Molecular modelling of the gastric barrier response, from infection to carcinogenesis.

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Abstract

The lining of the stomach is a tight monolayer of epithelial cells performing functions in digestion and a protective barrier against gastric acid, toxic metabolites and infectious agents, including *Helicobacter pylori.* The response of the epithelial barrier to infections underlies gastric pathologies, including gastric cancer. *H. pylori* has the unique capacity to colonise the gastric mucosa while evading the immune system. The colonised mucosa initiates an inflammatory response to fight the infection and a strong regenerative program to avoid barrier failure and ulceration. This response changes the morphology and cell composition of the gastric epithelium and in parallel it might contribute to the accumulation of somatic mutations leading to cellular transformation. Genetically modified mice, cell lines and human-derived organoids are the main biological models to study the gastric epithelial barrier. With these models it is possible to dissect the stepwise process of tissue adaptation to infection that places the epithelium at risk of malignant transformation.

Keywords

Epithelium, *Helicobacter pylori*, adhesion, colonisation, inflammation, regeneration, carcinogenesis, immune evasion, organoids, mucosoids

Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide, and it is the cause of 8.8% of all cancer related deaths [1]. Cancer generally arises in the elderly (median age at diagnosis is 70) and it is often discovered in later stages, implicating a poor prognosis. It has been shown that risk factors for gastric cancer development are high-salt and meat-rich diets, alcohol consumption, cigarette smoke, but also family history and gastro-oesophageal reflux [2, 3]. Notwithstanding the importance of these risk factors, *Helicobacter pylori* (*H. pylori*) infection is still considered the main one for stomach cancer initiation. Although there has been a remarkable decrease of *H. pylori* infection during the last decade, more than half of the world population is still affected, but not all patients develop symptoms and only 1-3% of *H. pylori*-infected patients develop GC [2-4]. Symptoms might depend on the virulence of specific strains, host genetic factors and diet [5]. Interestingly, more recent evidence suggests that the disease starts when the bacterium colonises deep in the gastric mucosal barrier [6-8] (Fig.1).

The gastric mucosa is organised in narrow invaginations called glands that are made of a simple columnar epithelium. The glands are supported by a scaffold of mesenchymal derived stromal tissue (*lamina propria*) (Fig.1). The upper part of the gland, called pits or foveola, is moisturised by abundant mucus produced by the epithelium and is the contact surface to the stomach content. The inner part of the gland contains the factory of the gastric mucosa, including chief and parietal cells that are responsible for digestive enzymes and gastric acid production, respectively. Secretion is coordinated by hormone-producing enteroendocrine cells and stem cells fuel the regeneration of all cell types (Fig.1).

The luminal content of the stomach is never in contact with the inner part of the gland, however, *H. pylori* has evolved strategies to penetrate inside to establish a protected niche where the infection can persist. The epithelial barriers are the first that react against the infectious agent. Pathogenic bacteria like *H. pylori* have evolved strategies to break this first line of defence and adhere to the gastric epithelium by harnessing specific host cell receptors. While inducing a strong inflammatory response,

H. pylori is also able to evade the immune system and to cause profound changes in tissue morphology as a response to the damage caused by the infection. *H. pylori* infection induces indeed genomic instability into the infected cells posing them at risk of somatic mutation.

Here we summarise how different models have been used to understand how *H. pylori* infection disrupts epithelial barriers and leads to carcinogenesis. This involves four stages: colonisation; inflammation and immune evasion; disruption of epithelial homeostasis; and DNA damage.

Biological models to understand epithelial barriers

Experimental mice and human cells lines are the most common tools to study epithelial barriers, but the recent development of human organoids is providing new prospects for understanding the healthy epithelium using a human relevant model.

Animal models

Mongolian gerbils can be successfully colonised with *H. pylori* and they eventually develop gastric adenocarcinoma but the lack of specific immunological reagents and genetic tools impairs the molecular characterisation of the disease. Mice do not develop cancer after infection probably because major virulence factors are lost due to the bacterial adaptation process to the mice [9]. However, the enormous variety of genetic tools available for mice makes this an attractive model for understanding the steps underlying gastric carcinogenesis. Transgenic mice can be used to understand the role of specific genes in virtually all cell types involved in host responses to infection [10]. Furthermore, Cre recombinase-based tracing approaches have illuminated epithelial cell fate in the healthy mucosa and during disease progression. However, direct transposition of findings from mice to human is still limited by genetic and anatomical differences between human and rodents and by the poor adaptation of a human specific pathogen to the murine stomach.

From human cell lines to stem cell driven culture models

Human gastric cell lines are derived from cancer resections and have been widely used to understand the consequences of infection on epithelial cells. Although some cell lines can be cultivated in monolayers as a surrogate of an epithelial barrier, they are clones of a single cell and, being derived from a cancer explant, they represent the endpoint of the process that we want to understand. A valid alternative to cell lines is the recently established human organoid technology [11]. Thanks to a better understanding of the factors involved in stem cell regeneration, it is now possible to propagate healthy epithelial barriers *in-vitro* [12]. These epithelial structures grow as cysts in extracellular gel matrixes and they maintain key features of the epithelium *in situ* like polarity, multi-lineage differentiation and regeneration. The regeneration of the gastric epithelium into organoids depends on the support of tissue specific growth factors [13, 14]. Infection of organoids with H*. pylori* can be performed by injecting the bacteria inside their lumen or by infecting dissociated organoids [15]. Alternatively, freshly dissociated epithelial cells from explants or organoids can be cultivated at an air-liquid interface, thereby obtaining a polarised monolayer which includes different cell types and that accumulates abundant mucus on the apical side [16]. Due to the similarity of the cells in this model to the gastric mucosa, this system is called "mucosoid culture". The accessible apical epithelial side allows infection as it would occur *in-vivo*, and the presence of mucus offers the possibility to model unexplored aspects of the bacterial colonisation *in-vitro*. In the following sections we consider how these models have provided insights into the interactions of *H. pylori* and the gastric epithelium.

Epithelial barrier colonisation

Following the apical route of infection, *H. pylori* swims through a thick mucus layer, enters the gland and adheres to the epithelium (Fig. 2). *In-vitro* models such as cell lines and organoids have been successfully used to understand the infection process at the mucus layer and on the epithelial surface. However, modelling glands penetration requires animal models as there are no 3D models available to replicate the glandular structure *in-vitro.*

Mucus: the forefront of mucosal defence

The gastric lumen is a hostile habitat for bacteria due to the secretion of proteolytic enzymes and low pH of the gastric acid. To protect the mucosa and insulate against acid corrosion, the epithelium produces mucus. The mucus is a viscoelastic fluid which possesses hydrogel-like properties, it is formed of 95% water, 3% mucin glycoproteins and other low-molecular weight molecules [17]. The stomach pH allows the mucins to aggregate in a network, determining viscosity.

Mucus represents the first physical and immunological barrier of the mucosa due to the presence of antimicrobials and specific immunoglobulins [18]. The stomach mucus can reach 700µm in thickness and is composed of two layers. Both layers are predominantly made of mucins: the internal layer is thick, firmly adherent to the epithelial surface, abundant in MUC5AC and normally sterile; the outer layer is made by MUC6 and is generally looser [17]. Mucus is denser at low pH and *H. pylori* flagella motion is drastically impaired at pH2-4 [19]. In addition, the MUC6 monosaccharide α(1,4)-linked N-acetylglucosamine terminal seems to play a role in the inhibition of *H. pylori* cell component assembly [20]. Mucus composition alters when the epithelium is inflamed [18] and epithelial cells of mucosoids primed with *H. pylori* secrete bactericidal mucus, probably containing antimicrobials, to control the infection [16]. Hence, the mucus is both a physical barrier and immunological defence via secretion of antimicrobials.

Notwithstanding these defence mechanisms, *H. pylori* has evolved strategies to penetrate the mucus and colonize the epithelial surface. The stomach epithelium secretes urea as a catabolite but *H. pylori* takes up urea through a proton-gated channel and hydrolyses it with urease, yielding NH_3 and CO_2 [21] [22] (Fig.2). This increases the local pH, enabling *H. pylori* to acquire higher mobility and reach the epithelial surface [22]. Indeed, *H. pylori* senses urea, via a chemoreceptor called TlpB, to find its way to the epithelium [23]. The metabolism of urea is used to maintain the gradient of this catabolite hence inducing the bacterium to swim toward the source [23] (Fig.2) especially in the case of an injured mucosa as demonstrated using organoids [24].

The epithelial surface

A fundamental component of *H. pylori* membrane is the core complex type IV secretion system (TIVSS), encoded by the cag-pathogenicity island. This is probably the most studied bacterial feature and a prominent virulence determinant of *H. pylori*. Gastric cancer (GC) has been associated with TIVSS positive *H. pylori* strains, and this structure is crucial for the translocation of CagA, a putative bacterial oncoprotein, into the host cells [25]. Translocation of CagA by the TIVSS requires contact with the epithelium. Using its arsenal of outer membrane proteins (OMPs), encoded by 30 genes, *H. pylori* can bind to the highly polymorphic epithelial surface receptors avoiding shredding [26] [27]. BabA, SabA and OipA are well known *H. pylori* OMPs [25] but recent attention has focused on HopQ which has been found to target CEACAMs - adhesion molecules - to adhere to the stomach cells, enabling the translocation of CagA [28] [29] (Fig.2). The CEACAM receptor family has variable membrane domains and they mediate signalling that regulates, apoptosis, proliferation, immunity and motility. In the healthy epithelium CEACAMs are not highly expressed but they are upregulated in gastritis and tumours; the phosphorylation of CEACAMs triggers inflammation [28].

In addition, *H. pylori* is also able to bind integrins which are adhesion molecules located at the basal side of the epithelium. To reach the integrins, *H. pylori* has evolved the ability to break the apical epithelial barrier integrity by using a protease called HtrA. This virulence factor lyses tight junction proteins including occluding, claudin-8 and E-cadherin [30, 31] (Fig.2) and upon getting access to the basal side, the TIVSS binds integrins and facilitates the translocation of CagA [31] (Fig.2).

The glands as infection niches

Adhesion to the epithelial surface might not be sufficient for mucosal colonization as surface foveolar cells are constantly shed into the lumen of the stomach and repopulated from the inner part of the gland. By ejecting the foveolar cells, the stomach detaches the bacteria from the mucosa. Notwithstanding the importance of *H. pylori* adhesion strategies on the cell surface, inflammation of the mucosa starts only when bacteria penetrate inside the glands [8]. A protein on the tip of the flagella

called ChePep enables the bacteria to swim inside the gland and colonize the mucosa [6]. These micro-niche settlements allow the bacterium to persist in the mucosa in more favorable conditions, including pH and reactive oxygen species [6]. The glands are a reservoir of bacteria and initial colonization of this niche by a small number of them is followed by expansion to neighbouring glands as a clonal population. The establishment of bacterial colonies in the glands in one hand modifies T cell responses leading to their tolerance and persistence [8], and in the other hand, it enhances stem cell turnover and proliferation [7] probably inducing the hypertrophy of the gastric mucosa observed during gastritis.

Inflammation, defence and immune evasion

Inflammation is the typical reaction of a mucosal barrier to infection and it is the result of chemokines coordination between the epithelium, stroma and immune cells that migrate into the mucosa to fight the infection. Strategies to defuse host defence allow *H. pylori* to persist lifelong if not eradicated with antibiotics.

Inflammation

Adhesion of the bacteria to the epithelial surface is only a first step to trigger inflammation, as the host is also equipped with an arsenal of bacterial sensors called pattern recognition receptors (PPR) that sense bacterial pathogen associated molecular patterns (PAMPs). Activation of PPRs by PAMPs initiates the transcription of pro-inflammatory genes and mediators. The main transcription factor that initiates this response is the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Activation of NF-κB leads to the production of inflammation-associated cytokines, such as IL1β, TNF-α IL-18 [32] and IL-8 and to the recruitment of innate immune cells, such as macrophages and neutrophils [33] . Toll-like Receptors and NOD-like receptors (TLRs and NLRs) are the most studied PPRs. For example: bacterial flagellin can be detected by TLR5; lipopolysaccharide (LPS) is detected by TLR4; and intracellular bacteria can be sensed by NLRs [34]. However, *H. pylori* has developed strategies to evade

detection: mutations in the recognition domain of *H. pylori* flagellin allow bacteria to avoid activation of TLR5 [35], while compensatory mutations in other regions retain its motility [36]. Indeed *H. pylori* LPS does not activate TLR4 and is less toxic compared to LPS from other bacteria [37].

How *H. pylori* still triggers activation of NF-κB was discovered only recently. A novel PRR in epithelial cells, Alpha-kinase 1 (ALPK1) detects Gram-negative bacteria and activates NF-κB *via* a novel ALPK1/TIFA/TAK1 signalling axis [38]. An LPS intermediate called ADP-heptose was identified to be the PAMP responsible for NF-κB activation *via* ALPK1 [39] (Fig.3). Interestingly, a functional TIVSS is necessary to deliver this sugar into infected cells, corroborating the importance of this secretion system in inflammation [39]. A further role of the TIVSS in the activation of the NF-kB inflammatory pathway depends on one of its subunits, CagL, which binds TLR5 and enhances secretion of several NF-kB pro-inflammatory chemokines [40]. The lack of a functional TIVSS has been associated with less severe pathology [41], indicating that, in addition to CagA, important pathogenic effects attributed to the TIVSS might be due to the translocation of ADP-heptose that induces inflammation.

The innate immunity of the epithelial barrier

The epithelial barrier is the first point of contact with pathogenic bacteria and thus is equipped with mechanisms of innate immunity to prevent bacteria from entering the body. These mechanisms are especially important as, in contrast to the intestine, the healthy stomach lacks adaptive immune surveillance. The epithelial barrier secretes factors such as antimicrobial peptides (AMPs), which are able to maintain immediate surrounding of the epithelial barrier clean of bacteria [42, 43] (Fig.3). Examples of AMPs include: defensins, which are small cationic proteins that increase bacterial membrane permeability; cathelidicin (LL-37), which is a broad spectrum antimicrobial found in the inflamed stomach of *H. pylori* infected individuals [44]; and Intelectin1 (Itln1) and Regenerating family member 3 gamma (Reg3γ) which impair motility of *H. pylori* and have been found at base of the gland in mice [45]. Human beta defensin 1, 2 and 3 (hBD1, hBD2, hBD3) are found in the stomach and they are effective antimicrobials *in-vitro* against *H. pylori*, their expression is stimulated by NF-κB (hBD1,2)

or IFNγ (hBD3). Whilst hBD2 is highly expressed in relation to the infection [46], the TIVSS and CagA can downregulate the expression of hBD1 and hBD3 [47] [48].

Escape from immunosurveillance

H. pylori is able to persist in the gastric mucosa for decades as it has evolved strategies to elude both innate and adaptive immune responses. The initial NF-κB-driven response and release of chemokines triggers recruitment of neutrophils, macrophages, dendritic cells, T and B lymphocytes to the site of infection. This condition might persist for years in patients with untreated chronic gastritis. Subsets of T cells, including CD4+ Th1, Th17 and Th22, are also recruited and their role is to control infection by contributing to the inflammatory milieu with the secretion of IFNγ and IL22 (Fig.3). Receptors on the epithelium detect these cytokines, whereby they stimulate the secretion of antimicrobials and chemokines. The assembly of those cytokine receptors on the epithelium is stabilised by cholesterol-rich micro-domains on the plasma membrane. *H. pylori* is auxotroph for cholesterol and upon binding it extracts cholesterol, which disturbs the micro-domains and dislocates the receptors. Therefore, as it has been proven using mucosoids, infected cells offer a unique protected niche for the bacteria as they are insensitive to crucial defensive signals like IFN-γ and IL-22 [49] (Fig.3). *H. pylori* is also able to disarm T cells by inhibiting their activation through the virulence factor VacA [50] and inducing the infected epithelium to secrete TGF-β [51] and PD-L1 [52], which reduces CD4+ T cell proliferation and induces development of regulatory T cells, favouring tolerance to the infection.

Gland epithelium homeostasis and niche signalling

Gastric epithelial homeostasis and cell composition of the gland depend on the coordination of signals and receptors orchestrating regulation in proliferation, regeneration, differentiation and apoptosis. These epithelial shaping signals – the gland "morphogens" - can be of epithelial or non-epithelial origin.

Potential sources of morphogens surrounding the gland include stromal, endothelial, neuronal and immune cells. During disease progression morphogenic signalling pathways are dysregulated and specific signals can be the driving force for carcinogenesis.

Healthy gland homeostasis

Homeostasis of the gastric epithelium depends on activation of regenerative cells that can proliferate and differentiate toward all the lineages of the gastric gland. Cre recombinase-based genetic tracing experiments in mice revealed that cells expressing markers including *Lgr5*, *Axin2*, *Mist1*, *Lrig1*, *Troy*, *Sox2*, *Bmi1*, *Stmn1* and *Aqp5* [53-61] can regenerate the whole gland. These markers are found on cells located throughout the gland, including cells with features of differentiation and different proliferation capacities [55, 62]. Indeed an unbiased lineage tracing approach (i.e. marker free) revealed that the murine gastric glands are repopulated from two distinct sites, one at the isthmus and one at the base of the gland [56]. Although regenerative cells at the isthmus are more actively cycling, the ones at the base of the gland can also regenerate entire glands, but at a slower pace [56]. Altogether these experiments suggest that cells with different identity, from different locations, and with distinct differentiation features, can act as regenerative cells or stem cells. Our interpretation is that the existence of multiple stem cells calls in to question the “unidirectional” regenerative model (eg. from one specific stem cells to all differentiated cells) that is focussed on determining the hierarchy of multipotency in the gastric gland. The concept of stem cell “identity” remains undefined and an alternative model to explain these data would rely on the notion that regeneration is a possible feature of almost all cell types and that cell differentiation depends on the combination of specific niche signalling factors instructing the cells to commit toward a specific lineage. Further investigations are needed to understand whether the commitment to differentiation is reversible, and to identify the signals (and their location) that shift the dynamic balance between regeneration and differentiation.

Retention of regenerative capacity largely depends on the activation of the WNT/β–catenin signalling pathway [13, 14, 16] (Fig.4). Sources of WNT ligands have been identified throughout the gastric gland,

but the stroma of the *muscularis mucosae* produces Rspondin (Rspo) a co-factor that potentiates WNT/βcatenin activation through a co-receptor called Lgr5 [53]. The Notch, BMP and EGFR signalling pathways also have an important role in the gastric gland morphogenesis. Activation of Notch receptor is essential for stem cell proliferation [63-65]. A role for BMP has been identified in a study of canine parietal cell differentiation [66] and indeed, overexpression of Noggin, a BMP inhibitor, causes the loss of parietal cells and activation of proliferation [67]. Activation of EGFR through the tumour growth factor α (TGF-α), is important for foveolar cell differentiation [68] and its over-activation influences gastric function in mice [69].

Signals in the gland in the transition from infection to carcinogenesis

Histological analysis of the progression of gastric disease to neoplasia has shown a defined series of pre-cancerous conditions characterised by distinct morphological and cell composition features, known as “Correa´s cascade” [70]. In the majority of the gastric cancers the course of these events is initiated by *H. pylori* infection. These histological changes can be considered as an adaptation of the tissue to infection. Alteration of the expression of growth factors and morphogens regulating the gastric glands might be responsible for the observed changes in tissue morphology.

A typical condition resulting from the infection with *H. pylori* is hypertrophy of the gastric mucosa. Herein, numerous immune cells migrate into the *lamina propria* next to the epithelium and the glands increase in height. Deep colonisation of bacteria inside the murine glands stimulates an expansion of the stem cell compartment at the base of the gland through the increase in expression of Rspo3 in the *muscularis mucosa* [7, 53]. This mechanism promotes the activation of the Wnt/β-catenin signalling pathway and cells at the base of the gland expands, probably causing the increase in gland height [53]. In addition, the *H. pylori* virulence factor CagA can directly activate the Wnt/β-catenin signalling pathway in epithelial cells [71] (Fig.4).

Mice infected with other non-*pylori* helicobacter species can develop metaplasia and dysplasia specifically when BMP receptor is deleted in Lgr5+ cells, suggesting the importance of BMP signalling factors in driving disease progression [72]. Moreover, BMP signalling inhibition leads to hyper-proliferation, probably partially due to higher expression of epidermal growth factor receptor (EGFR) ligands and ERK activation [67] (Fig.4).

Active Kras expression in specific cells in mice has been shown to recapitulate gastric disease progression to dysplasia similarly to that caused by *H. pylori* infection in humans. While constitutively active Kras expression in Lrig1+ stem cells induces a hyper-proliferation of surface mucous cells [73], the same transgene expressed in chief cell precursors (Mist1+) induces a trans-differentiation of these cells into metaplastic cells [74] (Fig.4). Inhibition of the kinase MEK, downstream the KRAS pathway, reduces the number of metaplastic cells *in-vitro* but it also selects for dysplastic cell population indicating the importance of KRAS signalling pathway in carcinogenesis only in specific cells [75] (Fig.4).

Manipulation of p53 signalling

Gastric cancer is characterised by numerous mutations in driver genes including *TP53*, *DRD2*, *CDH1*, *AKAP9* and *ATM*, and the tumour suppressor gene *TP53* is the most frequently mutated [76]. In host cells, CagA activates multiple signalling pathways leading to oncogene upregulation and inactivation of tumour suppressors [77]. For example, CagA activates the serine/threonine kinase AKT1, resulting in phosphorylation and activation of the ubiquitin ligase MDM2 which induces proteasomal degradation of p53 [78]. Thus, *H. pylori* is able to alter the levels of p53, leading to an initial increase and followed by a rapid decrease. It has been shown that CagA also controls the activity of p53 through interaction with the tumour suppressor apoptosis-stimulating protein of p53 (ASPP2) [79]. In the presence of genomic damage, ASPP2 binds and activates p53 leading to apoptosis; however, during *H. pylori*-mediated infection, interaction with CagA modifies ASPP2 activity so it becomes an inhibitor of p53 and a promoter of cell survival [79]. Moreover, *H. pylori* leads to an upregulation of p53 isoforms

Δ133p53 and Δ160p53 which obstruct p53 and p73 activity [80]. Thus, *H. pylori* leads to survival and proliferation of damaged cells, increasing the chances of tumorigenesis (Fig.4).

From DNA damage to somatic mutations

Gastric adenocarcinoma is characterised by genomic mutations, including in specific driver genes [76, 81]. There is accumulating evidence that bacterial infections cause DNA damage in host cells [82], and in this section we consider the ways *H.pylori* might promote mutagenesis.

Infection and DNA damage

DNA damage is increased by oxidative stress in *H. pylori*-infected gastric epithelium [83] – due to increased expression of spermine oxidase, which produces H_2O_2 production – increasing risk of neoplastic transformation [84]. Further direct interaction of *H. pylori* with host cells causes DNA double strand breaks (DSBs), independent from the major virulence factors CagA or VacA [85]. Proteins involved in homologous recombination, like MRE11 and NBS1, are downregulated during infection of organoid derived human primary cells with CagA-positive *H. pylori* [86], suggesting the involvement of the less accurate non-homologous end joining (NHEJ) repair pathway [87] (Fig.5). A genome-wide screen for factors inducing DSBs revealed that NF-κB is recruited to the chromatin together with nucleotide excision repair nucleases (XPF and XPG), causing DSBs in a TIVSS-dependent manner [87] (Fig.5). This link between infection-induced inflammation and DNA damage was further corroborated by the finding that *H. pylori*-induced DNA damage depends on co-transcriptional RNA/DNA hybrids (R-loops) that form as a consequence of β-ADP-heptose/ALPK1/TIFA/NF-κB signalling [88] (Fig.5). β-ADP-heptose is probably injected via the TIVSS of *H. pylori*, thus shedding new light on the role of this bacterial injection machinery in increasing the risk of cellular transformation.

Mutations

Although recent studies suggest that cells infected with *H. pylori in-vitro* experience DNA damage, it is not known if this damage is repaired or if error prone repair machinery might be responsible for the introduction of somatic mutations. It has recently been observed that other oncogenic bacteria such as *pks+ Escherichia coli* leave a mutational signature in the infected host cells, which is seen in colon cancer [89]. The Pks pathogenicity island of this specific strain of *E. coli* encodes a toxin responsible for both DNA damage and mutagenesis. Other enteric bacteria possess similar genotoxins, but they have not yet been identified in *Helicobacter*.

Summary

Taking advantage of the knowledge acquired by studying *H. pylori* and gastric cancer it is possible to define the hallmarks that make a bacterium, in specific conditions, a pathogen and a carcinogen: 1) ability to colonise and persist in the mucosa; 2) triggering inflammation and immune evasion; 3) alteration of epithelial barrier homeostasis; and 4) DNA damage and mutagenesis. These are all necessary steps observed in the transition between an infected epithelium and an adenocarcinoma. Epidemiological evidence and microbiota sequencing from cancer samples are strongly suggesting that bacteria such as typhoidal *Salmonella*, *Pks+ Escherichia coli* or *Fusobacterium nucleatum,* and others, might also be causative agents in cancer. However, the steps of molecular events from infection to cancer are still unknown. Often, pathogenic bacteria are found in the microbiota of healthy individuals (as in the case of *H. pylori*), suggesting that host and determinant must also contribute to the definition of pathogenesis.

Research point

- Human cancer-causing bacteria are exquisitely adapted to the human body, and thus animal models often do not recapitulate the human disease stages. Human-derived models, like organoids, offer a new opportunity to understand human physiology and pathology.

- Rebuilding the complexity of the human mucosa *in-vitro* is a technological and scientific challenge that has seen recent advancement, especially in the gastrointestinal tract. By combining bioengineering and stem cell technology, it is becoming possible to regenerate complex 3D tissue structure mimicking the mucosa *in situ* [90].
- A stem cell-driven 3D model that includes elements of the stroma and the immune system would be of enormous value to dissect the role of each cell type in the complex network of the mucosa to understand mucosal homeostasis in health and disease.

Conflict of interest

No conflict of interest has been declared by the authors

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Figure 1

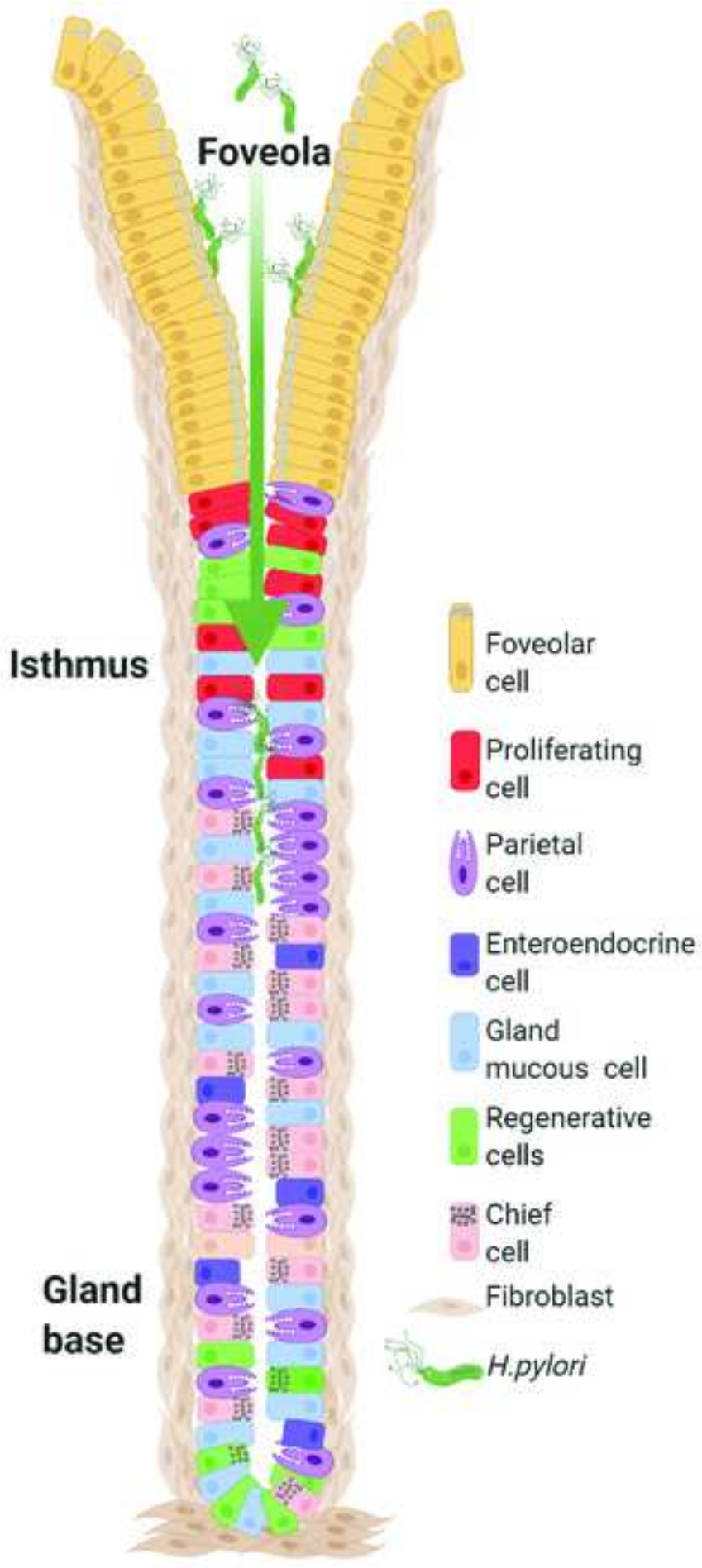
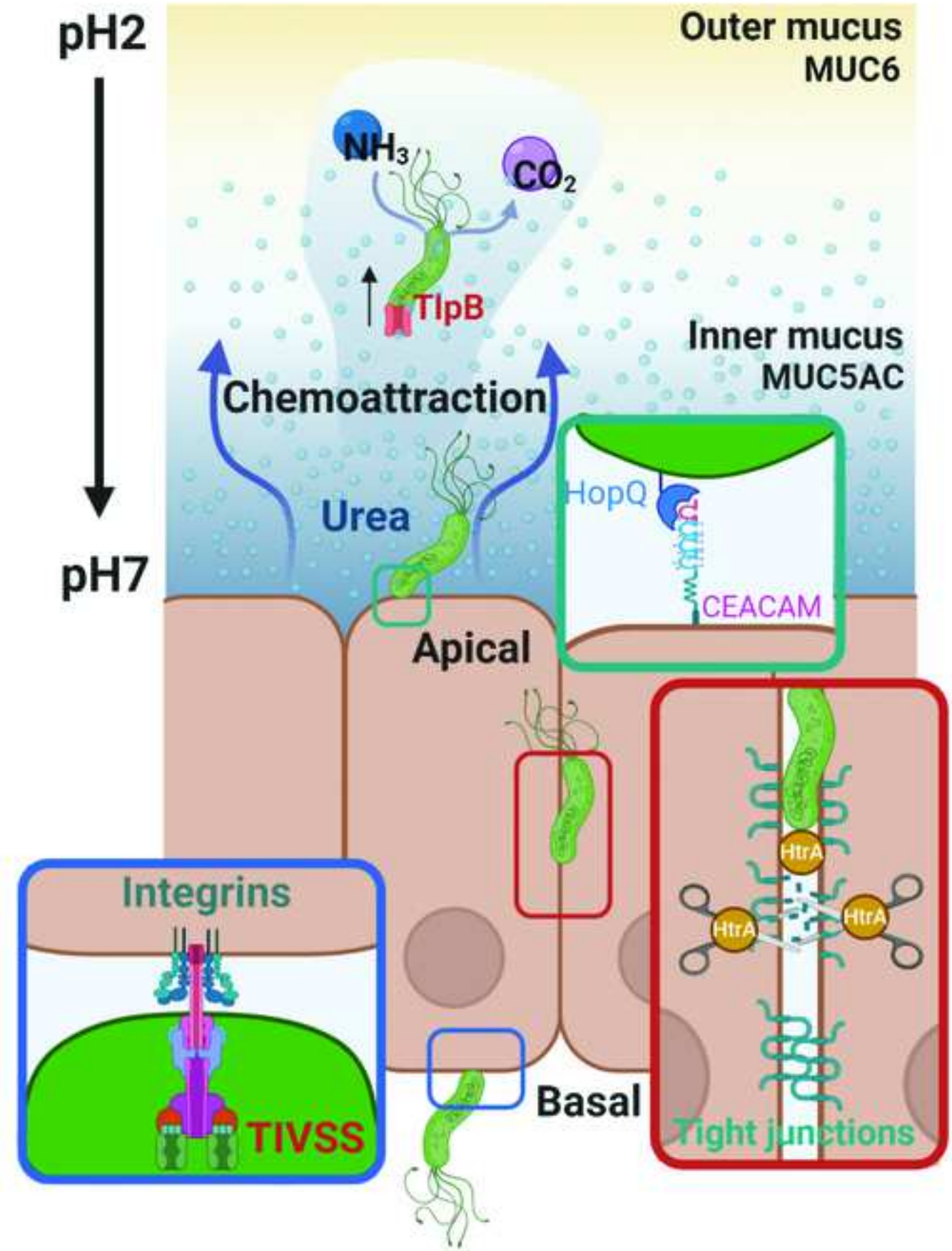


Figure 2



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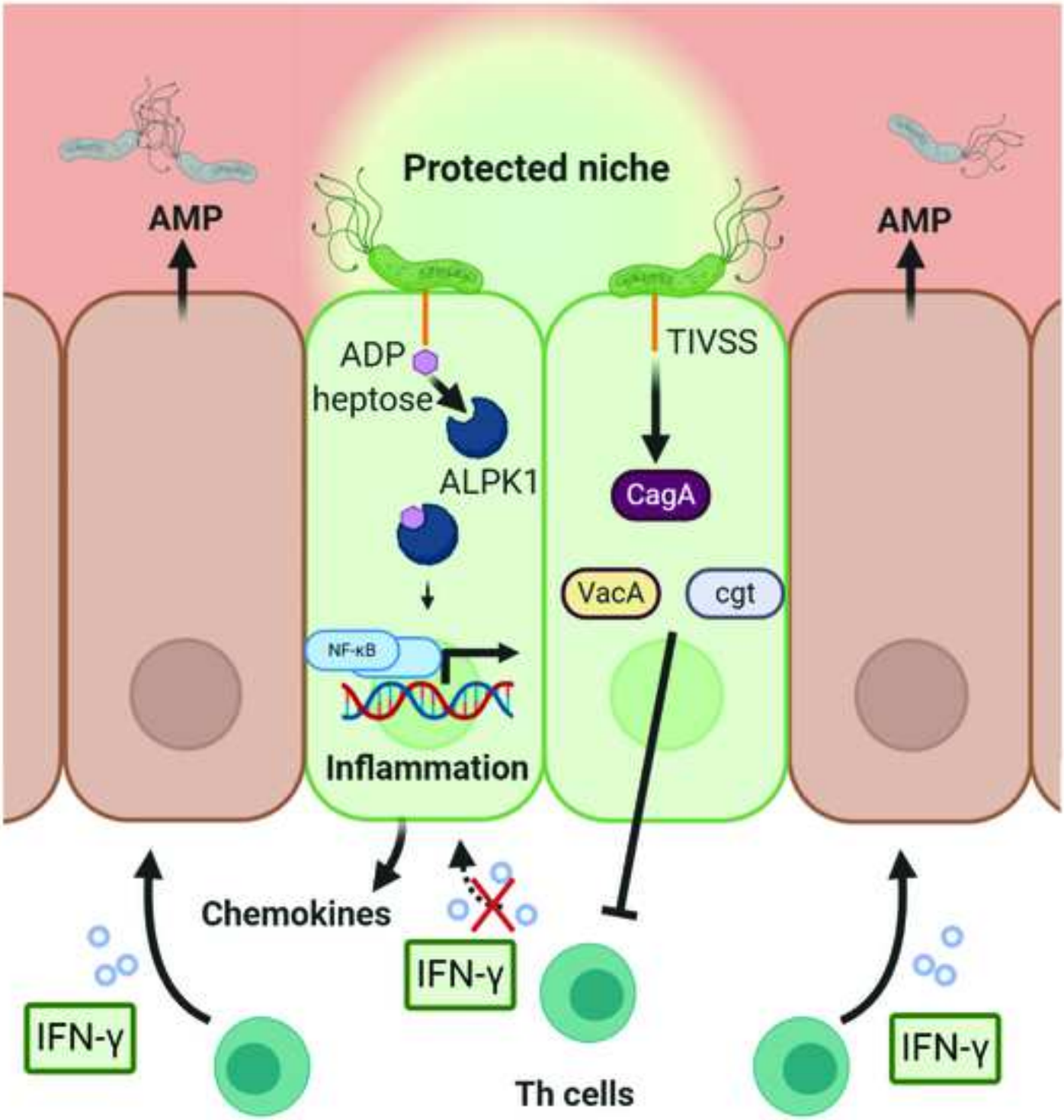

Figure 4

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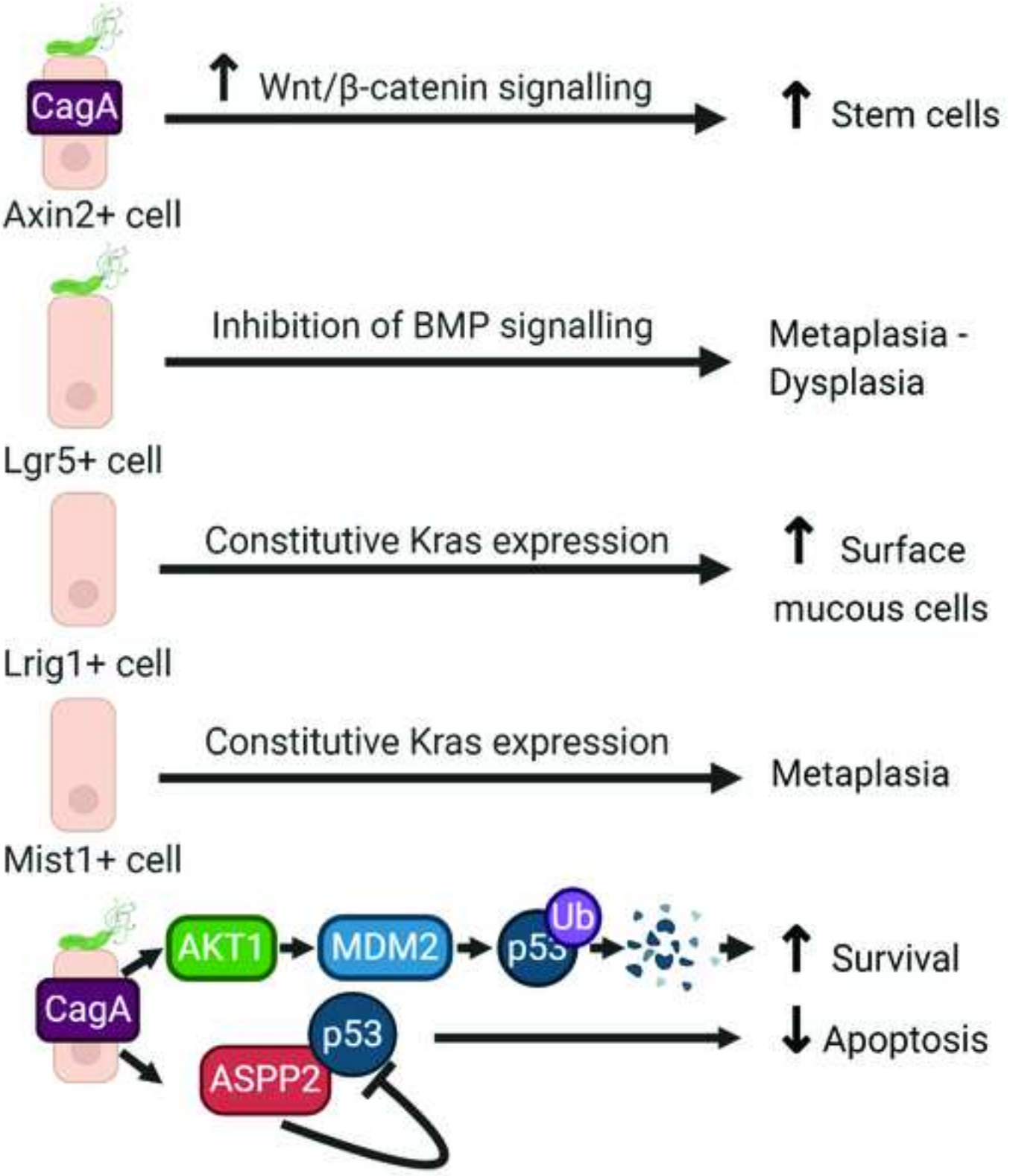

Figure 5

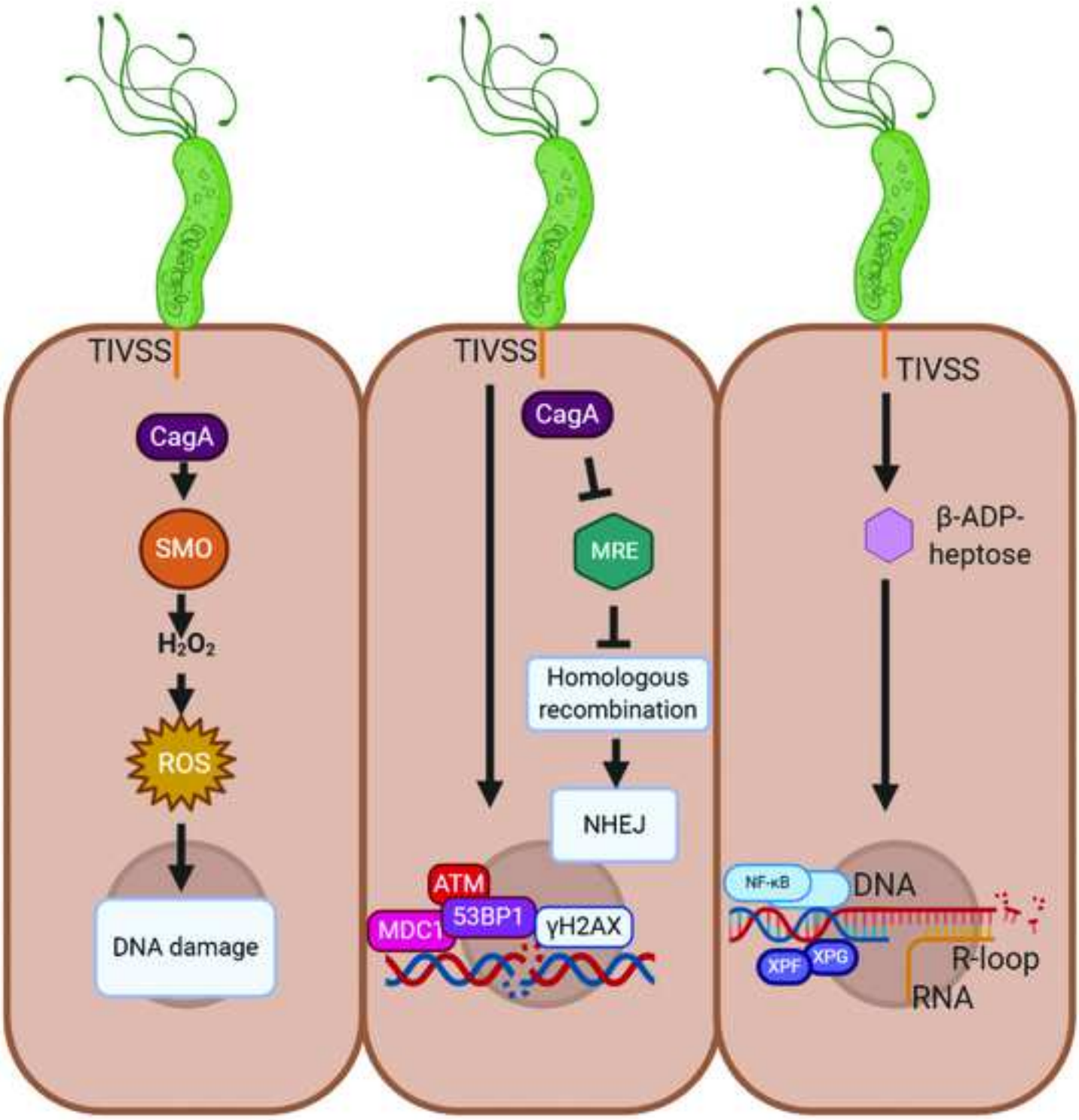

Figure legend

Figure 1. Schematic of an infected human gastric gland

A gastric gland comprising: foveolar cells producing mucus enriched in MUC5AC; gland mucous cells producing MUC6; proliferating cells typical of the isthmus; cells with regenerative capacity or stem cells; parietal cells producing gastric acid; chief cells producing zymogenic enzymes; hormone producing enteroendocrine cells; supporting fibroblast of the *muscularis mucosa* and of the *lamina propria.* The schematic shows that *H.pylori* can colonise the inner part of the gland.

Figure 2: Adhesion and Colonisation

H. pylori is guided towards the epithelium *via* the chemoreceptor TlpB sensing urea produced by the epithelial cells. Utilizing TlpB, *H. pylori* penetrates the thick mucus layer of the stomach by hydrolysis of urea which lead to pH increase and enhanced bacteria mobility. *H. pylori* can bind the apical cellular site by epithelial receptors (CEACAMs) via HopQ but can also access basal integrins by disrupting tight junctions through HtrA secretion. Upon binding, the bacterial type 4 secretion system (TIVSS) gets in contact with the cell and enables translocation of bacterial proteins such as CagA, which is involved in carcinogenesis.

Figure 3: Inflammation and immune evasion

A bacterial intermediate of LPS, ADP heptose, binds the intracellular receptor kinase ALPK1 thereby inducing NF-κB nuclearisation and transcription of pro-inflammatory mediators including chemokine for the recruitment of immune cells and antimicrobial (AMP) for controlling the infection in the mucus. Among the immune cells, T helper type 1 cells plays a critical role as they secrete IFNγ. Interferon receptors are located on the epithelium and its activation contributes to the production of further cytokines and antimicrobial peptides. *H.pylori* has evolved strategies to hijack this defence mechanism. A bacterial virulence factor called cgt mediate the delocalization of IFNγ on the epithelial cells while other two important virulence factors, CagA and VacA can inhibit activation of T helper cells.

Figure 4: Gland niche signalling from infection to carcinogenesis

D Homeostasis of a healthy gastric gland is orchestrated by a variety of morphogens, which shape the gland by inducing differentiation of progenitor into specialized cell types. *H. pylori* infection disrupts this homeostasis modifying the levels and activation of the stomach gland morphogens, leading to changes in tissue morphology as increase in stem cells, transition into dysplastic/metaplastic lineages, hyper-proliferation of surface mucous cells, and promoting cell survival.

Figure 5: Bacterial induced DNA damage and DNA damage response

H. pylori infection leads to DNA damage through production of ROS and specifically of double stranded brakes (DSBs). The inflammatory mediator ADP heptose seems to play a relevant role in the generation of (DSBs) specifically in the regulatory R-loops that are RNA-DNA triple stranded structures formed during transcription.

List of abbreviations

ALPK1	Alpha-kinase 1
AMPs	Antimicrobial peptides
Aqp5	Aquaporin-5
ASPP2	Apoptosis-stimulating protein of p53
ATM	Ataxia telangiectasia mutated
BabA	Blood group antigen-binding adhesin A
Bmi1	Polycomb complex protein BMI-1
BMP	Bone morphogenetic protein
CagA	Cytotoxin-associated gene A
CEACAMs	Carcinoembryonic antigen-related adhesion molecules
DSBs	Double strand breaks
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
GC	Gastric Cancer
H. pylori	*Helicobacter pylori*
hBD	Human beta defensin
IFN-γ	Interferon γ
IL-18	Interleukin 18
IL1β	Interleukin-1 β
IL22	Interleukin 22
Itln1	Intelectin1
Lgr5	Leucine-rich repeat-containing G-protein coupled receptor 5
LPS	Lipopolysaccharide
Lrig1	Leucine-rich repeats and immunoglobulin-like domains protein 1
MEK	Mitogen-activated protein kinase kinase
Mist1	Class A basic helix-loop-helix protein 15
MUC5AC	Mucin 5, subtype AC
MUC6	Mucin 6
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHEJ	Non homologous end joining
NOD	Nucleotide Oligomerization Domain
OipA	Outer inflammatory protein A
OMPs	Outer membrane proteins
PAMPs	Pathogen associated molecular patterns
PD-L1	Programmed Death Ligand 1
PPR	Pattern recognition receptors
Reg3γ	Regenerating family member 3 gamma
ROS	Reactive oxygen species
Rspo	Rspondin
SabA	Sialic acid-binding adhesin A
Stmn1	Stathmin
TAK1	Mitogen-activated protein kinase kinase kinase 7
TGFα	tumour growth factor α
TGFβ	tumour growth factor β
TIFA	TRAF-interacting protein with FHA domain-containing protein A
TIVSS	Type IV secretion system
TLRs	Toll-like receptors
TNFα	Tumour necrosis factor alpha
Troy	Tumor necrosis factor receptor superfamily member 19
VacA	Vacuolating cytotoxin A
WNT	Wingless and Int-1 / Wingless-related iNTegration site

Molecular modelling of the gastric barrier response, from infection to carcinogenesis.

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Conflict of interest

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