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Recapitulating Human Gastric Cancer Pathogenesis: Experimental Models of Gastric Cancer

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Overview

Gastric cancer has been traditionally defined by the Correa paradigm as a progression of sequential pathological events that begins with chronic inflammation [1]. Infection with *Helicobacter pylori* (*H. pylori*) is the typical explanation for why the stomach becomes chronically inflamed. Acute gastric inflammation then leads to chronic gastritis, atrophy particularly of acid-secreting parietal cells, metaplasia due to mucous neck cell expansion from trans-differentiation of zymogenic cells to dysplasia and eventually carcinoma [2]. The chapter contains an overview of gastric anatomy and physiology to set the stage for signaling pathways that play a role in gastric tumorigenesis. Finally, the major known mouse models of gastric transformation are critiqued in terms of the rationale behind their generation and contribution to our understanding of human cancer subtypes.

Comparative Gastric Anatomy and Physiology

Gastric Anatomy

The stomach is surrounded by the greater and lesser omenta, which both provide conduits for draining lymph nodes and lymphatic vessels, blood vessels, and nerves. The lesser omentum supports the lesser curvature of the stomach and anchors it to the liver. The greater omentum emerges to overlie the small intestinal tube and supports the greater curvature of the stomach. Cancer cells can therefore drain into the supporting lymph nodes or can be transported through the gastric and/or gastro-omental veins, which all lead to the hepatic portal vein into the liver. There are multiple clusters of lymph nodes draining the stomach, which are supported by the omenta. For example, pathogenic antigens from *Helicobacter pylori* or Epstein Barr Virus (EBV) in theory drain from the mucosa into lymph nodes via

the afferent lymphatics or post-capillary high-endothelial venules to activate B cell germination, plasma cell generation, and antibody production. Concurrently, the gastric mucosa and submucosa are invaded by a large influx of immune cells including monocytes, macrophages, dendritic cells, neutrophils, B and T effector cells, T-regulatory cells, and mast cells. However, the relationship between the initiation of gastric inflammation in the mucosa and its dependence on antigen presentation in the lymph nodes is poorly understood and might contribute to the difficulty in generating a cancer-preventing vaccine.

Histology

It is important to outline the cellular layers of the gastric tube in order to understand the pre-malignant developments that were outlined by Correa [1]. The human stomach is divided into four parts which display different histological characteristics: (1) cardia, (2) fundus, (3) corpus or body, and (4) antrum/pylorus. Mice lack a cardia but contain two different glandular domains (the body and the antrum). The gastric tube is composed of mucosa (inner epithelial lining facing the lumen), a submucosa formed of dense connective tissue, three layers of muscle (inner oblique, middle circular, and outer longitudinal), and serosa. The muscularis mucosa is a thin layer of smooth muscle that separates the mucosa from submucosal layers (Fig. 22.1). The epithelial mucosa is organized into glands, which vary in their cellular composition between different parts of the stomach.

Gastric Cardia

The gastric cardia lies adjacent to the gastroesophageal junction and consists of tortuous glands populated by mucous-secreting pit cells and scattered oxyntic and chief cells in a 1:1 pit to gland ratio. The main function of the cardia is to neutralize the acidic content of the stomach adjacent to the gastroesophageal junction.

This function depends on mucin- and bicarbonate-rich secretions by the mucous pit and neck cells. The gastric cardia is associated with gastroesophageal acid reflux disease (GERD) and gastric cardia cancer [3]. Gastric cardia cancer is currently on the rise in the US for unknown reasons, but epidemiologically this cancer correlates with inflammation-driven gastric atrophy and acid-bile reflux [4, 5].

Gastric Fundus and Corpus

These two anatomical regions display a more heterogeneous composition than the cardia. The epithelial mucosa consists of a mixture of glands that exhibit a shorter pit cell region with a pit to gland ratio of 1:4 or 1:5, respectively. The fundus and corpus contain several major cell types: (1) acid-secreting parietal cells spanning the entire central gland region, (2) pit cells (mucus-secreting), (3) neck cells (mucus-secreting), (4) zymogenic or chief cells (pepsinogen and lipase-secreting), and (5) endocrine cells that secrete various bioamines or peptide hormones (Fig. 22.1). These cells play several physiological roles.

The parietal cells exchange hydrogen for potassium ions using ATP (H^+, K^+ -ATPase) from abundant mitochondria that fill their cytoplasm. Parietal cells contain a tubulovesicular membrane network available to increase the plasma membrane surface area at the apical surface upon secretagogue stimulation. During secretion, the tubulovesicular membrane

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organizes into apically directed canaliculi simultaneously with insertion of the H⁺,K⁺-ATPase enzyme. The rich membranous content and mitochondrial overabundance imparts to parietal cells their distinctive eosinophilic hue on H&E stains and coupled with their large size (~10 µm) gives these cells a “fried egg” appearance (Fig. 22.1). Pathologically, these cells are very important in the innate mucosal protection against pathogens due to their acid-secreting capabilities. It is therefore not surprising that their loss (atrophy) signals one of the earliest events during *H. pylori*-induced chronic gastritis. Whether triggered by a pathogen or a chronic immunological defect, parietal cell atrophy is a common occurrence that precedes malignant development (Figs. 22.1 and 22.2).

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The fundic surface pit and neck cells are mucus-secreting, and like the cardia, these cells secrete large amounts of mucins and bicarbonate-rich secretions to neutralize the effects of stomach acid. These cells expand in response to chronic inflammation at the expense of parietal cell atrophy (Fig. 22.1). In mice, they arise from cryptic progenitor stem cells residing in the chief cell layer at the base of the fundic gland [6]. Transdifferentiation of these cells into hybrid chief/mucous cells signals the development of gastric metaplasia, which is believed to precede the development of the differentiated gastric cancer subtype. In mice, the metaplasia expresses trefoil factor 2 (TFF2), also known as spasmodic polypeptide. Therefore the mouse form of gastric metaplasia is called SPEM for SP-Expressing Metaplasia [7]. SPEM also develops in the human stomach, but more typically is described as intestinal metaplasia in which the gastric metaplasia resembles goblet cells of the small intestine (complete intestinal metaplasia) or colon (incomplete metaplasia) [8, 9].

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The chief or zymogenic cells located at the base of fundic glands secrete lipase-and pepsinogen. Acid produced by parietal cells stimulates the activation of zymogenic enzymes produced by chief cells, for example by hydrolysis of pepsinogen to pepsin. Due to their protein-secreting properties, chief cells contain a large amount of rough endoplasmic reticula, giving these cells a strong basophilic appearance with H&E staining (Fig. 22.1). Electron microscopy of these cells shows an abundance of secretory vesicles at the apical surface indicating luminal secretion. In addition, a subset of zymogenic cells harbors a cryptic progenitor or “stem cell” that transdifferentiates into SPEM during chronic gastric inflammation [6]. Indeed another report showed that a subset of zymogenic cells express the stem cell marker Troy and give rise to entire gastric units thereby confirming their progenitor capability [10].

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The endocrine cells of the fundus/corpus consist of the Delta (D) and Enterochromaffin-like (ECL) cells, which express muscarinic M₃ receptors. Acetylcholine directly stimulates the D, ECL, and parietal cells to secrete somatostatin, histamine, and acid respectively. Somatostatin from D cells also indirectly regulates parietal cell acid secretion through paracrine stimulation of ECL cells to produce histamine. Thus ECL cells express somatostatin receptors while parietal cells express histamine receptors [11]. Endocrine cells play an important role in gastric pathology by regulating the output of acid secretion, and therefore affect development of hypochlorhydria produced during chronic gastritis.

Gastric Antrum

The gastric antrum displays a more homogenous composition of mucous glands with a 2:1 pit to gland ratio. Antrum function is epitomized by its prominent endocrine role due to the presence of the gastrin-producing G and somatostatin-producing D cells. Unlike the D cells of the fundus (closed-type), the D cells of the antrum are open to the gastric lumen (open-type). The antral D cells therefore sense the acidic-luminal content which stimulates the paracrine release of somatostatin. Activated somatostatin receptors on the antral G cell inhibit gastrin gene expression and secretion [12, 13]. G cells are only present in the antrum where their apical surface faces the lumen to sense digested amino acids in the gastric chyme, probably through primary cilia [14, 15]. G cells respond to several stimuli including: (1) luminal content, (2) parasympathetic stimulation by gastrin-releasing peptide (GRP) secreted from postganglionic fibers of the vagus nerve and, (3) somatostatin inhibition. G cells secrete gastrin basolaterally into the circulation, which then targets cells in the fundus, e.g., stem cells, parietal cells, and D cells in the antrum to complete the negative feedback loop. The importance of gastrin in gastric cancer has been exploited in mouse models of hypergastrinemia, which develop pre-malignant lesions due to chronic stimulation by high gastrin levels which exerts a proliferative effect on mucous pit, parietal and ECL cells in the fundus, but not the antrum [16].

Histologic and Molecular Classification of Gastric Cancer

EBV-associated cancers exhibit higher CpG island methylation associated with mutations in the alpha subunit of the PI3K enzyme (*PI3KCA*). Growth factor pathways, e.g., EGFR and mitotic pathways were commonly perturbed in the MSI cancers; whereas p53 was the most prominent gene abnormality in CIN tumors. Ninety percent of gastric cancers are adenocarcinomas (American Cancer Society: Cancer Facts and Figures 2015; <http://www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf>). However, other cell types can develop into cancer including a B cell lymphoma called mucosa-associated lymphoid tissue (MALT), or neuroendocrine-related tumors arising from ECL cells due to hypergastrinemia (type 1 and 2 gastric carcinoids) [17, 18]. Gastric adenocarcinomas are histologically classified into two types according to the Lauren classification: differentiated or diffuse [19]. Cancers classified as differentiated or intestinal-type arise in the setting of chronic inflammation as described by Correa [1]. On the other hand, the diffuse type exhibits *dis-cohesive* expansion of mucus-secreting cells and is poorly differentiated (lack organized glandular features). In some instances of diffuse gastric cancer, mucus is retained within the tumor cell and displaces the nucleus to the periphery, producing what is known as signet-ring cell carcinoma.

Although the mechanisms leading to the different types of adenocarcinoma remain unclear, recent studies have reclassified gastric cancers according to their molecular signatures [20, 21]. For example, The Cancer Genome Atlas (TCGA) classified gastric adenocarcinomas as: (1) EBV-associated (EBV); (2) microsatellite instability (MSI); (3) genomically stable (GS); (4) chromosomal instability (CIN) [20]. Moreover, these analyses provided valuable insight into some of the molecular mechanisms that underlie different histological subtypes. For example, the diffuse gastric cancer subtype was enriched in the GS group, which contains mutations in the *RHOA*, *CDH1* genes, or a *CLDN18-ARHGAP26* translocation, all loci

associated with the cell cytoskeleton [20]. By contrast differentiated gastric cancer subtypes are enriched in the EBV, MSI, and CIN subgroups [20]. Recently, the Asian Cancer Research Group reported gastric cancer classification based upon p53 activity (MDM2 and p21^{Waf1} expression) [22]. Although their whole genome sequencing of 251 gastric cancers validated the TCGA classifications, their collection of added clinical data permitted further correlation of genotypes with p53 status. Interestingly, subjects with microsatellite stable (MSS) versus microsatellite instability (MSI) tumors showed worse survival. Within the MSS group, those cancers that loss p53 activity or exhibited epithelial–mesenchymal transition (EMT) exhibited the poorest survival. Thus, determining the underlying mechanisms for each molecular subtype is now important to further understand the etiology of these cancers.

Contribution from Invertebrate Biology

Drosophila midgut flexibly enables the genetic modeling of gastric stem cells. The *Drosophila* gut contains a region of low pH (<3) that resembles the mammalian stomach. This area was initially identified by its characteristic ability to accumulate copper [23, 24], and hence the designation “copper cells region” (CCR). The CCR contains three different cell types: (1) copper cells, (2) interstitial cells, and (3) enteroendocrine cells. Copper cells resemble parietal cells in their structure and function: they contain an invaginated apical membrane with microvilli and H⁺-ATPase pumps (that recapitulates parietal cell canaliculi), with an abundance of mitochondria [25, 26]. Recent studies in *Drosophila* have shown that a common progenitor gastric stem cell produces the copper, interstitial, and enteroendocrine cells of the stomach [27, 28], and that this progenitor cell is maintained by Wnt signaling [27]. This *Drosophila* model is useful for studying the molecular aspects of gastric stem cells given the flexibility of generating *Drosophila* genetic mutants.

C. elegans is also a useful model for genetic manipulation. The *C. elegans* stomach also bears resemblance to the mammalian stomach in its functional and molecular composition, such as the existence of a vacuolar-type H⁺-ATPase genes and proteins [29]. *C. elegans* has also been used to elucidate molecular pathways associated with gastric cancer such as RUNX/CBF β homologues [30], which provide an accessible framework to genetically model cancer-associated pathways in vivo.

Signaling Pathways in Gastric Cancer

The recent publication of the TCGA data has been important in re-refocusing the typing of human gastric cancers according to genetic alterations rather than by histology. In some instances, now we interpret mouse models of cancer will be reinterpreted. There are no mouse models of gastric cancer if one follows the strict definition of cancer that entails demonstration of cell-autonomous growth and spreading to distant organs (metastasis). Specifically, there are no examples of using soft agar assays, cell line generation, or distant metastatic lesions to verify that the aggressive-appearing lesions found in the mouse stomach and labeled severe dysplasia or carcinoma in situ progress past this stage by demonstrating true malignant capability. However, mouse models remain useful for testing the in vivo role of individual genes either alone or in combination with other loci or

environmental agents. There are models in which hyperplastic, metaplastic, and dysplastic lesions are observed, some with submucosal tissue invasion. The TCGA database only examines the primary cancer and does not compare these genetic changes with those present in metastatic lesions and, those that change after chemotherapy, which can favor the emergence of a variety of malignant new clones from multiple heterogeneous driver and passenger mutations. Moreover, only mouse models are able to track preneoplastic changes from known or hypothetical triggers to better define the timeline of molecular changes. In addition, mouse modeling of specific signaling pathways can provide insight into the relative strength of the mutations with respect to driving the neoplastic process and partnership with synergistic pathways.

Signaling Pathways in the Proximal Versus Distal Stomach

Although it is often difficult to identify the site of the original cancer in humans, gastric cancer is thought to arise in three major sites, the antrum, corpus, and cardia. Since the mouse does not have a clearly defined cardia region, most mouse models of gastric cancer exhibit dysplastic tumors in the gastric body or distal stomach. This result suggests that different regions of the stomach depend on different signaling pathways to drive the hyperplastic phenotype. Therefore the current section will focus on the implications for understanding gastric cancer using rodent models that target major signal transduction pathways. A recent report using a mouse model of IL-1 β overexpression from the EBV-L2 promoter, which typically targets squamous mucosa, revealed hyperplastic changes in the first gland of the mouse corpus, which progressed to metaplasia reminiscent of Barrett's esophagus. There was the suggestion that the gland adjacent to the mouse forestomach might mimic the human gastric cardia [31]. Nevertheless, the authors indicated that this approach is a model for esophageal as opposed to gastric cardia cancer [32]. Since the promoter is expressed in squamous cells, the tumor arising in the adjacent columnar epithelium of the first gland likely represents a non-cell autonomous effect.

EGR/Ras/MapK

K-Ras mutations account for about 10–15% of the genetic aberrations in gastric cancers [33]. Overexpression of oncogenic K-ras^{G12D/+} driven conditionally from the ubiquitin Ubc9 promoter resulted in depletion of parietal cells (atrophy) and metaplastic changes in the fundic glands [34]. Overexpression of the EGF receptor ligand TGF α produces foveolar hyperplasia reminiscent of Menetrier's Disease [35]. Thus the surface pit cell layer in the corpus appears to be more susceptible to EGFR and *ras* signaling than the antrum.

Notch Signaling

The Notch signaling pathway is one of several cell–cell communication mechanisms initially identified using fly mutagenesis [36]. In mammals, the multiple ligands (Delta, (DLL) 1–4; Delta-like (DLK) 1,2 and Jagged (JAG) 1, 2) are produced by cells adjacent to the cells that express the receptor (NOTCH1–4). In addition, the pattern of ligand and receptor expression is tissue-dependent. Engagement of the ligand with its receptor initiates proteolysis via a two-step process, which involves ADAM proteases and γ -secretase and ultimately releases the NOTCH intracellular receptor domain (NICD) [37]. This C-terminal NICD migrates to the nucleus where it forms a complex with NOTCH-related transcription factors, e.g.,

Mastermind (MAML1-3) and RBPJ that subsequently activate canonical target genes HES, HEY, and HEYL [38]. In addition, NOTCH can modulate cell adhesion and NF κ B gene targets through the ability of the NICD to partner with R-Ras and IKK α respectively [37]. Consistent with the tissue specificity of this pathway, it has been reported that NOTCH signaling is oncogenic in gastric and colon cancers, but anti-neoplastic in squamous cell carcinoma of the esophagus [39]. NOTCH signaling is typically required to maintain the stem cell niche, deep in the crypt zone of the intestine; while a similar function for NOTCH is implied for the gastric antrum due to its similarity to the small bowel in terms of the position and expression of Lgr5+ stem cells at the crypt base [40, 41]. Nevertheless, there are no reports yet demonstrating the effects of modulating this pathway in the glandular stomach.

One possible exception is a mouse model of Barrett's esophagus. Quante et al. overexpressed the IL-1 β cytokine in squamous mucosa from the EBV promoter LD2 and observed tumors in the initial gastric gland at the gastroesophageal junction within 12–15 months [31]. In addition, 0.2% bile acids accelerated the histologic changes, which correlated with increased *Notch* ligand expression. Indeed increased expression of Notch signaling components is consistent with maintenance of the self-renewing stem cell compartment and has been observed in human gastric [42]; esophageal adenocarcinoma [43] and esophageal squamous carcinoma [44]. Thus the Notch signaling pathway clearly contributes to foregut transformation, but overexpression of this pathway has not been performed directly in the gastric epithelium to determine if it is sufficient to drive transformation.

Hedgehog Signaling

Like the discovery of Notch signaling, the Hedgehog pathway in mammals has been associated with cell growth and development as well as neoplastic transformation [45–17]. The three hedgehog ligands (Sonic, Indian, and Desert) are typically expressed and then after release from the epithelium modulate cells in the stroma. Thus normal hedgehog signaling is typically paracrine [48]. Recipient cells express the canonical ligand binding receptor Patched (Ptch1, 2), which normally represses pathway activation in the absence of ligand via the G-protein coupled receptor Smoothened (Smo) [49]. Upon Smo de-repression, the inactive cytoplasmic glioma-associated transcription factors (Gli 2,3) are released from an inhibitory complex and undergo limited proteolysis revealing positive and negative regulatory domains. Afterwards, the factors translocate to the nucleus to regulate canonical target genes such as Gli1, a third family member of the Gli family and ligand receptors Ptch and Hedgehog Inhibitory Protein (HhIP) [50].

In the stomach, several labs have examined the location and function of sonic hedgehog (Shh) expression. The primary ligand expressed in the stomach is Shh [48, 51]. Although all gastric epithelial cells express Shh, the highest levels in the uninfected stomach occur in parietal cells where it appears to be required for H⁺,K⁺-ATPase expression and acid production [51–55]. The initial infection of the stomach by *Helicobacter* initiates recruitment of bone marrow-derived immune and mesenchymal cells to the stomach presumably with the intent to initiate repair [56–58]. However, the inflammatory milieu

hastens hypochlorhydria within a few months that segues to parietal and zymogenic cell atrophy [51, 59]. Once gastric atrophy sets in as a consequence of the chronic inflammation, hedgehog-dependent immune cells acquire a phenotype sufficient to initiate gastric metaplasia and in some instances dysplasia [60] (Fig. 22.3). Although mouse models do not progress to dysplasia and frank cancer, current studies indicate that the bacterial infection and inflammatory response cooperates with hedgehog signaling to create a micro-environment sufficient for epithelial transformation. Moreover, this conclusion is consistent with prior studies of Hedgehog expression in human gastric cancers and cells lines [61–63].

Wnt/ β catenin

Gastric adenomas and carcinomas in the stomach of FAP patients does not occur frequently [33]. Yet when FAP dependent gastric polyps occur they are associated with pyloric gland and fundic gland polyps [64]. Similarly, examination of mice carrying a truncated APC gene show a predilection for polyps and dysplasia in the antral pyloric glands, suggesting a predisposition of this gastric region to elevated Wnt signaling [65].

Akt/PI3K

There are no direct mouse models of the PhosphoInositol-3 kinase/Akt pathway for gastric cancer. Nevertheless, the pathway appears to be activated in human gastric cancers as a result of chronic EBV infection [20]. Indeed, the initial cloning of the retroviral *v-akt* oncogene in 1987 was initially linked to its cellular homologues AKT1 and AKT2 in primary gastric adenocarcinomas [66]. AKT1 and 2 are primarily cytoplasmic while AKT3 resides in the nucleus [67]. PIK3CA mutations and gene amplification are the two major mechanisms through which the pathway becomes overly active and associated with gastric cancer and poor survival [20, 68]. PI3Ks are found in the nucleus associated with nuclear speckles implicating their role gene expression; while, the oncogene AKT is the canonical effector of PI3K that activates growth pathways, e.g., inhibiting GSK3 β [67]. A recent study that examined the role of trefoil factor 1 (TFF1) in gastric tumorigenesis revealed that loss of this factor stabilized β -catenin through reduced GSK3b and Akt phosphorylation [69].

Mouse Models of Gastric Cancer

Rodent models have been used to elucidate details of the molecular mechanisms of various cancers in ways that cannot be ethically studied in humans. It is particularly vital to the study of gastric carcinogenesis, where the host factors, infectious agents and environment individually and combinatorially influence disease outcome. Although rodents especially mice rarely develop spontaneous gastric cancer, cotton rats (*Sigmodon hispidus*) and *Mastomys natalensis* exhibit a genetic propensity to develop gastric carcinoids [70–72]. Thus, researchers using animal models have focused on the development of chemical, infectious, or genetic tools to experimentally induce gastric cancer in rodents. The paucity of inbred strains of rats, gerbils, and *Mastomys* has limited the use of these rodent models in the study of gastric carcinogenesis. Therefore, most investigators have chosen to use mouse models because of the widespread availability of multiple inbred strains, genetically engineered variants, short breeding cycles, and the accessibility of experimental reagents. A large number of transgenic and knockout mouse models of gastric cancer have been

developed using genetic engineering (Table 22.1). A combination of carcinogens and genetic manipulation has been applied to facilitate development of advanced gastric cancer. Therefore, we have focused primarily on the current mouse models of gastric carcinogenesis by comparing their pathological phenotype, experimental limitations, and applications to improve our understanding of the neoplastic process in the stomach. A schematic overview chemical, infectious, and dietary manipulation of the mouse models to generate gastric cancer is depicted in Fig. 22.4.

Chemical Carcinogen-Induced Models of Gastric Cancer

N-Nitroso Compounds (MNNG and MNU)—Prior to the widespread acceptance of *Helicobacter* infection, researchers tested the utility of several chemical carcinogens such as benzo-a-pyrene, 3-methylcholanthrene and 2-acetyl aminofluorene in animals starting in the 1930s, but the incidences of chemically induced stomach cancer were low. In 1967, Sugimura and Fujimura were able to report higher yields of adenocarcinomas in the glandular stomachs of rats treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) [73]. Under acidic conditions, MNNG is converted to *N*-methyl-*N'*-nitroguanidine, which is capable of alkylating the purine bases of DNA, RNA, and some amino acids leading to subsequent mutations. Moreover, MNNG was found to be a very potent gastric carcinogen in Mongolian gerbils. Treatment with 400 ppm of MNNG in drinking water for 50 weeks resulted in 64% gastric adenocarcinomas in the gerbils. However, the mouse glandular stomach was found to be relatively resistant to MNNG. Administration of MNNG in the drinking water of rats over their life span resulted in adenomatous tumors only in the glandular epithelium of the stomach [74].

The utility of another N-nitroso compound, *N*-methyl-*N*-nitrosourea (MNU) as a gastric carcinogen was tested in mice and found to be more effective. Weekly gavage with 0.5 mg MNU in the Balb/c mice resulted in premature death due to squamous cell carcinoma in the forestomach. Surgical removal of the forestomach prior to MNU treatment leads to 100 % development of glandular cancer by 40 weeks [75]. Thus, the glandular stomach is sensitive to the MNU, but the greater sensitivity of the forestomach to MNU obscured the phenotype. Further testing by Tatematsu et al. revealed that low dose (30–120 ppm) administration in the drinking water proved more effective in targeting the glandular stomach without tumors in the forestomach [76]. The same group further demonstrated that the induction efficiency of adenocarcinomas in the glandular stomach depended on MNU concentration rather than total quantity. As a result, they established a protocol of 240 ppm MNU in the drinking water biweekly for 5 weeks as the standard method to induce gastric carcinogenesis in mice [77]. This MNU mouse model opened up new approaches for using transgenic and knockout mice to investigate various signaling pathways or transcription factors in gastric carcinogenesis. It should be noted that MNNG- and MNU-induced tumors primarily in the antral mucosa and rarely in the normal fundic mucosa, like the tumor types found in humans [1].

Dietary Salt—A high salt intake has been implicated in a number of human case-control and ecological studies from various geographical regions as a risk factor for stomach cancer [78, 79]. This phenomenon has been addressed in rodents by assessing the effects of sodium

chloride administration. Sodium chloride possibly decreases the viscosity of gastric mucins and might reduce the protective mucous barrier. Acute exposure of rats to a single dose of hypertonic sodium chloride immediately damages the surface mucous cell layer and then stimulates regenerative cell proliferation returning the mucosa to homeostasis within 24–18 h after the exposure [80, 81].

The effect of chronic sodium chloride administration in high-salt diets has been evaluated in a number of rodent studies. When given alone, a high-salt diet causes atrophic gastritis in gerbils [82] and C57BL/6 mice [83, 84], but no evidence of tumors. When administered with MNNG or Nitroquinolone-1-oxide (NQO), sodium chloride promotes stomach carcinogenesis in the rats [85, 86] in a dose-dependent manner [87]. A high-salt diet also enhances the multiplicity of gastric tumors in MNU-treated mice and synergizes with the transforming effects of a *Helicobacter* infection [88]. Therefore the available data from experimental rodent models clearly supports the concept that high salt intake alone does not induce but rather increases the risk for gastric neoplasia.

Other Environment-Related Agents—Several other environment-related agents thought to promote the onset of gastric carcinogenesis have also been tested in mouse models. Administration of catechol (a phenol in cigarette smoke, perfumes, and insecticides) in the diet has been shown to enhance preneoplastic and neoplastic lesions in the Balb/c mouse glandular stomach with MNU treatment in a dose-dependent manner [89]. The same group also demonstrated that low dose catechol at predicted human exposure levels has a limited effect on MNU-induced cancers [90]. However, it should be noted that 0.8% catechol alone is sufficient to induce adenocarcinomas in the rat stomach. However the incidence varied with the rat strain suggesting that genetic background influences susceptibility to catechol carcinogenicity [91, 92].

Butylated hydroxyanisole (BHA), an antioxidant commonly used in food preservatives was fed at a concentrate of 0.5–2.0 % to Fisher 344 rats, Syrian golden hamsters, and B6C3F1 mice for 2 years, and caused increased forestomach hyperplasia and papillomas in all three species. Nevertheless, forestomach (squamous cell) carcinoma was only observed in rats and hamsters underscoring that variations in the genome modulate carcinogen susceptibility [93–95].

Ethylene dibromide (EDB) a soil, grain fumigant, chemical intermediate, and solvent increased forestomach cancer when administered to rats and B6C3F1 mice by gavage but not when given by inhalation [96].

Bacterial Models

Helicobacter

Helicobacter is thought to account for about 80 % of gastric cancers. Although many animals have been successfully infected with human *H. pylori*, none of these early models proved sufficiently similar to the situation with human *H. pylori* infection and pathology. Then in 1998, Watanabe et al. published the first successful experiment of gastric cancer induced by *H. pylori*. After 62 weeks of infection, 10 of 27 (37 %) infected Mongolian

gerbils developed gastric tumors with histological similarity to human intestinal-type gastric cancer. In general mice, especially the C57BL/6 strain, were remarkably resistant to colonization with various *H. pylori* strains [97,98] until Lee et al. successfully adapted a clinical Cag A and Vac A-expressing strain called SS1 (Sydney strain) that efficiently colonized the mouse stomach [99]. High levels of colonization have been achieved in C57BL/6, while colonization levels in Balb/c, DBA/2, and C3H/HeJ strains were lower. Although the SS1 strain causes chronic active gastritis and atrophy after 8 months of infection, it does not induce gastric carcinoma in C57BL/6 wild type mice even after 2 years [100].

Thus, alternative mouse models of gastric *Helicobacter* infection were explored. In 1990, *Helicobacter felis*, a close relative of *H. pylori* was isolated from the cat stomach and shown to efficiently colonize the mouse stomach causing more severe gastritis than that induced by *H. pylori* [101–103]. *H. felis*-infected mice show gastric metaplasia, dysplasia, and eventually progress to invasive cancer after extended periods of infection [104, 105]. However despite extensive submucosal cystic lesions observed in the *H. felis*-infected mice, no metastasis was reported, nor were other means of assessing malignant potential performed, suggesting that the model still falls short of mimicking true carcinoma. In fact a surprising finding was the observation that infecting mice heterozygous for p53, mitigated the appearance of invasive lesions. This result seems counter-intuitive given the recent observation that subjects with p53 mutant gastric cancer have worse survival [22].

Following *H. felis* or *H. pylori* infection, the immune response in the C57BL/6 strain is predominantly Th1-skewed with low bacterial loads and high levels of epithelial cell damage whereas, the Th2-predominant Balb/c strain exhibits higher bacterial load and less evidence of cell damage [99,103,104,106]. Both *H. pylori*-infected C57BL/6 and Balb/c mice show a marked influx of mononuclear cells [103,107].

Much of the research now focuses on the cancer-preventive effect of *H. pylori* eradication. Several cohort studies and randomized controlled trials have shown that *H. pylori* eradication can halt the histological progression from chronic gastritis to gastric adenocarcinoma and even induce regression of atrophy in patients with tumor-associated infection [108–112]. However, the effect of most of these interventions is less evident. The striking observation was that those gastric cancers that occurred after eradication treatment were confined to those subjects who already had atrophic gastritis and intestinal metaplasia at baseline. It suggests that there may be a “point of no return” beyond which the precancerous cascade can no longer be reversed. Antimicrobial treatment studies have been conducted in mouse models and might help us address the uncertain questions in this field. In *H. felis*-infected C57BL/6 mice, eradication of *Helicobacter* at early (2 months post infection) or at later (6 months) intervals led to regression of inflammation, restoration of parietal cell mass, and reestablishment of normal architecture. Late eradication (1 year) restricted the progression to dysplasia [113]. In *H. pylori*-infected hypergastrinemic INS-GAS mice treated with antibiotics, the progression of gastric lesions after curative treatment for *H. pylori* was significantly less than without eradication [114]. In *p27^{-/-}* mice, *H. pylori* eradication during the early (15 weeks post infection) and late (45 weeks) periods of infection effectively reduced development of gastric transformation even though mice had

already showed pseudopyloric metaplasia. These studies suggest that *Helicobacter* eradication might be beneficial for gastric cancer prevention in humans even when given relatively late in the natural history of the disease [115].

***Helicobacter* Coinfection with Other Microorganisms**

In C57BL/6 models in which immune response was shifted toward a Th2-polarized response by coinfection with an intestinal helminth attenuated *Helicobacter*-dependent atrophy and metaplasia without reducing inflammation, which suggested that the epithelial changes were not directly related to the severity of the inflammatory response [116]. A recent study showed that helminth infection reduces *H. pylori*-induced gastric lesions while inhibiting changes in gastric flora [117]. Conversely, shifting the immune cytokine profile of resistant host Balb/c mice toward a Th1-polarized response by prior infection with the Th1-provoking protozoan *Toxoplasma gondii* confers susceptibility to chronic active gastritis and dysplastic lesions after *H. felis* infection [118]. These two models offer a potential explanation for the “African enigma,” which infers a high *H. pylori* prevalence with relatively low gastric cancer burden, especially in countries with frequent endogenous parasitic diseases [119, 120].

Epstein-Barr Virus

It is likely that some cases of gastric cancer might be attributable to other infectious agents. For example, Epstein-Barr virus (EBV) has been linked to 6–16 % of gastric cancer cases worldwide [121–123]. EBV-associated gastric cancer (EBV-GC) has unique morphologic and phenotypic features, and might also differ considerably from EBV-negative gastric cancers [124]. However, research regarding the role of EBV in gastric carcinoma has been hampered by the absence of a suitable model system. To investigate the mechanism of EBV-induced gastric cancer (EBV-GC), researchers have explored models of EBV engraftment using infected epithelial cell lines. SNU-719 is a gastric carcinoma cell line established from a Korean patient that shows modified latency of EBV infection closely resembling EBV-GC [125]. After subcutaneous injection of the SNU-719 gastric cancer cell line into athymic nude mice (BALB/c nu/nu) with the Matrigel substrate as an irritant, all mice developed tumors, which showed characteristics of moderately differentiated carcinoma with no gland formation and areas of necrosis [124].

Genetically Engineered Mouse Models (GEMMs)

Transgenic mouse models have proved to be the most powerful tool for dissecting the importance of individual host susceptibility genes and signaling pathways. These have included abnormal expression of growth factors and cytokines, as well as mutations in oncogene and tumor suppressor gene loci. Most of these models were developed on the *C57BL/6* genetic background. Representative highlights of the use of mouse models is shown in Fig. 22.3 with a more detailed list of mouse models listed in Table 22.1.

Gastrin Mutants

Gastrin is a crucial peptide hormone released by G cells located in the antrum that stimulates secretion of gastric acid (HCl) by the parietal cells and aids in gastric motility. Altered gastrin gene expression and secretion leads to disturbances in gastric epithelial cell dynamics potentially promoting gastric cancer, as revealed by the various mouse models described below.

INS-GAS Mice

Given the known properties of gastrin as a mucosal growth factor, hypergastrinemia was postulated to be a factor promoting the development of gastric cancer. The insulin-gastrin (INS-GAS) mice were engineered as a transgene in the FVB/N strain, which overexpress the human gastrin gene under the control of the mouse insulin promoter [16, 126]. These mice have elevated serum levels of human amidated gastrin (sustained hypergastrinemia) and spontaneously develop gastric atrophy, metaplasia, dysplasia, and eventually progress to invasive gastric tumors in the corpus (submucosal cysts) by 20 months of age without lymph node invasion or distant metastasis [16, 127, 128]. Amidated gastrin in the corpus up-regulates growth factors [16] in combination with induction of apoptosis in gastric epithelial cells, particularly parietal cells [129], both of which may trigger Correa's cascade and lead to gastric cancer. Due to its lower threshold for carcinogenesis, the INS-GAS mouse has proven to be a valuable model of gastric cancer development when used in combination with other agents. Infection of INS-GAS mice with *H. felis* or *H. pylori* led to accelerated carcinogenesis (7 months after infection) [16,130], and more severe lesions were observed in the male INS-GAS mice [130]. However tumor development was delayed for months in gnotobiotic INS-GAS mice mono-infected with *H. pylori* compared to INS-GAS mice colonized with *H. pylori* and complex enteric microbiota [131]. A most recent study demonstrated that gnotobiotic INS-GAS colonized with *H. pylori* and three bacterial members of Altered Schaedler Flora, developed gastritis and premalignant gastric lesions equivalent to *H. pylori*-infected INS-GAS mice with complex microflora [132]. These data support the notion that hypergastrinemia and *Helicobacter*-induced tumors require additional microflora. The metaplasia induced in the INS-GAS mouse also involve reactivation of the Hedgehog pathway [133] whereas, inhibition of the gastrin/CCK2 and histamine H2 receptor limits the development of gastric neoplasia in these mice [134].

Gastrin-Deficient Mice

Gastrin-deficient mice (*Gast*^{-/-}) on a mixed C57BL6/129Sv background are hypochlorhydric and develop spontaneous gastric antral tumors at 12 months of age [135, 136]. Tumors in this mouse model are associated with bacterial overgrowth [137] and inflammation [136, 138]. We reported multiple inflammatory mediators, such as IL-1 β , IL-11, and the Tgf β pathway components activin A and follistatin with epithelial Gli2 appear to be important epithelial drivers of the histologic changes during antral transformation in the *Gast*^{-/-} mice [139, 140]. Takashi et al. investigated the role of gastrin in *H. felis*-infected hypergastrinemic transgenic (INS-GAS) mice, GAS-KO mice, and C57BL/6 wild-type mice on a uniform C57BL/6 genetic background housed under specific-pathogen-free conditions [141]. Their results showed that gastrin has a distinct effect on the

gastric corpus and antrum in the setting of chronic gastric *Helicobacter* infection. While gastrin is possibly an essential cofactor for gastric corpus carcinogenesis, gastrin deficiency can predispose animals to antral tumorigenesis, and thus any imbalances in gastrin physiology may represent a risk for gastric transformation.

The Trefoil Factor 1 (Tff1) and gp130 Mutants

The tumor suppressor Trefoil factor 1 (TFF1) protein is normally expressed by the surface pit cells and is abnormally expressed in gastrointestinal diseases and various cancers [142–144]. *Tff1*^{−/−} mice on a 129/Svj mixed genetic background develop antropyloric adenomas and 30% develop multifocal intraepithelial or intramucosal carcinomas [145]. A recent study has shown that loss of *Tff1* leads to activation of β -catenin signaling and gastric tumorigenesis through induction of PP2A, a major regulator of AKT-GSK3 β signaling [69]. Genetic deletion of *cyclooxygenase-2* (*Cox-2*) in the *Tff1*^{−/−} mice resulted in reduced adenoma size and ulceration with a chronic inflammatory reaction at the site of the adenoma. Moreover, selective inhibition of *Cox-2* resulted in regression of established gastric adenomas in *Tff1*^{−/−} mice [146].

Tff1 expression is strongly suppressed in gp130-mutant mice (*gp130*^{F/F}). Interestingly, the phenotype of *gp130*^{F/F} mice in many ways mimics that of *TFF1*^{−/−} and *Gast*^{−/−} mice. Glycoprotein 130 (gp130) is a ubiquitously expressed, signal-transducing receptor that forms part of the receptor complex for the interleukin-6 (IL-6) family of cytokines. IL-6 and IL-11 are the dominant IL-6 family cytokines in the gastric mucosa, and the only cytokines of the family that exclusively utilize gp130 homodimers. Homeostatic gp130 signaling following receptor activation can trigger three alternate signaling cascades: the JAK/STAT, SHP-2/ERK/MAPK, or Src/PI3/AKT pathway. Under homeostatic conditions, these three gp130 pathways are tightly controlled by multiple negative feedback mechanisms [147]. *Gp130*^{F/F} mice generated by a knock-in point mutation that converts a tyrosine (Y) to a phenylalanine (F) blocking phosphorylation at the receptor site recognized by the SHP-2/SOCS3 signaling complex [148]. The mutant receptor was generated to examine the role of gp130 ligands and their signaling pathways in hematopoiesis and inflammation. The knock-in mutation prevents SHP-2 from docking and ablates signal transduction through the SHP2/ERK/MAPK cascade. The absence of one of the three signaling pathways transduced by the gp130 receptor subsequently creates a signal imbalance which favors the JAK/STAT1/3 pathway in the absence of negative feedback from SHP2. A principle feature of *gp130*^{F/F} mice is the phenotypic change in the distal stomach of these mice characteristic of human gastric adenocarcinoma, including rapid development of gastritis, atrophy, intestinal metaplasia and SPEM, dysplasia and submucosal invasion by 20 weeks of age [149], making them an excellent model to study gastric cancer progression. In a mouse model with compound *gp130*^{F/F}/STAT3^{+/-} mutants, mice have significantly smaller tumors and reduced gastric inflammation, proinflammatory cytokines, and chemokines. Chronic treatment of the *gp130*^{F/F} mice with antibiotics reduced tumor mass by reducing activated macrophages in the gastric mucosa [150]. Moreover on the *gp130*^{F/F} background, neither *Rag1*^{−/−} mice, which lacks T, B, and NKT cells, nor the *Perforin*^{−/−} mice, which have diminished cytotoxic T cell function, reduce tumor development [151], suggesting that macrophages might play an aggressive tumor-promoting role.

Parietal Cell Mutants

Parietal cells in the fundus or corpus stomach secrete hydrochloric acid (HCl) to maintain a highly acidic environment and promote the activation of stomach enzymes for digestion such as pepsin. Three different signaling pathways—a bioamine (histamine), a neurotransmitter (acetylcholine), and a hormone (gastrin) regulates parietal cell acid secretion. Loss of parietal cells or their ability to secrete acid predisposes the gastric epithelium to metaplasia and cancer.

The enzyme hydrogen potassium ATPase (H^+,K^+ -ATPase) is unique to the parietal cell and is the most critical component of the ion transport system mediating acid secretion in the stomach. The enzyme consists of two subunits, a 114-kDa α -subunit (Atp4a) and a 35-kDa (protein moiety) β -subunit (Atp4b). Mice homozygous null for the α -subunit (*Atp4a*^{-/-}) alleles exhibit normal systemic electrolyte and acid-base status but are achlorhydric and hypergastrinemic [152]. Chronic achlorhydria and hypergastrinemia in aged *Atp4a*^{-/-} mice produced progressive hyperplasia, mucocystic and incomplete intestinal metaplasia, and induction of growth factors without histological evidence of neoplasia [153].

The potassium channel is crucial for H^+,K^+ -ATPase activity. The KvLQT1 gene encodes a voltage-gated potassium channel. KvLQT1 knockout mice display a threefold enlargement of the stomach resulting from mucous neck cell hyperplasia (SPEM) by 3 months of age [154].

Histamine H2 receptor (H2R) is expressed on parietal cells and functions to stimulate gastric acid secretion. The H2R-deficient mice exhibit hypergastrinemia and marked hypertrophy due to an increase in the numbers of parietal cells, ECL cells, and other types of cells. It should be noted that the morphological characteristics of the parietal cells were remarkably altered in H2R-deficient mice. The size of parietal cells in these mice was significantly smaller despite increased cells numbers [155].

Oncogene and Tumor Suppressor Gene Mutants

COX-2

Overexpression of cyclooxygenase 2 (COX-2) is involved in gastric cancer and is highly induced in *H. pylori* infection. There are compelling epidemiological data to suggest that long-term use of nonsteroidal anti-inflammatory drugs is associated with a significant reduction in gastric cancer risk, largely attributed to the inhibition of COX-2 enzymes [156–158]. COX-2 transgenic mice, generated on a C57BL/6 genetic background expressing full-length human COX-2 cDNA, showed an increased frequency of MNU-induced gastric cancer [159]. Treatment with celecoxib, a specific COX-2 inhibitor, prevents MNNG-induced gastric cancer in a rodent model [160]. Transgenic mice (*K19-C2mE*) simultaneously expressing *COX-2* and the microsomal *prostaglandin E synthase (mPGES)-1* develop metaplasia, hyperplasia, and tumorous growths at 48 weeks with heavy macrophage infiltrations after *Helicobacter* infection [161], through a tumor necrosis factor- α (TNF- α) dependent pathway [162]. Treatment of K19-C2mE mice with NS-398, a COX-2 selective inhibitor, for 4 weeks completely suppressed gastric hypertrophy, reducing mucosal

thickness to that found in the age-matched wild type. These results clearly indicate that increased levels of COX-2 are essential for the gastric pathology in K19-C2mE mice [161]. Thus, COX-2 is a prime target for chemoprevention of stomach cancer.

K-ras

One oncogene that has been strongly linked to the development of chronic inflammation and a variety of human cancers has been *K-ras*. Activating *K-ras* mutations are found in approximately 5–20 % of gastric cancers [163], and are more prevalent in intestinal-type gastric cancers [164]. A transgenic model (K19-K-ras-V12) in which the cytokeratin 19 (K19) promoter targets *K-ras-V12* mutant gene expression to the gastric mucus neck cells was used to analyze the function of K-ras on the stomach carcinogenesis [165]. Activated K-ras in this context increased recruitment of bone marrow-derived inflammatory cells that contribute to the stromal microenvironment and causes gradual parietal cell loss and mucous neck cell hyperplasia, comparable to *H.felis* infection [166]. Introduction of *K-ras^{G12D}* mutation controlled by inducible, Cre-mediated recombination in the K19 expressing lineage in another mouse model (*CK19CreERT; LSL-Kras^{G12D}* mice) led to numerous hyperplasias, metaplasias, and adenomas in the stomach as well as in the oral cavity, colon, and lungs [167]. The effects in mice of ubiquitous activation of *K-ras* were determined in a mouse model created by cross *UBC9-CreERT* mice with *LoxP-STOP-LoxP-Kras^{G12D}* mice. Systemic activation of K-ras leads to rapid changes in gastric cellular homeostasis, and resulted in activation of the MAPK pathway and hyperproliferation of squamous epithelium in the forestomach and metaplasia in the glandular stomach, resembling the preneoplastic changes that take place during gastric carcinogenesis in humans [34]. It suggests mutant *K-ras* signaling modulates important molecular events in the initiating gastric carcinogenesis.

p27^{Kip1}

The cyclin-dependent kinase (CDK) inhibitor p27^{Kip1} has an important role in cell cycle regulation and is associated with many malignancies, including gastric cancer [168]. *Helicobacter* infection is associated with complete loss of p27^{Kip1} or cytoplasmic p27^{Kip1} retention [169,170]. p27^{Kip1} mislocalization to the cytoplasm blocks its ability to suppress nuclear cell cycle events. *p27^{Kip1}* knockout mice develop mild gastric hyperplasia, random foci of moderate metaplasia and atypia or low-grade dysplasia. After *H. pylori* infection, these mice show intestinal metaplasia, high-grade gastric intraepithelial neoplasia, and polypoid adenomas and, in some cases, in situ or intramucosal carcinoma, all of which were more advanced than in WT mice [168]. Thus, the *p27^{Kip1}*-deficient mouse is a useful model to examine the pathogenesis of *H. pylori* in gastric carcinogenesis and to test eradication and chemopreventive strategies [115].

Inflammation Mediators and Cytokine Mutants

Inflammatory mediators and cytokines are vital for developing a tumor microenvironment, which further stimulate tumor progression. The following GEMMs have been useful for separating events due to an overactive immune system versus parietal cell atrophy.

TGF- β Signaling

Transforming growth factor beta (TGF- β) is a cytokine that controls proliferation, cellular differentiation, and other functions in most cells. TGF- β exists as three isoforms designated TGF- β 1, TGF- β 2, and TGF- β 3. All three isoforms bind to the TGF- β receptor II that recruits and phosphorylates TGF- β receptor I. The TGF- β 1 signaling pathway is commonly altered in gastric cancer [171–173]. TGF- β 1 null mice develop a severe wasting syndrome from inflammatory cell infiltration into various tissues including the stomach [174], which eventually exhibits gastric epithelial hyperplasia and SPEM, contributing to the early lethality [175]. To circumvent early demise, Ota et al. generated mice encoding a TGF- β 1 mutant that prevents ligand binding to the latent TGF- β binding protein (*Tgfb1*^{-C33S}) [176]. About 55 % of these mice survived at 12 weeks and displayed multi-organ inflammation and an elevated incidence of various types of gastrointestinal solid tumors. A *Tgfb1*^{-C33S}; *Rag2*^{-/-} chimeric mouse line that lacks mature lymphocytes was generated to further investigate the relative contribution of TGF- β 1 to lymphocyte-mediated inflammation in gastrointestinal tumorigenesis. No tumors were found in the stomachs of these mice, demonstrating that active TGF- β 1 enhanced the levels of lymphocyte-dependent inflammation and gastric epithelial proliferation, while blocking TGF β -mediated immune cells can impede tumor development [176].

A dominant-negative transgenic model (pS2-dnRII) of the TGF- β receptor II was expressed under the control of the TFF1 promoter to restrict expression of the transgene to the stomach. These mice showed a higher proliferation index and a higher incidence of gastric adenocarcinoma after *H. pylori* infection [177]. SMAD proteins are downstream effectors of the TGF- β signaling pathway. Smad3-null mice develop gastric tumors in the fundus initiated from the forestomach/glandular transition zone along the lesser curvature [178]. Similarly, heterozygous Smad4 knockout mice develop gastric cancer spontaneously [179, 180]. However, selective loss of Smad4-dependent signaling in T cells leads to spontaneous epithelial cancers throughout the gastrointestinal tract in mice, with induction of abundant Th2 and Th17 type cytokines, suggesting that Smad4 signaling in T cells is required for suppression of gastrointestinal cancer [181, 182].

Autoimmune Model (TxA23 Mice)

Autoimmune gastritis also triggers a chronic state of gastric inflammation. Individuals with severe autoimmune gastritis exhibit an increased risk of gastric cancer [183, 184]. A model of autoimmune gastritis (TxA23 mice) was developed to investigate how to suppress chronic inflammation in the gastric mucosa. These mice mimic many aspects of the corresponding human condition and develop gastric lesions in accordance with the Correa paradigm [185, 186].

Interleukin-1 β

Interleukin-1 β (IL1- β) is a pleiotropic proinflammatory cytokine that has profound effects on inflammation and immunity. The polymorphism of IL1- β has been shown to increase the risk of gastric cancer [187, 188]. Transgenic mice with stomach-specific human *IL1- β* expression (*H⁺,K⁺-ATPase-hIL-1 β*) develop spontaneous gastric inflammation and marked gastric hyperplasia, parietal cell loss, metaplasia, and dysplasia, all of which are accelerated

by *H. felis* infection. Transgenic overexpression of *IL-1 β* in the stomach mobilizes myeloid-derived suppressor cell (MDSC) recruitment at the earliest histopathologic stages of progression from gastric inflammation to cancer. This mouse line was crossed to *Rag2*^{-/-} mice to generate lymphocyte deficient *IL-1 β* transgenic mice, which displayed the spontaneous development of atrophic gastritis, metaplasia, and dysplasia, accompanied by a marked increase in the number of MDSCs in the stomach, blood, and spleen, suggesting that MDSCs are a critical mediator of early stages of gastric transformation [189]. On the contrary, *IL-1 β* null mice show decreased recruitment of neutrophils and macrophages, which suppress the multiplicity of gastric tumors in the setting of *H. pylori* infection [190].

Models of Precancerous Change

In addition to mouse models of gastric cancer, there are a number of models that show precancerous lesions. Most of these models do not progress to neoplasia. Goldenring and coworkers developed two short-term SPEM models by using DMP-777 and L-635, both are chemical protonophores that elicit a rapid loss of parietal cells followed by the emergence of foveolar hyperplasia and SPEM [191, 192]. DMP-777 is a neutrophil elastase inhibitor. As a result, mice treated with DMP-777 for 14 days develop SPEM in the absence of significant inflammation. By contrast, the DMP-777 enantiomer called L-635 is a structurally related β -lactam compound that develops more advanced proliferative SPEM lesions associated with an intestinal metaplastic phenotype and more prominent inflammatory infiltrate in just 3 days of treatment. Thus L-635 rapidly induces the mucosal phenotype associated with 6 (or more) months of *H. felis* infection. These results indicate that the SPEM phenotype per se might be driven primarily by the inflammation than by parietal cell atrophy. Moreover, a recent study showed that M2 macrophages are the critical immune cell driver of this rapidly generated metaplastic change after loss of parietal cells [193]. Thus, strategic use of these two compounds in mice could help attribute the origin of the mucosal effects induced by parietal cell atrophy versus the inflammatory response.

Model of Bone Marrow-Derived Gastric Cancer Stem Cells

The main function of gastric stem cells is to maintain the integrity of the gastrointestinal epithelium and replenish all of the mature cell lineages. Recent advances in gastric stem cell biology have lead to a new paradigm in which chronic inflammation causes tissue injury and local tissue stem cell failure, followed by recruitment and permanent engraftment of circulating bone marrow-derived stem cell (BMDC) in the tissue stem cell niche. In this way, BMDC essentially take over the function of the tissue stem cell in a severely damaged mucosa [194–196]. To test the role of BMDC in tissue repair after treatment with gastric carcinogens, mice were myelo-ablated via irradiation and then transplanted with gender-mismatched BM. To further facilitate tracking of the BMDC, BMDCs carried a reporter (GFP or β -gal). After recovery of immune function, mice were infected with *H. felis* and after a period time, engraftment of BMDCs into the stomach was detected and found to differentiate into a range of epithelial cells [197]. After 30 weeks of engraftment, antralized glands and metaplastic cells at the squamo-columnar junction were entirely replaced by BMDCs. One year of infection, most mice developed invasive neoplastic glands, which arose from donor marrow cells. However, neither acute ulceration by cryo-injury or acetic

acid nor selective but reversible parietal cell ablation required BMDCs for repair and neither condition was associated with any evidence of marrow engraftment into the gastric epithelium [197].

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Fig. 22.1.

Histological structures of the gastric body of the mouse in normal and inflamed mucosal epithelia. Cell types resemble those of the human stomach. *Left panel*, normal uninflamed gastric mucosa. *Right panel*, chronically inflamed mucosa after 6-month *Helicobacter felis* (*H. felis*) infection. Annotated are the gastric pits and glands, and constituent cell types, including: (1) pit cells (foveolar cells), (2) parietal cells (large eosinophilic cells which are “fried egg”-shaped), (3) chief/zymogenic cells (basophilic cells at base of the gland), (4) mucous neck cells, and (5) smooth muscle cells of the muscularis mucosa

Fig. 22.2.

Loss of parietal cells (*pink*) following chronic (>6 months) *H. felis* infection.

Immunofluorescent photomicrographs of gastric corpus mucosa showing parietal cells.

Parietal cells stained in pink in normal uninfected gastric mucosa (*left panel*) and chronically (>6 months) infected mucosa with *H. felis* (*right panel*)

Fig. 22.3.

Timeline of chronic gastritis to dysplasia in experimental mouse models. Schematic depiction of *Helicobacter* infection leading to chronic gastritis and ultimately gastric dysplasia. Shown is the two-phase development observable in mice. The first phase indicates chronic-active inflammation after *Helicobacter* infection. The second phase is labeled metaplasia/dysplasia and involves a change in the microenvironment. Dysplasia/cancer in situ is observed in the antrum for *Gastrin^{-/-}* and *GP130^{F/F}*. Tumors are present in the corpus for the other models. The L-635 model is also shown as a rapid (chemical) model for the induction of SPEM. Note that human subjects develop chronic gastritis over months to years and cancer (CA) over decades

Fig. 22.4. Modifying factors for mouse models of gastric carcinogenesis. Drinking water containing chemical carcinogens MNU (a) or *H. felis* inoculation (b) strongly enhances stomach carcinogenesis in combination (c). Long-term administration of a COX-2 inhibitor (nimesulide) shows strong chemopreventive action against *H. pylori*-associated gastric transformation (d). Early, middle, or late eradication of *H. felis* reduces risk of gastric carcinogenesis in mice (e, f, g). Similar increased risk is observed in INS-GAS or p27^{-/-} mice (h–k). A high-salt diet further increases the incidence of gastric cancer (l)

Table 22.1

Overview murine gastric cancer models

Model	Incidence, %	Duration or age of onset	Location	Phenotype	References
MNU	18–60	50 Weeks	Antrum	Adenocarcinoma, dysplasia	[77, 198]
MNU + <i>H. pylori</i>	80	50 Weeks	Antrum	Adenocarcinoma, dysplasia, metaplasia, atrophy	[77, 199]
<i>H. felis</i>	80	15 Months	Corpus	Adenocarcinoma, dysplasia, metaplasia, atrophy	[104, 105]
MNU + <i>H. felis</i>	100	36 Weeks	Antrum	Adenocarcinoma, dysplasia, metaplasia, atrophy	[200]
MNU + high salt	50	40 Weeks	Antrum	Adenocarcinoma	[88]
MNU + <i>H. felis</i> + high salt	100	40 Weeks	Antrum	Adenocarcinoma	[88]
DMP-777	100	7–14 Days	Corpus	Rapid loss of parietal cells, atrophy, SPEM	[191, 192]
L635	100	7 Days	Corpus	Rapid loss of parietal cells, atrophy, high proliferative SPEM	[193]
Tamoxifen	100	3 Days	Corpus	Rapid, reversible atrophy and metaplasia	[201]
INS-GAS	75	20 Months (7 months w/ <i>H. felis</i>)	Corpus	Adenocarcinoma, dysplasia, metaplasia, atrophy, accelerated by <i>H. felis</i>	[127, 128, 202]
GAS ^{-/-}	60	12 Months	Antrum	Dysplasia, metaplasia, atrophy	[136]
TEF1 ^{-/-}	30	5 Months	Antrum	Multifocal intraepithelial or intramucosal carcinomas	[145]
Gp130 ^{FF}	100	20 Weeks	Antrum	Atrophy, IM, and SPEM, dysplasia and submucosal invasion	[149]
Atp4a ^{-/-}	100	12 Months	Corpus	Progressive hyperplasia, mucocystic and incomplete IM	[153]
Potassium channel	100	3 Months	Corpus	Mucous neck cell hyperplasia	[154]
COX-2 + MNU	48	50 Weeks	Antrum	Atrophy, IM and carcinoma	[159]
COX-2 (K19-C2mE)	100	48 Weeks	Corpus	Metaplasia, hyperplasia and tumor	[161]
K-ras (K19-K-ras-V12)	100	3–20 Months	Corpus	3 Months: mucus metaplasia <20 Months: dysplasia and carcinoma	[165, 166]
K-ras (ubiquitous)	100	18 Days	Junction of forestomach and glandular stomach	Rapid loss of parietal cell, hyperplasia, IM	[34]
P27 ^{-/-} + <i>H. pylori</i>	60	60 Weeks	Corpus	IM, Intraepithelial neoplasia and Polypoid adenomas, and in situ or intramucosal carcinoma	[115, 168]
Tgfb1 ^{-/-} C33S	40	16–19 Weeks	Stomach and rectal-anal	Well differentiated invasive adenocarcinoma	[176]
TGF- β R II (pS2-dnRII) + <i>H. pylori</i>	ND	36 Weeks	Corpus	Adenocarcinoma	[177]
Smad3 ^{-/-}	100	10 Months	Corpus	Metaplasia and Invasive tumor	[178]
Smad4 ^{+/-}	100	>12 Months	Corpus and antrum	Polyposis, hyperplasia, dysplasia, in situ and invasive carcinoma	[180]

Model	Incidence, %	Duration or age of onset	Location	Phenotype	References
RUNX3 ^{-/-}	70	52 Weeks	Corpus and antrum	Adenocarcinoma, IM, SPEM, dysplasia, loss of chief cells	[203]
MT-TGF α	ND	4-6 Weeks	Corpus	Foveolar hyperplasia, loss of parietal cell and chief cell	[204, 205]
TxA23	88	12 Months	Corpus	Oxyntic atrophy, hyperplasia, SPEM, dysplasia, intraepithelial neoplasias	[185]
H/K-ATPase/hIL-1 β	>70	>12 Months	Corpus	Well-differentiated adenocarcinoma, dysplasia, metaplasia, atrophy, increased MDSCs	[189]
H/K-ATPase/hIL-1 β + <i>H/felis</i>	10	12 Months	Corpus	Invasive adenocarcinoma	[189]
Myd88 ^{-/-} + <i>H. felis</i>	100	25 Weeks	Corpus	Atrophy, IM, dysplasia	[206]
Menin ^{FL/FL} ;Villin-Cre	11	12 Months	Antrum	Antral tumors	[207]
MTH1 ^{-/-}	14	18 Months	Antrum	Adenomatous hyperplasia, adenoma, or adenocarcinoma	[208]
Atp4b ^{cre} ; Cdh1 ^{FL/FL} /p53 ^{FL/FL}	69	12 Months	Corpus	Invasive cancer; lymph node metastasis (40 %)	[209]
Atp4b/SV40	100	12 Months	Corpus	Cancer with lymphatic-vascular invasion, lymph node and hepatic metastasis	[210]

Intestinal metaplasia, *SPEM* spasmolytic polypeptide-expressing metaplasia, *MDSCs* myeloid-derived suppressor cells