Review Article

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Gastric stem cell research and gastric organoids

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The stomach is a complex organ lined with ordered epithelium consisting of different adult stem cell (ASC) pools. In the previous decade, research into gastric epithelial stem cells has been performed using lineage tracing methods, and several putative ASC markers in the gastric gland have been identified, although their roles in homeostasis maintenance and the origin of cancer remain to be clarified. With advances in gastric stem cell research, 3-dimensional (3D) organoid culture has been developed on the basis of in-depth insights into the control of stem cell self-renewal, proliferation, and differentiation. Since the initial report that single intestinal stem cells have the ability to generate long-lived 3D structures that exhibit budding forms and self-renewal, tissue-specific adaptations of this method have been established in various organs, such as the small intestine, colon, liver, and stomach. In the murine stomach, putative ASCs isolated from the corpus and antrum generate gastric organoids that can simulate organ-specific cells to some extent. In addition, a few trials have been conducted to generate long-lived 3D organoids using human-derived ASCs and pluripotent stem cells. We hope that this review will provide comprehensive knowledge on gastric stem cell research and gastric organoids.

Keywords: Stem cells; Genetic markers; Stomach; Organoids; *Helicobacter pylori*

Introduction

The stomach is a digestive organ responsible for the mechanical and chemical digestion of food. Various physiological events, including acid secretion, mucin secretion, and hormone production, occur in specialized cells of the stomach [1]. Two glandular units, the corpus and antrum, harbor functional cells and constitute the epithelial layer of the stomach (Fig. 1). Unlike in humans, the murine stomach contains a non-glandular structure, called the forestomach, with stratified epithelial cells in the upper part of the corpus [1,2]. Varying types of mature cells are sequentially differentiated and make up the corpus and antrum (Fig. 1B). Mucin5AC (MUC5AC)-secreting pit cells, trefoil factor family 2 (TFF2), and GSII from *Griffonia simplicifolia* (GSII)-expressing mucous neck cells, H/K ATPase positive parietal cells, zymogenic granule-secreting chief cells, and enteroendocrine cells (G-cells, D-cells, and enterochromaffin-like cells) contribute to the physiological function of the gastric glands [3,4]. Those differentiated epithelial cells emerge from adult stem cells (ASCs) that exhibit diverse markers in specialized locations (Fig. 1B).

Since Hans Clevers' group introduced intestinal organoid culture from single leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)+ stem cells in 2009 [5], this novel method has been utilized in studies of various organs, including the colon [6], tongue [7], brain [8], gut [9], liver [10], and kidney [11]. Furthermore, organoid technology is being applied in regenerative medicine and drug discovery as a replacement for clinical research [12,13].

The gastric epithelium must self-renew its own structure be-

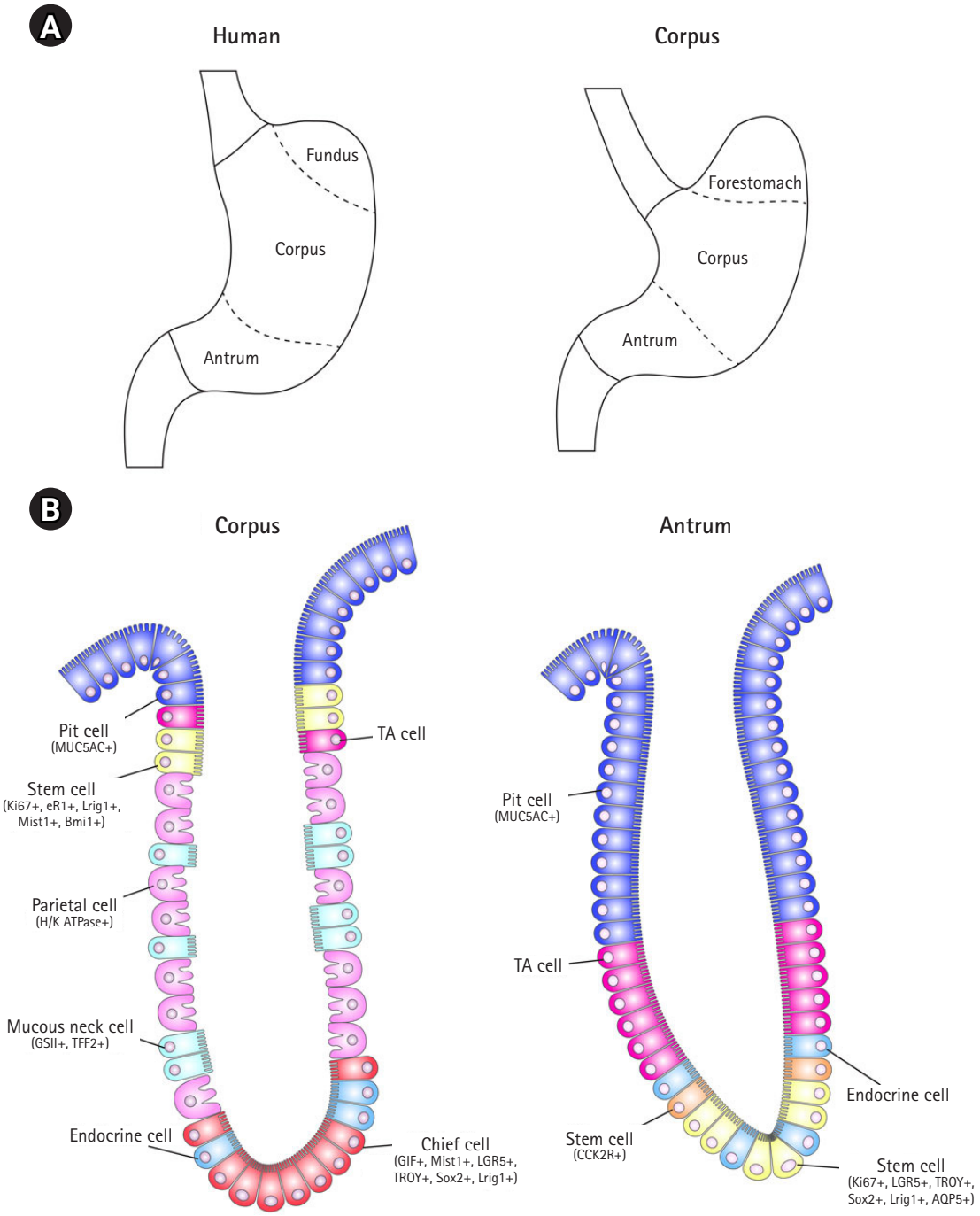

Fig. 1. Illustrations of the human and mouse stomach. (A) Anatomy of the human and mouse stomach. (B) Images show the distribution of adult stem cells and functional units in the corpus and antrum. The defined molecular markers for adult stem cells and gastric lineage cells are marked in parentheses. Transit-amplifying (TA) cells are an undifferentiated subset in transition between stem cells and differentiated cells. eR1, Runx1 enhance element; GSII, lectin GS-II from *Griffonia simplicifolia*.

cause it constantly encounters a harsh environment [14]. Thus, several studies have focused on the identification of gastric stem cells and the construction of gastric organoids using populations thereof. The human and mouse stomach consists of 2 representative glandular structures that have distinct cellular lineages: the corpus and antrum [15]. The corpus is the main acid-producing region of the stomach, and gastric acid-secreting parietal cells are ablated during *Helicobacter pylori* or *Helicobacter felis* infection [16] or treatment with metaplasia-inducing chemicals (DMP-777, L-635, high-dose tamoxifen [HDT]) [17–19]. These injuries activate the ASCs responsible for mucosal recovery. The antrum gland contains gastrin-secreting endocrine

cells, and gastric tumors predominantly develop in the antrum in response to treatment with a carcinogenic agent (N-methyl-N-nitrosourea, MNU) [20,21].

Stem cells are characterized by self-renewal and multi-potency. These specialized cells are crucial for maintaining tissue homeostasis and response to injury [22,23]. Gastric stem cells reside in the base of the antrum in both humans and mice, expressing distinct molecular markers such as LGR5, cholecystokinin 2 receptor (CCKR2), axis inhibition protein 2 (AXIN2), aquaporin-5 (AQP5), and leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1). Barker et al. [9] found that single LGR5+ cells from the antrum gland could generate gastric organoids, generating most antrum cell lineages. By contrast, the location of ASCs that maintain tissue homeostasis and contribute to gastric cancer in the corpus remains a matter of debate [3]. In the corpus, 2 known populations have been identified as putative ASCs. One is located in the isthmus region, where highly proliferating cells reside, and the other is located in the base of the gland, where a subset of mature chief cells act as quiescent "reserve" stem cells. A recent lineage tracing study demonstrated that single stem cells derived from both regions have the capacity to generate all corpus lineages.

Cancer stem cells (CSCs) are defined by their ability to self-renew and maintain a malignant tumor. Their characteristics are highly similar to the properties of stem cells in normal tissues. It has been believed that CSCs originate from stem cells rather than differentiated cells [24,25]. In gastric cancer progression, gastric stem cells act as the origin of cancer [26], developing spasmolytic polypeptide expressing metaplasia (SPEM) and intestinal metaplasia toward gastric cancer. Due to the low cost of breeding and high homologies with human genes, murine models have been broadly utilized in stem cell research as well as cancer research [27,28]. Indeed, SPEM has been noted in mice models, including those with *H. pylori* or *H. felis* infection [29,30]. Furthermore, human-resembling cancer could be recapitulated in stomach-specific transgenic mice [31]. A recent study also established metaplastic organoids using the murine dysplasia model [32], suggesting that organoids are a promising tool for studying gastric carcinogenesis. Collectively, this review aims to present a comprehensive range of knowledge on gastric stem cell research and stem cell-derived organoids in humans and mice.

Identification of gastric stem cell populations

A subset of antrum basal cells is responsible for self-renewal, proliferation capacity, and differentiation potential. Putative ASCs expressing distinct molecular markers are present in the base of the gland, and previous studies investigated their potential stem cell activity. In an early study, Barker et al. [9] found that LGR5, which is an R-spondin receptor and WNT target gene, was expressed in the very base of the gland and marked ASCs in the antrum. Using *Lgr5*-driven LacZ systems, they proved that single LGR5+ cells constructed the entire antrum gland within 7 to 10 days and their daughter cells expressed MUC5AC and gastrin. This tracing event persisted for 620 days, revealing the self-renewal activity and multi-potency of LGR5+ cells. Furthermore, considering the high expression of LGR5 in ASCs, researchers developed gastric disease models using an *Lgr5*-driven Cre-LoxP system [33,34]. A recent report showed that a subpopulation of LGR5+ cells strongly expressed AQP5. Of note, AQP5+ cells serve as potential CSCs in humans and mice [35]. Although LGR5+ cells play critical roles in maintaining tissue homeostasis [36], several studies have demonstrated the existence of LGR5-independent stem cell populations in the antrum. SRY-box transcription factor 2 (*Sox2*)+ cells also possess multi-potency, with the capability of generating all lineages of the corpus and antrum, but they do not co-localize with Lgr5 [37]. Recently, antrum stem cells controlled by gastrin-secreting G-cells were identified. This CCK2R+ stem cell pool resides in +4 antrum loci from the base of the gland (+0), is not co-localized with LGR5 (Fig. 1B), and has quiescent characteristics [20,38]. In addition, *Lrig1*, which was initially identified as a stem cell marker along with *Lgr5* [39–41], also marks the quiescent stem cell pool in the lower part of the antrum gland [42,43].

Defining the ASCs and the origin of cancer is more complicated in the corpus than in the antrum because molecular makers overlap in 2 distinct regions (Fig. 1B) [3,44–46]. Reliable reports have shown that there are 2 distinct compartments where ASCs reside in the corpus gland and demonstrated that these cells contribute to regeneration and cancer development. As the cellular origin of gastric stem cells in the corpus remains a matter of debate, researchers are trying to identify a molecular marker that can distinguish the 2 compartments (Table 1) [9,20,34,35,37,38,42,43,47–50]. Previous studies have employed lineage tracing methods to investigate the existence of multipotent ASCs in the corpus epithelium.

It is generally believed that stem cells are the source of cancer due to their properties [51]. Since cells need to acquire multiple mutagenic events to convert to malignant cells, long-lived stem cells have a greater chance to become CSCs. Considerable evidence has suggested that mature chief cells trans-differentiate into pre-neoplastic cells and can be the origin of gastric cancer

Table 1. Characteristics of stem cell-derived gastric organoids

Molecular marker	Study	*In vivo* location	Organoid formation	Constructed lineage in organoid	Culture medium
Lgr5	Barker et al. [9], 2010	Antrum (base)	Yes	Undefined	WENRF
	Leushacke et al. [34], 2017	Corpus (chief cells)	Yes	Pit cells	WENRF
Sox2	Arnold et al. [37], 2011	Corpus and antrum	Undefined	-	-
Troy	Stange et al. [47], 2013	Corpus (chief cell)	Yes	Mucus neck cells, chief cells, pit cells	WENRF
Mist1	Hayakawa et al. [49], 2015	Corpus (isthmus)	Yes	Parietal cells, ECL cells	WENR or ENJ
Bmi1	Yoshioka et al. [50], 2019	Corpus (isthmus) and Antrum (base)	Yes	Undefined	WENR
Cck2r	Hayakawa et al. [38] 2015	Antrum (base)	Yes	Undefined	WENR
	Chang et al. [20], 2020	Antrum (base)	Yes	Pit cells, endocrine cells, *Lgr5*+ cells	WENRF
Aqp5	Tan et al. [35], 2020	Antrum (base)	Yes	Pit cells	WENRF
Lrig1	Schweiger et al. [42], 2018	Corpus (base) and antrum (base)	Yes	Undefined	WENRF
	Choi et al. [43], 2018	Corpus (isthmus) and antrum (base)	Undefined	-	-
eR1	Matsuo et al. [48], 2017	Corpus (isthmus)	Yes	Mucus neck cells, chief cells, pit cells	WENR or ENJ

W, Wnt3A or Wnt; E, epidermal growth factor; N, Noggin; R, R-spondin; F, fibroblast growth factor-10; ECL, enterochromaffinlike; J, Jagged-1.

[19,45,52,53]. Supporting this, stem cell markers such as TNF receptor superfamily 19 (*Troy*) and *Sox2* are strongly expressed in chief cells of the corpus [37,47]. Stange et al. [47] found that *Troy*+ chief cells act as "reserve stem cells" characterized by slow clonal expansion. The cells reproduce the entire gastric unit under conditions of both homeostasis and injury. Unlike the antrum, *Troy*+ chief cells in the corpus exhibited strong expression of *Lgr5* in comparison with *Troy*-cells. Furthermore, basic helix-loop-helix family member a15 (*Mist1*), a granule maturation factor, marks mature chief cells as well as quiescent stem cells in the base of corpus [47]. Interestingly, *Lgr5*+ cells, which are known as a subpopulation of antrum stem cells, exist in the corpus gland of humans and mice [34,36]. *Lgr5* is expressed in a subpopulation of chief cells; this specific subset acts as ^{2}reserve^{1} stem cells, contributing to the regeneration of the gland after HDT-induced injury rather than in a homeostatic state [34]. Additionally, metaplastic lesions were promoted in *Lgr5*-driven *Kras* proto-oncogene, GTPas (*Kras*) mutant mice (*Lgr5*-2A-creERT2/*Kras*^{G12D}), suggesting that *Lgr5*+ chief cells may be the origin of gastric cancer.

By contrast, several reports proposed the presence of ASCs in the isthmus region along with putative stem cell markers. In particular, *Runx1* enhancer element (*eR1*)+ marks the proliferating progenitors in the isthmus, contributing to the maintenance of the gastric gland unit [48]. Moreover, a recent study revealed the existence of a unique ASC population expressing BMI1 proto-oncogene, polycomb ring finger (*Bmi1*) in the isthmus region. In the corpus, *Bmi1*+ cells represent the ASC population independent of *Lgr5* and *eR1* [50]. Hayakawa et al. [49] stated that quiescent stem cells exist in the isthmus cell region, but not in the chief cell region. In *Mist1*-driven confetti mice, a few *Mist1*+ isthmus cells expand their population and still produce gland units even 18 months after tamoxifen injection, and also act as the cellular origin of cancer. This is a contradictory result to previous reports that *Mist1*+ chief cells were responsible for maintaining homeostasis and developing cancer [45,47,52,53].

Han et al. [54] recently found that both isthmus stem cells and chief cells contributed to the maintenance of the corpus unit using marker-free lineage tracing (*Rosa26*-CreERT2; Confetti). Although basal corpus cells are slowly recycled, this ASC pool has the capacity to generate a mature gland. Thus, the isthmus region seems to be the central zone of proliferation, but it is apparent that there is another zone with the capability of self-renewal and multi-potency.

Taken together, ASCs exist in the gastric corpus and antrum in different regions, contributing to the maintenance of homeostasis, response to injury, the development of gastric cancer, and organoid formation. It is noteworthy that the ASC populations of the gastric unit share the same molecular markers or express distinct markers (Table 1).

Murine-derived gastric organoids

Unlike two-dimensional culture, 3-dimensional (3D) organoid culture has the advantage of reproducing functional units under *in vitro* conditions. Therefore, many approaches have been used to construct murine-derived gastric organoids using ASCs. As shown in Table 1, each organoid from diverse ASCs contains different gastric cell lineages (Table 1). Barker et al. [9] first reported that organoids derived from single LGR5+ cells in antrum could form a budding-structure and persist for 9 months. LGR5+ cells from the corpus and AQP5+ cells from the antrum successfully reproduced MUC5AC+ pit cells in a conditioned culture medium [34,35]. Among the cultured organoids, the corpus organoids derived from *Troy*+ chief cells and *eR1*+ isthmus cells exhibited the most diverse corpus cell lineages [47,48]. Lectin GSII+ mucus neck cells, gastric intrinsic factor (GIF)+ chief cells, and *Muc5ac*+ pit cells were generated from *Troy*-derived corpus organoids, and the differentiation was confirmed by immunofluorescence staining and reverse-transcription quantitative polymerase chain reaction [47]. In contrast to mucus neck cells and chief cells, gastric pit cell development was triggered in a medium without Wnt, noggin, and fibroblast growth factor (FGF)-10. Moreover, it is noteworthy that single CCK2R-derived organoids gave rise to *Lgr5*+ cells as well as differentiated cells [20].

Gastric parietal cells are unique cells in the corpus and play a critical role in gastric mucosal homeostasis through the secretion of growth factors and gastric acid [55]. Despite the physiological impact of parietal cells, regeneration of parietal cells in organoid culture has rarely been observed. Surprisingly, Hayakawa et al. [49] showed cultured parietal cells in a 3D organoid system. *Mist1*+ isthmus cells generated H/K ATPase+ parietal cells after 20 days of culture under epidermal growth factor (EGF), Noggin, and jagged canonical notch ligand 1 (Jagged-1), called "ENJ" medium. However, it is uncertain whether cultured parietal cells persist after passaging.

Maintenance of stemness and differentiation in organoid culture are controlled by the established factors such as Wnt, EGF, Noggin, R-spondin, FGF-10, Notch ligand, and Jagged-1 [5,56–59]. It seems that Wnt3A plays a crucial role in maintaining LGR5-derived organoid formation [9].

Single *Troy*+ chief cells isolated from the corpus gland can maintain their stemness and generate long-lived organoids under Wnt, EGF, Noggin, and R-spondin (WENR) conditions. For differentiation into pit cells, *Troy*-derived organoids are cultured in Wnt-, FGF-10-, and Noggin-free conditions [47]. Single *Mist1*+ cells can construct gastric organoids, containing parietal cells and enterochromaffin-like cells, and this phenomenon is dependent on Notch signaling (+ENJ medium) [49]. In WENR medium, *Mist1*+ isthmus cells do not survive and degenerate [49]. By contrast, *Bmi1*+ isthmus cells can successfully form organoids even in WENR medium. Ablation of *Bmi1*+ cells by treatment of diphtheria toxin (DT) significantly reduced organoid counts compared to a non-DT-treated control in the same condition. These results demonstrated that diverse subsets responding to different factors exist in the ASC zone [50].

Nowadays, organoids are considered novel models for improving regenerative medicine. In murine models, emerging results have demonstrated that multiple types of organoids could regenerate injured tissues, including the colon [60], intestine [61], lung [62], and liver [63]. Additionally, cultured gastric organoids exhibited therapeutic effects in mice. Organoid transplantation in injured mice promoted wound healing, and transplanted cells expressed metaplastic cell markers [64], revealing that gastric organoids originating from chief cells can be utilized as a therapeutic strategy.

Human-derived gastric organoids

Organoids can be constructed from 2 sources: pluripotent stem cells (PSCs) or ASCs. ASCs can only generate the specific cells from their tissue of origin, whereas PSCs have the potential to reproduce any cell type. Thus, PSC-derived organoid culture needs a step-wise method to control PSC differentiation into target cells. Some studies have been conducted to develop gastric gland organoids using human ASCs or PSCs.

McCracken et al. [65] proposed a step-wise differentiation approach to reproduce human gastric organoids using PSCs. First, human PSCs were differentiated into endoderm by supplementation of FGF4 and WNT. To acquire foregut from definitive endoderm, Noggin was additionally added to suppress BMP signaling. Finally, the antrum structure was reproduced by EGF and retinoic acid treatment. This organoid method is the first human PSC-derived antrum organoid that recapitulates the hostÉs physiology [66,67]. The method of generating corpus organoids from PSCs is more complicated because the signal cascade required for differentiation is obscure [57]. The cultured organoid contains parietal cells, mucus neck cells, endocrine cells, and chief cells. PSC-derived gastric organoids have been employed to understand the gastric development, physiological mechanism, and host response to pathogens [57,68,69].

Bartfeld et al. [67] developed a long-term culture system using human gastric organoids derived from surgical specimens

of the corpus. By inhibiting transforming growth factor-beta signaling, the ASC-derived organoids reproduced the molecular characteristics of their origin site. Tan et al. [35] recently isolated *AQP5*+ ASC cells from human antrum specimens and maintained *AQP5*+-derived organoids for more than 3 months. Of note, depletion of Wnt family member 3A (WNT3A), Noggin, and FGF-10 induced differentiation into mucous lineages. According to previous results, the maintenance and differentiation of ASC-derived organoids need simpler steps than those used for PSC-derived organoids. In PSCs, sequential signaling with diverse factors is needed in a timely manner to culture organoids. This is due to the pluripotency of PSCs, which can differentiate into multiple organs. Although the culture process is less complex in ASCs, ASC-derived human organoids have possible defects because ASCs are mostly derived from surgical specimens that are likely to harbor abnormal cells. Therefore, we assume that PSC-derived organoids would be suitable for patient treatment if the culture method is optimized.

H. pylori infection in gastric organoids

A vast variety of organoid models have been established to understand host-microbe interactions, including *H. pylori*, *Cryptosporidium parvum*, *Salmonella enterica*, and *Clostridium difficile* [70,71]. *H. pylori* is the primary etiological agent for gastric cancer and infection of Helicobacter species leads to atrophic gastritis and dysplasia in rodent models [16,21]. Mice infected with *H. pylori* or *H. felis* developed metaplasia accompanied by parietal cell loss within 20 weeks. In human, *H. pylori* infection give rise to chronic gastritis and pre-neoplastic metaplasia in the early stage of cancer progression, and infected patients are at greater risk of developing gastric cancer [72]. While *in vitro* culture methods for *H. pylori* are well established [73,74], the infection system for host cells still has limitations. Since most gastric cancer cell lines are derived from late stages of cancer, they are not sufficient to represent *H. pylori*-induced pathogenesis. Animal models are also susceptible to *H. pylori*, but they take a long time to develop gastric lesions and cannot show real time-pathogenesis. To satisfy the requirements for models in this field, organoid methods have been utilized to study *H. pylori* and its interaction with carcinogenesis.

Nuclear factor kappa B (NF-*H. pylori*-induced gastritis and is related to chronic infection [75,76]. Bartfeld et al. [67] generated human-derived gastric organoids and microinjected *H. pylori* into the established organoids. They investigated the primary response of gastric organoids to *H. pylori* infection and found that interleukin 8 levels, attributable to NF-κB was also highlighted in another previous study using murine-derived gastric organoids-increased in gastric organoids. The upregulation of NF-κB was also highlighted in another previous study using murine-derived gastric organoids-[77]. *H. pylori* infection induced sonic-hedgehog (Shh) expression in gastric organoids. This upregulation was suppressed by blockage of NF-Additionally, microinjected *H. pylori* in human gastric organoids induced an acute host response, including c-Met phosphorylation and proliferation [65].

Real-time screening revealed that injected *H. pylori* could adhere to the apical cell-cell junctions of human gastric organoids by sensing urea concentrations [78]. After adhesion of *H. pylori* in gastric organoids, the pathogens promoted proliferation of host epithelial cells via interaction with CD44 [79]. While *H. pylori* can be grown on human blood plates and in liquid media, the bacteria could not expand their population and ceased proliferation in a conventional eukaryotic culture system [80]. However, *H. pylori* can expand its population in organoid systems, suggesting that gastric organoids may produce niche factors for *H. pylori* growth [67,79]. Collectively, these studies indicate that gastric organoids are a useful tool for studying *H. pylori* infections and simulate important hallmarks of infection.

Conclusion and remarks

Given the results of the past decade, the definition of stem cells and the origin of cancer are more complicated in the corpus than in the antrum. This is because conflicting reports regarding corpus stem cells have been published. We feel that this debate stems from issues in animal models and reagents, such as tamoxifen. Although tamoxifen is a useful reagent for inducing cell-specific lineage tracing, this chemical can elicit severe injury in the corpus and may cause unexpected genetic changes [18]. Recent reports have recognized this problem and used doxycycline-inducible transgenic mice instead of tamoxifen-inducible mice to trace stem cell activity. Nevertheless, contradictory results were still observed. In addition to the tamoxifen issue, the overlapping markers such as *Mist1* between isthmus stem cells and chief cells make it difficult to define stem cell zones causing different tracing events in the corpus. We assume that confusing consequences in homeostatic status may be related to the use of different reporter systems [47,49]. However, in terms of tumorigenesis, we speculate that the origin of cancer may be chief cells because pre-neoplastic markers including CD44v9 and WFDC2 are expressed from the base immediately after injury [19,81]. Supporting this, *H. pylori* shows a strong tropism for metaplastic cells in the base of the corpus [82].

Organoid culture is an emerging tool for research on development, cancer, translational clinical applications, regenerative medicine, and infection biology [83]. Using lineage tracing, researchers have found various markers of gastric stem cells. Presumably, there are more specific markers to be uncovered. Advances in single-cell analysis technology will make it possible to identify more specific subsets of gastric stem cells. Indeed, increasingly many results support the presence of cellular heterogeneity in the stem cell population [84–86]. In light of previous studies, we assume that some subsets derived from chief cells or isthmus cells could generate all lineages of the gastric corpus, including gastric-secreting parietal cells and endocrine cells. Hence, future studies must be conducted using unique subsets and niche factors to better understand gastric physiology and pathogenesis. A few studies have exhibited the presence of cultured parietal cells in organoid systems, but they may not survive and persist after passaging. Since oxyntic atrophy is an initial step in gastric carcinogenesis, it is an urgent task for gastric organoid technology to generate parietal cells in 3D culture systems.

Current organoid technology is still incomplete. Cultured organoids mostly consist of an epithelial layer without essential components of the tissue microenvironment, such as stromal cells and tissue-resident immune cells, which are critical for maintaining homeostasis in the gastrointestinal tract. Despite recent advances in culture protocols, organoid culture systems are still complex and cultured cells have the possibility of transforming after several passages, losing their original characteristics. Furthermore, Matrigel-based organoid culture confers a limitation because the reagent is produced from mouse tumor lines. It remains unclear whether Matrigel has detrimental effects on organoids, which is a barrier to the utilization of organoids as regenerative medicine. Further efforts are needed to solve current hurdles.

Nevertheless, this promising technology has great potential to overcome disease and study pathogenesis. In particular, many stomach cancer patients still undergo surgery and removal of a large portion of the tissue. Patients who have undergone surgery suffer from digestive defects because their tissues do not fully regenerate. Studying the pathogenesis of *H. pylori* is critical to understand the development of gastric cancer. However, it takes a considerable time (at least 16 weeks after infection) until gastritis emerges in *in vivo* models. Due to their relatively simple culture systems and regenerative potential, we expect that gastric organoid systems will help to solve these current problems.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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