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Review Article

Prostaglandin E_2, Wnt, and BMP in gastric tumor mouse models

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The development of gastric cancer is closely associated with *Helicobacter pylori* (*H. pylori*) infection. The expression of cylooxigenase-2 (COX-2), a rate-limiting enzyme for prostaglandin biosynthesis, is induced in *H. pylori*-associated chronic gastritis, which thus results in the induction of proinflammatory prostaglandin, PGE_2. The COX-2/PGE_2 pathway plays a key role in gastric tumorigenesis. On the other hand, several oncogenic pathways have been shown to trigger gastric tumorigenesis. The activation of Wnt/β-catenin signaling is found in 30–50% of gastric cancers, thus suggesting that Wnt signaling plays a causal role in gastric cancer development. Mutations in the bone morphogenetic protein (BMP) signaling pathway are responsible for the subset of juvenile polyposis syndrome (JPS) that develops hamartomas in the gastrointestinal tract. BMP suppression appears to contribute to gastric cancer development because gastric cancer risk is increased in JPS. Wnt signaling is important for the maintenance of gastrointestinal stem cells, while BMP promotes epithelial cell differentiation. Accordingly, it is possible that both Wnt activation and BMP suppression can cause gastric tumorigenesis through enhancement of the undifferentiated status of epithelial cells. Recent mouse model studies have indicated that induction of the PGE_2 pathway is required for the development of both gastric adenocarcinoma and hamartoma in the Wnt-activated and BMP-suppressed gastric mucosa, respectively. This article reviews the involvement of the PGE_2, Wnt, and BMP pathways in the development of gastric cancer, and gastric phenotypes that are found in transgenic mouse models of PGE_2 induction, Wnt activation, BMP suppression, or a combination of these pathways. (*Cancer Sci* 2009; 100: 1779–1785)

Epidemiological studies indicate that the regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) lowers the mortality rate of gastrointestinal cancer.[1] The major target of NSAIDs is cyclooxygenases (COXs), COX-1 and COX-2, which are rate-limiting enzymes for prostaglandin biosynthesis (Fig. 1). COX-1 is constitutively expressed in most tissues and it is considered to be responsible for physiological levels of prostaglandin.[2] In contrast, COX-2 is induced in inflammation by various stimuli including cytokines and growth factors.[3–5] The induction of COX-2 expression is also found in a variety of cancer tissues. Mouse genetic studies have demonstrated that disruption of the *Ptgs2* gene encoding COX-2 results in the suppression of tumor development in the intestine and skin.[6,7] Moreover, various animal studies have confirmed that treatment with NSAIDs or COX-2 selective inhibitors (COXIBs) suppressed chemically induced tumor formation and xenografted tumor growth.[8] These results, taken together, indicate that the COX-2 pathway plays an essential role in cancer development.

However, the mechanism of the COX-2 pathway underlying gastric tumorigenesis has not yet been fully elucidated. To investigate the possible crosstalk between the COX-2 pathway and oncogenic activation in gastric carcinogenesis, a series of mouse models have been constructed and examined as discussed in this review.

Induction of the COX-2/PGE_2 pathway in gastric cancer

Regular use of NSAIDs is associated with a decreased incidence of gastric cancer.[9–12] Induction of COX-2 is found in approximately 70% of gastric cancer, whereas the expression of COX-1 is not elevated.[13,14] Gastric cancer can be divided into two histological subtypes: intestinal and diffuse types, and the expression of COX-2 is found predominantly in the intestinal-type gastric cancer.[15] These results suggest that the COX-2 pathway plays a role in the development of intestinal-type gastric cancer.

Infection with *Helicobacter pylori* (*H. pylori*) causes chronic gastritis, which is associated with gastric carcinogenesis.[16] The expression of COX-2 is significantly induced in the *H. pylori*-infected gastric mucosa, and that COX-2 expression is suppressed by the eradication of *H. pylori*.[17] Although the molecular mechanism for COX-2 induction in tumors has not been elucidated, it is possible that the cytokine network is activated by infection and induces the expression of COX-2. *H. pylori* can stimulate Toll-like receptors (TLRs), leading to activation of the nuclear factor-κB (NF-κB) pathway that induces the expression of COX-2 (Fig. 1).[18,19] Moreover, TLR2/TLR9 signaling by *H. pylori* activates mitogen activated protein kinases (MAPK) including p38, resulting in the activation of CRE and AP-1 elements on the COX-2 gene promoter.[19,20]

Microsomal PGE synthase-1 (mPGES-1), a PGE_2 converting enzyme is functionally coupled with COX-2.[21] Simultaneous induction of COX-2 and mPGES-1 is observed in gastric cancer tissues suggesting induction of the PGE_2 pathway in gastric tumors (Fig. 1).[22,23] The level of mPGES-1 also decreases after the eradication of *H. pylori*,[24] thus indicating that *H. pylori* infection induces the PGE_2 pathway through induction of both COX-2 and mPGES-1. The PGE_2 level significantly increases in gastric cancer,[25] and the level is associated with the *H. pylori* infection status.[26]

Gastric tumor development in mouse and rat models induced by chemical carcinogens or *Helicobacter* infection is suppressed by treatment with NSAIDs or COXIBs.[27–29] *H. pylori* infection in Mongolian gerbils induces gastric tumorigenesis, which is quite similar to the course of human gastric carcinogenesis. Importantly, treatment with *H. pylori*-infected and chemical carcinogen-treated Mongolian gerbils with a COXIB suppressed gastric carcinogenesis.[30,31] These animal studies suggest that the COX-2 pathway thus plays an essential role in *H. pylori* infection–associated gastric tumorigenesis.

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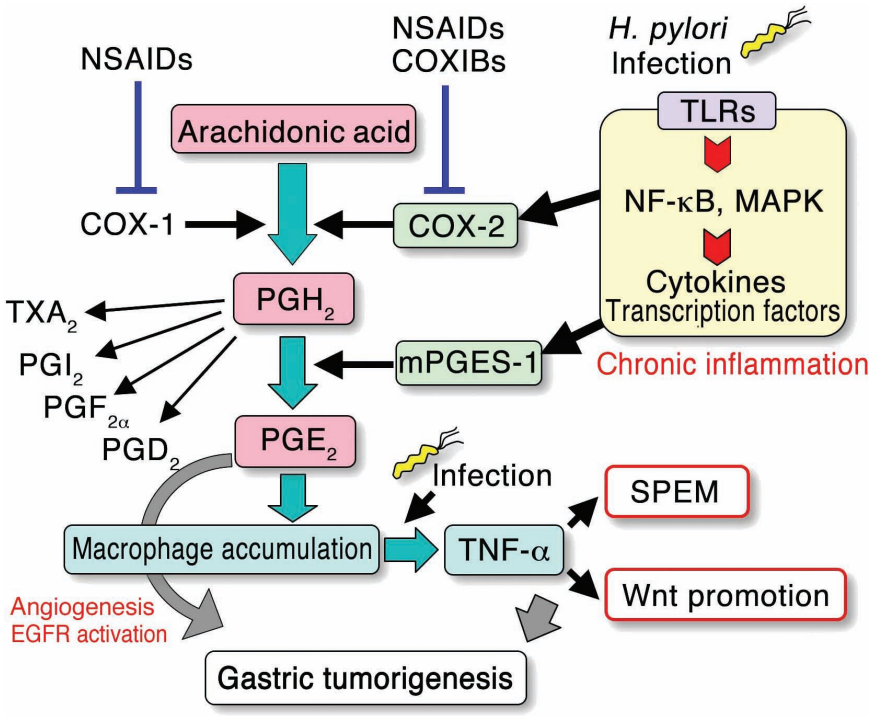

Fig. 1. Schematic presentation of arachidonic acid metabolism in the context of gastric tumorigenesis. The expression of cyclooxygenase (COX)-2 and microsomal PGE synthase-1 (mPGES-1) is induced by *Helicobacter pylori* (*H. pylori*)-associated inflammatory responses. The simultaneous expression of both COX-2 and mPGES-1 leads to induction of the prostaglandin PGE_2 pathway, which results in macrophage accumulation. These macrophages are activated by infectious stimuli, resulting in the induction of tumor necrosis factor (TNF)-α-dependent SPEM development and the promotion of Wnt signaling, which may contribute to gastric tumorigenesis. The induction of angiogenesis and activation of epidermal growth factor receptor (EGFR) signaling are also possible mechanisms of PGE_2 in tumorigenesis. COXIBs, COX-2 selective inhibitors; NF-κB, nuclear factor-κB; NSAIDS, nonsteroidal anti-inflammatory drugs; SPEM, spasmolytic polypeptide/TFF2-expressing metaplasia; TLRs, Toll-like receptors.

K19-C2mE transgenic mice: A model for PGE_2 induction in the stomach

K19-C2mE transgenic mice express both COX-2 and mPGES-1 in gastric epithelial cells, which results in induction of the PGE_2 pathway in the stomach (Fig. 2).[32] *K19-C2mE* mice develop hyperplastic lesions in the glandular stomach. Histologically, the major cell type of hyperplasia is the mucous cell, which is similar to that found in the spasmolytic polypeptide/TFF2-expressing metaplasia (SPEM; Fig. 2).[33] The development of SPEM is associated with an *H. pylori* infection and gastric adenocarcinoma, thus suggesting that SPEM is an *H. pylori*-induced precancerous lesion.[34] It is thus possible that the PGE_2 pathway induced by *H. pylori* infection is responsible for the development of SPEM. Treatment with *N*-methyl-*N*-nitrosourea (MNU) to the *H. pylori*-infected mice causes gastric tumor development. Notably, the multiplicity of gastric tumors induced by *H. pylori* infection and MNU treatment was significantly higher in *K19-C2mE* mice compared with wild-type mice.[35] These results suggest that PGE_2-induced metaplastic hyperplasia is a precursor for chemical carcinogen-induced gastric tumor.

Notably, macrophages infiltrate and are activated in the gastric mucosa of *K19-C2mE* mice.[32] The activation of these macrophages is suppressed by treatment with antibiotics, thus indicating that infectious stimuli activate the accumulated macrophages. Importantly, the development of SPEM is also suppressed by antibiotic treatment, thus suggesting that bacterial infection activates macrophages, which is required for SPEM development. Consistently, disruption of the tumor necrosis factor (*TNF*)-α gene in *K19-C2mE* mice results in the suppression of SPEM development, thus suggesting that TNF-α derived from activated macrophages plays an essential role in SPEM formation.[33] Moreover, it is conceivable that the induction of PGE_2 signaling is the primary cause for the mucosal macrophage accumulation, because the treatment of *K19-C2mE* mice with a COXIB, but not with antibiotics, inhibits macrophage infiltration. A possible mechanism for the processes from *H. pylori* infection to SPEM development through PGE_2 induction and macrophage activation is depicted in Figure 1.

Activation of Wnt signaling in gastric cancer

Canonical Wnt signaling (Wnt/β-catenin signaling) is a critical pathway in the regulation of development as well as in tumorigenesis.[36] In the absence of the Wnt ligand, cytoplasmic β-catenin is phosphorylated by GSK-3β within a complex containing adenomatous polyposis coli (APC) and Axin, thus resulting in the degradation of β-catenin through the ubiquitin proteasome pathway.[37] When Wnt ligands bind Frizzled receptors, phosphorylation of β-catenin is suppressed, leading to stabilization and nuclear translocation of β-catenin (Fig. 3). Nuclear β-catenin interacts with T-cell factor/lymphocyte enhancer factor (TCF/LEF) to induce transcription of Wnt target genes. *APC* or β-catenin mutation causes tumor development by activation of the canonical Wnt signaling.

Patients with germ-line mutations in the *APC* gene have an increased risk of gastric cancer.[38] Moreover, β-catenin accumulation, a hallmark of Wnt activation, is found in 30–50% of gastric cancers.[39,40] These results suggest that Wnt activation is one of the major causes of gastric cancer development. In gastric cancer, mutations in β-catenin are reported, while *APC* mutations are rarely detected.[41–43] However, the incidence of β-catenin mutations is less than 30% in the Wnt-activated gastric cancers,[39] thus suggesting mechanism(s) other than *APC* or β-catenin mutation for activation of the Wnt pathway. It has been suggested that the cytoplasmic β-catenin level is increased by E-cadherin downregulation or β-TrCP mutation through decrease of E-cadherin-bound membrane β-catenin or inhibition of β-catenin ubiquitination, respectively (Fig. 3).[44,45] These mechanisms may thus contribute to the activation of Wnt signaling in gastric tumorigenesis. On the other hand, the expression of the *SFRP1*, *-2*, and *-5* genes are silenced by promoter methylation in gastric cancer cells.[46] SFRPs are secreted endogenous antagonist of the Wnt ligands. Accordingly, it is possible that *SFRP* methylation is also an

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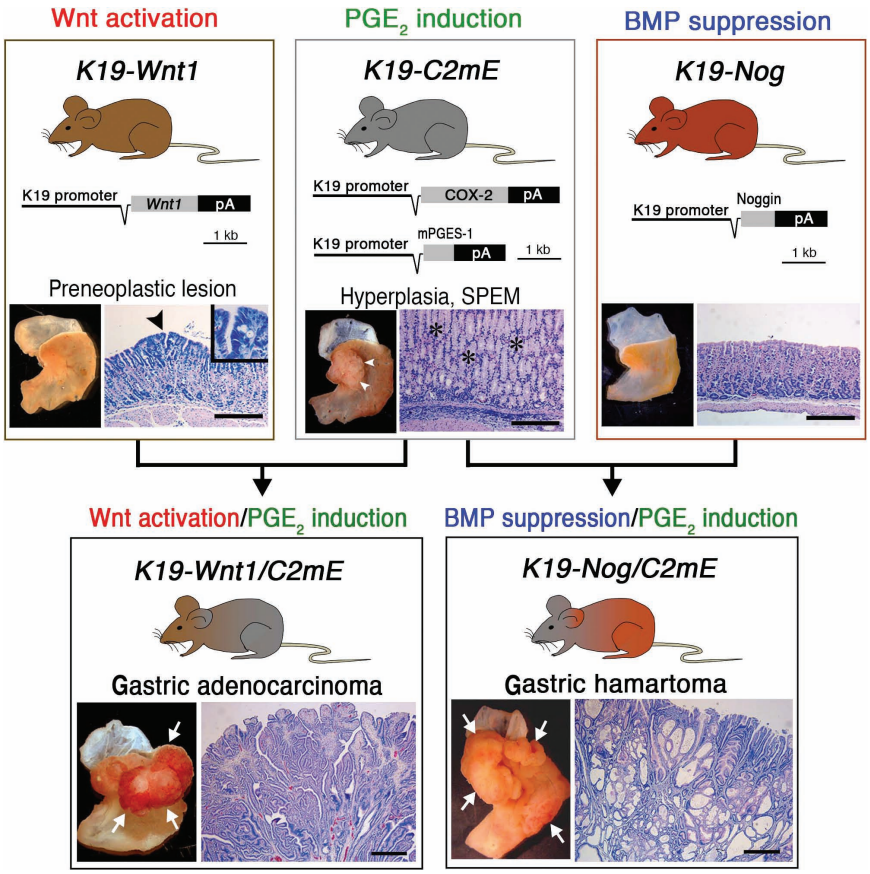

Fig. 2. Transgenic mouse models of gastric tumorigenesis. Transgenic vector construction(s) and representative macroscopic and microscopic photographs of the stomach are shown for each line. *K19-Wnt1/C2mE* and *K19-Nog/C2mE* are compound transgenic mice of *K19-Wnt1* and *K19-C2mE*, and *K19-Nog* and *K19-C2mE*, respectively. The arrowhead in the *K19-Wnt1* mouse stomach indicates a preneoplastic lesion. The arrowheads and asterisks in the *K19-C2mE* mouse stomach indicate gastric hyperplasia and mucous metaplasia (SPEM), respectively. The arrows in *K19-Wnt1/C2mE* and *K19-Nog/C2mE* indicate gastric tumors. Note that the histology of the *K19-Wnt1/C2mE* mouse shows dysplastic adenocarcinoma, while that of the *K19-Nog/C2mE* mouse shows hamartoma with dilated cystic structure. Bars indicate 100 μm. (Reproduced from Oshima *et al*. *Cancer Res*, 69: 2729–33, 2009.) BMP, bone morphogenetic protein; COX-2, cyclooxygenase-2; SPEM, spasmolytic polypeptide/TFF2-expressing metaplasia.

important mechanism for Wnt activation in gastric tumorigenesis (Fig. 3).[46]

K19-Wnt1 transgenic mice: A model for Wnt activation in the stomach

K19-Wnt1 transgenic mice express Wnt1, one of the canonical Wnt ligands, in the gastric epithelial cells, which results in the activation of Wnt signaling in the stomach (Fig. 2).[40] The number of undifferentiated epithelial cells increases in the *K19-Wnt1* mouse stomach, thus suggesting that Wnt signaling keeps gastric epithelial cells in an undifferentiated status. Small preneoplastic lesions spontaneously develop in the gastric mucosa of *K19-Wnt1* mice, which consist of dysplastic epithelial cells (Fig. 2). However, gastric tumors do not develop in *K19-Wnt1* mice. It is thus possible that the activation of Wnt signaling can trigger tumorigenesis and form small preneoplastic lesions; however, Wnt activation alone is not sufficient for tumor development (Fig. 3).

To examine the effect of the PGE_2 pathway in the Wnt-activated gastric mucosa, *K19-Wnt1* mice were crossed with *K19-C2mE* to construct compound transgenic mice (*K19-Wnt1/C2mE* mice), in which both the Wnt and PGE_2 pathways were activated in the stomach simultaneously. Importantly, *K19-Wnt1/C2mE* mice developed gastric adenocarcinoma (Fig. 2).[40] The tumors consisted of dysplastic epithelial cells, which sometimes invade the smooth muscle layers. These results clearly indicate that the simultaneous activation of the Wnt and PGE_2 pathways is responsible for the development of gastric adenocarcinomas. Importantly, the gene expression profile of *K19-Wnt1/C2mE* mouse gastric tumors is similar to that of human intestinal-type gastric cancer (Hiraku Itadani *et al*., submitted manuscript, 2009). Therefore, *K19-Wnt1/C2mE* mice recapitulate human intestinal-type gastric cancer not only in the molecular etiology, but also in the pathological and molecular characteristics of tumors.

These results of mouse model studies suggest the following possible scenario for gastric tumorigenesis (Fig. 3): *H. pylori* infection causes the induction of the COX-2/PGE_2 pathway, which thus leads to SPEM development. The activation of Wnt signaling in the normal gastric mucosa causes small preneoplastic lesions, but is not sufficient for tumor formation. When Wnt signaling is activated in the PGE_2 induction–associated inflamed mucosa, gastric adenocarcinomas develop through cooperation of the Wnt and PGE_2 pathways.

Suppression of BMP signaling in gastric tumors

Juvenile polyposis syndrome (JPS) is characterized by hereditary gastrointestinal hamartomatous polyposis,[47] a subset of which is caused by germline mutations in the BMP receptor type IA gene (*BMPR1A*).[48] BMP ligands bind to a complex of the BMP receptor type II and I, leading to phosphorylation of Smad1,5,8, thereby allowing them to form a complex with Smad4.[49,50] These Smad complexes translocate to the nuclei and function as transcription enhancers. BMP signaling inhibits epithelial proliferation and promotes differentiation.[51,52] The suppression of BMP signaling in the mouse intestine results in hamartomatous polyp development,[52,53] elongated villi, and crypt fission.[54] These results suggest that the suppression of BMP signaling causes tumorigenesis by the inhibition of epithelial cell differentiation. Although the main affected site in JPS patients is the intestine, gastric polyps also develop in JPS, and the cancer risk in JPS patients increases both in the colon and stomach.[55,56] Moreover, the expression of BMP-2 is suppressed by promoter methylation

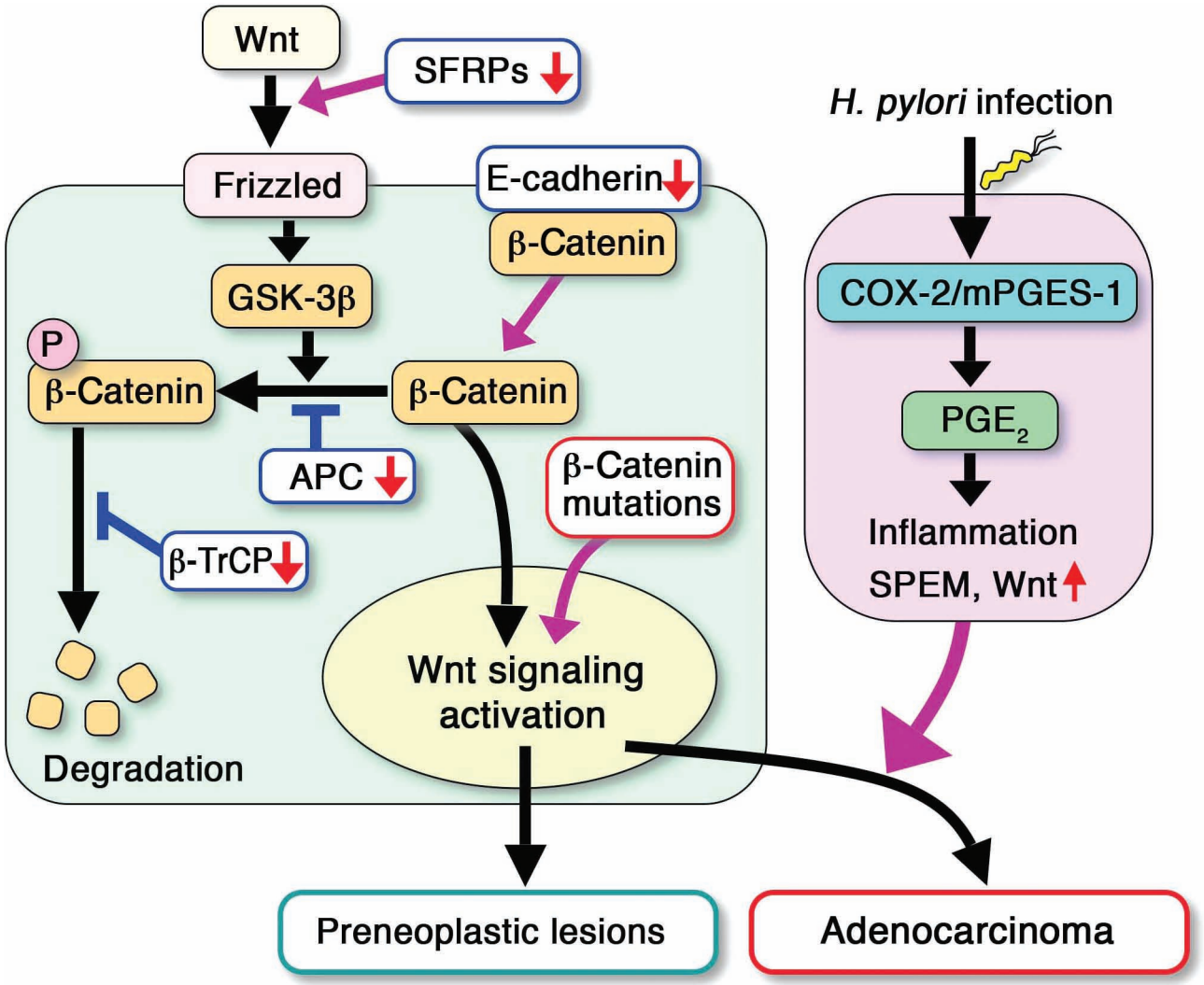

Fig. 3. Schematic presentation of the canonical Wnt signaling and cyclooxygenase (COX)-2/prostaglandin PGE_2 pathway in gastric tumor development. β-Catenin mutations, *SFRPs* methylation, and downregulation of E-cadherin or β-TrCP can activate Wnt signaling in gastric cancer. Cooperation of the *Helicobacter pylori* (*H. pylori*)-induced COX-2/PGE_2 pathway with Wnt activation leads to the development of gastric adenocarcinoma. Without the induction of the PGE_2 pathway, Wnt activation alone does not cause gastric cancer development. mPGES-1, microsomal PGE synthase-1.

in gastric cancer cells,[57] and stimulation of gastric cancer cells with BMP-2 suppresses proliferation.[58] These results suggest that the inhibition of BMP signaling contributes to gastric tumorigenesis through the suppression of differentiation.

K19-Nog transgenic mice: A model for BMP suppression in the stomach

K19-Nog mice express noggin, an endogenous BMP antagonist, in the gastric epithelial cells, thus resulting in the inhibition of BMP signaling in the stomach (Fig. 2).[59] Noggin is a polypeptide that inhibits BMP signaling by binding the BMP ligands.[50] In the *K19-Nog* mice, the phosphorylation of Smad1,5,8 is suppressed in the gastric gland by BMP inhibition. However, *K19-Nog* mice do not develop gastric lesions, and the histology of the gastric mucosa is normal (Fig. 2). To examine the effect of cooperation of BMP suppression and PGE_2 induction, *K19-Nog* mice were crossed with *K19-C2mE* to construct compound transgenic mice (*K19-Nog/C2mE* mice), in which BMP signaling is suppressed and the PGE_2 pathway is induced in the gastric mucosa. Importantly, *K19-Nog/C2mE* mice develop large tumors in the glandular stomach (Fig. 2). These results indicate that the suppression of BMP signaling is insufficient for gastric tumorigenesis; however, the induction of the PGE_2 pathway does promote tumor formation in the BMP-suppressed gastric mucosa.

Histologically, *K19-Nog/C2mE* mouse tumors are not dysplastic, but consist of irregular branching of epithelial cell layers, combined with dilated cysts (Fig. 2). Such histological characteristics are distinct from adenocarcinomas of *K19-Wnt1/C2mE* mice, but are typical of the hamartomas of JPS patients.[55,56,60] These results indicate that the suppression of BMP signaling associated with PGE_2 induction causes gastric hamartoma development. Accordingly, it is possible that types of genetic alterations determine the histological types of tumors, e.g. adenocarcinoma by Wnt activation or hamartoma by BMP suppression. Furthermore, the induction of the PGE_2 pathway promotes tumor formation regardless of the histological types (Fig. 2). Accordingly, it is thus possible that *H. pylori* infection contributes to development of both types of gastric tumors through PGE_2 induction.

It has been reported that BMP signaling negatively regulates Wnt signaling in the intestinal crypt.[53] Namely, suppression of BMP signaling enhances Wnt activity through the activation of PI3K/Akt pathway. However, the β-catenin level in the *K19-Nog/C2mE* hamartomas is the same as that in the wild-type

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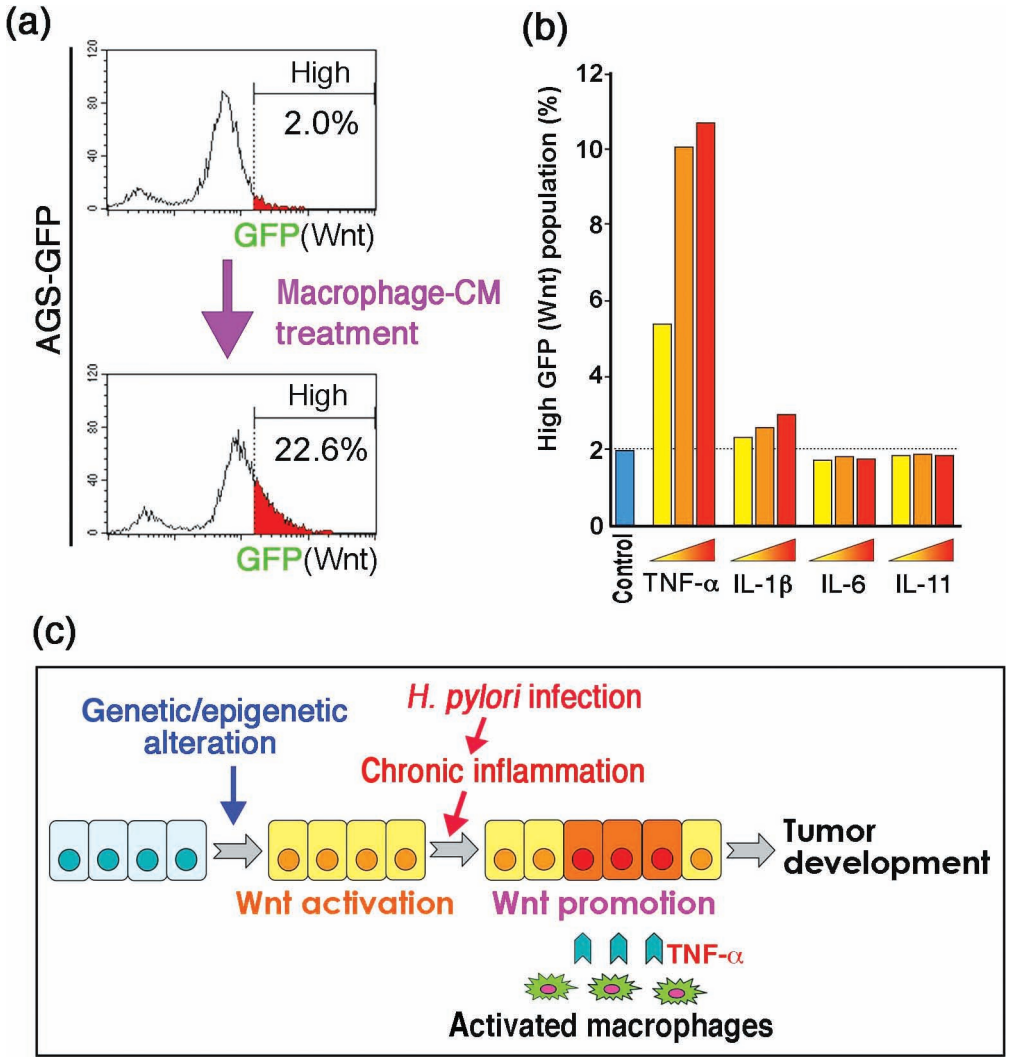

Fig. 4. Promotion of Wnt signaling by macrophage-derived tumor necrosis factor (TNF)-α in gastric cancer cells. (a) Representative FACS analyses of Wnt-reporter gastric cancer cells, AGS-GFP, in which GFP expression is regulated by β-catenin/TCF. GFP intensity increased significantly when cells were treated with conditioned medium (CM) from activated macrophages. (b) GFP intensity of reporter cells treated with indicated cytokines are shown in the bar graph. Note that Wnt activity is elevated by treatment with TNF-α in a dose-dependent manner. (c) Hypothesis for gastric tumor development. The level of Wnt signaling activated by genetic/epigenetic alteration is not sufficient for tumorigenesis. However, *Helicobacter pylori* (*H. pylori*) infection-induced inflammation promotes the Wnt activation level through macrophage-derived TNF-α, which contributes to gastric tumorigenesis. (a and b, reproduced from Oguma *et al*. *EMBO J*, 27: 1671–81, 2008, with permission from the Nature Publishing Group.)

mouse stomach, while it is markedly elevated in *K19-Wnt1/C2mE* gastric tumors.[59] These results indicate that gastric hamartomas develop in *K19-Nog/C2mE* mice due to a Wnt-activation independent mechanism.

Possible mechanisms of the PGE_2 pathway in gastric tumorigenesis

There are four G protein-coupled receptors for PGE_2, EP1–EP4. Among these receptors, the expression of EP4 increased significantly in gastric tumors of both *K19-Wnt1/C2mE* mice and *K19-Nog/C2mE* mice.[59] It is thus possible that PGE_2 signaling through EP4 plays a role in the development of adenocarcinoma and hamartoma in the Wnt-activated and BMP-suppressed gastric mucosa, respectively.

The promotion of the Wnt signaling activity beyond the basal activation level may be important for malignant progression.[61] For example, increased accumulation of β-catenin is found in the invasion front of colon cancer, suggesting that the increased Wnt activation level contributes to tumor invasion.[62] Hepatocyte growth factor (HGF) and platelet-derived growth factor (PDGF) increase the Wnt signaling activity in colon cancer cells, suggesting that these factors function as Wnt promoters.[63,64] Notably, the level of Wnt signaling activity in gastric cancer cells significantly increases when the cells are stimulated with a conditioned medium from activated macrophages (Fig. 4a).[65] Moreover, TNF-α, but not other proinflammatory cytokines, caused an increase in the Wnt signaling activity in gastric cancer cells (Fig. 4b).[65] These results suggest the hypothesis that Wnt activation, due to either genetic or epigenetic alterations in normal epithelial cells, is not sufficient for gastric tumor development. However, the Wnt activation level increases further in the inflamed mucosa by macrophage-derived TNF-α, and such Wnt promotion contributes to gastric cancer development (Fig. 4c). The induction of the PGE_2 pathway leads to macrophage accumulation in the gastric mucosa. It is therefore conceivable that the induction of Wnt promotion is one of the important mechanisms of the PGE_2 pathway in gastric tumorigenesis (Fig. 1).

Conclusions

Studies with mouse models have elucidated the roles of the PGE_2 pathway in gastric tumorigenesis in the Wnt-activated and BMP-suppressed gastric mucosa. Alterations in morphogen signals, such as the Wnt and BMP pathways, can therefore trigger gastric tumorigenesis by the suppression of epithelial differentiation. However, alterations of these signals in the non-inflamed stomach do not cause gastric tumor formation. In contrast, alterations of these signals in the inflamed gastric mucosa lead to the development of gastric tumors through cooperation with the PGE_2 pathway. Moreover, mouse studies show the possible mechanisms of PGE_2 in gastric tumorigenesis, i.e. macrophage accumulation and activation, subsequent SPEM formation, and Wnt signaling promotion. Considering the multifunctional nature of PGE_2, it is possible that other mechanisms triggered by the PGE_2 pathway may also contribute to gastric tumorigenesis, which should be further elucidated using mouse models in the future. These studies will provide a rationale for the inhibition of the PGE_2 pathway as a possible preventative strategy against gastric tumorigenesis.

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