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Mouse Models of Gastric Carcinogenesis

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Gastric cancer is one of the most common cancers in the world. Animal models have been used to elucidate the details of the molecular mechanisms of various cancers. However, most inbred strains of mice have resistance to gastric carcinogenesis. *Helicobacter* infection and carcinogen treatment have been used to establish mouse models that exhibit phenotypes similar to those of human gastric cancer. A large number of transgenic and knockout mouse models of gastric cancer have been developed using genetic engineering. A combination of carcinogens and gene manipulation has been applied to facilitate development of advanced gastric cancer; however, it is rare for mouse models of gastric cancer to show aggressive, metastatic phenotypes required for preclinical studies. Here, we review current mouse models of gastric carcinogenesis and provide our perspectives on future developments in this field.

Key Words: Stomach neoplasms; *Helicobacter* infections; Mouse model

Introduction

Gastric cancer is the second leading cause of death from cancer worldwide and is associated with a poor prognosis and a high incidence of drug resistance.[1,2] The molecular mechanisms that promote gastric carcinogenesis are not yet fully understood. Gastric carcinomas can be divided into intestinal and diffuse types according to histological characteristics.[3] Intestinal-type carcinomas, which are thought to be derived from gastric mucosa cells, are histologically differentiated and exhibit well-defined glandular structures with expanding growth patterns developing through sequential stages, including chronic gastritis, atrophy, intestinal metaplasia (IM), spasmolytic polypeptide-expressing metaplasia (SPEM), dysplasia, and submucosal invasion; these changes are typical of precancerous epithelium.[4] On the other hand, diffuse-type carcinomas are histologically undifferentiated and have a diffuse infiltrative growth pattern, with tumor developing through a shorter, less well-characterized sequence of events from gastric epithelial cells.[5]

Abate-Shen[6] suggested an association between development and gastric carcinogenesis. Inappropriate activation of specific developmental pathways seems to be involved in the development of IM and intestinal-type gastric carcinomas. An appropriate animal model needs to be developed in order to improve our understanding of the mechanisms involved in gastric cancer and to promote the discovery of novel therapeutic interventions. The gastric anatomy of mice is different from that of humans. In mice, the squamo-columnar junction does not universally approximate the gastro-esophageal junction as it does in normal human anatomy. Moreover, rodents rarely develop spontaneous gastric cancer, although cotton rats (*Sigmodon hispidus*) and the Z strain of the African rodent *Mastomys natalensis* exhibit enterochromaffin-like cell carcinoids and develop gastric tumors more frequently.[7-11] Thus, studies have concentrated on the development of chemical, infectious, or genetic tools to induce gastric cancer in animals.

Here, we review chemically induced, *Helicobacter* infection-induced, and genetic models of gastric carcinogenesis and compare their pathological patterns, limitations, and applications to improve

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our understanding of gastric carcinogenesis.

Chemical Carcinogen-Induced Models of Gastric Cancer

Establishment of adequate mouse models of gastric cancer is necessary for exploring the mechanisms of gastric tumorigenesis. To this end, researchers have tested the utility of various chemical carcinogens to induce gastric cancer in mice. N-nitroso compounds (NOCs), which are generated in the stomach by anaerobic bacteria following ingestion of nitrates and nitrites, have been studied as cancer inducers. N-methyl-N-nitro-N-nitrosoguanidine (MNNG) has been used to induce stomach tumors in rats. For example, Schoental et al.[12] treated rats with MNNG using a stomach tube to induce formation of squamous cell carcinoma in the rat forestomach. Additionally, Sugimura and Fujimura[13] generated antropyloric adenocarcinomas with high frequency by administering MNNG orally to rats in drinking water. MNNG was found to be a very potent gastric carcinogen in Mongolian gerbils.[14,15] Treatment with 400 ppm MNNG in drinking water for 50 weeks resulted in the development of gastric adenocarcinomas in 63.6% of gerbils.[15] However, because of the lack of genetic models using these animals, rats and gerbils have limited applications as model systems, and therefore, the effects of oral administration of nitrosamines has been investigated in inbred strains of mice. However, mice have been shown to have resistance to MNNG-induced gastric carcinogenesis. Indeed, when Balb/c mice were infected with *H. heilmannii* and administered MNNG in drinking water for 38 weeks, squamous cell carcinomas were found in the mouth and forestomach, but adenocarcinoma was not observed in the glandular stomach.[16] The ability of N-methyl-N-nitrosourea (MNU) to induce gastric carcinogenesis in mouse models has also been explored. Biweekly intragastric intubation with 0.5 mg MNU resulted in death of most Balb/c mice due to squamous cell carcinoma in the forestomach. Operative removal of the forestomach prior to MNU treatment helped to promote the development of well-differentiated adenocarcinoma in the glandular stomach, with a 100% incidence rate after 40 weeks of treatment.[17]

Therefore, while glandular stomach is sensitive to the carcinogenic effects of MNU, this phenotype was not the result of the greater sensitivity of the forestomach to MNU under the investigated treatment conditions (dose and route of administration). Tatematsu et al.[18] demonstrated that low-dose MNU (30-120 ppm) given in drinking water was effective without the induction of tumors in the forestomach. The efficiency of tumor induction by MNU depends on its concentration rather than the total intake,[19] and MNU in the drinking water at 240 ppm for 5 weeks (every other week) has been shown to induce gastric cancer in six strains of mice.[20] Consequently, this protocol is used currently as a standard method for induction of gastric carcinogenesis in mice. MNU-induced tumors in mice are located mainly in the gastric antrum and are uniformly well-differentiated adenocarcinomas.[21]

The MNU mouse model of gastric cancer has been used for studying various signaling pathways in gastric carcinogenesis, including the roles of p53,[22] nuclear factor-kappaB (NF-κB),[23] the mitogen-activated protein kinase (MAPK) pathway,[24,25] Cox-2,[26,27] β-catenin,[26] E-cadherin,[28] and Kruppel-like factor 4 (KLF4).[29] MNU is known to modify amino acids in histone proteins, especially histone H3 lysine residues, leading to chromatin remodeling.[30] MNU treatment in mice induces altered expression of Trefoil factor 1 (TFF1), a gastric-specific tumor suppressor gene, through epigenetic modifications similar to those observed in human gastric cancer.[31] This suggests that epigenetic effects are likely to constitute a key mechanism of NOC-induced carcinogenesis. While the MNU model does not progress through a classical atrophy-metaplasia-dysplasia sequence, this latter *H. pylori*-dependent pathway results in achlorhydria with subsequent bacterial overgrowth. NOCs may be generated from nitrates and nitrites in this setting, and thus the argument can be made that the generation of NOCs may play a role in *Helicobacter*-associated carcinogenesis. Recent studies used a combination of MNU and *H. felis* infection achieved a very rapid induction of antral gastric cancer[31] and induced a high frequency of gastric cancer in *H. pylori*-infected Mongolian gerbils compared to gerbils receiving MNU only.[32,33] Thus, the combination of *Helicobacter* infection and MNU treatment may, in some ways, mimic the proposed pathogenesis of human antral carcinogenesis.

Helicobacter Infection Models

H. pylori are thought to be the main cause of chronic gastritis. The first animal models of *Helicobacter* infection-induced carcinogenesis in the gastric mucosa was the ferret model.[34-36] Ferrets with *H. mustalae* infection exposed to 100 mg/kg MNNG developed gastric cancer, while *H. mustalae*-infected ferrets did not,[34] although MNNG-induced gastric cancer from *H. mustalae*-free SPF ferrets was not proven because these animals were not available. *H. mustelae*-infected ferrets have been shown to develop gastritis, dysplasia, and gastric adenocarcinoma with aging.[37] Several *H.*

pylori strains, including G1.1,[38] TN2,[39] and B128,[40] have the ability to colonize Mongolian gerbils and induce gastric adenocarcinoma.

The mouse C57BL/6 strain is remarkably resistant to colonization with various *H. pylori* strains.[41,42] Thus, alternative mouse models of gastric *Helicobacter* infection have been established using *H. felis*, a close relative of *H. pylori*. This strain was isolated from the cat stomach and was shown to readily colonize the mouse stomach.[43] Several papers have reported that *H. felis* can induce severe gastritis and atrophy in mice.[43-45] *H. felis*-infected mice show gastric SPEM, dysplasia, and invasive cancer with long observation periods.[46,47] Extensive dysplastic lesions are observed in the gastric corpus at the squamocolumnar junction (SCJ) along the lesser curvature, and large polypoidantral tumors develop.[48,49]

In the *H. felis* infection model in mice, eradication studies have revealed that inflammation, metaplasia, and dysplasia are reversible when early eradication therapy is applied and that progression to dysplasia can be restricted with eradication therapy at a later time point.[50,51] This phenomenon is linked to an epidemiological decrease in the incidence of gastric adenocarcinoma in humans.[52,53] Gerbils and INS-GAS mice treated with antibiotics to eradicate *H. pylori* exhibit arrested progression of gastric lesions.[43,54]

The Sydney strain of *H. pylori* (SS1) has been adopted as a useful strain in murine model systems.[55] High levels of colonization have been achieved in inbred C57BL/6 mice, while colonization levels in Balb/c, DBA/2, and C3H/He strains were lower. Active gastritis and severe atrophy have been observed with detectable levels of bacteria after 8 months of infection. In a 2-year follow-up, infection with SS1 or other strains (i.e., 119p and G50) did not progress to gastric cancer, although some mice developed gastric lymphoma.[56] Infection with *H. pylori* SS1 did result in the development of carcinomas *in situ* in C57BL/129 mice after 15 months of infection.[49] Moreover, *H. pylori* infection can also cause gastric cancer in genetically modified mice, as shown in a study of INS-GAS mice.[57]

Vacuolating cytotoxin (Vac), encoded by the *vacA* gene, facilitates the pathogenesis of stomach dysplasia. Infection with an adapted *H. pylori* strain lacking VacA reduces the incidence of gastric carcinoma in a Mongolian gerbil model. While CagA deprivation protects against cancer development by enhancing inflammation, ablation of VacA does not affect inflammation.[58] The genetic loci *cag* and *vacA* are thought to be related to crucial virulence factors in gastritis. In *H. pylori*, a 40-kb genomic fragment known as the *cag* pathogenicity island (*cag*-PAI) encodes a type IV secretion (TFSS) apparatus for the bacterial protein CagA, particularly in high-risk intestinal-type adenocarcinoma.[49] After invasion of CagA into host cells, CagA phosphorylation by host cell kinases induces activation of SHP-2 tyrosine phosphatase, NF-κB signaling, and MAPK signaling.[59-61]

CagA and/or the *cag*-PAI may play a key role in gastric carcinogenesis. Nevertheless, in mice, *cag*-negative strains, such as *H. felis*, display carcinogenic abilities similar to *cag*-positive *H. pylori* strains. Moreover, while inactivation of the *cagE* gene encoding TFSS delays the progression to carcinoma in an *H. pylori* strain, neoplasia ultimately develops in all INS-GAS mice infected with *H. pylori* mutant.[57] Thus, these results suggest that gastric preneoplasia occurs in mice through host-related factors, such as inflammation or other genetic factors. For example, the C57BL/6 strain is more sensitive to *H. felis*-induced gastric atrophy than the Balb/c strain because C57BL/6 mice show much higher levels of pro-inflammatory cytokines, such as interferon (IFN)-γ due to increased T-helper-1 (Th1)-dependent immune responses in C57BL/6 mice compared to the Th2-dominant immune response in Balb/c mice.[47]

Mouse models of *Helicobacter* infection have been studied to determine the effects of other cofactors in gastric carcinogenesis, such as gender, diet, and co-infection. Gender may be an important factor, as gastric cancer is much more prevalent in men than in women. However, C57BL/6 mice infected with *H. felis* did not exhibit significant gender-related differences in the incidence of gastric carcinoma.[46,57] These data suggested that these models have distinct mechanisms of carcinogenesis. High-salt diets and diets rich in nitrates and nitrites have been associated with an increased risk of gastric cancer. Treatment with MNU prior to *H. pylori* infection induces more severe preneoplastic changes and increased incidence of gastric cancer.[62,63] C57BL/6 mice with SS1 infections and consuming a high-salt diet develop more pronounced gastric atrophy and hyperplasia.[64] Concurrent parasitic infection may alter the effects of *Helicobacter* infection. Indeed, co-infection of C57BL/6 mice with *H. felis* and the helminth *Heligmosomoides polygyrus* reduces the severity of gastric atrophy and preneoplastic lesions observed following infection with *H. felis* alone.[65] This response has been shown to be associated with a switch from the usual Th1 immune response to a polarized Th2 response.

Murine models of chronic *Helicobacter* infection are definitive and reproducible models that can be used to investigate the molecular mechanism of gastric carcinogenesis. However, there are limitations to *Helicobacter* mouse models, including the limited number of *H. pylori* strains available, the slow time course for the progression of tumors, the low incidence rate of advanced gastric

Table 1. Mouse models of gastric cancer by chemical treatment or/and infections of helicobacter and viruses

Model	Incidence (%)	Duration	Location	Phenotype				State	References
				Atrophy	Metaplasia	Dysplasia	Adenocarcinoma		
MNU	<70	12 months	Antrum	+	-	+	+		17~20
Helicobacter felis	80	18 months	SCJ/transition	+	+	+	+		46
MNU+*H. pylori*	80	12 months	Antrum	+	+	+	+		66, 67
MNU+*H. felis*	100	36 weeks	Antrum	+	+	+	+		31
$CDH1^{+/-}$+MNU	45.8	40 weeks	Antrum	-	-	-	+	Signet-ring cell Ca	28
$RUNX3^{-/-}$+MNU	71	52 weeks	Corpus/antrum	-	+	+	+		68
CEA/SV40	100	50 days	Antrum	-	-	+	+	Invasion to submucosa and duodenum	69
MMTV/Ad12	82 (male)	3~4 months	SCJ	-	-	-	+	Adenosquamous Ca	70
HPV-16	100	246~352 days	Antrum					Carcinoid, metastasis to LN and liver	71

MNU = N-methyl-N-nitro-N-nitrosoguanidine; CEA = carcinoembryonic antigen; HPV = human papillomavirus; SCJ = squamocolumnar junction; Ca = cancer; LN = lymph node.

cancer, and the anatomical differences between humans and mice (Table 1).[17-20,28,31,46,66-71]

Genetically Engineered Mouse Models

Most genetic models in mice have been established on the C57BL/6 or mixed C57BL/6/129SvJ background. In the models discussed below, there are differences in tumor progression and phenotypes that are dependent on genetic background and gender. As expected for inbred mouse populations, genetic variability in mouse strains produces different susceptibilities to the development of gastric cancers. Therefore, when using particular genetic models of gastric cancer, the use of wild-type control mice with genetic backgrounds identical to the mutant mice is crucial.

The epithelium of the mouse stomach comprises the proximal fundus and the distal antrum, and these tissues have distinct functions. The fundus produces acid for digestive enzymes, while the antrum has an endocrine and mucus-secretory role. Gastric tumors progress independently in separate regions under the control of different genetic triggers. Most of the models summarized in Table 2[57,70,72-141] produce various stages of tumors in either the fundus or the antrum, while some model produce tumors in both tissues.

1. The Trefoil factor $1^{-/-}$ mutant

TFF1 (also called pS2) is a member of the trefoil domain peptide family, which also includes TFF2 and TFF3. These peptides are highly expressed in the gut, and TFF1 and TFF2 are synthesized and secreted by surface/pit mucus and mucus neck/astral gland cells, respectively. $TFF1^{-/-}$ mice have gastric astral/pylorus-specific hyperplasia by 1 week of age, and one-third of mice develop dysplasia and multifocal intra-epithelial carcinomas by 20 weeks.[72]

$TFF1^{-/-}$ tumors have two phenotypes. Tumors with the first phenotype are located in the distal stomach, supporting a role for TFF1 as a stomach-specific tumor-suppressor gene.[73] Secondary phenotypes show increased lengths of small intestinal villi with associated lymphocytic infiltrate[72] and the loss of neutral glycoprotein from surface and pit cells of the stomach. This phenotype suggests a role for TFF1 in regulating gastric differentiation pathways.

The genes exhibiting the highest levels of overexpression in the stomachs of $TFF1^{-/-}$ mice are claudin 7 (encoding a tight junction protein), early growth response 1 (encoding a nuclear transcription factor), and epithelial membrane protein 1 (encoding a junctional membrane protein).[74] Upregulation of claudin 7 has also been observed in preneoplastic lesions in human stomachs and in gastric adenocarcinoma, thus underscoring the utility of the $TFF1^{-/-}$ mouse model in the discovery of genes related to gastric cancer progression.

Table 2. Pathologic development in the stomach examplified by various genetic models of gastric hyperplasia or tumoriogenesis in mice

Model	Age of onset	Fundus	Antrum	Carcinoma *in situ*	Invasion	Metastasis	References
TFF1$^{-/-}$	1 week	++	++	Y	Y	N	72~74
gp130^{757F757F}	3~4 weeks	+++	+++	Y	Y	N	75~77
Cdx2 transgenic	12 weeks	++	-	Y	Y	N	78~80
INS-GAS	24 months	+++	-	N	N	N	57, 81, 82
ACT-GAS	20 months	+++	-	Y	N	N	83, 84
Gastrin$^{-/-}$	12 months	ND	+++	Y	N	N	85~90
H/K-ATPase α subunit$^{-/-}$	3 months	+++	-	Y	N		91
H/K-ATPase β subunit$^{-/-}$	20 months	+++	-	N	N		91
NHE2$^{-/-}$	3 months	++	-	N	N	N	92
NHE4$^{-/-}$	3 months	++	-	N	N	N	93
Kv1qt1$^{-/-}$	ND	+++	-	N	N	N	94
KCNE2$^{-/-}$	ND	+++	-	N	N	N	
Kcnq1$^{-/-}$	ND	+++	-	N	N	N	95
Histamine H2 receptor$^{-/-}$	17 months	++	-	N	Herniation	N	96, 97
IQGAP1$^{-/-}$	24 months	+	-	N	N	N	98
TGF β1$^{-/-}$	0.5 month	ND or +++	-	N	N	N	99, 100
SMAD4$^{+/-}$	18 months	-	ND or +++	Y	Y	N	101
ELF$^{+/-}$SMAD4$^{+/-}$	ND	-	ND or +++	Y	Y	N	102
Runx3$^{-/-}$	8 months	+++	ND	Y	Y	N	103~105
β-Catenin transgenic (D)	ND	ND or +++	-	N	N	N	106~110
MTH1$^{-/-}$	ND	-	ND or +	Y	ND	ND	111, 112
K19-C2mE transgenic	5 weeks	++	-	N	N	N	113~115
TSP-1$^{-/-}$	ND	ND	ND or +	ND	ND	ND	99, 116, 117
TGF α transgenic	4~6 week	++	-	N	N	N	118~124
AhR transgenic	12 months	+++	-	Y	Y	N	92, 125, 126
Klf4 conditional$^{-/-}$	6 months	+++	+++	N	N	N	127, 128
p27Kip1$^{-/-}$	12 months	ND or +++	-	N	N	N	129
MHC Class II$^{-/-}$	6 months	++	-	N	N	N	130
CA IX$^{-/-}$	ND	ND or ++	-	N	N	N	131, 132
CEA SV40 transgenic	5 months	-	ND or +++	Y	Y	N	70, 133
H^+/K^+-ATPase β subunit SV40transgenic	12 months	ND or +++	-	Y	Y	Y	134~136
Fkh6$^{-/-}$	0.1 month	ND or ++	-	ND	ND	ND	137
Shh$^{-/-}$	18.5 day embryo	ND or ++	ND or ++	ND	ND	ND	138
Occludin$^{-/-}$	10 months	ND or ++	-	ND	ND	ND	139
CCR7$^{-/-}$	12 months	ND or +++	-	N	Y	N	140
NF-κB2$^{-/-}$	12 months	-	+++	ND	ND	ND	141

TFF1 = Trefoil factor 1; NHE = Na^+/H^+ exchanger; TGF = transforming growth factor; TSP = thrombospondin; AhR = aryl hydrocarbon receptor; Klf4 = Kruppel-like factor 4; MHC = major histocompatibility complex; CA = carbonic anhydrase; CEA = carcinoembryonic antigen; Fkh6 = forkhead homolog 6; Shh = Sonic hedgehog; NF-κB2 = nuclear factor-kappa B2; ND = not detectable; Y = yes; N = no.

2. The gp130^{757F757F} knock-in mutant

gp130^{757F757F} mice were generated by knock-in mutation of the SHP2/SOCS3 binding site on the interleukin (IL)-6 family coreceptor gp130 in order to genetically dissect the independent contribution of the two proteins downstream of the signal transducing receptor.[75] The tyrosine (Y) residue at position 757 in the intracellular domain of gp130 was changed to a phenylalanine residue in both alleles (757F757F), thereby preventing SHP2 (and SOCS3) docking after ligand binding with the receptor complex and blocking signal transduction through the Ras/extracellular signal-regulated kinase (ERK)/AP-1 signaling pathway. Inhibition of this pathway prevents activation of target genes by AP-1 and promotes signaling via an alternate pathway including IL-6 cytokines, which involves the transcription factor STAT3. Thus, loss of feedback inhibition of STAT3 activation by SHP-2/Ras/ERK and SOCS3 results in constitutive oncogenic signaling by STAT3 dimers. The resulting phenotype is characterized by splenomegaly and rapid gastric tumorigenesis, with downregulation of genes regulated by IL-6 via the SHP-2/Ras/ERK/AP-1 pathway and upregulation of genes mediated by STAT3, including growth factors like Reg1[76] and anti-apoptotic, pro-angiogenic, and cytostatic genes.[77]

A principle feature of this model is the phenotypic changes in the intestine characteristic of human gastric adenocarcinoma, including gastritis, atrophy, intestinal-type mucus metaplasia and SPEM, dysplasia, and submucosal invasion, but without metastasis. This tumorigenesis is independent of *H. pylori* infection, hypergastrinemia (mice are hypogastrinemic), and constitutive activation of epidermal growth factor receptor,[76] as required for many other stomach cancer models, and highlights the importance of IL-6 signaling in the maintenance of gastric homeostasis. The timing and site of tumor initiation and dysplastic changes are consistent in all mice; development is rapid, such that initiation of antral tumors with transmural gastritis is observed by 4 weeks of age, and tumor growth then progresses rapidly along the lesser curvature of the stomach to encompass the entire secretory mucosa by 20 weeks of age.[76]

3. Cdx1 and Cdx2 transgenic models

Cdx1 and Cdx2 are adult intestine/colon-specific transcription factors that play roles as caudal-related homeobox genes during development. These proteins are involved in IM, the intestinalization of the gastric mucosa associated with progression to intestinal-type gastric adenocarcinoma in both mice and humans. When housed under specific pathogen-free (SPF) conditions, Cdx2 transgenic mice in the C57BL/6 background exhibit incomplete IM throughout gastric fundic glands at 12 weeks of age,[78] coincident with hypergastrinemia, achlorhydria, and SPEM.[79,80] The stomachs from Cdx1 transgenic mice show rapidly expanding IM, similar to that observed in Cdx2 transgenic mice, but with a variety of differentiated cell types, including Paneth cells and hormone-expressing endocrine cells.[79,80]

4. Gastrin mutants

Gastrin, which is produced by G cells in the antral mucosa, is a crucial regulatory hormone in the gastric mucosa and can regulate cell division, invasion, angiogenesis, and anti-apoptotic activity at the transcriptional level.[142,143] It functions to regulate acid secretion in response to feeding and in maintaining developmental epithelial cell homeostasis in the fundic and antral mucosa. A failure to strictly regulate gastrin expression will induce perturbations in gastric epithelial cell dynamics and potentially promote gastric cancer. Such dysregulation of gastrin has been used in various mouse models of stomach cancer.

1) Insulin-gastrin transgenic mice

Expression of the human gastrin transgene is induced by a mouse insulin promoter, and processed forms of gastrin are found in the pancreas, stomach, and colon.[144,145] In 1-year-old INS-GAS mice, marked thickening of the fundic mucosa and multifocal hyperplasia are observed in the stomach in the context of gastrin overexpression,[145] and these mice spontaneously develop atrophy and cancer by 2 years of age.[81] INS-GAS mice can be used in combination with other agents as a model of gastric cancer development due to the lower threshold for carcinogenesis. At 7 months after infection with *H. pylori* or *H. felis*, male INS-GAS mice develop atrophy, IM, dysplasia, and finally, gastric adenocarcinoma.[57,81] Cancers develop *in situ* or intramucosal carcinoma[57] by reactivation of sonic hedgehog expression.[82] Inhibition of the gastrin/CCK2 and histamine H2 receptors limits the development of gastric cancer in these mice.[146]

2) Actin-gastrin transgenic mice

The actin promoter has been used to drive gastrin expression in the Act-Gas transgenic model. Mutation of the gastrin gene allows the expression of processed forms of gastrin by nonendocrine cells.[83] By 16 weeks of age, mice develop mucosal hypertrophy, consisting mainly of foveolar hyperplasia, accompanied by parietal cell atrophy.[84] Similar to the TFF1$^{-/-}$ model, treatment with a selec-

tive COX-2 inhibitor reduces cell proliferation and foveolar thickness, suggesting that COX-2 and prostaglandin E2 (PGE2) might function downstream of gastrin.[84]

3) Gastrin-konckout mice

Gastrin-deficient mice are hypochlorhydric due to the absence of the gastrin hydrochloric acid secretory pathway.[85,86] The absence of gastric acid provides permissive conditions for bacterial overgrowth in hypochlorhydric mice.[87] This overgrowth with inflammation was recovered by treatment with antibiotics. By 12 months of age, hypochlorhydric mice develop chronic gastritis, atrophy, metaplasia, dysplasia, and intramucosal carcinoma in the antral mucosa,[88] dependent on mucosal inflammation. The metaplasia that develops in this model is not a true IM (marked by the presence of goblet cells), but is instead a naturalization caused by inflammation-dependent expansion of a mucous cell lineage often seen in mouse gastric metaplasia (SPEM).[89,90] Moreover, in this model, the development of carcinomas is independent of gastrin, but is related to increases in the amount of activated STAT3 and loss of RUNX3 expression.[88]

5. Parietal cell mutants

Parietal cells of the fundic stomach secrete hydrochloric acid to sterilize gastric contents and promote the activation of stomach enzymes for protein digestion. Physical loss of parietal cells or their acid synthetic function promotes bacterial colonization of the gastric lumen, and this, along with the constitutive inflammatory response of the host, likely predisposes the cells to gastric pathology, including cancer. Therefore, it follows that a variety of mutations affecting the acid secretion function of parietal cells will contribute to improving our understanding of the mechanisms predisposing the gastric tissue to metaplasia and cancer in mouse models.

1) H^+/K^+-ATPase-knockout mice

Gastric acid plays a role in minimizing infection and the subsequent inflammatory response in the stomach. The H^+/K^+-ATPase expressed by fundic parietal cells is responsible for acidification of gastric contents. The mice deficient for the H^+/K^+-ATPase α subunit exhibit progression of fundic hypertrophy to hyaline transformation, mucocystic and ciliated metaplasia, and chronic gastritis at 20 months of age, particularly in female mice.[91]

2) Na^+/H^+ exchanger-knockout mice

Na^+/H^+ exchangers (NHEs) are proteins in the basolateral membrane of gastric epithelial cells, particularly parietal cells, and are known to be involved in mediating acid secretion and in maintaining epithelial cell viability. Knockout of NHE2[92] or NHE4[93] induces fundic atrophy, parietal cell loss, achlorhydria, hypergastrinemia, and glandular hyperplasia.

3) Potassium channel-knockout mice

Due to the high activity of the H^+/K^+-ATPase in gastric parietal cells, potassium channels have important roles in the maintenance of ion homeostasis. The fundic mucosas of these mice morphologically resemble those of the H^+/K^+-ATPase knockout mice because these mice develop achlorhydria, hypergastrinemia, and hyperplasia, although the mechanism has not been directly tested.[94] Targeted mutation of Kcnq1 in mice leads to an expanded fundic proliferation zone with severe hyperplasia, achlorhydria, and hypergastrinemia. Several mouse lines with a defective *Kcnq1* locus (14Gso) have been generated using random mutagenesis induced by X-ray irradiation of spermatogonia.[95] The products of these mutations are similar to those in H^+/K^+ ATPase α- or β-null mutant mice.

4) Histamine receptor-knockout mice

The histamine histamine receptor (H2R) is expressed on acid-secreting parietal cells of the gastric mucosa and functions to stimulate gastric acid secretion. H2R-knockout mice are viable, fertile, and have normal basal gastric acid secretion, maintained by muscarinic receptors.[96] Fundic hyperplasia occurs as a direct product of increased numbers of parietal and enterochromaffin-like cell until 17 months of age, after which the pathology worsens to include mucocystic metaplasia, with a proportion of the mice developing herniation of the epithelium penetrating the muscularis mucosa and producing a phenotype closely mimicking Ménétrier's disease in humans.[97]

5) IQGAP1-knockout mice

Parietal cells in the gastric mucosa show subcellular reorganization upon activation, leading to secretion of gastric acid. Through its F-actin-binding function, IQGAP1 is involved in this subcellular reorganization event, which is dependent on the precise formation of F-actin structures by the Rho family of Ras-related GTPases.[98]

6. Transforming growth factor beta, transforming growth factor beta receptor, and signaling mutants

There are three isoforms of transforming growth factor beta (TGF β): TGF β1, -2, and -3, and all three isoforms can bind to

TGF β receptor II, although TGF β1 is most frequently altered in tumorigenesis. Upon ligand binding to TGF β receptor II, heterodimerization and activation of TGF β receptor I occur. Activation of the SMAD complexes via activation of TGF β receptor I can activate TGF β-responsive genes in the nucleus. Many of the downstream components of the TGF β signaling pathway are thought to act as tumor-suppressor proteins, including TGF β receptor I, TGF β receptor II, SMAD2, and SMAD4. Changes in TGF β signaling can stimulate tumor growth, invasion, and metastasis.[147,148]

1) Transforming growth factor beta1-knockout mice

TGF β1 suppresses cell growth and tumor development by reducing the expression or activity of TGF β receptors and by altering downstream signaling pathways. This resistance to TGF β1 signaling may represent a significant step in the process of carcinogenesis.[99,100] TGF β1 also functions to control the production and degradation of extracellular matrix proteins, as well as cellular differentiation. About 20 days after birth, mice homozygous for the TGF β1-null mutation develop a severe wasting syndrome resulting from multifocal, mixed inflammatory cell infiltration in a variety of tissues, including the stomach. In particular, ulceration, hyperplasia, and nodule formation have been observed in the mucosa of the stomach.[99,100] Because this mutation results in early lethality, the effects of TGF β1 knockdown on gastric carcinogenesis have not yet been assessed.

2) Transforming growth factor beta type II receptor dominant-negative transgenic mice

A dominant-negative transgene of the TGF β type II receptor was produced under control of the TFF1 promoter to direct stomach-specific expression.[149] These transgenic mice did not respond to TGF β ligands in the stomach, but also did not exhibit gastric abnormalities. However, infection with *H. pylori* induced the acquisition of a more severe phenotype in the fundus and antrum including greater hyperplasia, inflammation, and dysplasia, as well as intramucosal carcinoma.

3) SMAD4 hemizygous knockout mice

SMAD4 belongs to a family of proteins involved in the TGF β signaling cascade. Homozygous SMAD4-knockout mice exhibit embryonic lethality, whereas heterozygotes appear normal up to 1 year of age. SMAD4 mutations have been observed in 50% of patients with familial juvenile polyposis. Moreover, mice in which SMAD4 is conditionally ablated in T cells or epithelia, including the intestinal epithelium, begin to display signs of illness by 3 months of age and exhibit shortened lifespans as a result of pathological changes initiated in the small and large intestine, which eventually develop into invasive and metastatic epithelial cancer.[101] However, specific deletion of SMAD4 in epithelial cells is not sufficient to induce these cancers in the gut. SMAD4-deficient T cells exhibit increased IL-6 receptor α expression in the gastric epithelium. In some epithelial cancers, the control of TGF β signaling in the inflammatory cells is crucial for regulating tumor development.

4) ELF and SMAD4 double hemizygous knockouts

Embryonic liver fodrin (ELF), a novel β-spectrin, is a membrane-associated cytoskeletal component in cellular differentiation. ELF plays a role as an adaptor for SMAD proteins. Loss of ELF disrupts nuclear translocation of SMAD3 and SMAD4. Mice with homozygous ELF4-deficiency die during embryonic development, and mice with heterozygous deletions for both SMAD4 and ELF show a higher incidence of severe gastric lesions than those with mutations in SMAD4 alone.[102]

5) Runx3-knockout mice

Runx proteins act as regulators of gene expression in developmental pathways. Runx3-knockout mice on a C57BL/6 background show reduced viability and do not survive beyond 10 days of age.[103] Runx3-knockout mice exhibit thickened gastric mucosa with increased proliferation and decreased apoptosis in the fundic and antral mucosa. In contrast, Runx3-knockout mice in an ICRxMF1 background survive for several months and do not develop gastric hypertrophy or carcinogenesis,[104] while mice on either a C57BL/6 or BALB/c background do not survive after birth.[105] At 8 months of age, Runx3 mice on an ICRxMF1 background develop marked hyperplasia, glandular atrophy, hyaline degeneration, hyperproliferation, and gastritis in the fundic mucosa.

7. *APC*$^{min/+}$ and Wnt signaling pathway mutants

Wnt signaling plays a major role in determining cell fate and morphogenesis during embryogenesis and maintaining homeostatic control of rapidly repaired tissues in adults. Transgenic overexpression of activated β-catenin, which is stabilized and translocated to the nucleus upon activation of Wnt ligands, is involved in tumorigenesis.[106,107] These models have shown that β-catenin is overexpressed in a large number of cells. In order to mimic the process of human carcinogenesis, in which tumors arise from a mutation in single cell, a transgenic model with overexpression of the acti-

vated form of β-catenin occurring in sporadic cells was established. However, in this model, few mice developed discrete multifocal dysplastic lesions in the gastric mucosa.

Loss of the *APC* tumor-suppressor gene is involved in the initiation of colorectal cancer in humans and mice via nuclear accumulation and activation of β-catenin/Tcf target genes. *APC* mutant mice (codon 1638) develop gastric tumors with low frequency during the aging process.[109,110] Gastric pathology associated with the $APC^{min/+}$/C57BL/6 background may be infrequently observed because of the shortened lifespan of these mice. Recently, $APC^{min/+}$/C57BL/6 mice were shown to develop gastric adenomas of the antrum by 20 weeks of age when maintained under SPF conditions. Microbial infection seems to play a role in the development of some types of pathologies in these mice. Moreover, antral adenomas show hyperplasia and nuclear atypia acquired from the strong expression of Myc, cyclin D1, and β-catenin.[110]

8. MTH1-knockout mice

MTH1 acts as a tumor suppressor by inhibiting the incorporation of 8-oxodGTPase residues into DNA, thereby promoting nucleotide oxidation and transversion during DNA synthesis and inducing carcinogenesis in susceptible tissues. Assessment of organ pathology using 18-month-old MTH1-knockout mice and littermate controls, revealed the presence of lung and liver tumors, stomach adenomatous polyps, and adenocarcinomas in 14% of $MTH1^{-/-}$ mice, but only 4% of wild-type controls.[111] Additionally, tumors were more prevalent in males than in females. Cai et al.[112] demonstrated that MTH2 has a similar activity profile as MTH1, suggesting the possibility that this gene family may contribute to the inhibition of tumorigenesis by mediating local oxidative damage.

9. K19-C2mE transgenic mice and genetic variants

K19-C2mE mice exhibit overexpression of COX-2 and microsomal PGE synthase 1 genes under the control of the cytokeratin 19 gene promoter.[113] In the context of PGE2 upregulation, this mouse model develops gastric hyperplasia in a macrophage-dependent manner. Although hyperplasia appears by 12 weeks of age, maximal tumorigenesis is not found until about 50 weeks of age or more. Loss of the pro-inflammatory cytokine IL-1β or the adaptive immune response ($Rag2^{-/-}$) in the K19-C2mE transgene does not affect tumorigenesis. However, depletion of tumor necrosis factor causes severe delays in inflammation, hyperplasia, and development of TFF2-associated mucous cell SPEM,[114] suggesting that pro-inflammatory cytokines are important in gastric metaplasia and oncogenesis. Compared with K19-C2mE mice alone, K19-C2mE×K19-Wnt1 transgenic mice exhibit accelerated formation of gastric tumors, concurrent with severe dysplasia, hyperplasia, inflammation, and submucosal invasion by 20 weeks of age.[115]

10. Thrombospondin 1-knockout mice

Thrombospondin (TSP) proteins are extracellular calcium-binding proteins that regulate cellular attachment, migration, differentiation, and proliferation.[116] Due to the TSP-1-dependent activation of TGF β1, TGF $\beta1^{-/-}$ and $TSP\text{-}1^{-/-}$ mice have similar phenotypes in various tissues, including IM and increased mitosis and hyperplasia of the gastric epithelium after postnatal days 17 to 21.[99] Compound TSP-1×aVb6 integrin-null mice develop stomach hyperplasia (21%), gastric papillomas, and squamous cell carcinomas.[117]

11. Transforming growth factor alpha transgenic mice

TGF α transgenic mice with transgene overexpression in fundic stomach mucus cells (MT-TGF α) exhibit a phenotype in which giant fundic mucosal folds form as a result of massive cellular hyperplasia and glandular cystic dilation.[118,119] This phenotype is reminiscent of the rare human condition Ménétrier's disease, which exhibits elevated TGF α expression.[118] In adult TGF α transgenic mice, the surface mucous cell population increases at the expense of both parietal and chief (zymogenic) cells, with the isthmus-located stem cell zone nearer to the base of the glands,[120-122] and the mucosa become much more fibrotic.[123] Thus, the fundic mucosa phenocopies the antralization observed in precancerous metaplasia accompanying antral expression of Pdx1.[124] However, invasive gastric tumors with IM are not observed.

12. Dioxin/aryl hydrocarbon receptor transgenic mice

Activation of aryl hydrocarbon receptor (AhR) by environmental stimuli, such as dioxins and biphenyls, results in transcriptional activation of genes encoding xenobiotic metabolizing enzymes.[125] Endogenous expression of AhR is observed predominantly in the lungs, although mice transgenic for AhR also show expression of the transgene in the thymus, spleen, liver, skin, and stomach. In AhR transgenic mice, cysts are grossly apparent in the fundic gastric mucosa at 3 months of age and develop into dysplastic structures that penetrated the muscularis mucosa into the submucosa and subserosa by 12 months of age, at which point trans-

gene expression results in lethality. The penetrating mucosal cells exhibit a well-differentiated, benign appearance and seem to be invasive rather than a result of herniation.[125] Similar lesions have also been observed in laboratory animals following treatment with AhR ligands.[125] As cause of lesions, decreased expression of osteopontin has been detected in these fundic lesions in differential gene expression analyses. Because osteopontin is involved in tissue remodeling, invasive lesions may be caused by its altered expression in this mouse model.[126] In contrast, H^+/K^+-ATPase α subunit-knockout mice have elevated levels of osteopontin compared with wild-type mice and exhibit reduced invasion of lesions into the gastric muscularis.[93]

13. Kruppel-like factor 4-knockout mice

Kruppel-like factor 4 (Klf4) is an epithelial-specific, zinc finger transcription factor that plays important roles in the regulation of cellular proliferation and differentiation. Klf4-knockout mice exhibit early lethality,[127] and mice with Klf4 deletions specifically in the glandular gastric mucosa show epithelial hypertrophy, hyperproliferation, mucous metaplasia, atrophy, and polypoid lesions in the fundus and antrum, but no inflammation, hypergastrinemia, dysplasia, or malignancies.[128]

14. p27Kip1-knockout mice

The p27Kip1 protein inhibits cyclin-dependent kinases to block cell cycle progression, playing vital roles in cell migration, apoptosis, differentiation, and inflammatory responses. p27Kip1-knockout mice develop mild epithelial hyperplasia at approximately 1 year of age, mucous cell metaplasia, and low-grade dysplasia. After *H. pylori* infection, these preneoplastic conditions facilitate the development of high-grade dysplasia or intramucosal carcinoma in p27Kip1 mice due to increased cell turnover and an exaggerated inflammatory response.[129]

15. Major histocompatibility complex class II knockout

Major histocompatibility complex (MHC) class II molecules have major roles in regulating the $CD4^+$ arm of the adaptive immune response. MHC class II-deficient mice are unable to produce a functional $CD4^+$-mediated immune response because MHC class II protein is required for the maturation of $CD4^+$ T cells in the thymus. At 6 months of age, these mice have fundic stomachs with gastrin-dependent mild hyperplasia, including infiltration of granulocytes and macrophages, but no epithelial cell atrophy.[130] Thus, these data have shown that persistent activation of the innate immune system can produce hyperplastic changes in the fundic mucosa.

16. Carbonic anhydrase IX-knockout mice

Carbonic anhydrases (CAs) are metalloenzymes containing zinc that are involved in pH regulation. CA IX has tumor-related expression and high catalytic activity and has been shown to function as an adhesion molecule. Moreover, CA IX may function as a pH regulator in the hypoxic tumor mass. CA IX-deficient mice have nonprogressive glandular expansion, restricted to foveolar hyperplasia in the fundus.[131] CA IX-knockout mice in the C57BL/6 or BALB/c background or feeding of a high-salt diet produces variations in the observed pathological changes.[132] However, deletion of CA IX does not affect gastric acid secretion, serum pH, electrolytes, or gastrin, suggesting that CA IX contributes to the hyperplastic phenotype.

17. p53 hemizygous knockout

p53 is a transcription factor that acts as a regulator of proliferation, apoptosis, and genomic repair. p53 hemizygous knockout mice have been shown to exhibit a low incidence of spontaneous carcinogenesis (2%) in organs. Infection with *H. felis* in hemizygous p53-knockout mice leads to an increased proliferative index and growth advantage compared with wild-type mice, but no obvious neoplasia was observed.[132] Moreover, wild-type C57BL/6 mice develop early invasive adenocarcinomas at 15 months after infection, with associated metaplasia and a greater inflammatory response. Therefore, hemizygosity of p53 appears to result in depressed Th1 immune responsiveness.[150]

18. SV40 T antigen transgenic mice

The SV40 T antigen from the simian virus is a potent transforming agent and oncogene. Its aberrant expression has been used to generate transformations in a number of different cells lines and tissues.

19. Carcinoembryonic antigen and SV40 T antigen transgenic mice

Carcinoembryonic antigen (CEA) is expressed during embryonic development and in various tumors, including colorectal, breast, lung, and pancreatic carcinomas. The CEA promoter has been used to drive the transgenic expression of the SV40 T antigen. Despite detectable levels of SV40 T antigen transgene expression only in the stomach, these animals develop carcinomas, lymphomas, and sarcomas with varying frequencies. Only one transgenic line has been shown to reproducibly develop tumors in the antral

stomach; these tumors were poorly differentiated adenocarcinomas that had lost gastric mucin expression. Additionally, these tumors were visible macroscopically from 5 weeks of age, and penetration of tumors in all tissue layers of the stomach invaded and blocked the duodenum, causing death of the mice at approximately 20 weeks of age.[70] A mouse adenocarcinoma cell line generated from these gastric adenocarcinomas is currently available.[133]

20. H^+/K^+-ATPase β subunit SV40 T antigen transgenic

When the promoter of the H^+/K^+-ATPase β subunit is used to direct transgenic expression of the SV40 T antigen, a dramatic increase in number of rare preparietal cells was observed by 12 weeks of age. However, such changes did not lead to differentiation into mature parietal cells.[134] Mice with this transgene exhibit hyperplasia in the stomach, accompanied by a reduction in the numbers of mature zymogenic cells and parietal cells. By 40 weeks of age, these mice exhibit various abnormal gastric phenotypes, with progressive hyperplasia, cystic dilations, and focal dysplasia. Moreover, by 1 year of age, all mice have invasive gastric cancer with lymphatic-vascular invasion and associated lymph node and hepatic metastasis. Invasive tumor cells were only weakly positive for SV40 T antigen and negative for the H^+/K^+-ATPase β subunit. However, typical mucous-glandular structures used as histopathological diagnostic criteria in adenocarcinomas of the human stomach were not maintained. Using transcriptome analysis, researchers have shown that invasive tumor cells can transdifferentiate into neuroendocrine cells based on their expression of dopa decarboxylase, chromogranin A, and tryptophan hydroxylase, as well as increased expression of Sox2, Hey1, and Neuro D1.[135] Moreover, use of the H^+/K^+-ATPase β subunit promoter to induce cultured progenitor cells into mature parietal cells at the nonpermissive temperature for the SV40 T antigen resulted in development of fundic hypertrophy in transgenic mice at 12 weeks of age.[136]

21. Forkhead homolog 6-knockout mice

Forkhead homolog 6 (Fkh6) is expressed in the gastrointestinal tract in the mesenchyme directly adjacent to the endoderm-derived epithelium. Fkh6-knockout mice have progressively worsening gastric pathology from 3 days of age. Stomachs from these mice display epithelial hyperplasia, cyst formation, mucous cell metaplasia, increased cell proliferation, and a diffuse submucosal mesenchyme, as well as a significant reduction in BMP4, which has been implicated in epithelial signaling processes. Thus, this result suggests that BMP4 may be a downstream target of Fkh6.[137] The dramatic and rapid changes in phenotype in this knockout mouse highlight the important role of mesenchymal-epithelial cell interactions in the growth and differentiation of the gastric mucosa.

22. Sonic hedgehog-knockout mice

Sonic hedgehog (Shh)-knockout mice die at or shortly after birth. Therefore, analysis must be performed before birth. Such analyses have shown that at 18.5 days of embryonic life, Shh-knockout mice exhibit hyperplastic gastric epithelium with no increase in cell proliferation and occlusion of the duodenum caused by overgrowth of villi. Additionally the stomachs of these mice show evidence of intestinalization due to increased expression of intestinal alkaline phosphatase, a marker of the brush border of enterocytes.[138]

23. Occludin-knockout mice

Occludin is a functional component of tight junctions, which are involved in cell-cell adhesion and maintaining the integrity of intercellular spaces. Occludin-knockout mice do not exhibit changes in viability, but have significant reductions in body weight. While deletion of occludin does not affect the morphology, protein content, or function of tight junctions in intestinal epithelial cells, occludin-knockout mice exhibit atrophy of the fundic mucosa by 3 to 6 weeks of age.[139] Moreover, gastritis develops progressively, and the fundic mucosa becomes hyperplastic with mucous cell metaplasia by 40 weeks of age.

24. CCR7-knockout mice

CCR7 is a chemokine receptor that regulates the trafficking and retention of leucocytes in secondary lymphoid organs. Loss of expression of CCR7 leads to a number of phenotypes, including accumulation of functional lymphoid follicles in the stomachs of mice at 8 to 10 week of age, with concomitant development of the gastric mucosa, including accumulation of cells in the mucous neck region.[139] At 12 month of age, profound hyperplasia is observed, with cystic dilatation reminiscent of Ménétrier's disease. As a result, differentiation and proliferation of fundic tissue are affected by the presence of nonspecific, noninflammatory lymphoid aggregates in the gastric mucosa.

25. Nuclear factor-kappaB2-knockout mice

Supporting the role of the inflammatory system in regulating the gastric mucosa, loss of the COOH terminus of NF-κB2, an important transcription factor mediating inflammatory signals, has been

shown to stimulate activation of Rel/NF-κB transcription factors.[141] These mice have antral epithelial tissue with severe hyperplasia at 3 weeks of age, resulting in premature death.[151-168] It is unclear which genes are affected by the increased activation of NF-κB, leading to this gastric phenotype. Additional studies are required to elucidate the details of these mechanisms.

Mouse Models of Preneoplastic Changes

H. pylori-related gastric cancer in humans is preceded by chronic gastritis, gastric atrophy, IM, and dysplasia. Thus, in addition to mouse models of gastric cancer, there are a number of genetically engineered models exhibiting decreased numbers of parietal cells or gastric atrophy, along with metaplasia. Several models of atrophy and metaplasia can be considered for use in experimental studies (Table 3).[49,80,94,121,123,124,132,151-162] However, most of these models do not show the progression to neoplasia and most have not been examined to assess susceptibility to cancer in response to carcinogens.

Table 3. Mouse models of precancerous changes

Model	Duration	Phenotype	References
Helicobacter pylori (SS-1)	6~9 months	Atrophy, SPEM	49
TGF-α transgenic	3 months	Atrophy	121, 123
H/K-ATPase/DT	28~80 days	Atrophy	151
H/K-ATPase/Tk	Ganciclovir-treatment	Atrophy	152
H/K-ATPase-$\alpha^{-/-}$	10 weeks	Atrophy	153
H/K-ATPase-$\beta^{-/-}$	17 days	Atrophy	154, 155
$NHE2^{-/-}$	17 days	Atrophy	94
$Car9^{-/-}$	4 weeks	Atrophy	132
$CCK2R^{-/-}$	18 weeks	Atrophy	156, 157
H/K-ATPase/$Shh^{-/-}$	3~8 months	Pit cell hyperplasia, loss of parietal cell function	158
DMP-777	7~14 days	Atrophy, SPEM	124, 159
L-635	7 days	Atrophy, SPEM	160
Cdx2 transgenic	120 days	Intestinal metaplasia	161, 162
Cdx1 transgenic	120 days	Intestinal metaplasia	80

TGF = transforming growth factor; Shh = Sonic hedgehog; SPEM = spasmolytic polypeptide-expressing metaplasia.

Table 4. Promoters for establishing gene expression in the stomach

Gene	Location	Lineage in the stomach	References
TFF1	Surface of stomach (pit cell area)		72~74
TFF2	Isthmus of corpus & base of antrum	Parietal, mucous neck, and chief cells	79
H/K-ATPase	Fundus (parietal cell)	All gastric lineages of the fundus glands with Notch activation	163
Foxa3	Whole stomach, other organ from endoderm		164
K19	Whole stomach, intestine, colon, etc.		164
Lgr5	Cardia, antrum, intestine, colon, etc	All major cell types in the cardia, antrum and transition zone	165
Sox2	Fundus, antrum, esophagus, forestomach, etc.	All major cell types in the fundus and the antrum	166
Mist1	Corpus (chief cell), Brunner gland, pancreas	Chief cell and drug-induced SPEM	160
Villin	Antrum, intestine, colon	All gastric lineages of the antral glands with IFN-γ treatment	167
Lrig1	Fundus, whole stomach		168

TFF1 = Trefoil factor 1; SPEM = spasmolytic polypeptide-expressing metaplasia; IFN = interferon.

Conclusions and Future Perspectives

Numerous mouse models with various gastric phenotypes are now available for studies of gastric carcinogenesis. These include transgenic mice, knockout mice, *Helicobacter* infection, and carcinogen (MNU) models. These models have demonstrated that gender, diet, bacterial flora, inflammatory cytokines, T helper immune response, acid secretion, virulence, colonization properties of *H. pylori* strains, and host genetic background may all have roles in mediating the development of gastric cancer.

Unfortunately, genetic models of metastatic gastric cancer similar to those developed for pancreatic cancer, comprising two or three mutations targeted to specific cell lineages, are not available. The major limitations of these models are minimal and include dispersion of promoter activity in the stomach and the lack of stomach-specific promoters that target antral progenitors only (Table 4).[72-74,79,160,163-168]

Reasonable mouse models of gastric cancer are available for studies of early-stage pathogenesis and cancer therapy, which have distinct mechanisms and different tumor phenotypes, with variations in the time course, location, and pathology of the disease. Thus, researchers are able to utilize appropriate mouse models for their studies. Newly suggested research methods, including lineage tracing or genome-wide analysis, should prove valuable for understanding the causes of gastric cancer, and thereby facilitating the discovery of a cure for this disease.

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