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Abstract

Accumulating evidence has suggested that cooperation of oncogenic activation and the host responses is important for cancer development. In gastric cancer, activation of Wnt signaling appears to be a major oncogenic pathway that causes tumorigenesis. In the chronic gastritis caused by *Helicobacter pylori* infection, cyclooxigenase-2 induces prostaglandin E_2 (PGE_2) biosythesis, which plays an important role in tumorigenesis. We constructed a series of mouse models and investigated the role of each pathway in the gastric tumorigenesis. Wnt activation in gastric epithelial cells suppresses differentiation, and induces development of preneoplastic lesions. On the other hand, induction of the PGE_2 pathway in gastric mucosa induces development of spasmolytic polypeptide-expressing metaplasia (SPEM), which is a possible preneoplastic metaplasia. Importantly, simultaneous activation of Wnt and PGE_2 pathways leads to dysplastic gastric tumor development. Moreover, induction of the PGE_2 pathway also promotes gastric hamartoma development when bone morphogenetic protein (BMP) signaling is suppressed. These results indicate that alteration in the Wnt or BMP signaling impairs epithelial differentiation, and the PGE_2 pathway accelerates tumor formation regardless of the types of oncogenic pathways. We review the phenotypes and gene expression profiles of the respective models, and discuss the cooperation of oncogenic pathways and host responses in gastric tumorigenesis.

Key words: gastric cancer, mouse model, Wnt, PGE_2

Gastric cancer is the second most common cause of cancer-related death worldwide.[1] Infection with *Helicobacter pylori* is associated with gastric cancer development, and the International Agency for Research on Cancer (IARC) classified *Helicobacter pylori* as a class I carcinogen.[2] Accumulating evidence has indicated that chronic inflammatory response associated with infectious disease is a critical component of tumor development.[3] Moreover, it has been shown that infections are responsible for more than 15% of all malignant cancers worldwide, including the association between *H. pylori* infection and gastric cancer.[4] Notably, host genetic variants in cytokine genes are related to responsiveness to *H. pylori* infection and the susceptibility to gastric cancer development.[5-7] Specific polymorphisms of interleukin (IL)-1β, an important inflammatory cytokine and a potent inhibitor of gastric acid secretion, contribute to intestinal-type gastric cancer progression.[8] Polymorphisms in tumor necrosis factor (TNF)-α, IL-1 receptor antagonist, and IL-10 also influence gastric cancer development,[8-10] while polymorphisms in the IL-8 promoter have been linked to diffuse-type gastric cancer.[11] These results suggest that the response of the host cytokine network to *H. pylori* infection is an important factor for gastric cancer development.

On the other hand, several somatic alterations that activate oncogenic pathways have been identified in human gastric cancer. For example, allelic loss or mutations in p53 are detected in 60% or 30-50% of gastric cancers, respectively,[12] while mutations in the β-catenin gene is detected in 30% of the Wnt-activated subgroup of gastric cancer.[13] TGF-β type II receptor gene is recognized as a tumor suppressor, and mutations have been found in gastric cancer associated with microsatellite instability (MSI).[14,15] Moreover, about 15% of gastric cancers show expression of both epidermal growth factor (EGF) and EGF receptor (EGFR),

suggesting activation of the EGFR signaling pathway.[16] Taken together, these results indicate that both infection-associated inflammatory responses and oncogenic activation by genetic alterations are required for gastric cancer development.

To date, many genetic mouse models have recapitulated some of the developmental stages associated with intestinal-type gastric cancer, such as gastritis, atrophy, mucous cell metaplasia, dysplasia, and invasion.[17] These models are useful for examining the phenotypic changes caused by individual genetic alterations. In addition to these models, we constructed a series of transgenic mouse models to investigate the role of oncogenic and inflammatory pathways, such as Wnt, BMP, and PGE_2 signaling, in gastric epithelial differentiation, inflammation, and tumor development.

Activation of Wnt signaling in gastric mucosa

Wnt activation in human gastric cancer

Canonical Wnt signaling (Wnt/β-catenin signaling) is a critical pathway in the regulation of development as well as in tumorigenesis.[18] When Wnt signaling is in a resting state, cytoplasmic β-catenin is phosphorylated by GSK-3β within a complex containing APC and Axin, resulting in the degradation of β-catenin through the ubiquitin proteasome pathway.[19] When the pathway is activated, the binding of Wnt ligands to Frizzled receptors leads to the suppression of the phosphorylation of β-catenin, resulting in the to stabilization and nuclear translocation of β-catenin. β-Catenin then interacts with T-cell factor/lymphocyte enhancer factor (TCF/LEF) to induce transcription of Wnt target genes. In the normal intestine, Wnt

signaling is important for maintaining the stem cell characteristics and undifferentiated status of the epithelial cells, whereas Wnt signaling is suppressed in the differentiated epithelia (**Fig. 1**)[19-21] Mutations in the *APC* or β-catenin genes constitutively activate Wnt signaling, which causes tumor development in the intestine.[22,23] In the gastric mucosa, epithelial cells expressing Lgr5, which is a target of Wnt signaling, show stem cell phenotypes, confirming the role of Wnt pathway in normal gastric stem cells.[24] Moreover, nuclear accumulation of β-catenin, a hallmark of Wnt signaling activation, is found in 30-50% of gastric cancers (**Fig. 2**),[13,25-27] and mutations in the β-catenin gene have also been detected,[13,25,28,29] which suggest that activation of Wnt signaling is a major cause of gastric cancer development. However, *APC* gene mutations are not common in gastric cancer, and β-catenin mutations are present in fewer than 30% of the Wnt-activated gastric cancers.[25] Accordingly, it is possible that other mechanism(s) may also activate Wnt signaling in gastric cancer. For example, it has been reported in gastric cancer cells that downregulation of E-cadherin is associated with β-catenin accumulation in gastric cancer,[30] somatic mutations in the ubiquitin ligase β-TrCP causes stabilization of β-catenin,[31] and the expression of the *SFRP1, 2* and *5* genes encoding the secreted endogenous antagonist of the Wnt ligands is silenced by promoter methylation in gastric cancer cells.[32] All of these alterations contribute to activation of Wnt signaling.

Gastric preneoplastic lesions in *K19-Wnt1* transgenic mice

To examine the role of Wnt signaling in gastric tumorigenesis, we constructed *K19-Wnt1* transgenic mice that express *Wnt1*, one of the canonical Wnt ligands.[27] The *K19* gene promoter was used to express *Wnt1* in gastric epithelial cells, including undifferentiated

isthmal cells.[33,34] The number of undifferentiated epithelial cells that express trefoil factor 2 (TFF2) increases significantly in the *K19-Wnt1* mouse glandular stomach, indicating that Wnt signaling functions to maintain the undifferentiated status of the gastric epithelial cells (**Fig. 3a, b**). Notably, aberrant cryptic foci were found on the mucosal surface of *K19-Wnt1* mice, which consist of dysplastic epithelial cells with irregular branching, and increased cell proliferation and β-catenin accumulation were also detected (**Fig. 3c-g**). We thus diagnosed these foci as preneoplastic lesions. However, gastric tumors do not develop in *K19-Wnt1* mice, indicating that activation of Wnt signaling alone is not sufficient for gastric tumor formation. Importantly, macrophages were infiltrated into the preneoplastic lesions, whereas tissue macrophages were sparsely scattered in the normal mucosa of the same mice. It is possible that local inflammatory responses caused by spontaneous physical insult or infection might promote proliferation of Wnt-activated dysplastic cells, resulting in formation of the preneoplastic lesions.

Induction of PGE_2 pathway in gastric mucosa

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

Epidemiological studies indicate that the regular use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a decreased incidence of gastric cancer.[35-38] NSAIDs inhibit the enzymatic activity of cyclooxygenases (COXs), which are rate-limiting enzymes for prostaglandin biosynthesis. COX enzymes catalyze synthesis of prostaglandin (PG)H_2, which is subsequently converted to various prostanoids, including PGE_2, by tissue-specific

converting enzymes. There are two COX isozymes, COX-1 and COX-2, which share a high degree of structural and enzymatic homology. COX-1 is constitutively expressed in most tissues and is considered to be responsible for maintaining physiological levels of prostaglandin biosynthesis,[39] while COX-2 expression is induced by inflammation and in tumor tissues by cytokines and growth factors.[40-42] Induction of COX-2 is found in approximately 70% of gastric cancer, predominantly in intestinal-type gastric cancer, whereas COX-1 expression is not elevated.[43-45] Moreover, the level of COX-2 expression in gastric cancer correlates with the tumor size, depth of invasion and lymph-node metastasis.[46-48] Microsomal PGE synthase-1 (mPGES-1) is an inducible enzyme that converts PGE_2 from PGH_2, and is functionally coupled with COX-2.[49] Simultaneous induction of COX-2 and mPGES-1 has been observed in a variety of cancers, including gastric cancer, suggesting that the inflammatory PGE_2 pathway is induced in these tumors (**Fig. 1**).[50-53] Consistently, the PGE_2 level is found to be significantly increased in gastric cancer[47], and the COX-2 and PGE_2 level is associated with the *H. pylori* infection status,[54,55] indicating that *H. pylori* infection causes induction of the PGE_2 pathway (**Fig. 1**). These results suggest that the COX-2/PGE_2 pathway plays a key role in *H. pylori* infection-associated inflammation in gastric cancer development.

Suppression of gastric cancer by COX-2 inhibition in animal models

Suppression of gastric tumorigenesis by treatment with COX-2 selective inhibitors (COXIBs) has been examined in several animal model experiments. Growth of gastric cancer cell xenografts was inhibited by treatment with COXIBs in immunodeficient mice.[56,57] Rat

gastric cancer induced by carcinogen *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) and mouse gastric tumors induced by *H. pylori* infection or a combination of *H. pylori* with *N*-methyl-*N*-nitrosourea (MNU) were suppressed by treatment with NSAIDs or COXIBs.[58-60] A significant decrease in the PGE_2 level and substantial induction of apoptosis were also found in the tumors of mice treated with NSAIDs or COXIBs. *H. pylori* infection of Mongolian gerbils is an established model to study gastric tumorigenesis by *H. pylori*. Treatment of the *H. pylori*-infected Mongolian gerbils with COXIBs suppressed the development of gastric cancer, as well as intestinal metaplasia.[61,62], These animal studies indicate that the COX-2 pathway plays a key role in gastric tumorigenesis.

Gastric metaplasia in *K19-C2mE* transgenic mice

To examine the effect of PGE_2 pathway activation in the gastric mucosa, we constructed another transgenic mouse model, *K19-C2mE*, which expresses both COX-2 and mPGES-1 simultaneously in gastric epithelial cells.[34] *K19-C2mE* mice developed hyperplasia in the gastric mucosa, which was suppressed by treatment of the mice with a COX-2 selective inhibitor, NS-398 or meloxicam (**Fig. 4a, b**).[34,63] Histologically, the major cell type involved in the hyperplasia were mucous cells, which was similar to the findings in the spasmolytic polypeptide/TFF2-expressing metaplasia or SPEM (**Fig. 4c, d**).[34,64] SPEM is characterized by the presence of TFF2 immunoreactive cells, which is morphologically similar to those of Brunner's gland. Since SPEM was associated with greater than 90% of the resected gastric cancers analyzed in three studies,[64-66] it is suggested that SPEM is a putative preneoplastic metaplasia in the stomach.[67] In other animal models, SPEM

development was also found in the stomach of *Helicobacter*-infected mice, gastrin gene knockout mice, and in Stat3-activating gp130 mutant mice.[68-70] Notably, SPEM in these mouse models was accompanied by inflammatory responses. Importantly, disruption of the TNF-α gene in *K19-C2mE* mice results in the suppression of inflammatory responses and SPEM development, suggesting that TNF-α-associated inflammation plays an essential role in SPEM formation (**Fig. 4e-g**).[63] In contrast, *Rag2* gene disruption did not suppress SPEM formation in the *K19-C2mE* mice, indicating that acquired immune responses are not involved in SPEM development.

Treatment with MNU to the *H. pylori*-infected mice led to the development of gastric tumors. Notably, multiplicity of gastric tumors induced by *H. pylori* infection and MNU treatment was significantly higher in *K19-C2mE* mice compared with wild-type mice.[71] These results suggest that PGE_2-induced SPEM is a precursor for chemical carcinogen-induced gastric tumors.

Oncogenic activation and PGE_2 pathway in gastric tumorigenesis

Gastric dysplastic tumors in *K19-Wnt1/C2mE* transgenic mice

Compound *K19-Wnt1/C2mE* transgenic mice were generated by crossing *K19-Wnt1* mice and *K19-C2mE* mice, in which both Wnt and PGE_2 pathways are activated simultaneously in the gastric mucosa.[27] *K19-Wnt1C2mE* mice develop large gastric tumors in the glandular stomach (**Fig. 5a, b**), while no such tumors are found in either simple *K19-Wnt1* or *K19-C2mE* transgenic mice. Histologically, gastric tumors in the *K19-Wnt1/C2mE* mice

(hereafter *Gan* mice for gastric neoplasia) consist of dysplastic epithelial cells with nuclear stratification and irregularly branched tubules (**Fig. 5c**). Tumor epithelial cells showed increased nuclear accumulation of β-catenin and increased Ki-67 labeling, indicating promotion of Wnt activity and an increased proliferation of tumor cells, respectively (**Fig. 5e**). Increased capillary vessels were also found in the tumor stroma, which was caused by enhanced angiogenesis through the tumor-stroma interaction (**Fig. 5f, g**).[72] Interestingly, part of the activated stromal fibroblasts in *Gan* mouse tumors were derived from bone marrow, thus suggesting that the bone marrow cells contribute to the formation of the gastric cancer microenvironment.[72]

Notably, TFF2-expressing SPEM was found adjacent to the dysplastic tumor tissue,[27] showing a similar histology to human gastric cancer (**Fig. 5h**). *K19-C2mE* mice start to develop SPEM lesions at 5 weeks of age, and the number of TFF-positive metaplastic cells increases with age. In the *Gan* mouse stomach, the same SPEM phenotype is found at 5 weeks of age, however, dysplastic tumor cells are also found beginning at 10 weeks of age. The number of dysplastic tumor cells then increases with age, leading to formation of gastric tumors around 20-30 weeks of age, with SPEM being found adjacent to the tumors. These results, taken together, indicate that the simultaneous activation of the Wnt and PGE_2 pathways causes gastric tumor development through the metaplasia (SPEM)-carcinoma sequence.

Gastric hamartomas in *K19-Nog/C2mE* transgenic mice

Juvenile polyposis syndrome (JPS) is characterized by hereditary gastrointestinal

hamartomatous polyposis,[73] and a subset of JPS is caused by germline mutations in the BMP receptor type IA gene (*BMPR1A*).[74] BMP signaling through its type I and II receptors leads to the phosphorylation of Smad 1,5, and 8, resulting in formation of a complex with Smad4, which induces transcription of target genes.[75] BMP signaling inhibits epithelial cell proliferation and promotes differentiation (**Fig. 1**), and suppression of BMP signaling in the mouse intestine results in JPS-type hamartomatous polyp development,[76-78] elongated villi and crypt fission.[79] Accordingly, it is possible that BMP suppression results in hamartoma formation by impairment of epithelial differentiation. Since the cancer risk in JPS patients increases in the gastrointestinal tract,[80,81] BMP suppression may also contribute to gastric cancer development.

To examine the effect of BMP suppression in gastric epithelial cells, we next constructed *K19-Nog* mice that express noggin, an endogenous BMP antagonist, in the gastric epithelial cells.[82] Although BMP signaling was suppressed in the stomach, *K19-Nog* mice do not develop any gastric lesions, and the histology of the gastric mucosa was normal. To examine the effect of cooperation of BMP suppression and PGE_2 induction, *K19-Nog* mice and *K19-C2mE* mice were crossed to construct compound *K19-Nog/C2mE* mice, in which BMP signaling is suppressed and the PGE_2 pathway is induced in the gastric mucosa. Importantly, the *K19-Nog/C2mE* mice developed large tumors in the glandular stomach (**Fig. 6a**), suggesting that induction of the PGE_2 pathway is required for tumor formation in the BMP-suppressed gastric mucosa. Histologically, *K19-Nog/C2mE* mouse gastric tumors are not dysplastic, but consist of irregular branching of the epithelial cell layers, combined with the formation of dilated cysts filled with mucin (**Fig. 6b, c**). Such histological

characteristics are distinct from the dysplastic gastric tumors of *Gan* mice (**Fig. 5c**), but are typical of the hamartomas of JPS patients.[80,81,83] These results indicate that the suppression of BMP signaling associated with PGE_2 induction causes gastric hamartoma development.

These results of compound mutant mice indicate that the type of genetic alteration, such as Wnt activation or BMP suppression, determines the histological type of tumors, such as adenocarcinoma or hamrtoma.[84] On the other hand, induction of the PGE_2 pathway promotes gastric tumor formation regardless of the genetic or histological types.

Gene expression profiles of mouse models and human gastric cancer

Gan mice and *K19-Nog/C2mE* mice develop gastric tumors caused by genetic alterations similar to those found in human gastric cancer and hamartomas, respectively. However, it is still important to compare gene expression profiles of mouse tumors with those of human cancer in order to examine whether these models really recapitulate human gastric tumors. We have measured mRNA expression levels using the Affymetrix GeneChip system, which includes 21,066 Entrez genes and 5,324 other sequences.[85] Genome-scale overview of the microarray data revealed that expression changes in the three models, *K19-C2mE* and *K19-Wnt1/C2mE* (*Gan*), and *K19-Nog/C2mE* mice are quite similar, whereas over-expression of *Wnt1* or *Noggin* in *K19-Wnt1* or *K19-Nog* mice, respectively, showed expression changes in a small portion of genes (**Fig. 7a**). These results suggest that most of the expression changes in *Gan* gastric tumors and *K19-Nog/C2mE* hamartomas are caused by induction of PGE_2 pathway, rather than by Wnt activation or BMP suppression. In other words, a small number of genes that are upregulated or downregulated by Wnt activation or BMP

suppression are important for determining the tumor phenotype.

Gene expression signatures of human gastric cancer[86] and breast cancer[87] retrieved from the Stanford Microarray Database,[88] colon cancer from the NCBI GEO (accession GSE5206), and lung tumors[90] retrieved from the United States National Cancer Institute website[91] can be plotted to distinct areas in a 3D figure based on the calculations of principal component analysis using the selected genes (**Fig. 7b**). Importantly, expression signatures of *Gan*, *K19-C2mE*, and *K19-Nog/C2mE* mice are clustered in a similar area as that of human gastric cancer, but not to the same area as cancers of other organs. These results indicate that *Gan* mouse tumors recapitulate human gastric cancer from the molecular etiology to histology and gene expression profiles. It is also possible that most of the changes in gene expression in human gastric cancer are attributable to *H. pylori* infection-associated inflammatory responses. Taken together, these results indicate that the *Gan* mouse model is a useful tool for studying the effects of oncogenic activation and inflammatory responses in human gastric cancer development and the evaluation of anti-gastric cancer drugs.

CONCLUSION

Wnt signaling functions to maintain the undifferentiated status of gastric epithelial cells. Activation of Wnt signaling by genetic or epigenetic alteration causes development of preneoplastic lesions. On the other hand, *H. pylori* infection induces expression of COX-2 and mPGES-1, resulting in induction of PGE_2 biosynthesis. Induction of the COX-2/PGE_2 pathway is responsible for SPEM development, which is a possible preneoplastic metaplasia of gastric cancer. Importantly, simultaneous induction of Wnt and PGE_2 pathways causes

development of dysplastic gastric tumors (**Fig. 1**). The results of mouse model studies reported herein suggest that oncogenic activation, such as activation of Wnt signaling by genetic or epigenetic alterations, triggers tumor initiation. However, initiated epithelial cells cannot continue proliferation in the non-inflamed gastric mucosa, thus indicating that Wnt signaling alone is not sufficient for tumor development. In contrast, if the oncogenic pathway is activated in the *H. pylori*-infected (and thus inflamed) stomach, the initiated cells proliferate to develop gastric cancer. Considering the relatively low frequency of somatic oncogenic activation, this hypothesis is consistent with the epidemiology of gastric cancer, in that only a small minority of the *H. pylori*-infected population develops gastric cancer, although *H. pylori* infection is an important risk factor for gastric cancer. It is possible that a similar mechanism underlies the development of hamartoma in the BMP-suppressed gastric epithelial cells (**Fig. 1**). Further studies using these mouse models will be useful for elucidating the role of oncogenic activation and host responses in gastric tumorigenesis at the molecular level.

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Figure legends

Figure 1 Schematic presentation of the connection of Wnt, BMP, and COX-2/PGE_2 signaling pathways in gastric tumorigenesis. "Wnt activation" or "BMP suppression" causes suppression of epithelial differentiation, which leads to transformation of epithelial cells. On the other hand, *H. pylori* infection induces expression of COX-2 and mPGES-1, resulting in activation of PGE_2 signaling pathway. PGE_2 pathway accelerates proliferation of the transformed cells through formation of tumor microenvironment.

Figure 2 Nuclear localization of β-catenin in human gastric cancer. (**a**) Representative histological sections of gastric cancer (H&E). (**b**) Immunostaining for unphosphorylated (active) β-catenin in the adjoining section of (*a*) showing nuclear β-catenin accumulation. (**c**) Total β-catenin immunostaining in the normal stomach of the same patient showing β-catenin localization on the cell membrane. Scale bars, 200 μm. (Reproduced from Oshima *et al*, *Gastroenterology* 2006; 131: 1086-95 with permission from Elsevier)

Figure 3 Gastric preneoplastic lesions in the *K19-Wnt1* mouse stomach. (**a**, **b**) TFF2-expressing undifferentiated epithelial cells (asterisks) in the glandular stomach of wild-type (*a*) and *K19-Wnt1* mice (*b*). Note that the number of TFF2 positive cells was increased in the *K19-Wnt1*mouse. Scale bars, 200 μm. (**c**, **d**) Toluisdine blue staining of the whole glandular stomach of wild-type (*c*) and *K19-Wnt1* mice (*d*). Arrows in (*d*) indicate preneoplastic lesions. Scale bars in (*c*, *d*), 0.5 mm. (**e**-**g**) Histology of preneoplastic lesion (H&E) (*e*), and Ki-67 staining (*f*) and β-catenin immunostaining (*g*) of

serial sections. Arrowheads indicate dysplastic epithelial cells. Scale bars in (*e*-*g*), 100 μm. (Reproduced from Oshima *et al*, *Gastroenterology* 2006; 131: 1086-95 with permission from Elsevier)

Figure 4 SPEM development in the *K19-C2mE* mouse stomach. (**a**, **b**) Representative photographs of the gastric mucosa of a meloxicam-treated *K19-C2mE* mouse (*a*) and a no-drug control *K19-C2mE* mouse (*b*). Arrows in (*b*) indicate hyperplastic lesions. (**c**, **d**) *Helix pometia* lectin staining (*c*) and TFF2 *in situ* hybridization (*d*) of *K19-C2mE* mouse stomach showing expansion of mucous cells expressing TFF2. Scale bars in (*c*, *d*), 200 μm. (**e**-**g**) Suppression of SPEM development in *K19-C2mE* mice by TNF-α gene disruption (*e*) but not by Rag2 knockout (*f*). Asterisks and arrows in (*f*) indicate SPEM and submucosal inflammatory infiltration, respectively. Bar graph indicates the mean mucosal thickness of the respective genotypes of mice (*g*). Asterisks, $P < 0.05$. (Reproduced from Oshima M *et al*, *Cancer Res* 2005; 65: 9147-51 with permission from American Association for Cancer Research, and Oshima H *et al*, *EMBO J* 2004; 23: 1669-78 with permission from Nature publishing group)

Figure 5 Gastric dysplastic tumors developed in the *K19-Wnt1/C2mE* (*Gan*) mice. (**a**, **b**) Representative photographs of the gastric mucosa of a *Gan* mouse (*a*) and a wild-type littermate mouse (*b*). Arrowheads indicate gastric tumors. (**c**-**e**) Histology of *Gan* mouse gastric tumors (H&E) (*c*), and β-catenin immunostaining (*d*) and Ki-67 staining (*e*) of the serial sections. Scale bars in (*c*-*e*), 100 μm. (**f**, **g**) Immunostaining for capillary vessels

(arrowheads) using anti-vWF antibody in a *Gan* mouse tumor (*f*) and a wild-type mouse stomach (*g*). Note that the number of vessels increased significantly in *Gan* mouse tumor. Scale bars in (*f*, *g*), 50 μm. (**h**) *In situ* hybridization for TFF2 to detect SPEM (arrows) adjacent to the dysplastic tumor area in the *Gan* mouse stomach. Scale bars in (*h*), 200 μm. (Reproduced from Oshima *et al*, *Gastroenterology* 2006; 131: 1086-95 with permission from Elsevier, and Guo *et al*, *J Biol Chem* 2008; 283: 19864-71)

Figure 6 Gastric hamartomas developed in the *K19-Nog/C2mE* mice. (**a**) Representative photograph of a *K19-Nog/C2mE* mouse gastric tumor (arrows). (**b**) Histology of a *K19-Nog/C2mE* gastric tumor showing a hamartomatous cystic structure (H&E). (**c**) PAS-Alcian blue staining of a *K19-Nog/C2mE* tumor showing a mucin-containing cystic structure. Scale bars in (*b*, *c*), 200 μm and 100 μm, respectively. (Reproduced from Oshima H *et al*, *Cancer Res* 2009; 69: 2729-33 with permission from American Association for Cancer Research)

Figure 7 Genome-scale expression pattern of the respective genotype mouse models. (**a**) Clustered in rows are 5,440 probe sets selected by genes whose expression levels were over 2-fold greater than the average of wild-type mice. Genotypes of mice are shown on the top. The red-green color scale indicates the log10 ratio of the average of wild-type samples. Red indicates “upregulated”, whereas green indicates “downregulated”. (**b**) The overall gene expression of human gastric (blue), colon (light blue), breast (green), and lung (red) cancers, and *K19-C2mE*, *K19-Wnt1/C2mE* (*Gan*), *K19-Nog/C2mE* mouse stomach (magenda). The

3D figure was plotted by principal component 1 to 3 calculated using 1,925 genes which were altered by more than 2-fold in more than 50 samples. (Reproduced from Itadani H *et al*, *BMC Genomics* 2009; 10: 615)

Figure 1

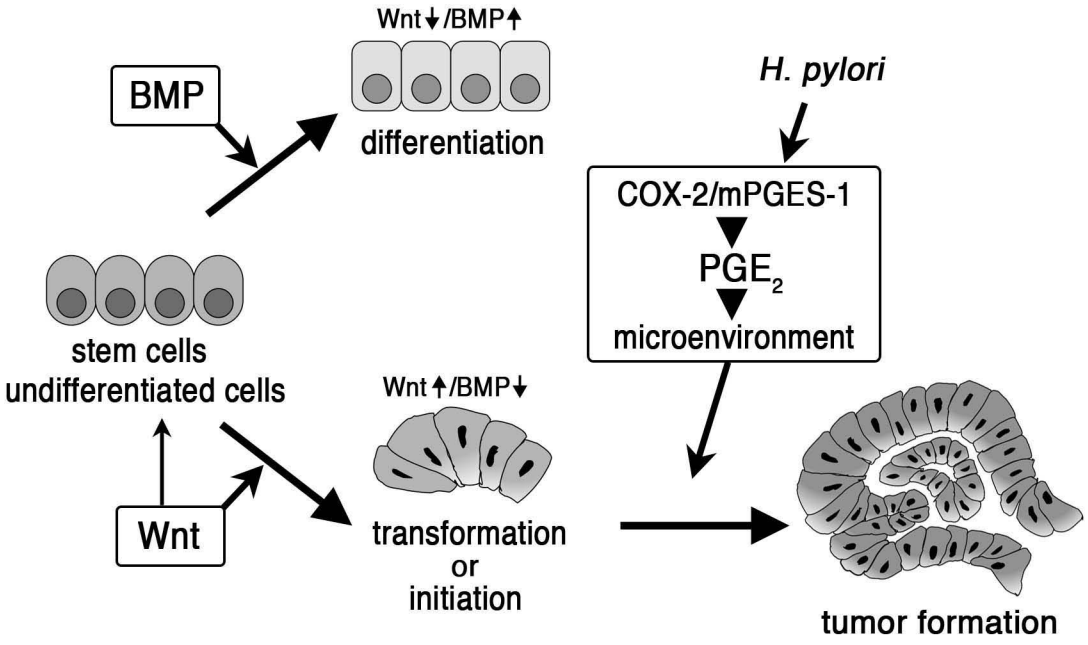

Wnt↓/BMP↑
BMP
differentiation
H. pylori
COX-2/mPGES-1
PGE2
microenvironment
stem cells
undifferentiated cells
Wnt↑/BMP↓
Wnt
transformation
or
initiation
tumor formation

Figure 2

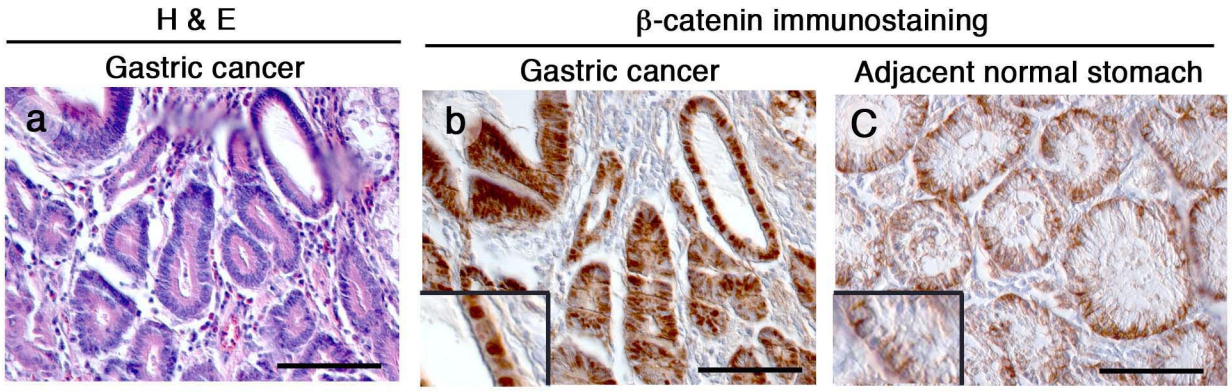

H & E
β-catenin immunostaining
Gastric cancer
Gastric cancer
Adjacent normal stomach
a
b
C

Figure 3

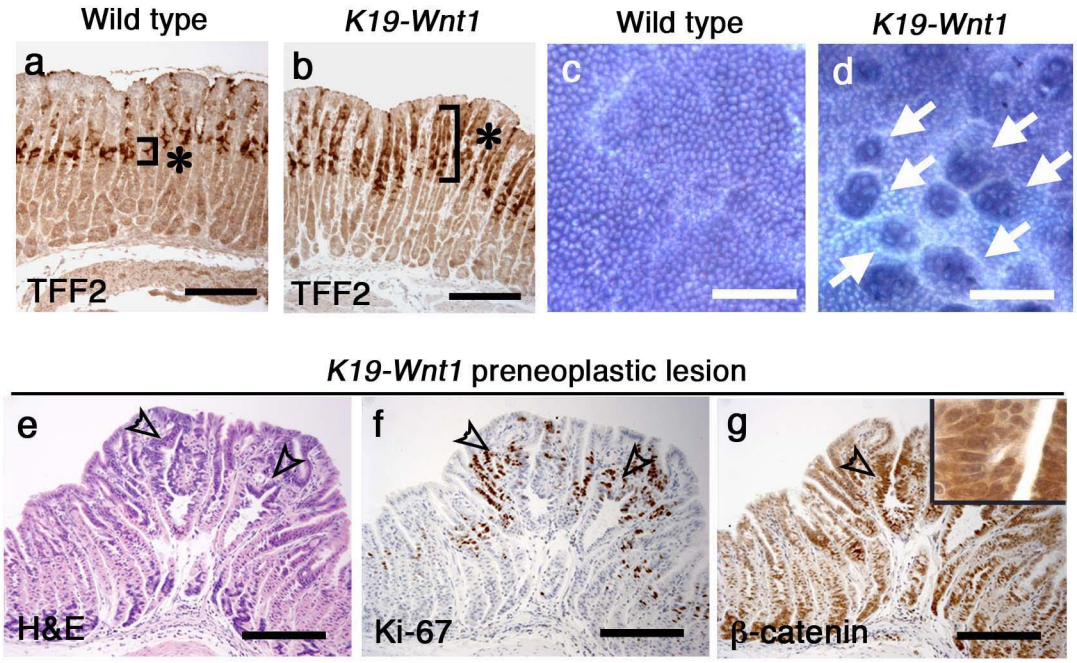

Wild type
K19-Wnt1
Wild type
K19-Wnt1
a
b
c
d
TFF2
TFF2
K19-Wnt1 preneoplastic lesion
e
f
g
H&E
Ki-67
β-catenin

Figure 4

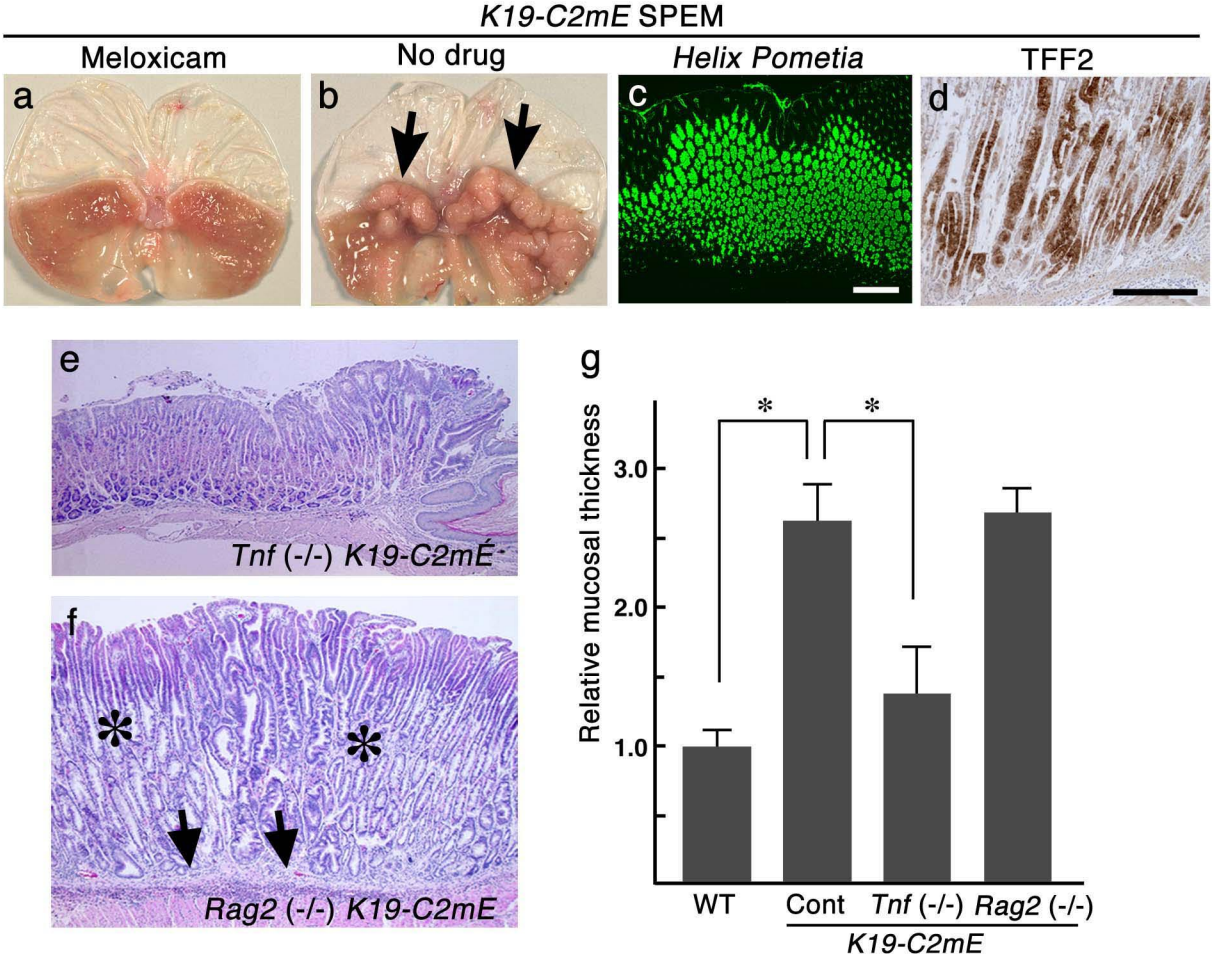

K19-C2mE SPEM
Meloxicam
No drug
Helix Pometia
TFF2
a
b
c
d
e
Tnf (-/-) K19-C2mE
f
Rag2 (-/-) K19-C2mE
g
Relative mucosal thickness
3.0
2.0
1.0
*
*
WT
Cont
Tnf (-/-)
Rag2 (-/-)
K19-C2mE

Figure 5

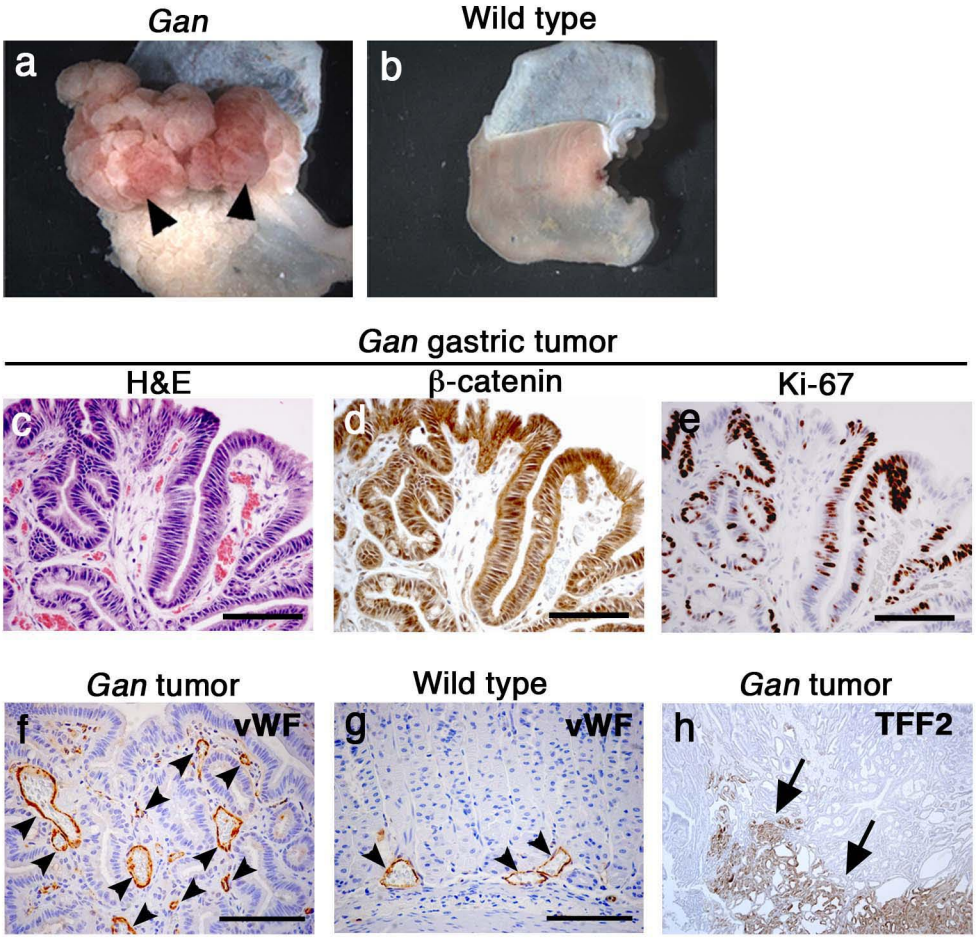

Gan
Wild type
a
b
Gan gastric tumor
H&E
β-catenin
Ki-67
c
d
e
Gan tumor
Wild type
Gan tumor
f
vWF
g
vWF
h
TFF2

FIgure 6

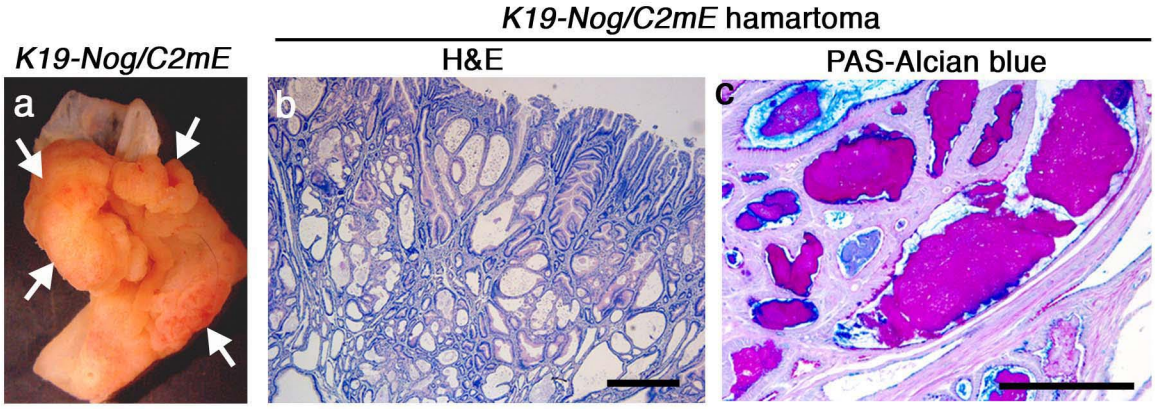
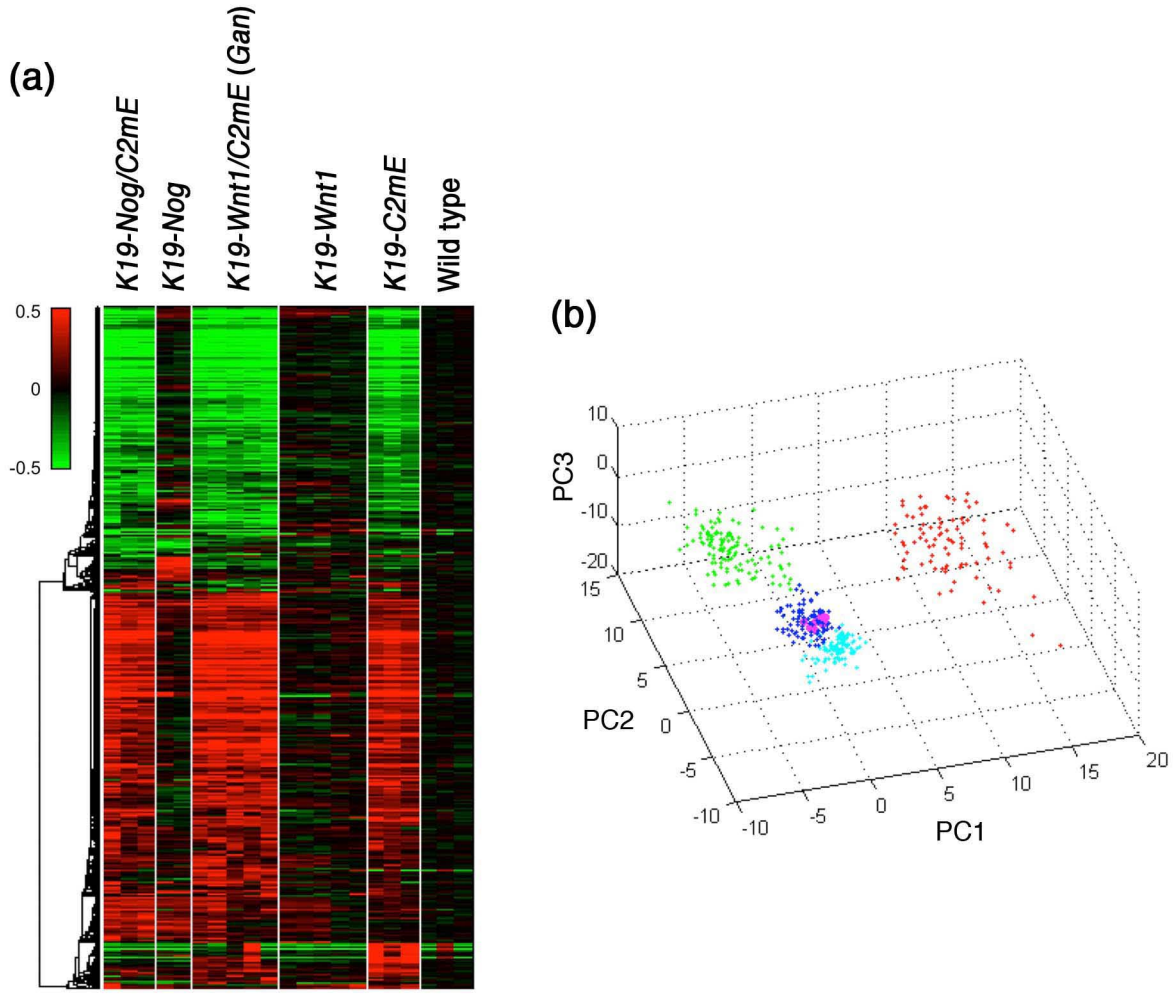


Figure 7


(a)
K19-Nog/C2mE
K19-Nog
K19-Wnt1/C2mE (Gan)
K19-Wnt1
K19-C2mE
Wild type
0.5
0
-0.5
(b)
PC3
PC2
PC1
10
0
-10
-20
15
10
5
0
-5
-10
-10
-5
0
5
10
15
20