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Genetically engineered mouse models in gastric precancerous lesions research

Quan Y *et al.* GEMMs and PLGC

Abstract

Precancerous lesions of gastric cancer (PLGC) are crucial for the progression to gastric cancer, and early intervention in PLGC is pivotal in preventing its development into gastric cancer. In order to illustrate the molecular mechanisms underlying PLGC and the roles of associated genes within these lesions, genetically engineered mouse models (GEMMs) have been developed. We systematically summarize the current GEMMs, and highlight the principal pathological mechanisms involved, including gastrin/gastric acid balance, inflammatory factors, the interplay between cancer-promoting and cancer-suppressing genes, and apoptotic pathways. We further discuss the mechanisms involved in the existing GEMMs of PLGC.

Key Words: Precancerous lesions of gastric cancer; Atrophic gastritis; Intestinal metaplasia; Dysplasia; Genetically engineered mice; Pathogenesis

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Core Tip: Precancerous lesions of gastric cancer refer to a series of pathological changes in the gastric mucosa, including atrophic gastritis, intestinal metaplasia, and dysplasia. These lesions are significant risk factors for the development of gastric carcinoma.

Genetically engineered mice, modified through techniques such as gene knockout, gene knock-in, transgenesis, and point mutation, are utilized to investigate gene function, disease mechanisms, and to develop novel therapeutic strategies. This review discusses the genetically engineered mouse models employed in the study of precancerous lesions of gastric cancer, with an emphasis on elucidating their pathological mechanisms.

INTRODUCTION

In 2022, there were over 968000 new cases of gastric cancer (GC) and nearly 660000 deaths worldwide, ranking it fifth in both incidence (4.9%) and mortality (6.8%) globally[1]. Due to the lack of evident symptoms in the early stages, GC is often diagnosed at a late stage, leading to a high mortality rate[2]. However, precancerous lesions of GC (PLGC) present a unique opportunity for monitoring and intervening in the development of GC, paving the way for potential early intervention and treatment. PLGC comprise a series of pathological changes in the gastric mucosa, including atrophic gastritis, intestinal metaplasia (IM), and dysplasia. The pathological mechanisms involve chronic inflammation, DNA damage, cell proliferation, and apoptosis[3].

Genomic sequencing study showed that the protein-coding genes of mice and humans share a high degree of similarity[4]. Through precise genetic manipulation, genetically engineered mouse models (GEMMs) can model the development of human PLGC, replicating key molecular events. Commonly use DNA pronuclear microinjection, site-specific nuclease technology, and the Cre/LoxP system. DNA pronuclear microinjection involves the injection of exogenous DNA into the pronucleus of fertilized eggs, effectively transferring the target gene into mice[5]. Site-specific nuclease technology is based on engineered nucleases composed of specific DNA-binding domains fused with nonspecific DNA-cutting modules. These chimeric nucleases induce target DNA double-strand breaks, which stimulate cellular DNA repair mechanisms, including non-homologous end joining and homology-directed

repair, thereby achieving gene knockout or insertion[6]. This category includes ZFNs, TALENs, and CRISPR/Cas, with CRISPR/Cas becoming the mainstream technology due to its high efficiency (Figure 1). In the Cre-LoxP system, a single Cre enzyme recognizes repeated LoxP sites, excising the flanked target DNA (Figure 2). Conditional knockout can be achieved by fusing the Cre enzyme with a drug-binding domain or placing it under the control of a specific tissue promoter[7].

This review systematically summarizes the application of GEMMs in the study of the pathological mechanisms of PLGC, with a focus on their role in gastrin/acid regulation, inflammatory factors, oncogenes/tumor suppressor genes, and apoptosis. It particularly emphasizes their unique value in simulating pathological processes and elucidating molecular mechanisms, as well as predicting disease progression and therapeutic responses.

GASTRIN/GASTRIC ACID DISORDER-INDUCED PLGC

INS-GAS mice

Gastrin is a gastrointestinal hormone secreted by G cells in the gastric antrum, promoting gastric acid secretion and participating in various normal and abnormal biological processes, including the maintenance of gastric mucosa, the proliferation of enterochromaffin-like cells, and tumorigenesis[8]. Infection with *Helicobacter pylori* (*H. pylori*) can result in hypergastrinemia, which is viewed as a contributing factor to the development of GC[9]. INS-GAS mice, under the control of the insulin gene promoter, overexpress the gastrin gene, resulting in high circulating gastrin levels and consistently developing atrophic gastritis with hypochlorhydria[10], can spontaneously develop atrophic gastritis, IM, dysplasia, and adenocarcinoma by approximately 20 months[11,12]. The INS-GAS mouse model is often used in combination with *H. felis* infection, after infection, INS-GAS mice exhibit accelerated carcinogenesis, severe atrophic gastritis, and gastric body dysplasia, without dysplasia observed in the antrum, indicating a differential carcinogenic effect of hypergastrinemia on the gastric body and antrum[13]. Additionally, INS-GAS mice exhibit strain and gender

differences, with FVB/N mice being more sensitive than C57BL/6 mice. Male gastric tissue tends to exhibit a more pronounced response to *H. pylori* infection, a high-salt diet, and their combination, resulting in more severe pathological changes, which is consistent with higher GC incidence in males compared to females[14]. Furthermore, ovariectomized (OVX) INS-GAS mice develop more severe gastric mucosal diseases than intact mice, suggesting that estradiol may have a protective effect on the gastric mucosa of female INS-GAS mice[15]. Further findings indicate that exogenous estradiol exerts a protective effect against PLGC by stimulating interleukin (IL)-10 activity, enhancing Th2-mediated immune responses, and inhibiting epithelial cell proliferation[16].

Due to its maturity, the INS-GAS mouse model has also been frequently used in recent years to study the effects of different drugs and strains on the gastrointestinal microbiota. This model accurately reproduces the gastric inflammation observed in human *H. pylori* infection and the gradual increase in FOXM1 expression (a GC-related protein). Using this model, thiopeptide-producing *C. acnes* strains can reduce *H. pylori*-induced gastric inflammation and FOXM1 expression[17]. Another study indicated that long-term vonoprazan treatment can promote gastric damage and gastrointestinal microbiota imbalance in male INS-GAS mice[18]. The research on the microbiota also confirmed the impact of sex differences on the development of PLGC: Male mice and OVX female mice exhibited more severe gastric lesions, while intact female mice had a higher abundance of beneficial bacteria. In contrast, pathogenic bacteria were more abundant in male mice and OVX female mice. Moreover, exchanging gut microbiota (through co-housing) significantly reduced the differences in gastric lesions between OVX and intact female mice[19].

***Gastrin*^{-/-} mice**

Gastrin^{-/-} mice spontaneously develop body atrophy and antrum hyperplasia, suggesting different regions of the stomach respond differently to the absence of gastrin[20]. One study revealed that TFF1 is reversibly decreased in Gastrin^{-/-} mice.

Gastrin activates TFF1 transcription through a Mek1-, Raf1-, and ERK-dependent but Ras-independent pathway and can also indirectly activate TFF1 through cell-cell interactions or soluble factors[21]. However, another study showed that TFF1 expression did not change significantly in young mice, but was significantly upregulated under long-term achlorhydric conditions. Additionally, achlorhydria in Gastrin-/- mice leads to the upregulation of immune defense genes and genes primarily expressed in the intestine. Exogenous gastrin can restore gastric acidity and reverse gene expression changes, but elderly Gastrin-/- mice develop irreversible IM, possibly related to CDX2 expression and CDX1 activation. Achlorhydria also promotes bacterial overgrowth, with these bacteria capable of forming carcinogenic N-nitrosamines[22]. The Gastrin-/- model combined with MNU and *H. felis* model replicates several key features of human GC, including a high mutational burden and increased programmed death-ligand 1 expression, making it a valuable model for studying the mechanisms of resistance to programmed death 1 inhibitors and the role of its ligand programmed death-ligand 1[23].

H⁺/K⁺-ATPase

H⁺/K⁺-ATPase, an enzyme predominantly located in the parietal cells of the stomach, utilizes the energy from adenosine triphosphate hydrolysis to exchange intracellular H⁺ for extracellular K⁺. The H⁺ then combines with Cl⁻ to form HCl, aiding in food digestion. The α subunit, encoded by the *ATP4A* gene, contains the catalytic site and mediates ion transport, while the β subunit, encoded by the *ATP4B* gene, stabilizes the α subunit[24]. In autoimmune gastritis, the gastric proton pump H⁺/K⁺-ATPase is the principal autoantigen recognized by autoreactive T cells[25]. Mice with knockout of either *ATP4A* or *ATP4B* genes exhibit achlorhydria and elevated gastrin levels.

***Atp4a*^{-/-} mice/*Atp4ap.R703C* mice**

Atp4a^{-/-} mice exhibit hyperplasia, and as age increases, the hyperplasia and metaplasia of the gastric mucosa progressively worsen, though GC does not develop. Additionally,

it was observed that female mice exhibit more pronounced hyperplasia than male mice, consistent with the higher incidence of autoimmune atrophic gastritis (AAG) in females[26,27]. Significant alterations in MUC2 expression in *Atp4a*^{-/-} mice were observed, suggesting that gastric acid levels may influence mucus expression. The absence of the α subunit leads to activation of the phosphatidylinositol 3-kinase/ protein kinase B/mammalian target of rapamycin signaling pathway, which subsequently promotes the Warburg effect. It was also found the lifespan of *Atp4a*^{-/-} mice does not differ significantly from wild-type mice, making them an excellent model for studying the progression from atrophic gastritis to IM[28]. Additionally, knockout of the α subunit does not impair the viability of parietal cells or the differentiation of chief cells, though parietal cells exhibit abnormal secretory membranes and tubulovesicular membranes[29,30].

When using this model to explore the treatment of IM with metformin, it was found that metformin significantly reduced the progression of IM lesions in the *Atp4a* mouse model, possibly by downregulating the nuclear factor kappa B and phosphatidylinositol 3-kinase/ protein kinase B/ mammalian target of rapamycin/HIF-1 α signaling pathways[31]. To better understand the relationship between impaired transmembrane proton export capability of parietal cells and tumorigenesis, researchers constructed an *ATP4A* mutant knock-in mouse model (*Atp4ap.R703C* mice). This model recapitulates atrophic gastritis and IM by affecting mitochondrial function and biosynthesis, thereby activating ROS signaling, triggering parietal cell death, and it suggests that autoimmune gastritis may not be caused by the conventional autoimmune response leading to parietal cell atrophy but rather by genetically mediated *ATP4A* dysfunction[32].

Atp4b^{-/-} mice

Atp4b^{-/-} mice exhibit elevated gastrin levels, marked gastric hypertrophy, submucosal cysts, and widespread expression of spasmolytic polypeptide-expressing metaplasia (SPEM) and NE cell markers in the corpus, while these phenomena are not observed in

the antrum. This finding is perfectly in line with the characteristics of human AAG[33]. Furthermore, in *Atp4b*^{-/-} mice, parietal cell development was delayed during embryogenesis, suggesting that H⁺/K⁺-ATPase may play a critical role in the developmental pathways of gastric mucosal cells. Moreover, parietal cells exhibit morphological abnormalities, including abnormally dilated tubules and a lack of typical tubulovesicular structures, which are associated with impaired acid secretion function[34]. Currently, there is limited research on the mechanisms of *Atp4b*^{-/-} mice, warranting further investigation.

Slc26a9^{-/-} mice

Slc26a9 belongs to the *Slc26a* family of anion transport proteins. In the stomach, it is primarily found on parietal cells and functions as a chloride channel/bicarbonate exchanger[35]. *Slc26a9*^{-/-} mice exhibited achlorhydria and a loss of tubulovesicular structures in parietal cells, indicating that *Slc26a9* is essential for gastric acid secretion by affecting the activity of tubulovesicular membranes and regulating chloride secretion in parietal cells[36]. Moreover, complete and parietal cell-specific knockout of *Slc26a9* in mice could both accelerate the carcinogenesis. Additionally, selective deletion of *Slc26a9* in parietal cells led to dysregulated differentiation of stem and progenitor cells, induced hyperproliferation, and inhibited apoptosis in an inflammatory environment[37].

INFLAMMATION-INDUCED PLGC

H⁺/K⁺-ATPase-IFN- γ mice

Interferon- γ (IFN- γ) is an inflammatory cytokine produced by activated immune cells such as T cells and NK cells. Some studies suggest that IFN- γ has antitumor properties, while others indicate it may promote tumor growth and progression[38]. One research showed that *H⁺/K⁺-ATPase-IFN- γ* mice overexpress IFN- γ under the control of the *H⁺/K⁺-ATPase* α subunit promoter, resulting in spontaneous inflammation, SPEM, atrophy of parietal and chief cells, gland atrophy, and dysplasia, with the development of adenomas and adenocarcinomas. IFN- γ can directly induce gastric epithelial cell

death, playing a crucial role in the progression from gastritis to atrophy and metaplasia, and is essential for the development of metaplasia[39]. Another research showed that H⁺/K⁺-ATPase-IFN- γ mice do not develop spontaneous atrophic gastritis or metaplasia, with histopathology resembling normal tissue. High levels of IFN- γ have been reported to inhibit gastric progenitor cell proliferation and reduce epithelial cell apoptosis through autophagy, thereby cooperatively inhibiting bacterial infection and carcinogenesis in the gastric mucosa[40]. In non-small cell lung cancer, studies have indicated that low levels of IFN- γ confer cancer stem cell-like properties, while high levels of IFN- γ induce apoptosis[41]. It is hypothesized that different levels of IFN- γ lead to distinctly different experimental outcomes.

K19-IL11^{Tg} mice

IL-11, as both an anti-inflammatory and pro-inflammatory cytokine, has been shown to be involved in various inflammation-associated cancers, primarily due to its ability to activate the Janus kinase-signal transducers and activators of transcription (STAT) 3 pathway[42]. To investigate whether locally elevated expression of the IL-11 ligand can initiate GC pathogenesis independently of gp130-Janus kinase-STAT pathway mutations, researchers developed K19-IL11^{Tg} mice. These mice specifically express IL-11 in both gastric corpus and antrum, with no expression in other tissues. And K19-IL11^{Tg} mice spontaneously develop PLGC, demonstrating that persistent abnormal elevation of IL-11 alone is sufficient to initiate the GC cascade[43]. However, in K19-IL11^{Tg} mice, high-grade dysplasia and malignant lesions are usually not observed, and the progression of carcinogenesis is slow, localized to the gastric corpus. Another study indicated that IL-11 is a cytokine secreted by parietal cells, it inhibits gastric acid secretion by downregulating the expression of ion transport genes in parietal cells, as well as the expression of CCKB and histamine H2 receptors[44].

Gp130^{757E/E} mice

Gp130 is not a pro-inflammatory cytokine, however, as the shared signaling subunit for IL-6 family cytokines, it participates in inflammatory responses and contains two distinct functional modules that signal through STAT1/3 and SHP-2/ERK pathways[45]. The Gp130^{757F/F} mouse is a knock-in mutant carrying Y757F and V760A mutations, which disrupt the pY757xxV760 SHP2 binding domain, inhibiting the SHP2-Ras-ERK signaling cascade while elevating IL-6 and IL-11 levels. At 6-8 weeks of age, Gp130^{757F/F} mice spontaneously develop hyperplastic lesions in the antral mucosa, and at 3 months, they spontaneously develop gastric adenomas. It is hypothesized that the hyperplastic lesions due to enhanced STAT3 activity, in the absence of negative feedback signaling from the SHP2-Ras-ERK pathway[46]. Moreover, knocking out one allele of STAT3 in Gp130^{757F/F} mice suppresses the growth of gastric adenomas, confirming this hypothesis[47]. Furthermore, to investigate the role of IL-6, Gp130^{757F/F} mice with IL-6 knockout were studied, revealing that the lack of IL-6 did not affect tumor development, providing further evidence that IL-11 alone is sufficient to initiate the GC cascade. Further crossing Gp130^{757F/F} mice with RAG1 mice showed that the lack of mature T, B, and NK T cells did not affect tumor incidence, size, or IL-6 and IL-11 synthesis, indicating that T, B, and NK T cells are not the primary sources of IL-6 and IL-11[48].

H⁺/K⁺-ATPase-IL-1 β mice

IL-1 β is a potent pro-inflammatory mediator, typically expressed at low levels by macrophages, and its production can be induced by *H. pylori* infection[49]. H⁺/K⁺-ATPase-hIL-1 β mice, driven by the H⁺/K⁺-ATPase promoter, expressing high IL-1 β level, parietal cells that continuously secrete IL-1 β , leading to spontaneous inflammation, atrophy, metaplasia, and dysplasia. The histopathological scores of H⁺/K⁺-ATPase-IL-1 β mice expressing high levels of IL-1 β were significantly higher than those of H⁺/K⁺-ATPase-IL-1 β mice expressing lower levels of IL-1 β . It was also found that IL-1 β overexpression accelerated the progression of gastritis and cancer development in *H. felis*-infected mice[50]. Conversely, in IL-1R knockout mice,

following *H. pylori* infection, gastric atrophy was more frequently observed in WT mice with functional IL-1 β signaling compared to IL-1R1 $^{-/-}$ mice. Additionally, E-cadherin methylation was not detected in IL-1R1 $^{-/-}$ mice, suggesting that reducing IL-1 β activity may alleviate the burden of inflammatory diseases[51]. Given that H $^{+}$ /K $^{+}$ -ATPase-IL-1 β mice continuously secrete IL-1 β through parietal cells, leading to the early mobilization and recruitment of myeloid-derived suppressor cells, this model has been employed to explore the involvement of myeloid-derived suppressor cells in GC.

PLGC INDUCED BY MUTATION OF ONCOGENES AND/OR TUMOR SUPPRESSOR GENES

K19-kras/Mist1-Kras mice

The *Kras* gene is a common oncogene, with clinical studies indicating mutations in the *Kras* gene in patients with gastritis and gastric adenoma[52]. K19-kras transgenic mice, utilizing the K19 promoter, overexpress the oncogene K-Ras in gastric epithelial cells. One research showed that K19-kras mice spontaneously develop gastric atrophy, metaplasia, and dysplasia similar to *H. felis* infection. *H. felis* infection in K19-kras mice does not accelerate the progression of GC, suggesting that *Kras* mutations can compensate for the lack of infectious stimuli to induce inflammation and carcinogenesis[53]. In another research, tamoxifen was used to induce the expression of the *Kras* gene in chief cells of Mist1-Kras mice. This model demonstrated all the PLGC of human, including the differentiation of chief cells into SPEM as the initial step of metaplastic changes, SPEM progressing to IM, and the development of carcinoma. Lineage tracing confirmed that chief cells are the direct origin of metaplasia, and sustained activation of Ras signaling drives the further progression of metaplasia. It was further proposed that using Mek inhibitors could reverse the metaplastic process and allow the normal gastric epithelium to repopulate the mucosa[54].

Car9 $^{-/-}$ mice

CAIX is mainly expressed on the basolateral membrane of surface cells in the gastric mucosa and is a membrane-bound carbonic anhydrase that plays a critical role in tumor cell metabolism, pH regulation, migration, and invasion[55]. *Car9*^{-/-} mice exhibit significant hyperplasia, an expanded area of cell proliferation, an increased number of mucus-secreting pit cells, and a decrease in chief and parietal cells. However, their gastric acid secretion, pH, and systemic plasma electrolytes do not differ significantly from those of normal mice. It is hypothesized that CAIX does not directly participate in gastric acid secretion but rather contributes to gastric mucosal morphogenesis and homeostasis by controlling cell proliferation and regulating differentiation programs related to migration[56]. However, another study indicated that the absence of CAIX does not affect the maintenance of cellular pH under neutral conditions, whereas under acidic conditions, the lack of CAIX significantly decreases the pH of gastric mucosal cells, particularly in the tight junction regions of gastric mucosal epithelial cells. It is suggested that CAIX plays a crucial role in regulating gastric mucosal pH and protecting the gastric mucosa from acid injury, especially in the apical membrane and tight junctions of surface cells. Additionally, CAIX enabling the extrusion of protons through its basal pH regulatory mechanisms. Additionally, *Car9*^{-/-} mice exhibit significant downregulation of claudin-18 (CLDN18), suggesting that the lack of CAIX leads to continuous acid reflux mediated by CLDN18 downregulation, resulting in the loss of parietal cells, hypergastrinemia, and gastric gland hyperplasia[57]. Lastly, strain differences was found in *Car9*^{-/-} mice, with a higher incidence of atrophy observed in mice with a C57BL/6 background compared to those with a BALB/c background under the same conditions[58].

Cdx1/2 transgenic mice

Under normal conditions, both CDX1 and CDX2 are primarily expressed in intestinal epithelial cells and are not expressed in the gastric mucosa. However, both can be detected in gastric mucosa with IM, with expression levels positively correlated with the degree of IM[59], suggesting that CDX1/2 may be crucial for the development of IM

in the human stomach[60,61]. In CDX1/2 transgenic mice, the CDX1/2 genes are typically expressed in parietal cells[62]. The specific manifestations of IM differ between the two, CDX1 transgenic mice exhibit IM replacing the gastric mucosa, involving all four types of intestinal epithelial cells, while CDX2 transgenic mice only exhibit pseudopyloric gland metaplasia[63,64]. Since metaplasia refers the transformation from one tissue type to another, researchers hypothesize that this process may be initiated through changes in cellular differentiation states. Further research found that CDX1-mediated induction of SALL4 and KLF5, both of which are related to lineage reprogramming and stem cell acquisition, plays a significant role in the differentiation of gastric epithelial cells into an intestinal phenotype[65]. During IM of the gastric mucosa in CDX2 transgenic mice, the CDX2 gene induces endogenous CDX1 binding to the unmethylated region of the CDX1 promoter, indicating that CDX2 may act as a trigger for the development of IM[66,67]. Additionally, CDX2 expression gradually decreases during IM, dysplasia, and carcinogenesis in the human stomach, suggesting that CDX2 have opposing roles at different stages of GC[68]. In the early stage, CDX2 drives the differentiation of the gastric epithelial phenotype to the intestinal epithelial phenotype, functioning as an oncogene. In the later stage, as the IM progresses to GC, CDX2 acts as a tumor suppressor gene, inhibiting the invasion and growth of GC[69].

Apc^{min/+} mice

APC is a large multifunctional protein involved in various cellular processes, including cell proliferation, cell migration, cell adhesion, cytoskeletal reorganization, and chromosomal stability. Disruption of specific interactions can result in the loss of one or more functions of APC, thereby promoting tumor formation[70]. APC plays a crucial role in the early stages of GC, and well-differentiated adenocarcinomas of the stomach exhibit mutations in the APC gene[71,72]. And high expression of APC is correlated with differences in genome-wide gene/miRNA/methylation expression and associated cellular functional pathways, suggesting that APC is an adverse prognostic factor in T4 stage GC patients[73]. *Apc^{min/+}* mice are an animal model for studying familial

adenomatous polyposis, carrying a dominant mutation in the APC gene induced by N-ethyl-N-nitrosourea mutagenesis, resulting in truncation of the gene product at amino acid 850. At 24 weeks of age, *Apc^{min/+}* mice display epithelial cell proliferation and inflammatory infiltration in the forestomach, glandular atrophy and IM in the corpus, and dysplasia in the antrum[74]. And loss of APC function subsequently activates β -catenin/Tcf transcription, the accumulation of β -catenin may be an initiating event in GC[75].

Tff1^{-/-} mice

TFF1 is a member of the TFF family and is abundantly expressed in the mucin-secreting foveolar cells throughout the stomach. It is packaged with the secreted mucin MUC5AC in the mucin granules of apical mucin secretory vesicles, ready to be released into the adherent mucus layer[76]. TFF1 participates in the formation of a protective mucus barrier, shielding the stomach from gastric acid and digestive enzymes. And TFF1 is upregulated during early *H. pylori* infection and can specifically bind to the bacterium, causing it to lose its helical shape and significantly slow its movement in the mucus[77]. TFF1 is frequently lost in GC, and the absence of TFF1 in TFF1^{-/-} mice results in the loss of mucosal protection and the activation of multiple pro-inflammatory and carcinogenic signaling pathways, including STAT3, thereby promoting the development and progression of GC[78]. One research indicated that the gastrin gene is one of the most downregulated genes in the low-grade dysplastic tissues of TFF1^{-/-} mice, suggesting that the knockout of TFF1 may affect normal gastric mucosal function by influencing gastrin gene expression[79]. Conversely, gastrin significantly inhibits antral lesions by positively regulating TFF1 gene expression and through epigenetic silencing[80].

CLDN18^{-/-} mice

CLDN18 is predominantly located on the basolateral membrane of gastric epithelial cells and is a key component of tight junctions in gastric epithelial cells[81]. CLDN18^{-/-} mice exhibit PLGC by 7 weeks of age, intraepithelial neoplasia with invasive

submucosal glands by 20-30 weeks, and develop dysplastic polypoid tumors by 2 years[82]. In the stomach, CLDN18 typically forms an intercellular barrier against H⁺, and its absence leads to intercellular H⁺ leakage, upregulation of pro-inflammatory cytokines, chronic recruitment of neutrophils, which is followed by atrophic gastritis and SPEM[83]. CLDN18^{-/-} mice can also independently form gastric tumors without *H. pylori* infection, with the tumorigenesis process partially resembling that of *H. pylori*-associated human carcinogenesis[84]. And in adult CLDN18^{-/-} mice, the gradual carcinogenesis process may be attributed to altered functions of transcellular anion (mainly Cl⁻) transporters[85].

APOPTOSIS-INDUCED PLGC

***Bak*^{-/-} mice**

BAK, a pro-apoptotic member of the Bcl-2 protein family, plays an important role in promoting cell apoptosis and is primarily distributed in gastric epithelial cells, particularly in the gastric pits and parietal cells. Compared to normal gastric epithelial cells, the expression levels of BAK are reduced in gastric tumors[86]. In *Bak*^{-/-} mice induced by gamma radiation, gastric epithelial apoptosis is reduced compared to that in wild-type mice, further demonstrating that the *Bak* gene promotes gastric epithelial cell apoptosis in mice[87]. Also, *Bak*^{-/-} mice exhibit reduced numbers of parietal and endocrine cells in the fundic glands and increased fundic gland length, a characteristic specific to *BAK* gene knockout, indicating that the loss of the *BAK* gene does not directly lead to significant inflammation. Additionally, long-term infection with *H. felis* in *Bak*^{-/-} mice leads to a higher propensity for developing gastric atrophy and dysplasia compared to wild-type mice, suggesting that BAK not only regulates apoptosis but may also play an important role in cell proliferation. However, no significant differences in strain, gender, or *H. felis* colonization were observed in *Bak*^{-/-} mice[88].

H. PYLORI

Extensive epidemiological indicated that *H.pylori* is closely associated with PLGC of the stomach and is a major cause of these lesions. It induces inflammation in the antrum and parts of the corpus, leading to DNA damage in epithelial cells and initiating the GC cascade[89,90]. Commonly used genetically engineered mice, when infected with *H. pylori* and *H. felis*, can develop precancerous PLGC. *H. felis*, a close relative of *H. pylori* that is isolated from cat stomachs, has been shown to readily colonize the mouse stomach[91]. C57BL/6 mice exhibit significant resistance to the colonization of various *H. pylori* strains, leading to the development of a more strongly colonizing strain - *H. pylori* SS1 - that can colonize the entire glandular area of the mouse stomach. This strain prefers the antral mucosa and the transition zone between the antrum and corpus, but shows lower colonization levels in Balb/c mice[92]. *H. pylori* accelerates the carcinogenesis process in genetically engineered mice, which provide high control and precision, enabling accurate study of the carcinogenic mechanisms induced by *H. pylori* infection in mice[93].

CONCLUSION

Many GEMMs of PLGC have been established (Table 1), recreating key molecular events of PLGC. These models help us gain deeper insights into the pathological mechanisms of PLGC and provide potential targets for early diagnosis and treatment of GC. Most GEMMs can spontaneously develop PLGC, and can be used to model high-risk populations for GC, allowing exploration of the role of genetic inheritance. The INS-GAS mouse model can stably develop PLGC, with predictable progression and high similarity to human lesions, making it a widely used model. Infection with *H.pylori* or *H.felis* accelerates the progression of PLGC or GC in GEMMs, which provide a controlled environment for investigating the interaction between genetic and environmental factors[94,95]. Notably, gender-specific disparities observed in GEMMs of PLGC align with human epidemiological data. While males exhibit higher susceptibility to most subtypes of gastric carcinoma and associated PLGC, a distinct reversal is evident in AAG, where female predominance is characteristically observed.

Furthermore, genetic engineering techniques allow us to create mouse models with specific pathogenic genes, enabling the study of the isolated effects of these genes in PLGC while effectively excluding the interference of other genes.

However, due to the anatomical, physiological, and immunological differences between mice and humans, GEMMs of PLGC may not fully replicate all features of human PLGC. Some GEMMs have shortened lifespans due to genetic defects, while others may develop diseases unrelated to PLGC. Both of which may potentially influence the research process and outcomes. Moreover, the occurrence of PLGC and the colonization ability of *H.pylori/H.felis* vary among different mouse strains and sexes., potentially affecting the generalizability and predictive value of the models. Thus, these factors should be carefully considered when designing experiments. And certain rare or complex genetic background tumors are difficult to establish using GEMMs. The emergence of patient-derived xenograft (PDX) models and patient-derived organoid (PDO) models has effectively addressed this issue. PDX and PDO can be directly obtained from patient tumor tissues, and they are capable of preserving the tumor's tissue structure and cell-to-cell interactions while partially simulating the tumor microenvironment. Moreover, they hold promise for evaluating treatment responses in individual patients, thereby facilitating the development of personalized treatment regimens. However, the considerable individual differences in PDX and PDO models may lead to inconsistencies between models, which in turn pose challenges for the reproducibility of experiments. In contrast, GEMMs do not have this issue. Additionally, there is a scarcity of GEMMs specifically designed for studying PLGC. The creation of GEMMs demands considerable time and resources and it cannot be guaranteed that all GEMMs will develop PLGC. To overcome this problem, some researchers have proposed combining PDOs with CRISPR screening. PDOs are rapid and efficient in assessing drug sensitivity, while CRISPR screening can identify novel therapeutic targets. The combination of these two approaches is expected to accelerate the development of more effective and personalized treatment strategies[96].

The development of more sophisticated GEMMs incorporating multiple genetic alterations and environmental determinants to mimic human conditions better will be critical for achieving a comprehensive understanding of PLGC. Moreover, there is a need to develop more humanized mouse models, especially in terms of simulating human immune responses, which will provide a valuable platform for immunotherapy and targeted therapy. Advancements in CRISPR/Cas9 technology and other genome-editing platforms will facilitate the generation of models with more sophisticated genetic configurations that better recapitulate the heterogeneity observed in human gastric carcinogenesis. Furthermore, the integration of multi-omics approaches (genomics, transcriptomics, proteomics) with GEMMs will enable deeper insights into the molecular mechanisms underlying PLGC pathogenesis. Moreover, the incorporation of microbiome investigations into GEMM-based studies could further elucidate the functional contributions of gastrointestinal microbiota to PLGC progression, thereby establishing a theoretical foundation for microbiota-targeted therapeutic interventions. Studying mouse models with these gene alterations aids in investigating the functions of these genes. Consequently, these new models will help unravel the complex molecular networks of PLGC and offer new strategies for the prevention and treatment of GC.