell population drives stomach patterning and lineage specification during development and continued homeostatic maintenance and regeneration in adulthood.

Abstract

The stem/progenitor cell pool is indispensable for the development, homeostasis and regeneration of the gastric epithelium, owing to its defining ability to self-renew whilst supplying the various functional epithelial lineages needed to efficiently digest food. A detailed understanding of the intricacies and complexities surrounding the behaviours and roles of these stem cells offers insights not only into the physiology of gastric epithelial development and maintenance but also into the pathological consequences following aberrations in stem cell regulation. Here, we provide an insightful synthesis of the existing knowledge on gastric epithelial stem cell biology, including the *in vitro* and *in vivo* experimental techniques that have advanced such studies. We highlight the contributions of stem/progenitor cells towards patterning the developing stomach, specification of the differentiated cell lineages and maintenance of the mature epithelium during homeostasis and following injury. Finally, we discuss gaps in our understanding and identify key research areas for future work.

Introduction

Stem cells (see **Glossary, Box 1**) are unspecialised cells characterised by their ability to self-renew and differentiate into diverse cell lineages. Early in development, pluripotent embryonic stem cells (ESCs) contribute to the multitude of cell lineages that build the developing embryo, whereas multipotent adult

stem cells (ASCs) residing in mature tissues have a more restricted differentiation potential and sustain the homeostatic maintenance of their host tissues (Barker et al., 2008; Barker et al., 2010b). Within the gastrointestinal tract, ASC populations have been identified within the stomach, small intestine, and colon, among others (Lin and Barker, 2011). Although the intestine is one of the most heavily studied models, recent studies have put the spotlight on the stomach with the identification of new gastric stem cell markers, establishment of genetic mouse models, and technological advances in organoid culture techniques that facilitate its study.

Anatomically, the human stomach can be subdivided into five main regions: cardia, fundus, corpus, antrum and pylorus (see **Glossary, Box 1**) (**Fig. 1A**). The cardia and fundus are both located proximally, where the oesophagus connects to the stomach. The corpus makes up the main body of the stomach, and the antrum and pylorus form the distal stomach. A similar structural pattern is found in the mouse stomach, which is subdivided into the forestomach, corpus and pyloric antrum (henceforth referred to in this Review as the pylorus) (**Fig. 1B**). With the exception of the non-glandular forestomach that is unique to mice, the mouse corpus and pylorus contain a glandular columnar epithelium that resembles the human equivalent, making the mouse stomach one of the preferred models for studying gastric biology.

A key function of the stomach is the chemical digestion of food. This is carried out by the glandular units in the corpus, which secrete acid and digestive enzymes, and those in the pylorus, which secrete mucus and hormones such as gastrin (Kim and Shivdasani, 2016). These glands are found within the stomach epithelium, which is continuously exposed to the highly acidic environment of the gut lumen and to external agents that travel through the gastrointestinal tract. To ensure the continued maintenance of an intact epithelial layer, gastric ASCs actively regenerate the mature cell lineages within the gut epithelium (Hoffmann, 2008).

This Review explores the recent major findings which collectively highlight the role of gastric epithelial stem/progenitor cells in driving both early developmental events and in maintaining tissue homeostasis and regeneration in the adult gut. We first discuss how endodermal and mesenchymal signals work together to shape stomach regionalisation and patterning during embryonic development. We highlight the contribution of these early gastric progenitors in giving rise to each of the differentiated cell lineages that make up the adult glandular stomach. Finally, we consider the role of various ASC populations and their niches in the corpus and pylorus in maintaining and regenerating the gastric epithelium during homeostasis and injury.

Stomach regionalisation

During embryonic development, the stomach is specified from the endoderm-derived foregut. An array of transcription factors then pattern the stomach into the forestomach, corpus and pylorus regions. SRY-Box transcription factor 2 (SOX2), which defines the foregut region during endoderm patterning, is expressed throughout the embryonic mouse stomach (Francis et al., 2019), with the highest levels detected in the anterior region (Que et al., 2007). In *Sox2* knockout mice, the forestomach marker transformation-related protein 63 (TP63) is lost while expression of the hindstomach marker pancreatic and duodenal homeobox 1 (PDX1) remains unaffected (Francis et al., 2019). Within the pylorus, PDX1 defines the gastrin-secreting G cell lineage present specifically in the posterior stomach (Larsson et al., 1996; Offield et al., 1996). The delineation of the glandular corpus and pylorus from the non-glandular forestomach also involves the transcription factor GATA binding protein 4 (GATA4), which is crucial for columnar epithelial development (DeLaForest et al., 2021).

Signals derived from the surrounding mesenchymal tissues are also involved in patterning the major stomach regions. GATA4 expression in the epithelium directs the upregulation of mesenchymal fibroblast growth factor 10 (FGF10) (Sankoda et al., 2021). FGF10 interacts with fibroblast growth factor receptor 2 (FGFR2) expressed in the SOX2⁺ forestomach epithelium, activating MAPK/ERK signalling at the boundary separating the forestomach and glandular stomach to maintain the squamous-columnar junction (Sankoda et al., 2021). In the distal stomach, loss of the mesenchymal bagpipe homeobox protein homolog 1 (BAPX1) (also known as NK3 homeobox2, NKX3.2) transcription factor results in a markedly reduced pylorus, highlighting its importance in driving distal stomach development (Verzi et al., 2009). NKX3.2 is suggested to act downstream of barH-like homeobox1 (BARX1), another mesenchymal transcription factor that maintains stomach identity via secretion of Wntless-related integration site (Wnt) antagonists (Kim et al., 2005). In *Barx1* knockout embryos, the squamous-columnar junction and the boundary between the corpus and pylorus regions are not properly defined (Kim et al., 2007).

Gastric lineage specification

Embryonic epithelial lineage specification commences in the mouse stomach at approximately E13.5 (Willet and Mills, 2016; Sayols et al., 2020). By E16.5, markers of the various differentiated cell types become expressed in the gut (Nyeng et al., 2007). Thereafter, the simple columnar epithelium invaginates into the mesenchyme to form the stereotypical glandular structures seen in the adult gut (Lee et al., 1982; Mills and Shivdasani, 2011). These invaginations, known as gastric pits, lead into glands in the corpus and pylorus (Mills and Shivdasani, 2011). Corpus glands consist of the pit, isthmus,

neck and base regions, where the differentiated lineages comprising mucin-secreting surface/pit mucous cells, hormone-secreting enteroendocrine cells, gastric acid-producing parietal cells, zymogenic chief cells and rare tuft cells reside (see **Glossary, Box 1**) (**Fig. 1C**). In the pylorus, each gland unit contains a pit, isthmus and base region, encompassing surface/pit mucous, enteroendocrine, tuft and basal gland cells (see **Glossary, Box 1**) (Kim and Shivdasani, 2016). While some of these cell types can be found throughout the gland, others, including the surface/pit mucous cells and chief cells, are restricted to certain gland regions (**Fig. 1C**). Notably, not all cell lineages are mature at birth - for instance, the surface/pit mucous cell lineage starts emerging after 3 weeks and chief cells become mature at 8 weeks of age in mice (Keeley and Samuelson, 2010).

FGF and Bone morphogenetic protein (BMP) signalling have been found to govern stem cell activity and lineage specification during murine stomach development (**Fig. 2**). FGF signalling is active in both the gut epithelium and mesenchyme, and loss of the mesenchymal ligand FGF10 or its receptor FGFR2B at E18.5 in mice impairs parietal and chief cell differentiation (Spencer–Dene et al., 2006). Similarly, inhibition of the ubiquitously expressed receptor BMPR1A at E13.5 to block BMP signalling reduces expression of markers for parietal cells (*Atp4a*), chief cells (*Gif*, which marks parietal cells in humans), mucous neck cells (*Tff2*) and endocrine cells (*Chgb*), while promoting upregulation of the surface/pit mucous cell marker *Muc5ac* (Sayols et al., 2020). In contrast, in the adult mouse stomach, epithelial BMP signalling blocks the specification of nearly all differentiated cell lineages while promoting parietal cell formation (Maloum et al., 2011), highlighting stage-specific roles of this pathway. Although the precise source of BMPs in the stomach remains undefined, recent studies have revealed changes in mesenchymal cell composition over time that indicate a role for factors such as BMP in modulating stem cell specification in the embryonic gut (Han et al., 2020; Loe et al., 2021).

Wnt/ β -catenin signalling has also been implicated in the specification of some corpus cell lineages during development (Sayols et al., 2020). *Wnt5a/b* is expressed in both mesenchymal and epithelial cells in the E13.5 mouse stomach, along with robust expression of the Wnt signalling enhancer *Respondin1/3* (*Rspo1/3*) in the mesenchymal compartment (Sayols et al., 2020). Interestingly, although suppression of Wnt signalling in early development directs endodermal precursors towards the gastric lineage (Kim et al., 2005), elevation of Wnt signals plays a different role in later development to specify the corpus, acting in concert with BMP inhibition to repress intestinal marker expression (McCracken et al., 2017). Indeed, global inhibition of Wnt signalling at these later developmental stages impairs chief and parietal cell differentiation, highlighting their role in corpus lineage specification (Sayols et al., 2020).

Endocrine lineage specification

Additional regulators work in concert with the aforementioned pathways to specify the endocrine cell lineage, which comprises gastrin-secreting G cells, somatostatin-secreting D cells, serotonin-secreting enterochromaffin (EC) cells, histamine-secreting EC-like (ECL) cells and ghrelin-secreting X- or A-like (X/A) cells (Solcia et al., 2000). The precise identities of the transcription factors driving each of these endocrine lineage fates remain poorly defined. Nevertheless, one major regulator of the endocrine lineage appears to be mammalian achaete-scute homolog 1 (*Mash1/Ascl1*), whose selective depletion in mice results in reduced numbers of multiple endocrine cell types (Kokubu et al., 2008). Cells labelled by another marker, neurogenin 3 (*Ngn3*), give rise to the five major endocrine cell lineages (Jenny et al., 2002); however, in the absence of *Ngn3*, only the differentiation of G and D cells is fully disrupted (Jenny et al., 2002), implying that G and D cells may share a common progenitor. Alternatively, *Ngn3* may label a heterogeneous cell population containing multiple progenitor pools for the different endocrine lineages. The majority of pyloric endocrine cells arise from *Ngn3*⁺ precursors (Schonhoff et al., 2004); however, fewer than 40% of X/A and ECL cells and none of the EC cells in the mouse corpus are derived from *Ngn3*⁺ progenitors (Li et al., 2014), highlighting the possibility of additional unidentified progenitor pools at play in specifying the corpus endocrine lineages. Interestingly, corpus EC cells are completely depleted in a mast cell-deficient (see **Glossary, Box 1**) mouse model (Li et al., 2014), suggesting that mast cells might function as a niche for EC progenitors. Further lineage tracing experiments would clarify whether mast cells could also serve as a cell of origin for the EC lineage. Other regulators such as PDX1 (Larsson et al., 1996), Homeobox protein Nkx family (Choi et al., 2008), Aristaless Related Homeobox (*Arx*) (Du et al., 2012), and Paired Box 4 and 6 (*Pax4* and *Pax6*) (Larsson et al., 1998) are also known to direct endocrine cell differentiation.

Chief cell lineage specification

Chief cells are found at the gland base of corpus glands and are responsible for secreting the zymogen pepsinogen and gastric lipases (Karam and Leblond, 1993a). During chief cell lineage differentiation, the chief cell progenitor migrates through the neck of the gastric unit towards the base (Ramsey et al., 2007; Bredemeyer et al., 2009). In mice, loss of *Mist1* depletes terminally differentiated chief cells and increases the transitional cell population (Ramsey et al., 2007). *Mist1* signalling has also been implicated in cytoskeletal rearrangement and secretory processes, thus potentially serving as a regulator of cell migration during chief cell differentiation (Pin et al., 2000; Johnson et al., 2004; Hess et al., 2016). Upstream of *MIST1*, X-Box binding protein 1 (*XBPI*) acts as a master transcription factor that directly induces *MIST1* expression in mouse adult gastric cells and promotes chief cell differentiation (Huh et al., 2010). Notably, loss of *XBPI* does not block *Mist1* signalling after the onset of *MIST1* expression, suggesting the existence of other regulators of *MIST1* expression (Huh et al., 2010). Interactions between parietal cells and chief cells can also contribute to chief cell differentiation (Bredemeyer et al., 2009). In the neck zone, parietal cells surrounding chief cell progenitors are known to secrete morphogens including sonic hedgehog (*SHH*) (Van Den Brink et al., 2001) and Transforming

growth factor α (TGF α) (Beauchamp et al., 1989), potentially providing a niche supporting chief cell differentiation. In line with this, parietal cell-deficient mice show decreased chief and neck cell marker expression and their Gif⁺ cells are highly proliferative rather than terminally differentiated (Bredemeyer et al., 2009).

One notable gap in our knowledge is the mechanisms guiding differentiation of surface/pit mucous and parietal cell lineages. Transcription factors including Forkhead box Q1 (FOXQ1), which regulates the expression of mucous cell marker *Muc5ac* (Verzi et al., 2008), and Kruppel-like factor 4 (KLF4), which guides parietal cell differentiation (Miao et al., 2020), have been implicated, but the underlying pathways driving the specification process remain unresolved. The identity of the progenitors for each of the differentiated lineages and the potential existence of intermediate progenitors also remain poorly defined. Moreover, due to the limitation of early lethality caused by gene depletion in many mouse models, many studies have been performed at the adult stage, raising the question of whether these models truly recapitulate the key niche factors present within the developing gut.

Recent technological advances in modelling gastric development

The challenges in dissecting the myriad of signalling pathways and transcription factors that regulate the complex morphogenetic changes occurring in the embryonic gut have catalysed recent technological advances and the development of more physiological models. These include human/mouse organoids and single-cell transcriptomic approaches, which facilitate the discovery of niche components required to support the activity of gastric stem/progenitor cells.

Gastric organoids

Organoids are 3D cellular models capable of recapitulating major molecular and functional features of their source tissues, including the capacity to self-renew and differentiate into multiple cell lineages (Fatehullah et al., 2016). Gastric organoids can be generated from ASCs (Barker et al., 2010a; Bartfeld et al., 2015), or through the differentiation of ESCs or induced pluripotent stem cells (iPSCs) towards specialised cell lineages (McCracken et al., 2014; McCracken et al., 2017) (**Fig. 3**).

ASC-derived organoids represent an accessible system to investigate the niche factors required for stomach patterning. These organoids can be formed from ASCs present within individual mouse or human epithelial glands (Bartfeld et al., 2015; Schlaermann et al., 2016), or via the enrichment of the gland base ASC pool using available markers such as *Lgr5* and *Troy* in mice (Barker et al., 2010a; Stange et al., 2013). More recently, methods to establish mouse isthmus-like 2D monolayer and 3D organoid cultures have also facilitated the study of isthmus-localized ASC populations *in vitro* (Huebner

et al., 2023). In the absence of other stromal cell types or extracellular matrix (ECM), these ASC-derived epithelial organoids are grown within artificial ECM-like matrices and supplied with growth factors that support stem cell activity, while withdrawal of WNT and FGF directs lineage differentiation in mouse corpus and pylorus organoids (Barker et al., 2010a; Stange et al., 2013). Similar differentiation trajectories are starting to be understood in human ASC-derived mucosal epithelial monolayers, also known as mucosoids, where BMP and EGF activation results in the enrichment of pit mucous cells, whereas loss of EGF promotes chief and parietal cell differentiation (**Fig. 3A**) (Wölffling et al., 2021).

In contrast, iPSC-derived organoids model the earlier stages of human gastric development through the key stages of endodermal foregut patterning, acquisition of gastric regional identities and specification towards differentiated cell lineages. This was established by pioneering studies that generated fundic (McCracken et al., 2017) and antral (McCracken et al., 2014) organoids from human iPSCs (hiPSCs) (**Fig. 3B**). In a manner bearing striking similarities to *in vivo* human gut development, hiPSCs were first differentiated into definitive endoderm by Activin A, then into the SOX2⁺ posterior foregut lineage via the combined activities of WNT and FGF to promote morphogenesis, the BMP inhibitor Noggin to repress posterior fate, and retinoic acid (RA) to promote patterning of the foregut (McCracken et al., 2014). These foregut spheroids could develop into mature SOX2⁺ PDX1⁺ antral organoids with a complex glandular epithelial layer in the continued presence of RA, Noggin, and high levels of EGF. Conversely, activation of Wnt/ β -catenin signalling caused the spheroids to acquire a SOX2⁺ PDX1⁻ fundic identity (McCracken et al., 2017). In all, the close parallels between the signalling events during human embryonic gut patterning and lineage specification and the factors used *in vitro* to differentiate hiPSCs into complex gastric organoids containing the full spectrum of mature cell types highlight the utility of this system as a robust model of human gut development. Nevertheless, it is worth noting that hiPSC-derived gastric organoids retain a foetal-like transcriptional profile (McCracken et al., 2014), suggesting that additional factors are still needed to drive the complete maturation of these organoids to reflect expression patterns present in the adult stomach.

Efforts to derive gastric organoids from mouse ESCs have similarly centred around the transcription factors involved in directing specification of the embryonic gut lineage in mice (**Fig. 3C**). Previous studies have identified BARX1 as a key factor expressed within the gut mesenchyme that suppresses Wnt/ β -catenin signalling in the epithelium, specifying the foregut progenitors that ultimately give rise to the stomach (Kim et al., 2005). Using a combination of SHH and the Wnt antagonist DKK1, robust expression of mesenchymal BARX1 could be induced within spheroids generated from ESC-derived embryoid bodies, along with patterning of the SOX2⁺ foregut progenitors (Noguchi et al., 2015). These spheroids can develop into mature glandular organoids that contain both corpus-specific chief and parietal cells and pylorus-specific G cells. It remains to be evaluated whether separate corpus or pyloric fates could be derived with differential Wnt activation as seen in hiPSC-derived organoids (McCracken

et al., 2014; McCracken et al., 2017). Such studies will complement our understanding of the mechanisms guiding early mouse gut development and highlight species-specific differences between mouse and human development.

One of the major challenges in modelling complex developmental processes using the *ex vivo* organoid system is the recapitulation of interactions and signalling crosstalk between different tissues within the organ. ASC-derived organoids retain only the epithelial component, but the use of ESCs or iPSCs facilitates the incorporation of stromal cell types that support the development and functions of the embryonic gut. This was most recently demonstrated by Eicher and colleagues, who independently generated human gastric epithelial organoids from foregut endoderm, smooth muscle from mesodermal progenitors and enteric neurons from ectoderm-derived neural crest cells, then combined them sequentially in 3D culture (Eicher et al., 2022). The resultant human antral or fundus organoids were surrounded by innervated smooth muscle that acted as a functional unit to promote gastric identity and morphogenesis. Surprisingly, in the absence of a mesenchymal component, enteric neurons cultured with human antral organoids led to organoid posteriorisation, likely through upregulation of BMP signalling in these neurons, offering a previously unappreciated perspective of the contribution of gut innervation towards gastric specification. With the advancement of methods to incorporate multiple cell types, ECM proteins, and hormonal cues, it is a matter of time before more complex combinatorial organoid models of the developing human gut can be constructed.

Single-cell transcriptomics

Recent studies utilising high-throughput single-cell transcriptomic approaches have similarly offered a more comprehensive picture of the transcription factors and signalling pathways involved in gut lineage specification. This approach has revealed region-specific transcription factor networks along the anterior-posterior axis of the gut tube that could play roles in the early patterning processes. In the E8.75 mouse gut endoderm, a core set of transcription factors was found to be expressed within defined expression domains, including NKX2-1 in the anterior region and HOXB9 at the posterior (Nowotschin et al., 2019). Single-cell RNA sequencing (scRNA-seq) performed on mouse embryonic foregut tissues collected at E8.5, E9.0 and E9.5, when foregut regions begin to develop into distinct organs, has validated many known organ-specific transcription factors and also revealed novel markers for the gastric endoderm and mesenchyme (Han et al., 2020). Strikingly, mapping of lineage trajectories of both the foregut endoderm and surrounding mesenchyme revealed broad, coordinated shifts in transcriptional profiles predictive of paracrine epithelial-mesenchymal signalling between the two tissue layers (Han et al., 2020). For instance, RA in the E9.0 foregut mesenchyme likely signals to the endoderm to upregulate *Shh* signalling specifically within the future gut region, but not in the pharynx or liver precursors (Han et al., 2020). In line with this prediction, RA induction in differentiating hiPSCs

in culture can ultimately direct gut tube formation via Hedgehog pathway activation, while repressing the induction of hepatic markers (Han et al., 2020). Such combinations of niche factors allow multiple mesenchymal-like cell types to be generated in culture, demonstrating the utility of such datasets in revealing the niche components regulating key patterning events in early development.

Epithelial-mesenchymal interactions continue to play a central role in the subsequent stages of stomach regionalisation and gastric lineage specification. scRNA-seq revealed transcriptional profiles unique to the mesenchymal cells of the foregut and hindgut from E9.5 to E11.5 (Zhao et al., 2022). The authors found that FGF10 was highly expressed in the foregut mesenchyme and RA synthesis enzymes were upregulated in the hindgut mesenchyme, and used organoids to confirm a role for these mesenchyme-derived niche factors in foregut and hindgut patterning (Zhao et al., 2022). Subsequently, at E13.5, when differentiated gastric cell types are formed, signals from several key developmental pathways including Wnt, Notch and BMP were found to be crucial for lineage specification (Sayols et al., 2020). Thus, gastric epithelial precursors present at E13.5 retain cellular plasticity that allows them to be directed along different lineages by the combined effect of multiple niche factors. A closer look into the transcriptional profiles of the mesenchymal populations at this stage may reveal additional roles for these cells as sources of some of these niche factors.

Given the scarcity of human samples, these mouse embryonic transcriptional datasets have proven to be a valuable resource. Nevertheless, clarifying species-specific differences remains important for our understanding of human stomach development and for clinical applications. An scRNA-seq study on 6- to 25-week human foetal gastrointestinal tissues identified signalling pathways that could be involved in regulating patterning events at different developmental stages (Gao et al., 2018). Other studies have profiled the adult human gastrointestinal tract (Kim et al., 2020; Busslinger et al., 2021). Busslinger and colleagues identified neuromedin U (NMU), a potent regulator of gastric acid secretion and feeding behaviour (Mondal et al., 2003; Jarry et al., 2019), as a marker uniquely expressed within human but not mouse isthmus cells in the adult corpus (Busslinger et al., 2021). This comparative analysis highlights expression profile differences between the two species that could reflect distinct stomach functions, diets, and microbiota.

As more large-scale transcriptomic datasets are generated, it becomes essential to put this information into perspective, and the use of animal models and organoid systems facilitates the functional validation of predicted pathways and regulators derived from these datasets. Moreover, transcriptomic data can be combined with spatial information to offer insights into cell-cell interactions at the tissue level (Lohoff et al., 2022), combined with proteomic data to correlate changes in both RNA transcripts and proteins (Li et al., 2018), and integrated with single-cell epigenomic datasets to reveal how changes in chromatin accessibility and transcription factor binding might drive lineage specification (Smith et al., 2022).

Together, these multi-omics datasets can provide additional layers of insight into the patterning and specification events during stomach development.

Gastric homeostasis and regeneration

Gastric homeostasis and regeneration are both crucial elements for the maintenance of a healthy and well-functioning stomach. Homeostasis refers to the continuous process of maintaining a steady-state internal balance and regular functioning of the stomach, while regeneration occurs following injury to repair any damage and restore homeostasis. Dysregulation of these processes can result in marked alterations to the gastric epithelium, including defective maturation of secretory cells (Todisco et al., 2015), or even polyp formation (Demitrack et al., 2015). Given the harsh conditions in the stomach, gastric epithelial cells are prone to injury, necessitating a rapid regeneration program to rebuild damaged tissues (Hoffmann, 2008). These mechanisms are tightly regulated by multiple populations of tissue-resident ASCs located within the corpus and pylorus (Barker et al., 2010b; Mills and Shivdasani, 2011).

Epithelial stem cells in the adult corpus

Isthmus stem cell population

Radiolabelling and electron microscopy first identified the presence of highly proliferative granule-free cells in the isthmus region of the corpus gland thought to mark a tissue-resident stem cell pool (Karam and Leblond, 1993b; Bjerknes and Cheng, 2002). Since then, numerous biomarkers for the isolation and characterisation of isthmus stem/progenitor cells have been proposed (**Table 1**). Most of these studies utilise mouse models to mark putative isthmus stem cell (IsthSC) populations and perform lineage tracing to track the activity and fate of these cells and their progeny. Such experiments have highlighted the role of IsthSCs during homeostasis to replenish the differentiated cells in the corpus gland (**Fig. 1C**).

One of the earliest proposed IsthSC markers is *Sox2*, a key pluripotency factor. *Sox2*-expressing cells are present throughout corpus glands, overlapping with the actively cycling isthmus region (Arnold et al., 2011). Lineage tracing shows that *Sox2*⁺ cells give rise to fully-traced glands encompassing each of the differentiated cell lineages, indicating that they contain a stem cell pool (Arnold et al., 2011). Similar lineage tracing experiments have confirmed that cell populations expressing leucine-rich repeats and immunoglobulin-like domain 1 (*Lrig1*) (Choi et al., 2018) and those with activated *Runx1* enhancer element (*eR1*) (Matsuo et al., 2017) also harbour stem cells, although these populations are

heterogenous and mark differentiated lineages as well (Schweiger et al., 2018). In contrast, trefoil family factor 2 (Tff2) mRNA expression serves as a marker of multipotent progenitor cells with a more restricted lineage potential, giving rise only to chief, mucous neck, and parietal cells but not pit or endocrine cells (Quante et al., 2010). It is possible that a hierarchy of stem/progenitor cells exists within these glands that includes both uncommitted stem cells and progenitors with more restricted differential potential.

Lineage tracing experiments have also provided insights into the direction and dynamics of clonal expansion of stem cell populations within these glands. Cells labelled by *Mist1*, which identifies a quiescent IsthSC population largely distinct from actively cycling Sox2⁺ and eR1⁺ cells, were found to give rise to fully-traced glands after an extended tracing period of 540 days, expanding bidirectionally towards both the pit and gland base (Hayakawa et al., 2015b). However, *Mist1* also marks chief cells (Hayakawa et al., 2015b), and the possibility of Mist1⁺ chief cells contributing to fully-traced glands should not be ignored. In contrast, long-term tracing of randomly labelled clones derived from a *Rosa26-CreERT2;R26R-Confetti* mouse line has revealed a model in which two distinct isthmus-localised and gland base-localised stem cell pools exist that maintain the upper pit-isthmus-neck and gland base regions respectively during homeostasis (Han et al., 2019). This model is also supported by an independent pulse chase study to track BrdU-labelled proliferating cells (Burclaff et al., 2020). Long-term tracing of proliferative Stmn1⁺ cells in the isthmus confirmed their contribution to the pit-isthmus-neck compartment, although a minor subset of tracing events was observed from rare proliferative chief cells at the gland bases (Han et al., 2019).

The limitations of many of these proposed IsthSC markers in unequivocally identifying distinct IsthSC pools, along with potential cell lineage plasticity between these stem-like populations, have made it challenging to define the precise contributions of the IsthSCs to the homeostatic renewal of entire corpus glands. Recently, the IQ motif containing GTPase activating protein 3 (*Iqgap3*) was identified as a highly specific marker of the proliferative IsthSC population (Matsuo et al., 2021). *Iqgap3*⁺ cells exhibit stem potential and can give rise to the pit mucous, mucous neck, and chief cell lineages *in vitro*, together with parietal cells *in vivo* (Matsuo et al., 2021). This variation in lineage differentiation could be due to the failure to fully recapitulate the complex *in vivo* microenvironment which supports stem cell differentiation in an *in vitro* epithelial system. Interestingly, long-term lineage tracing of *Iqgap3*⁺ cell-derived progeny using the *Iqgap3-2A-CreERT2;Rosa-tdTomato* mouse model shows that ultimately not all chief cells at the gland bases are *tdTomato*⁺ (Matsuo et al., 2021), suggesting that these isthmus cells only partially contribute to the maintenance of gland base cells during homeostasis. Alternatively, the lack of tracing observed in a subset of chief cells could be attributed to the slow turnover rate of chief cells (Magami et al., 2002) extending beyond the 1 year duration of lineage tracing documented in this study.

Other putative IsthSC markers appear to label non-proliferative populations within the corpus. Mist1⁺ and approximately half of Sox2⁺ IsthSCs were found to be quiescent during homeostasis (Arnold et al., 2011; Hayakawa et al., 2015b). It is worth noting that the nature of the lineage tracing method biases the discovery of proliferative stem cell populations over quiescent populations that do not actively give rise to labelled clones under homeostatic conditions, or do so at very slow rates. Thus, alternative unbiased methods such as scRNA-seq that profile the cellular heterogeneity present within these glands under both homeostatic and injury/stress conditions could be used to identify quiescent populations.

IsthSCs have also been implicated in injury response and regeneration. Both acute acetic acid injury and chronic injury from *Helicobacter felis* infection increase proliferation of the originally quiescent Mist1⁺ IsthSCs at the injury site, pointing to the contributions of Mist1⁺ cells in regeneration (Nienhüser et al., 2021). Other acute injury models utilising high-dose tamoxifen (HDT) or the parietal cell protonophore DMP-777 to deplete parietal cells have also been used to generate short-term injury to the corpus (Manning et al., 2020). In one study, HDT treatment was used to simultaneously ablate the parietal cell population and initiate tracing from Iqgap3⁺ cells in *Iqgap3-2A-CreERT2;Rosa-tdTomato* mice (Matsuo et al., 2021). 14 days after HDT induction, patches of tdTomato tracing were observed in the regenerating glands, suggesting that Iqgap3⁺ cells play a role in the formation of new parietal cells and other epithelial lineages (Matsuo et al., 2021). However, HDT injury triggers the upregulation of Iqgap3 expression throughout the isthmus and base, making it unclear whether the traced progenies are derived from homeostatic Iqgap3⁺ populations or from the injury-activated Iqgap3⁺ cell pool. Similarly, Lrig1⁺ progenitor cells can give rise to all gland lineages following DMP-777-induced parietal cell loss, and are highly proliferative during the recovery phase, although the LRIG1 protein is not necessary for regeneration (Choi et al., 2018). Across these diverse injury models, IsthSC populations have been found to regenerate the epithelium and restore its integrity.

Chief cells as a quiescent/reserve stem cell population

Despite being a terminally differentiated lineage, chief cells reportedly make up an independent stem cell pool at the base of the corpus gland (Stange et al., 2013). One subset of chief cells in mice is labelled by the Wnt target gene *Troy* (Wilhelm et al., 2017). Lineage tracing performed over a period of 1.5 years revealed that Troy⁺ chief cells undergo a slow clonal expansion, giving rise to all differentiated cell types in the mouse corpus (Stange et al., 2013). Separately, marker-free tracing confirmed that gland base cells can expand clonally, albeit only to the basal region and not the entire gland (Han et al., 2019). These findings suggest that chief cells present at corpus gland bases are involved to some degree in homeostatic renewal, although their relative contributions to the various regions of the glandular units remain unclear. In contrast, a subpopulation of chief cells expressing *Lgr5*, a well-defined marker of

multiple Wnt-responsive stem cell populations throughout the gastrointestinal tract (Barker et al., 2007; Barker et al., 2010a), is known to be dispensable for epithelial homeostasis (Leushacke et al., 2017). Under homeostatic conditions, Lgr5⁺ chief cells do not proliferate or contribute to the different lineages in the corpus gland, even over a lineage tracing period of 6 months (Leushacke et al., 2017). Indeed, although selective ablation of Lgr5-expressing cells severely impairs the structure of the corpus glandular epithelium, the tissue does eventually regenerate, suggesting that non-Lgr5-expressing populations contribute to the repair process (Leushacke et al., 2017). Lgr5⁺ cells are more restricted to the gland base region, whereas Troy is expressed in a wider population of cells within the lower third of the corpus gland, overlapping with both the Lgr5⁺ chief cells and the parietal cell lineages (Stange et al., 2013; Leushacke et al., 2017). Thus, the Troy⁺Lgr5⁻ population could be responsible for the homeostatic stem cell behaviour of Troy⁺ cells in the corpus. Even so, the slow clonal expansion dynamics exhibited by Troy⁺ cells together with the complete absence of tracing from Lgr5⁺ cells suggest that corpus chief cells in general do not actively contribute to rapid epithelial turnover and remain in a relatively quiescent state during homeostasis.

During injury, chief cells play a more pronounced role in directing tissue regeneration. Lgr5⁺ chief cells actively proliferate during parietal cell atrophy-induced injury of the epithelium (Leushacke et al., 2017). Lineage tracing following injury reveals that Lgr5⁺ chief cells give rise to the different epithelial lineages throughout the whole gland unit (Leushacke et al., 2017). Similarly, upon 5-fluorouracil (5-FU)-mediated ablation of proliferative cells, Troy⁺ chief cells begin to proliferate and undergo clonal expansion at a much higher rate than under homeostatic conditions, with some clones regenerating the entire gastric unit within 1 month (Stange et al., 2013). Mist1⁺ cells, which include both quiescent chief cells and quiescent IsthSCs, also enter the cell cycle following acetic acid injury and generate tracing units around the healing zone of the resultant gastric ulcers (Nienhüser et al., 2021). Loss of Lgr5⁺ chief cells in this injury context does not affect ulcer size or perturb the regenerative capacity of the tissue, implying that Lgr5⁺ chief cells are not essential for the regenerative process, although the substantial *Lgr5* expression that remains in the injured tissue indicates that a minor surviving pool of unablated Lgr5⁺ cells could be sufficient to maintain the regenerative potential of the epithelium following injury (Nienhüser et al., 2021). Collectively, these studies confirm that the differentiated chief cell population has the capacity to exit the quiescent state and actively proliferate during injury conditions to regenerate the tissue. However, the precise contributions of each chief cell population and the influence of the specific injury model used remain unclear.

Further investigations have uncovered possible mechanisms by which chief cells switch into a more stem cell-like state for regeneration. During injury, upregulation of matrix metalloproteinase-7 (*Mmp7*) (a target gene of β -catenin/TCF-4) (Brabletz et al., 1999) and downregulation of the Wnt inhibitor sclerostin domain containing 1 (*Sostdc1*) (Ahn et al., 2010; Ahn et al., 2013) within Lgr5⁺ chief cells

promote elevated Wnt signalling, a key driver of stem cell activity (Leushacke et al., 2017). More recently, cyclin-dependent kinase inhibitor 1C p57^{Kip2} was found to act as a molecular switch, with the loss of p57 during injury converting chief cells from a quiescent state into a stem cell-like state to regenerate the damaged tissue (Lee et al., 2022a), although the mechanisms through which injury induces these signalling changes remain unclear. This process of chief cell transdifferentiation also involves a stepwise program known as pangenesis, beginning with DNA damage induced transcript 4 (DDIT4)-driven autophagy of differentiated cell structures followed by mTORC1-regulated cell cycle re-entry that promotes proliferation (Willet et al., 2018; Miao et al., 2020b; Miao et al., 2021). This chief cell plasticity can lead to the development of spasmolytic polypeptide-expressing metaplasia (SPEM), a precancerous stage of gastric cancer (Nam et al., 2010; Caldwell et al., 2022). Activation of the oncogenic Kras G12D mutation in Lgr5⁺ chief cells promotes the development of metaplastic corpus lesions, highlighting their role as a cell-of-origin of early-stage gastric cancer in the corpus (Leushacke et al., 2017), with a similar phenotype observed in Ras-activated Mist1⁺ chief cells (Choi et al., 2016). In contrast, an independent study found that chief cells marked by GPR30 were not activated but instead outcompeted during metaplasia development (Hata et al., 2018). It is possible that isthmus cells, including those labelled by Mist1 (Hayakawa et al., 2015b), or neck progenitor cells (Hata et al., 2018), also act as sources of SPEM, adding further complexity to these proposed models.

The studies discussed above have failed to provide unifying models of homeostasis or regeneration in the corpus. This is likely due to differences in the physiological accuracy of the reporter models used to mark prospective stem cell populations, the potential use of damaging Tamoxifen doses for some homeostatic lineage tracing studies and highly context-dependent differences in the response of plastic “reserve” stem cells to specific types of injury. The development of tamoxifen-independent Tet-On systems and the identification of more defined markers should address the limitations of the above models (Gossen and Bujard, 1992; Das et al., 2016). Despite these often confounding observations, it is clear that the corpus epithelium harbours active stem cell and progenitor pools responsible for driving tissue renewal throughout life, and highly plastic differentiated populations capable of switching to proliferating stem cells that actively contribute to regeneration following injury.

Epithelial stem cells in the adult pylorus

The adult pylorus is maintained by gastric epithelial stem/progenitor cells that are thought to reside in the isthmus and base regions of the glandular units (**Fig. 1C**). As in the corpus, multiple markers that purportedly identify independent stem cell populations responsible for epithelial homeostasis have been reported. One such marker is Lgr5, whose expression is restricted to the base of pyloric glands. Lineage tracing and organoid initiation assays of mouse pyloric stem cells have identified this Lgr5⁺ population

as active stem cells contributing to the rapid renewal of the gastric epithelium that occurs every 7-10 days (Barker et al., 2010a). The pyloric gland bases also harbour a population of stem/progenitor cells marked by Lrig1 capable of differentiating into various epithelial lineages (Choi et al., 2018). Lrig1+ cells are found to overlap with Lgr5+ cells in the pyloric gland, although Lrig1 has a broader expression than Lgr5 (Schweiger et al., 2018). It remains to be seen whether the Lrig1+Lgr5- compartment also harbours a stem cell pool.

In contrast, mouse pyloric stem cells expressing the gastrin receptor Cck2r are predominantly located above the Lgr5+ compartment, with little to no co-localisation with Lgr5-GFP+ cells (Hayakawa et al., 2015a; Chang et al., 2020). Cck2r+ cells can give rise to fully-traced glands encompassing the differentiated epithelial cell lineages (Hayakawa et al., 2015a). Notably, Lgr5+ cell ablation in *Lgr5-DTR-GFP;Cck2r-CreERT;R26-tdTomato* mice induces proliferation of Cck2r+ stem cells and does not appear to significantly disrupt homeostasis of the pyloric epithelium (Chang et al., 2020). These findings hint at the plasticity of the Cck2r+ population to compensate for the loss of the Lgr5+ stem cell pool and maintain the pyloric epithelium (Chang et al., 2020), although it is unclear whether the reduced efficacy of the Lgr5-DTR-GFP model in ablating Lgr5+ cells relative to the published Lgr5-2A-DTR model could have influenced this outcome (Tan et al., 2021). It will be worth exploring the underlying molecular mechanism by which Cck2r+ stem cells detect dysregulations in homeostasis and alter their state to meet the homeostatic need of the stomach, potentially through the downregulation of gastrin which normally maintains quiescence of Cck2r+ stem cells (Chang et al., 2020), and whether other stem cell pools possess such plasticity.

Stem cell populations have also been identified in the pyloric isthmus. eR1+ and Bmi1+ cells within the isthmus generate progeny spanning entire pyloric glands and initiate organoids *in vitro* (Matsuo et al., 2017; Yoshioka et al., 2019). Bmi1+ cells are reported to not co-localise with eR1+ or Lgr5+ cells, suggesting that these represent distinct cell populations (Yoshioka et al., 2019). Ablation of Bmi1+ cells leads to prominent signs of injury in both the corpus and pyloric epithelium that are rapidly restored to homeostatic conditions after 1 week (Yoshioka et al., 2019), although it is unclear whether this regeneration is mediated by remaining, unablated Bmi1+ stem cells or through the activation of other stem cell populations. Therefore, while the various stem cell populations identified within the pylorus play a pronounced role in homeostasis, their relative contributions and importance remain elusive.

Many candidate biomarkers of pyloric stem cells are shared with corpus stem/progenitor cells found within the same gastric tissue, or are incompatible with antibody-based isolation methodologies (**Table 1**), hindering the ability to isolate and characterise these stem cells in the mouse and human stomach. For instance, Lgr5 marks both pyloric stem cells and chief cells (Leushacke et al., 2017). Despite Lgr5 being a cell-surface marker, its limited expression on homeostatic stem cells makes it extremely

challenging to isolate endogenous Lgr5⁺ stem cells using available antibodies (Barker et al., 2013). Therefore, specific surface biomarkers are needed. Recently, comparative transcriptomic profiling of Lgr5⁺ cells along the gastrointestinal tract identified the water channel protein Aquaporin 5 (Aqp5) as a highly selective surface marker of Lgr5⁺ pyloric stem cells, both in mice and humans (Tan et al., 2020). Apart from Aqp5, differential gene expression analysis of pyloric Aqp5⁺ and Aqp5⁻ cell transcriptomes has revealed additional markers specific to the pyloric stem cell population facilitating their isolation and further characterisation (Tan et al., 2020). Interestingly, Aqp5 was also found to mark an early pool of chief cells transitioning into SPEM (Lee et al., 2022b), possibly representing a corpus stem-like population in this context.

Studies have also suggested that “reserve” stem cells also exist in the pyloric glands to effect rapid epithelial regeneration following injury. In mice, rare pyloric epithelial cells located close to the isthmus region that display Villin promoter activity contribute to glandular regeneration following inflammation (Qiao et al., 2007), although the underlying mechanism driving the activation of this Villin⁺ population remains unknown. In addition, Bmi1⁺ pyloric cells contribute progeny to regenerating glands following whole-body irradiation and acetic acid injury to the pylorus (Yoshioka et al., 2019). Given that Bmi1⁺ cells have roles in both homeostatic renewal and regeneration, it remains unclear whether the dynamics and contribution of this cell pool is altered under these different contexts, and if specific subpopulations of Bmi1⁺ cells might play distinct roles during homeostasis and regeneration. The contributions of the Lgr5⁺/Aqp5⁺ gland base stem cell population to epithelial repair following injury have yet to be evaluated. Differences in the local tissue microenvironment and niche factors supporting these isthmus and gland base stem cells could serve as potential regulators of their functions in both homeostasis and regeneration.

Adult Gastric Stem Cell Niche

Several pathways are known to regulate the maintenance and activity of the tissue-resident gastric ASCs, which together make up the stem cell niche. Notch signalling, a highly conserved pathway whereby a receptor-ligand interaction triggers the nuclear translocation of the Notch intracellular domain (NICD), has been implicated in promoting proliferation of both corpus IsthSCs and pyloric stem cells while inhibiting their differentiation (Demitrack et al., 2015; Demitrack and Samuelson, 2016; Demitrack et al., 2017; Gifford et al., 2017). In the pylorus, this signalling is mediated by the Notch1 receptors expressed on gland base stem cells binding to the Notch ligand Delta-like 1 (DLL1) on adjacent GS-II⁺ deep mucous cells (Horita et al., 2022). Another major signalling pathway active within the niche is the Wnt pathway. Within the gastric glands, expression of Wnt target genes such as Axin2 and Lgr5 presents as a gradient with the highest Wnt activity at the gland base (Sigal et al., 2017).

Unlike Notch, the source of Wnt signalling has been largely attributed to the stromal populations surrounding gastric ASCs. In particular, Rspo3, which is secreted by subepithelial myofibroblasts directly adjacent to the stem cell compartment, promotes the proliferation of the Axin2+/Lgr5- stem cells at the pyloric gland bases (Sigal et al., 2017) and regulates the balance between chief/parietal and pit cell lineage differentiation in the corpus (Fischer et al., 2022). Indeed, exogenous manipulation of Wnt activity in mouse and human gastric organoids was sufficient to direct acquisition of lineage fates in a bimodal axis, with high Wnt levels promoting gland base and neck cells and low Wnt levels favouring the pit mucous lineages (Barker et al., 2010a; Bartfeld et al., 2015; Fischer et al., 2022). This concentration of Wnt activity within the stem cell compartment is also driven by Wnt5a produced by Cxcr4+ innate lymphoid cells, which, together with Cxcl12+ endothelial cells make up a perivascular niche for IsthSCs in the corpus (Hayakawa et al., 2015b). While Cxcr4+ immune and basal epithelial cells are also present in the pyloric mucosa (Sakitani et al., 2017), it is unclear whether they play similar roles in supporting Wnt-driven stem cell activation. Additional niche pathways are just starting to be elucidated, including the activation of a RAS-ERK-CD44-STAT3-Cyclin D1 axis by the IsthSC marker IQGAP3 implicated in driving IsthSC proliferation (Khurana et al., 2013; Matsuo et al., 2021), and cholinergic signalling mediated by acetylcholine from nerve fibers within the submucosal layer that binds to the acetylcholine receptors present on Lgr5+ stem cells in the pylorus, promoting their expansion (Hayakawa et al., 2017). Finally, in addition to these signalling pathways, parietal cells in the isthmus zone were found to physically impede the lateral expansion of IsthSCs, resulting in a “punctuated” neutral drift model of stem cell proliferation (Han et al., 2019).

Conclusion

This Review explores the crucial role that gastric epithelial stem cells play during development, homeostasis and regeneration. In early stomach development, an interplay of transcription factors and signalling pathways is required for stomach patterning and the differentiation of stem cells into various epithelial cell lineages. Recent technological advances in organoid systems coupled with high-throughput (spatial) transcriptomics offer new insights into the niche factors required for development. In the mature stomach, multiple gastric ASC populations maintained by their niche within corpus and pyloric glands continuously sustain the homeostasis and regeneration of the epithelium. Although conflicting findings on the identity and roles of these ASC populations still exist, newer unbiased transcriptomic and lineage tracing approaches have facilitated an in-depth characterisation of these stem cells and emphasised the need for more specific markers to better isolate, study, and target them.

These studies in gastric epithelial stem cells offer great clinical relevance. The identification of Aqp5 as a human pyloric stem cell marker (Tan et al., 2020) has facilitated the isolation and study of this

population for the first time from native human tissues. Stem cells are known to be a major source of cancer, in light of their self-renewal properties that allow for the long-term accumulation of cancer-promoting mutations. Indeed, many of the stem cell populations highlighted in this review are also implicated in carcinogenesis and cancer progression (Khurana et al., 2013; Hayakawa et al., 2015a; Hayakawa et al., 2015b; Leushacke et al., 2017), including differentiated chief and isthmus cells both proposed to give rise to SPEM upon injury (Hayakawa et al., 2015b; Caldwell et al., 2022), although the precise origins of SPEM have not yet been fully resolved. A molecular understanding of their regulation will therefore facilitate advances in targeted cancer therapeutics. Although the majority of gastric stem cell markers have been identified using mouse models, studies that profile human gastric tissues spatially and at single-cell resolution (Busslinger et al., 2021; Dong et al., 2022) can offer more clinically relevant insights. These findings can be coupled with the creation of complex mouse and organoid models that closely recapitulate human gastric development and homeostasis while remaining highly accessible and experimentally tractable. The combination of these approaches will facilitate an exhaustive characterisation and validation of the niche factors and regulators of stem/progenitor cells at all developmental stages and adulthood.

Glossary

Antrum: Distal non-acid-secreting region of the human stomach that connects to the pylorus.

Basal gland cells: Mucus-producing cells located at the base of pyloric glands.

Cardia: Proximal region of the human stomach that connects to the oesophagus.

Chief cells: Differentiated epithelial cells found at the gland base of corpus glands responsible for secreting the zymogen pepsinogen and gastric lipases.

Corpus: Main body of both the human and mouse stomach.

Corpus glands: Tubular glands in the corpus that comprise mucus-, endocrine- and acid-secreting epithelial cells.

Enteroendocrine cells: Differentiated hormone-secreting epithelial cells found throughout corpus and pyloric glands.

Fundus: Proximal region of the human stomach that lies between the cardia and the corpus.

Mast cells: Immune cells that release granules upon activation by inflammatory signals.

Mucous neck cells: Mucus-producing cells that primarily line the surface of the gastric neck region.

Parietal cells: Differentiated epithelial cells found at the neck of corpus glands that secrete gastric acid.

Progenitor cell: An undifferentiated cell capable of self-renewal and differentiation. Compared to stem cells, progenitor cells are limited in the cell types they can differentiate into.

Pyloric glands: Tubular glands in the pylorus that comprise mucus- and endocrine-secreting epithelial cells.

Pylorus: Distal region of the human and mouse stomach that connects to the duodenum of the small intestine. In mice, this is also known as the pyloric antrum.

Stem cell: A relatively undifferentiated cell capable of self-renewal and differentiation into all cell types.

Surface / pit mucous cells: Differentiated mucus-producing cells that primarily line the pit region of the corpus and pyloric glands.

Tuft cells: Rare differentiated chemosensory epithelial cells found in corpus and pyloric glands.

Competing interests

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Corpus		Pylorus		References
Isthmus	Gland Bases	Isthmus	Gland Bases	
-	-	-	Aqp5	Tan et al., 2020
Bmi1	-	Bmi1	-	Yoshioka et al., 2019
CD44	-	-	CD44	Khurana et al., 2013; Takaishi et al., 2009
eR1	-	eR1	-	Matsuo et al., 2017
Iqgap3	-	-	-	Matsuo et al., 2021
-	Lgr5	-	Lgr5	Barker et al., 2010; Leushacke et al., 2017
Lrig1	-	-	Lrig1	Choi et al., 2018
Mist1	Mist1	-	-	Hayakawa et al., 2015b; Nienhüser et al., 2021
Sox2	Sox2	-	Sox2	Arnold et al., 2011
Stmn1	-	-	-	Han et al., 2019
TFF2 mRNA	-	-	-	Quante et al., 2010
-	Troy	-	-	Stange et al., 2013
-	-	Villin	Villin	Qiao et al., 2007

Table 1: Comparison of stem cell markers in the corpus and pylorus.

Figure Legends

Fig. 1: Stomach morphology and gland structures of the corpus and pylorus.

(A and B) Diagram of the major regions in human (A) and mouse (B) stomachs. (C) Illustration of the gland structure and cell populations within a stereotypical mouse corpus (top) and pylorus (bottom) gland. The corpus gland contains the pit, isthmus, neck, and base regions, while the pyloric gland contains the pit, isthmus, and base regions. Stem cells (green) and progenitor cells (light purple) residing in the isthmus region of the corpus gland regulate epithelial homeostasis and regeneration, whereas chief cells (dark purple) at the corpus gland base function as reserve stem cells that are activated following injury to regenerate the epithelium. In the pyloric gland, stem cells (green) are found throughout the isthmus and base regions, contributing to homeostasis and regeneration.

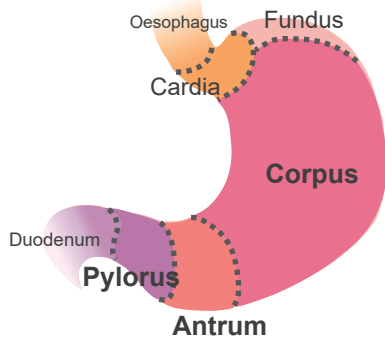
Fig. 2: Lineage specification of gastric progenitors during early mouse gastric development.

Schematic illustration of the gastric epithelial lineage specification process whereby solid lines mark a well-defined route and dotted lines represent proposed routes that warrant further investigation. ASCL1 is thought to serve as the master regulator for all endocrine lineages (yellow) in the stomach. Ngn3-expressing progenitors specify the G and D cell lineages, whereas other unidentified progenitor populations likely specify the EC, ECL, and X/A lineages. The neck progenitor cells (dark purple) differentiate into mucous neck cells (grey) which give rise to chief cells (light purple), regulated by XBP1 and Mist1 signalling. Transcription factors specifying the parietal cell lineage remain unclear; however, factors such as TGF α and SHH secreted by parietal cells are thought to influence chief cell specification (blue dotted line). FOXQ1 is reported to regulate pit progenitor (pink) differentiation. Factors required for the specification of each lineage are shown in blue text, and cell type markers are shown in brackets. Please refer to the main text for more details on the different factors. ASCL1: Achaete-scute homolog 1, ARX: Aristaless Related Homeobox, D: Delta, EC: Enterochromaffin, ECL: Enterochromaffin-like, FOXQ1: Forkhead box Q1, G: Gastrin, MIST1: Muscle intestine stomach expression 1, NGN3: Neurogenin 3, NKX6-3: NK6 homeobox 3, PAX4: Paired Box 4, PDX1: Pancreatic and duodenal homeobox 1, SHH: Sonic hedgehog, TGF: Transforming growth factor, X/A: X- or A-like, XBP1: X-box binding protein 1.

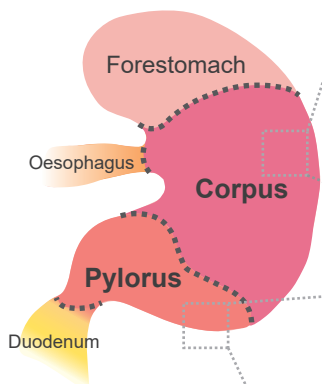
Fig. 3: Organoid modelling systems for gastric development studies. (A) Generation of human mucosoids from adult corpus gastric glands. Mucosoids are maintained in culture with Noggin, Wnt, RSPO1, EGF, FGF and Gastrin. Different combinations of EGF and BMP are used to derive the pit, parietal, and chief cell lineages in the mucosoids. (B) Generation of fundic and antral organoids from human induced pluripotent stem cells (hiPSCs). Treatment with Activin A promotes definitive endoderm formation. Subsequent treatment with EGF, RA, FGF, Wnt and Noggin leads to the formation of posterior foregut spheroids (grey circles). The presence or absence of the Wnt activator CHIR99021

determines their subsequent differentiation into fundus or antral human gastric organoids (hGO) respectively. (C) Mouse embryonic stem cells (mESC) give rise to definitive endoderm following FBS induction. KSR, DKK and SHH growth factors promote the formation of foregut spheroids. Medium containing Rspo1, FGF, Wnt and Noggin further promotes the establishment of mouse gastric organoids (mGO) presenting both corpus and antral lineage markers. Factors required for the specification of each lineage are shown in grey text, and cell type markers are shown in blue text. Please refer to the main text for more details on each study. BMP: Bone morphogenetic protein, EGF: Epidermal growth factor, FBS: Fetal bovine serum, FGF: Fibroblast growth factor, RSPO1: R-spondin 1, RA: Retinoic acid, KSR: Knockout serum replacement, DKK: Dickkopf, SHH: Sonic hedgehog.

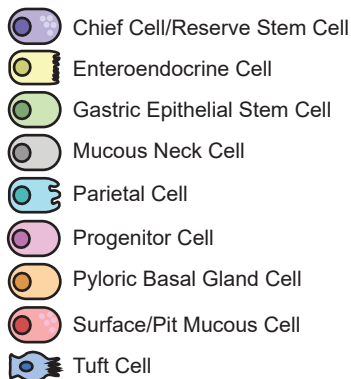
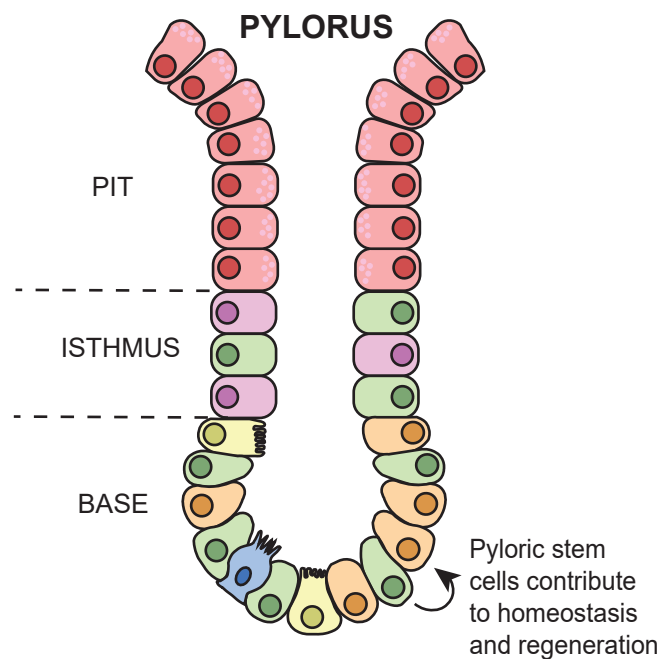
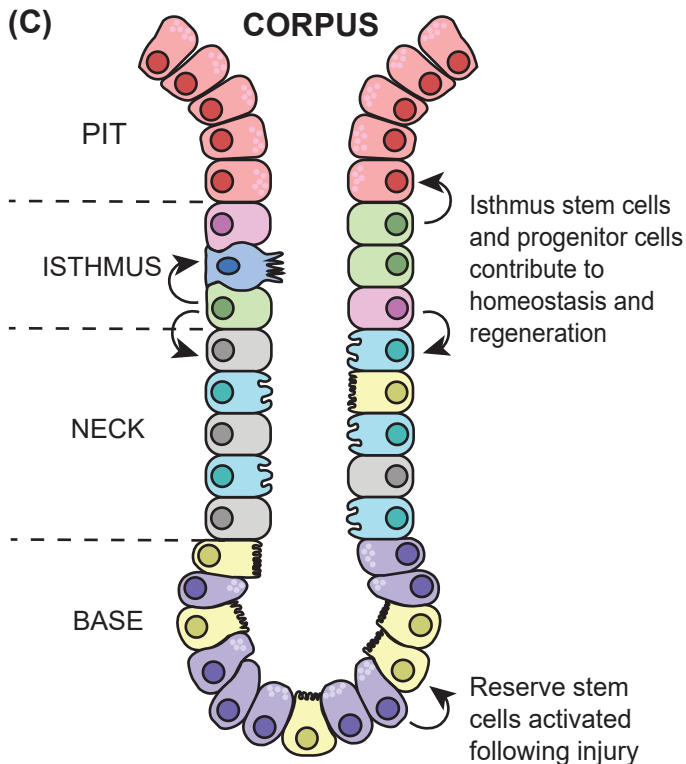
(A) Human stomach



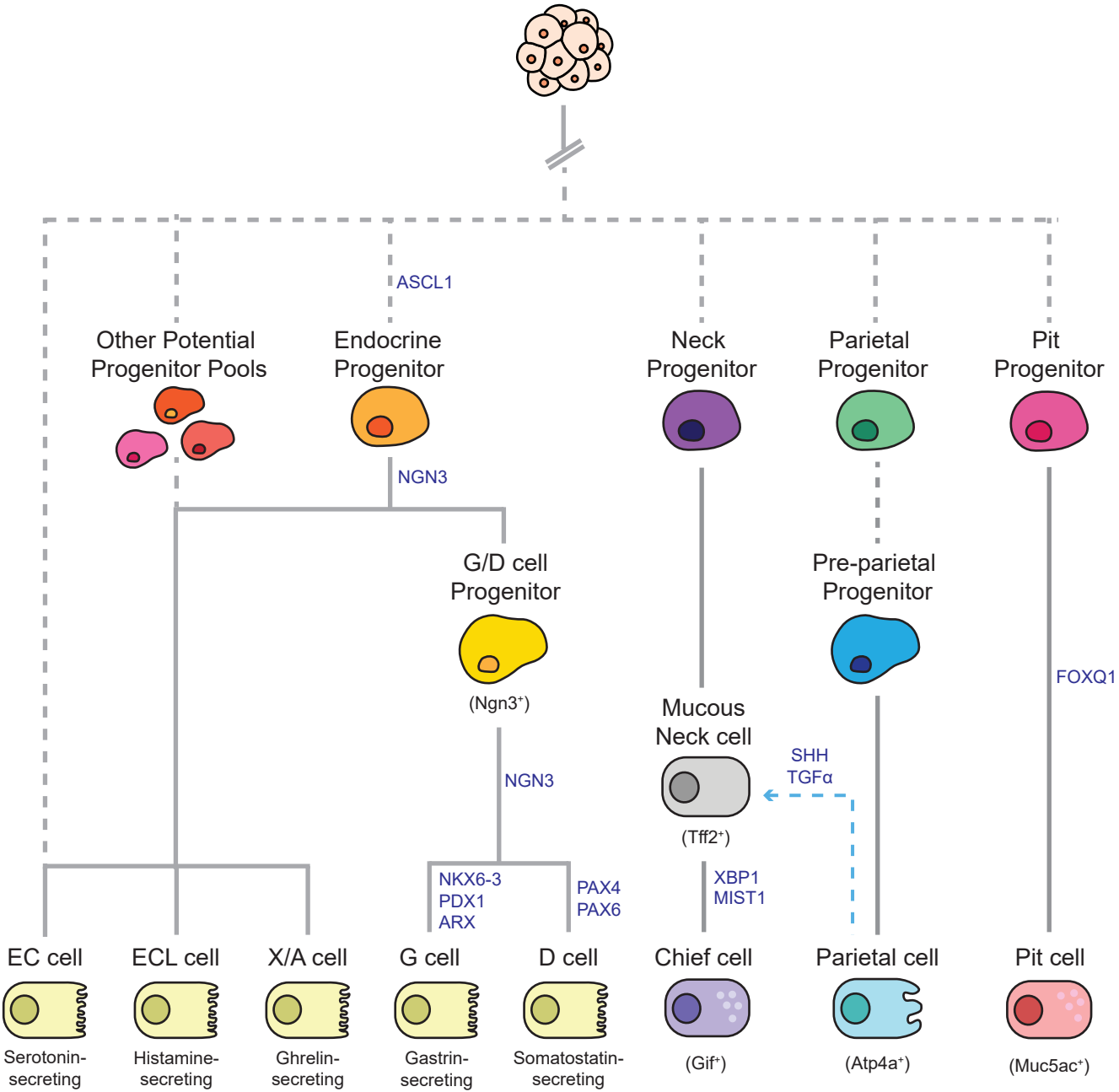
(B) Mouse stomach



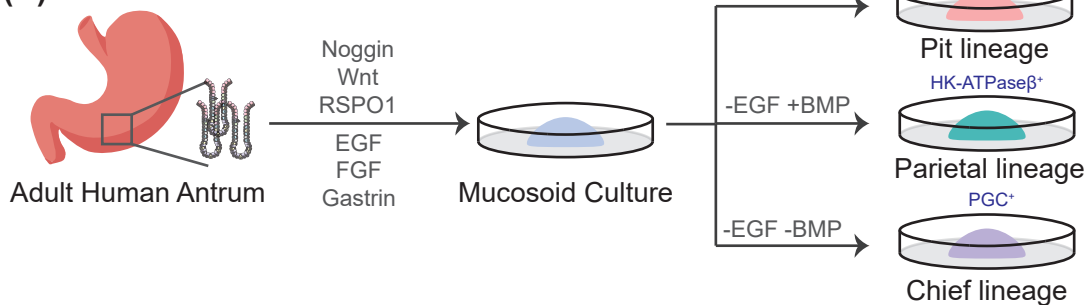
(C)



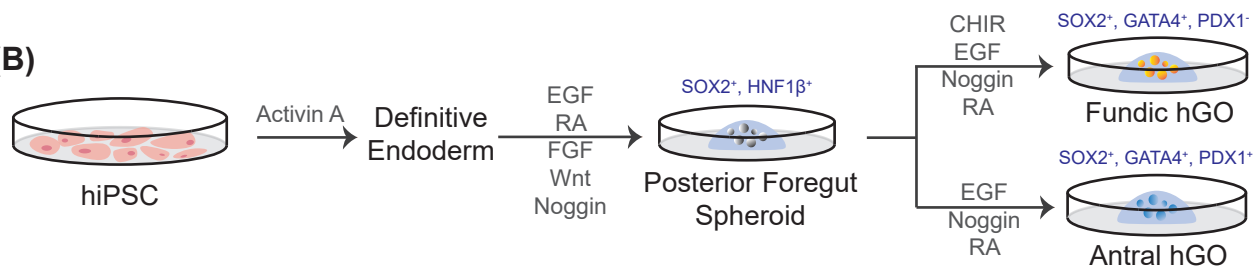
Mouse Embryonic Stem Cells



(A)



(B)



(C)

