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# **PGE<sub>2</sub>-associated inflammation and gastrointestinal tumorigenesis**

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## **Summary**

Accumulating evidence has indicated that chronic inflammation is associated with a variety of diseases, including cancer, heart attacks, Alzheimer's and other diseases. In the cancer research field, the association of inflammatory infiltration with cancer has been known histologically for a long time. Recent studies have indicated that macrophages and other immune cells infiltrate cancer tissues, expressing cytokines, chemokines and growth factors, thereby constructing an inflammatory microenvironment. In such microenvironment, NF- $\kappa$ B is activated, which contributes to the growth and survival of cancer cells. Moreover, it has also been shown that NF- $\kappa$ B activation is associated with the acquisition of stem cell properties by cancer cells. Using inflammation-associated gastric cancer model mice, *Gan* mice, we have shown that TNF- $\alpha$  signaling is activated in inflammatory microenvironment,

and plays a tumor promoting role by inducing Noxo1 in tumor cells. Taken together, these results indicate that regulation of chronic inflammation in tumor tissues would be an effective preventive and/or therapeutic strategy against cancer development and malignant progression.

## 1. Introduction

It has been shown that chronic inflammatory diseases are associated with malignant diseases. For example, reflux esophagitis, inflammatory bowel disease, ulcerative colitis, and chronic pancreatitis are linked to cancers of the esophagus, colon and pancreas, respectively. Moreover, chronic infections with *Helicobacter pylori*, hepatitis viruses or papilloma viruses are associated with gastric cancer, hepatocellular carcinoma and cervical cancer, respectively. These findings indicate that chronic inflammation and infection are important risk factors for cancer development. Among the various inflammatory signaling pathways, mouse genetic studies and clinical studies have indicated that prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) signaling plays an essential role in inflammation and cancer development. In this chapter, we discuss the role of PGE<sub>2</sub>-associated inflammation and the role of the TNF- $\alpha$  induced by the PGE<sub>2</sub>-related microenvironment in gastric and intestinal cancer development, which were elucidated by mouse genetic studies.

## 2. Non-steroidal anti-inflammatory drugs (NSAIDs) and cancer development

Epidemiological studies indicated that the incidence and mortality rates of colorectal cancer were decreased significantly in population who takes aspirin, one of the NSAIDs, compared with non-aspirin users (Thun et al. 1993). These results strongly suggested that inflammation is not just associated with cancer, but that it actually promotes cancer development. The target molecules of NSAIDs are cyclooxygenase (COX)-1 and COX-2, which are the rate-limiting enzymes for prostaglandin (PG) biosynthesis. COX-1 is constitutively expressed in most tissues, and is responsible for the physiological basal level of

prostaglandin production, whereas COX-2 expression is induced in inflamed tissues and plays a critical role in inflammatory responses. COX-2 catalyzes the synthesis of PGH<sub>2</sub> from arachidonic acid, which is further converted to PGE<sub>2</sub> by mPGES-1. Notably, the expression of COX-2 and mPGES-1 is induced simultaneously in both inflammatory and cancer tissues, including colon cancer and gastric cancer. These results strongly suggested that COX-2 and the downstream product, PGE<sub>2</sub>, are important for cancer development through the induction and maintenance of inflammatory responses (Wang and DuBois 2010).

### **3. The COX-2/PGE<sub>2</sub> pathway and intestinal tumorigenesis**

It is well established that the accumulation of mutations in oncogenes and tumor suppressor genes causes cancer development and malignant progression, which is known as “multistep tumorigenesis”. Among these cancer-related genes, an *APC* gene mutation is found in more than 80% of colon cancer cells, and is responsible for the initial step of tumorigenesis, *i.e.* the development of adenomatous polyps in the intestine. We have previously constructed *Apc* gene mutant mice, *Apc*<sup>A716</sup> mice, which develop numerous polyps in the entire intestinal tract (Oshima et al. 1995). In the *Apc*<sup>A716</sup> mouse polyp tissues, COX-2 expression is induced in stroma cells, including both macrophages and fibroblasts (Sonoshita et al. 2002). Accordingly, the tumor microenvironment consisting of COX-2-expressing stromal cells comprises the early stage of tumorigenesis. The expression of mPGES-1 is also induced in the tumor stroma, indicating that PGE<sub>2</sub> is produced in the microenvironment of tumor tissues (Fig. 1).

Importantly, disruption of the COX-2 gene or treatment with COX-2-selective inhibitors

resulted in significant suppression of the polyp formation in *Apc*<sup>A716</sup> mice (Oshima et al. 1996; Oshima et al. 2001). Consistently, disruption of the mPGES-1 gene also suppressed intestinal polyposis in other *Apc* gene mutant mice (Nakanishi et al. 2008). Moreover, blocking PGE<sub>2</sub> signaling through the EP2 receptor led to significant suppression of polyp formation in *Apc*<sup>A716</sup> mice (Sonoshita et al. 2001). These results clearly indicate that the induction of COX-2/PGE<sub>2</sub> signaling in the microenvironment is required for tumor development.

Many studies have shown the functions of PGE<sub>2</sub> in tumorigenesis; *i.e.*, angiogenesis through the induction of VEGF and bFGF (Seno et al. 2002), and suppression of apoptosis by the activation of PPAR $\delta$  (Wang et al. 2004). Moreover, PGE<sub>2</sub> signaling induces DNA methylation that silences tumor suppressor and DNA repair genes (Xia et al. 2012) (Fig. 1). However, the main mechanism by which PGE<sub>2</sub> promotes cancer is still unclear. We have constructed a new mouse model, *K19-C2mE* mice, which express COX-2 and mPGES-1 simultaneously in the stomach, which induces chronic PGE<sub>2</sub>-associated inflammation in the gastric mucosa (Oshima et al. 2004) (Fig. 2). Therefore, one of the important mechanisms by which the COX-2/PGE<sub>2</sub> pathway is involved in tumorigenesis is by generating an inflammatory microenvironment.

#### **4. NF- $\kappa$ B signaling and inflammation-associated colon cancer**

In the tissue exposed to chronic inflammation, lipid mediators like PGE<sub>2</sub>, chemokines and cytokines are induced, resulting in the recruitment of bone marrow-derived cells such as macrophages, and activation of these cells leads to the development of a cytokine network.

Several transcription factors, such as NF- $\kappa$ B and Stat3, are activated in the inflammatory lesions. NF- $\kappa$ B is activated by TNF- $\alpha$  signaling and Stat3 is activated by IL-6/IL-11, and these transcription factors both play a role in tumor promotion (Oshima and Oshima 2012). The role of Stat3 induction by IL-6 or IL-11 in intestinal tumorigenesis has been shown by genetic studies (Bollrath et al. 2009; Grivennikov et al. 2009; Putoczki et al. 2013). In this chapter, we discuss the role of NF- $\kappa$ B in the promotion of colon cancer development.

The treatment of mice with chemical mutagen azoxymethane (AOM) induces  $\beta$ -catenin gene mutations, and treatment with dextran sodium sulfate (DSS) in the drinking water induces ulcerative colitis. It is well established mouse model of colitis-associated colon cancer can be induced by treatment with a combination of AOM and DSS. Genetic inactivation of NF- $\kappa$ B in the bone marrow cells of AOM/DSS-treated mice resulted in a decrease in the growth factor expression by stromal cells and suppression of colon tumor development (Greten et al. 2004). Notably, inactivation of NF- $\kappa$ B in intestinal epithelial cells also suppressed colon tumor development by leading to decreased expression of anti-apoptotic factors. These results indicate that inflammatory responses promote tumorigenesis through the activation of NF- $\kappa$ B in both bone marrow-derived cells, as well as tumor epithelial cells, by inducing the expression of growth factors and suppressing apoptosis (Fig. 1).

Recently, it has been reported that NF- $\kappa$ B is important for the stem cell phenotype. Normal intestinal epithelial cells on villi are terminally differentiated and never proliferate. When Wnt signaling is activated in mouse villous epithelial cells by conditional mutagenesis in the  $\beta$ -catenin gene, the cells do not proliferate, indicating that Wnt activation cannot reset the

terminal differentiation. However, if NF- $\kappa$ B is activated together with Wnt, the epithelial cells acquire stem cell properties and start proliferating (Schwitalla et al. 2013a). These results suggest that inflammatory responses contribute to the development or maintenance of an undifferentiated status or stemness of cancer cells through NF- $\kappa$ B activation.

Although infection contributes to the development of an inflammatory microenvironment, it has not been elucidated how chronic inflammation is induced in non-infectious cancer tissues. Recent results have suggested the possibility that there may be oncogene-induced inflammation in tumor tissues. A loss or mutation of the p53 gene accelerates NF- $\kappa$ B activation in the microenvironment, resulting in an acceleration of colitis-associated tumor development in a mouse model (Schwitalla et al. 2013b; Cooks et al. 2013). Moreover, *KRAS* mutation causes TBK1-dependent NF- $\kappa$ B activation, which is required for the survival of cancer cells (Barbie et al. 2009). Accordingly, genetic alterations in oncogenes or tumor suppressor genes promote tumorigenesis not only by its intrinsic oncogenic activations, but also by inducing inflammatory responses.

## **5. *Gan* mice, a model of inflammation-associated gastric cancer**

*Helicobacter pylori* infection is strongly associated with the development of gastric cancer. *H. pylori* infection induces activation of the COX-2/PGE<sub>2</sub> pathway, which plays a role in infection-associated gastritis. We have constructed transgenic *K19-Wnt1* mice that express Wnt1, a ligand for canonical Wnt signaling, specifically in the gastric epithelial cells (Oshima et al. 2006; Oshima et al. 2009). These *K19-Wnt1* mice develop small preneoplastic lesions in the gastric mucosa, but they do not develop tumors (Fig. 2). We crossed *K19-Wnt1* mice

and *K19-C2mE* mice to induce activation of both Wnt signaling and the COX-2/PGE<sub>2</sub> pathway simultaneously in the stomach (Oshima et al. 2006; Oshima et al. 2009). Importantly, these double transgenic mice, *Gan* mice, develop large gastric tumors with a 100% incidence, indicating that the cooperation of Wnt signaling and the COX-2/PGE<sub>2</sub> pathway causes gastric tumor development (Fig. 2). Moreover, the gene expression profiles of *Gan* mouse tumor tissues are similar to those of human intestinal-type gastric cancer (Itadani et al. 2009). Accordingly, it can be concluded that *Gan* mice develop gastric cancer by the same mechanism as human gastric cancer; the induction of oncogenic activation and inflammatory responses, and the expression profiles also reflect those of human gastric cancer.

## **6. TNF- $\alpha$ signaling in *Gan* mouse gastric tumorigenesis**

In the gastric tumors of *Gan* mice, macrophages infiltrate into the tumor stroma, and they are activated to express proinflammatory cytokines, chemokines, growth factors and proteases (Oshima et al. 2011a). We found that the expression of EGFR ligands, including amphiregulin and epiregulin, and ADAM family proteases, is upregulated in *Gan* mouse tumors by an inflammation-dependent mechanism (Oshima et al. 2011b). The ADAM family proteases activate EGFR ligands by exodomain shedding, resulting in the activation of EGFR signaling, which may be one of the mechanisms by which inflammation leads to tumor promotion.

Among the various proinflammatory cytokines, we have been focusing on TNF- $\alpha$ , because it has been shown that disruption of TNF- $\alpha$  or the TNF- $\alpha$  receptor gene resulted in suppression of chemical carcinogen-induced tumor development in mouse models (Oshima

et al. 2013). To examine the role of TNF- $\alpha$  in gastric tumorigenesis, we crossed *Gan* mice with TNF- $\alpha$  gene (*Tnf*) knockout mice to generate *Tnf*<sup>-/-</sup> *Gan* mice (Oshima et al. 2013). Notably, gastric tumorigenesis was significantly suppressed in *Tnf*<sup>-/-</sup> *Gan* mice, and the tumor volume decreased to 18% of the size of the tumors in the *Tnf* wild-type *Gan* mice (Fig. 3). Notably, COX-2 and mPGES-1 are constitutively expressed in the *Tnf*<sup>-/-</sup> *Gan* mouse stomach because they are expressed by exogenous promoters, thus, it is possible that TNF- $\alpha$  signaling is required for tumor promotion even if the PGE<sub>2</sub> pathway is activated.

Of note, bone marrow transplantation into X-ray-irradiated *Tnf*<sup>-/-</sup> *Gan* mice from wild-type mice rescued the gastric tumor phenotype, indicating that the TNF- $\alpha$  expressed by bone marrow-derived cells (BMDCs) is important for tumor promotion (Oshima et al. 2013). Furthermore, bone marrow transplantation into *Gan* mice from TNF- $\alpha$  receptor *TNFR1* gene knockout mice resulted in significant suppression of the gastric tumor growth. Taken together, these results indicate that macrophage-derived TNF- $\alpha$  promotes gastric tumorigenesis by stimulating BMDCs in the tumor microenvironment in an autocrine or paracrine manner (Fig. 4). It has also been reported that TNF- $\alpha$  stimulates cancer cells directly, thus leading to the induction of IL-6 and CXCL12, and resulting in an inflammatory network (Kulbe et al. 2012). Therefore, it is conceivable that TNF- $\alpha$  promotes tumorigenesis by stimulating both stromal BMDCs and cancer cells.

## **7. Maintenance of the undifferentiated status of cancer cells by inflammation**

A microarray analysis using tumor tissues developed in *Tnf*<sup>-/-</sup> *Gan* mice and *Tnf* wild-type *Gan* mice revealed that over 150 genes are upregulated in the tumor tissues in a

TNF- $\alpha$ -dependent manner (Oshima et al. 2013). Interestingly, the genes specific for intestinal stem cells, such as CD44, CD133 and SOX9, were included in the upregulated genes, suggesting that TNF- $\alpha$  signaling in BMDCs plays a role in the maintenance of the undifferentiated status of tumor cells. By siRNA screening, we selected candidate genes that are important for tumorigenesis (Oshima et al. 2013). Among these genes, the inhibition of Noxo1 expression resulted in significant suppression of the tumorigenicity of gastric cancer cell lines. Noxo1 is a component of the NADPH oxidase NOX1 complex that regulates reactive oxygen species (ROS) production. A recent report indicated that Rac1, another member of the NOX1 complex, is activated by Wnt signaling in the intestinal tumor cells, and that Rac1 activation is required for the undifferentiated status of tumor cells induced by ROS production together with NF- $\kappa$ B activation (Myant et al. 2013). Moreover, Noxo1 expression is upregulated also in the normal gastric epithelial stem cells, suggesting a role of Noxo1 in maintenance of stem cells (Barker et al. 2010). These results strongly suggest that TNF- $\alpha$  signaling promotes tumor development by regulating the maintenance of the undifferentiated status of tumor cells through Noxo1 induction-associated ROS production.

## **8. Conclusion**

The COX-2/PGE<sub>2</sub> pathway is induced in most cancer tissues, and it has been established that PGE<sub>2</sub> plays an important role in cancer development. PGE<sub>2</sub> promotes tumor development through a variety of mechanisms, including the development of an inflammatory microenvironment. Accumulating evidence has indicated that activation of NF- $\kappa$ B, an important transcription factor involved in inflammation, is also essential for tumor promotion

via its induction of growth factors, protection of tumor cells from apoptosis and acquisition of stem cell properties. Moreover, the TNF- $\alpha$  that is induced in the inflammatory microenvironment is required for tumor promotion. TNF- $\alpha$  activation in the BMDCs in the tumor stroma induces Noxo1 expression in tumor cells, which may be important for the maintenance of the undifferentiated status of tumor cells by ROS production. Taken together, these results indicate that the regulation of PGE<sub>2</sub>-associated inflammatory responses and TNF- $\alpha$  signaling may be an effective preventive or therapeutic strategy against the development of gastrointestinal cancer.

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## Figure Legends

**Figure 1.** A schematic drawing of the inflammatory microenvironment in gastrointestinal tumors. The COX-2/PGE<sub>2</sub> pathway is important for the development and maintenance of the inflammatory microenvironment, including BMDCs. PGE<sub>2</sub> signaling also accelerates angiogenesis, cell survival as well as induces DNA methylation. In the activated macrophages, NF-κB induces the expression of growth factors and cytokines. The activation of NF-κB and Stat3 in tumor cells suppresses the apoptosis of tumor cells. NF-κB activation also induces the acquisition of stem cell properties. (Modified from Oshima and Oshima, 2012 with permission from Springer)

**Figure 2.** Transgenic mouse models used to examine gastric tumorigenesis. Transgenic vector constructs and representative macroscopic and microscopic photographs of the stomach are shown for each line. *Gan* mice are compound *K19-Wnt1* and *K19-C2mE* transgenic mice. The arrowhead in the *K19-Wnt1* mouse stomach indicates a preneoplastic lesion. The arrowheads in the *K19-C2mE* mouse stomach indicate inflammatory infiltration. The arrows in the *Gan* mouse indicate gastric tumors. Bars indicate 100 μm. (Modified from Oshima et al, 2009 with permission from Wiley)

**Figure 3.** Suppression of gastric tumor development by TNF-α gene disruption. (a) Representative histological photographs of whole views of *Tnf+/+ Gan* (top) and *Tnf-/- Gan* mouse (bottom) gastric tumors (H&E). The arrows indicate suppressed tumor lesions in *Tnf-/- Gan* mice. Bars indicate 5 mm. (b) The gastric tumor size of *Tnf+/+ Gan*, *Tnf+/- Gan*

and *Tnf-/- Gan* mice relative to the mean level of *Tnf+/+ Gan* mouse tumors (set at 100%).

Asterisks,  $P < 0.05$ . (Reproduced from Oshima H, 2013 with permission from Nature

Publishing Group)

**Figure 4.** A schematic drawing of the role of TNF- $\alpha$  signaling in gastric tumorigenesis.

BMDCs, including macrophages, are recruited to the inflammatory microenvironment and

express TNF- $\alpha$ , which further activates the TNFR1 receptor on BMDCs, which is important for

inducing tumor-promoting factors, including *Noxo1*, in tumor epithelial cells. *Noxo1*

expression may induce ROS production, which is required for the undifferentiated status of

tumor cells. (Reproduced from Oshima H, 2013 with permission from Nature Publishing

Group)

Figure 1

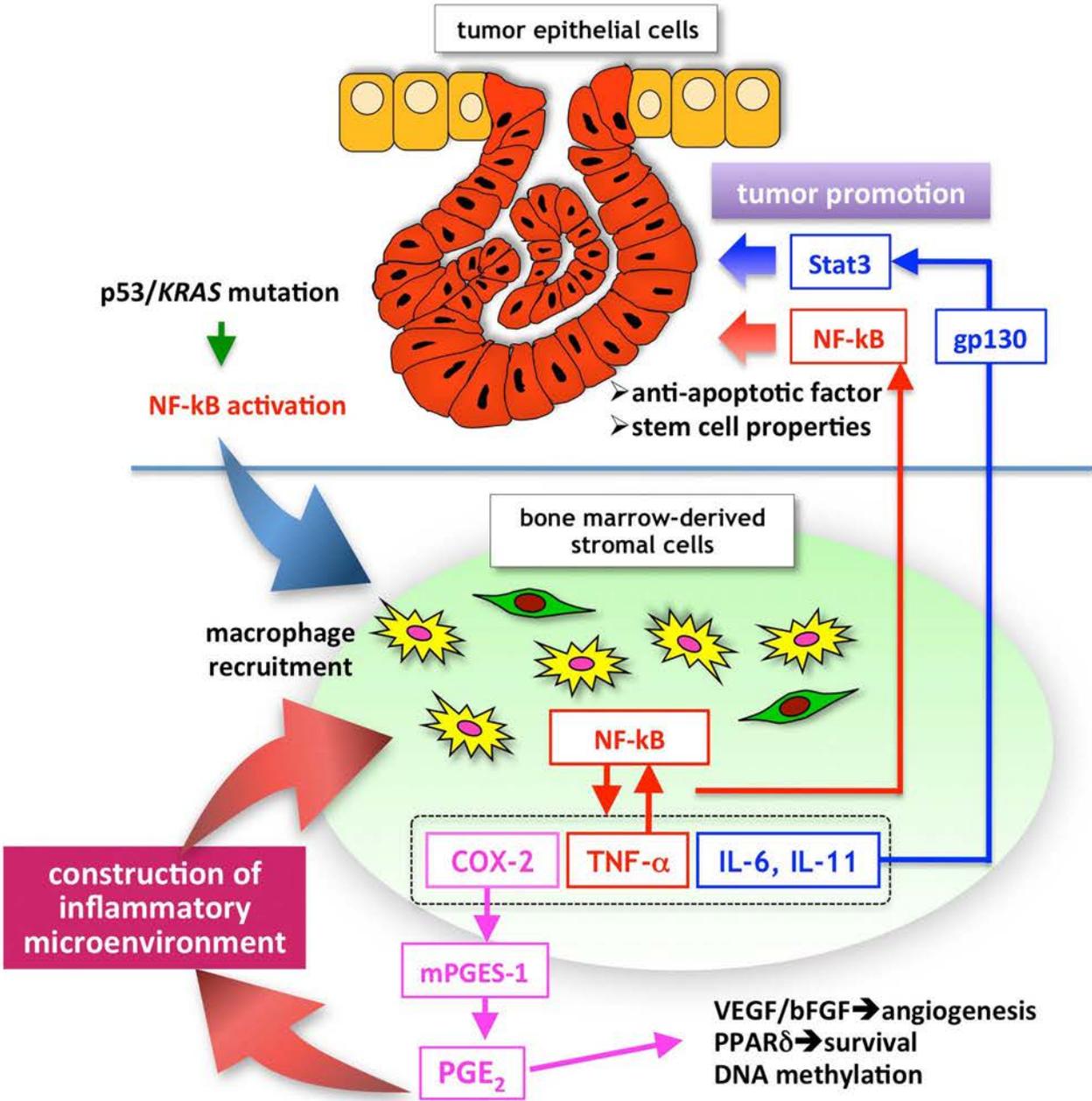


Figure 2

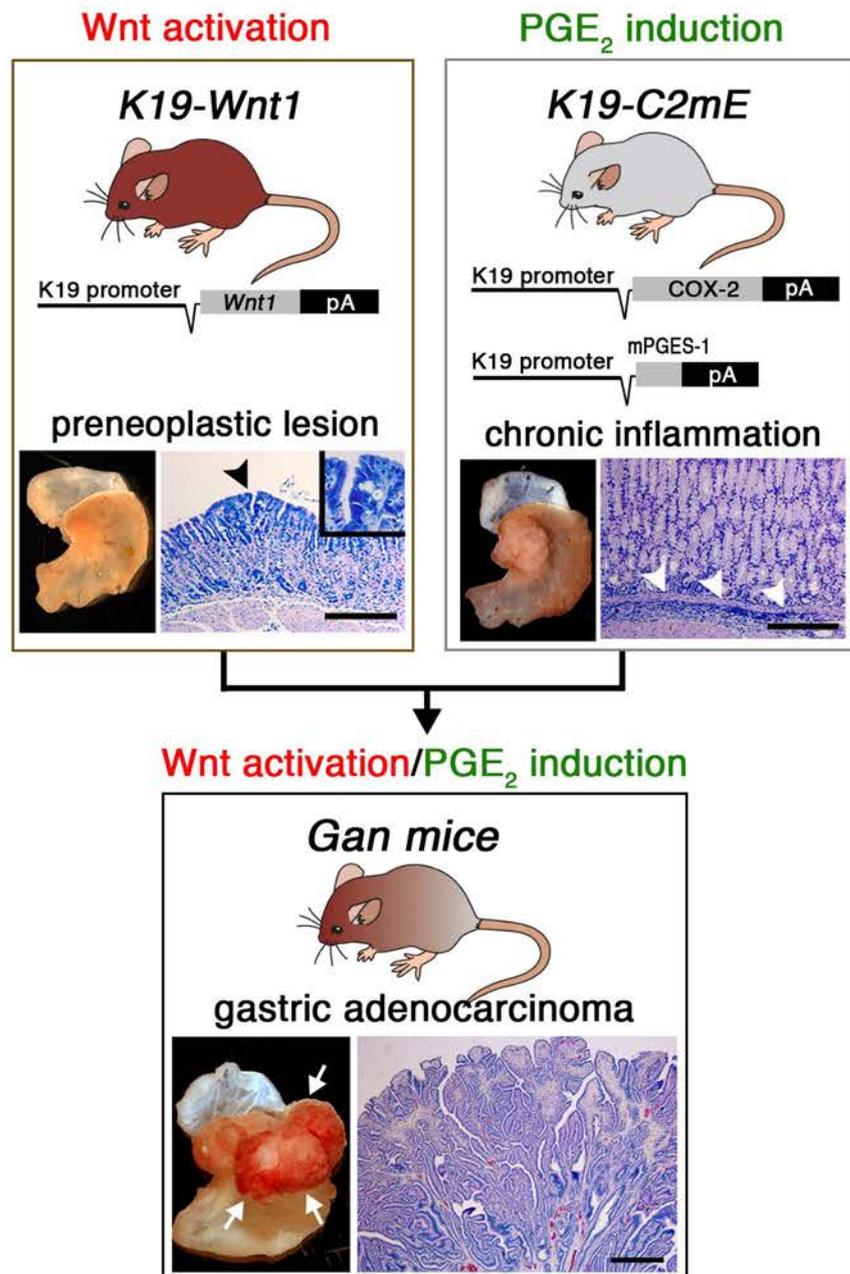
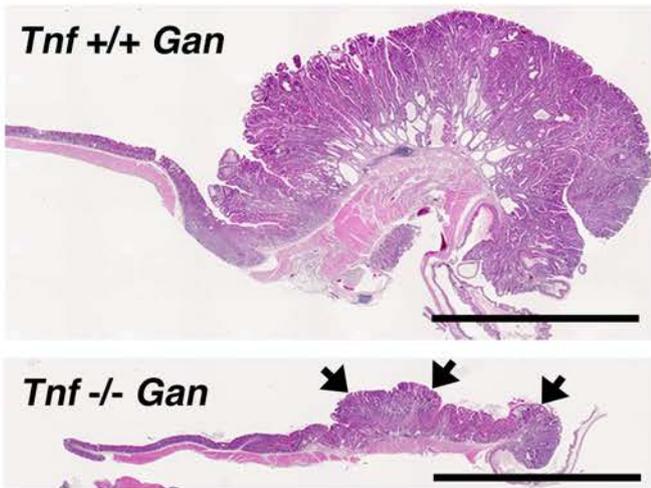


Figure 3

**A**



**B**

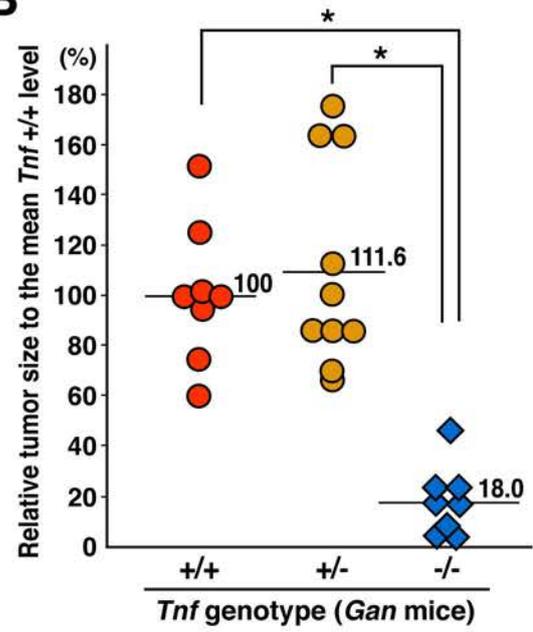


Figure 4

