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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-κB is activated, which contributes to the growth and survival of cancer cells. Moreover, it has also been shown that NF-κB 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-α 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_2 (PGE_2) signaling plays an essential role in inflammation and cancer development. In this chapter, we discuss the role of PGE_2-associated inflammation and the role of the TNF-α induced by the PGE_2-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_2 from arachidonic acid, which is further converted to PGE_2 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_2, are important for cancer development through the induction and maintenance of inflammatory responses (Wang and DuBois 2010).

3. The COX-2/PGE_2 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, $Apc^{\Delta 716}$ mice, which develop numerous polyps in the entire intestinal tract (Oshima et al. 1995). In the $Apc^{\Delta 716}$ 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_2 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*$^{\Delta 716}$ 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_2 signaling through the EP2 receptor led to significant suppression of polyp formation in *Apc*$^{\Delta 716}$ mice (Sonoshita et al. 2001). These results clearly indicate that the induction of COX-2/PGE_2 signaling in the microenvironment is required for tumor development.

Many studies have shown the functions of PGE_2 in tumorigenesis; *i.e*., angiogenesis through the induction of VEGF and bFGF (Seno et al. 2002), and suppression of apoptosis by the activation of PPARδ (Wang et al. 2004). Moreover, PGE_2 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_2 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_2-associated inflammation in the gastric mucosa (Oshima et al. 2004) (Fig. 2). Therefore, one of the important mechanisms by which the COX-2/PGE_2 pathway is involved in tumorigenesis is by generating an inflammatory microenvironment.

4. NF-κB signaling and inflammation-associated colon cancer

In the tissue exposed to chronic inflammation, lipid mediators like PGE_2, 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-κB and Stat3, are activated in the inflammatory lesions. NF-κB is activated by TNF-α 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-κB in the promotion of colon cancer development.

The treatment of mice with chemical mutagen azoxymethane (AOM) induces β-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-κB 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-κB 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-κB 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-κB 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 β-catenin gene, the cells do not proliferate, indicating that Wnt activation cannot reset the

terminal differentiation. However, if NF-κB 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-κB 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-κB 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-κB 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_2 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_2 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_2 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-α 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-α, because it has been shown that disruption of TNF-α or the TNR-α receptor gene resulted in suppression of chemical carcinogen-induced tumor development in mouse models (Oshima

et al. 2013). To examine the role of TNF-α in gastric tumorigenesis, we crossed *Gan* mice with TNF-α gene (*Tnf*) knockout mice to generate *Tnf*-/- *Gan* mice (Oshima et al. 2013). Notably, gastric tumorigenesis was significantly suppressed in *Tnf*-/- *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*-/- *Gan* mouse stomach because they are expressed by exogenous promoters, thus, it is possible that TNF-α signaling is required for tumor promotion even if the PGE_2 pathway is activated.

Of note, bone marrow transplantation into X-ray-irradiated *Tnf*-/- *Gan* mice from wild-type mice rescued the gastric tumor phenotype, indicating that the TNF-α 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-α receptor *TNFR1* gene knockout mice resulted in significant suppression of the gastric tumor growth. Taken together, these results indicate that macrophage-derived TNF-α 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-α 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-α 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*-/- *Gan* mice and *Tnf* wild-type *Gan* mice revealed that over 150 genes are upregulated in the tumor tissues in a

TNF-α-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-α 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-κB 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-α 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_2 pathway is induced in most cancer tissues, and it has been established that PGE_2 plays an important role in cancer development. PGE_2 promotes tumor development through a variety of mechanisms, including the development of an inflammatory microenvironment. Accumulating evidence has indicated that activation of NF-κB, 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-α that is induced in the inflammatory microenvironment is required for tumor promotion. TNF-α 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_2-associated inflammatory responses and TNF-α signaling may be an effective preventive or therapeutic strategy against the development of gastrointestinal cancer.

References

Barbie DA, Tamayo P, Boehm JS et al (2009) Systematic RNA interference reveals that oncogenic *KRAS*-driven cancers require TBK1. Nature 462:108-112

Barker N, Huch M, Kujala P et al (2010) Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell 6:25-36

Bollrath J, Phesse TJ, von Burstin VA et al (2009) gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell 15:91-102

Cooks T, Pateras IS, Tarcic O et al (2013) Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. Cancer Cell 23:634-646

Greten FR, Eckmann L, Greten TF et al (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118:285-296

Grivennikov S, Karin E, Terzic J et al (2009) IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 15:103-113

Itadani H, Oshima H, Oshima M et al (2009) Mouse gastric tumor models with prostaglandin E_2 pathway activation show similar gene expression profiles to intestinal-type human gastric cancer. BMC Genomics 10:615

Kulbe H, Chakravarty P, Leinster DA, Charles KA, Kwong J, Thompson RG, Coward JI, Schioppa T, Robinson SC, Gallagher WM et al (2012) A dynamic inflammatory cytokine network in the human ovarian cancer microenvironment. Cancer Res 72:66-75

Myant KB, Cammareri P, McGhee EJ et al (2013) ROS production and NF-κB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. Cell Stem Cell 12:761-773

Nakanishi M, Montrose DC, Clark P et al (2008) Genetic deletion of mPGES-1 suppresses intestinal tumorigenesis. Cancer Res 68:3251-3259

Oshima M, Oshima H, Kitagawa K et al (1995) Loss of Apc heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. Proc Natl Acad Sci U S A 92:4482-4486

Oshima M, Dinchuk JE, Kargman SL et al (1996) Suppression of intestinal polyposis in *Apc*$^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87:803-809

Oshima M, Murai N, Kargman S et al (2001) Chemoprevention of intestinal polyposis in the *Apc*$^{\Delta 716}$ mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. Cancer Res 61:1733-1740

Oshima H, Oshima M, Inaba K et al (2004) Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. EMBO J 23:1669-1678

Oshima H, Matsunaga A, Fujimura T et al (2006) Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E_2 pathway. Gastroenterology 131:1086-1095

Oshima H, Oguma K, Du YC et al (2009) Prostaglandin E_2, Wnt, and BMP in gastric tumor mouse models. Cancer Sci 100:1779-1785

Oshima H, Hioki K, Popivanova BK et al (2011a) Prostaglandin E_2 signaling and bacterial infection recruit tumor-promoting macrophages to mouse gastric tumors. Gastroenterology 140:596-607

Oshima H, Popivanova BK, Oguma K et al (2011b) Activation of epidermal growth factor receptor signaling by the prostaglandin E_2 receptor EP4 pathway during gastric tumorigenesis. Cancer Sci 102:713-719

Oshima H, Oshima M (2012) The inflammatory network in the gastrointestinal tumor microenvironment: lessons from mouse models. J Gastroenterol 47:97-106

Oshima H, Ishikawa T, Yoshida GJ et al (2013) TNF-α/TNFR1 signaling promotes gastric tumorigenesis through induction of Noxo1 and Gna14 in tumor cells. Oncogene doi: 10.1038/onc.2013.356. [Epub ahead of print]

Putoczki TL, Thiem S, Loving A et al (2013) Interleukin-11 Is the Dominant IL-6 Family Cytokine during Gastrointestinal Tumorigenesis and Can Be Targeted Therapeutically. Cancer Cell 24:257-271

Schwitalla S, Fingerle AA, Cammareri P et al (2013a) Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 152:25-38

Schwitalla S, Ziegler PK, Horst D et al (2013b) Loss of p53 in enterocytes generates an inflammatory microenvironment enabling invasion and lymph node metastasis of carcinogen-induced colorectal tumors. Cancer Cell 23:93-106

Seno H, Oshima M, Ishikawa TO et al (2002) Cyclooxygenase 2- and prostaglandin E_2 receptor EP_2-dependent angiogenesis in *Apc*$^{\Delta 716}$ mouse intestinal polyps. Cancer Res 62:506-511

Sonoshita M, Takaku K, Sasaki N et al (2001) Acceleration of intestinal polyposis through prostaglandin receptor EP2 in *Apc*$^{\Delta 716}$ knockout mice. Nat Med 7:1048-1051

Sonoshita M, Takaku K, Oshima M et al (2002) Cyclooxygenase-2 expression in fibroblasts and endothelial cells of intestinal polyps. Cancer Res 62:6846-6849

Thun MJ, Namboodiri MM, Calle EE et al (1993) Aspirin use and risk of fatal cancer. Cancer Res 53:1322-1327

Wang D, Wang H, Shi Q et al (2004) Prostaglandin E_2 promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor δ. Cancer Cell 6:285-295

Wang D, Dubois RN (2010) Eicosanoids and cancer. Nat Rev Cancer 10:181-193

Xia D, Wang D, Kim SH et al (2012) Prostaglandin E2 promotes intestinal tumor growth via DNA methylation. Nat Med 18:224-226

Figure Legends

Figure 1. A schematic drawing of the inflammatory microenvironment in gastrointestinal tumors. The COX-2/PGE_2 pathway is important for the development and maintenance of the inflammatory microenvironment, including BMDCs. PGE_2 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-α signaling in gastric tumorigenesis. BMDCs, including macrophages, are recruited to the inflammatory microenvironment and express TNF-α, 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

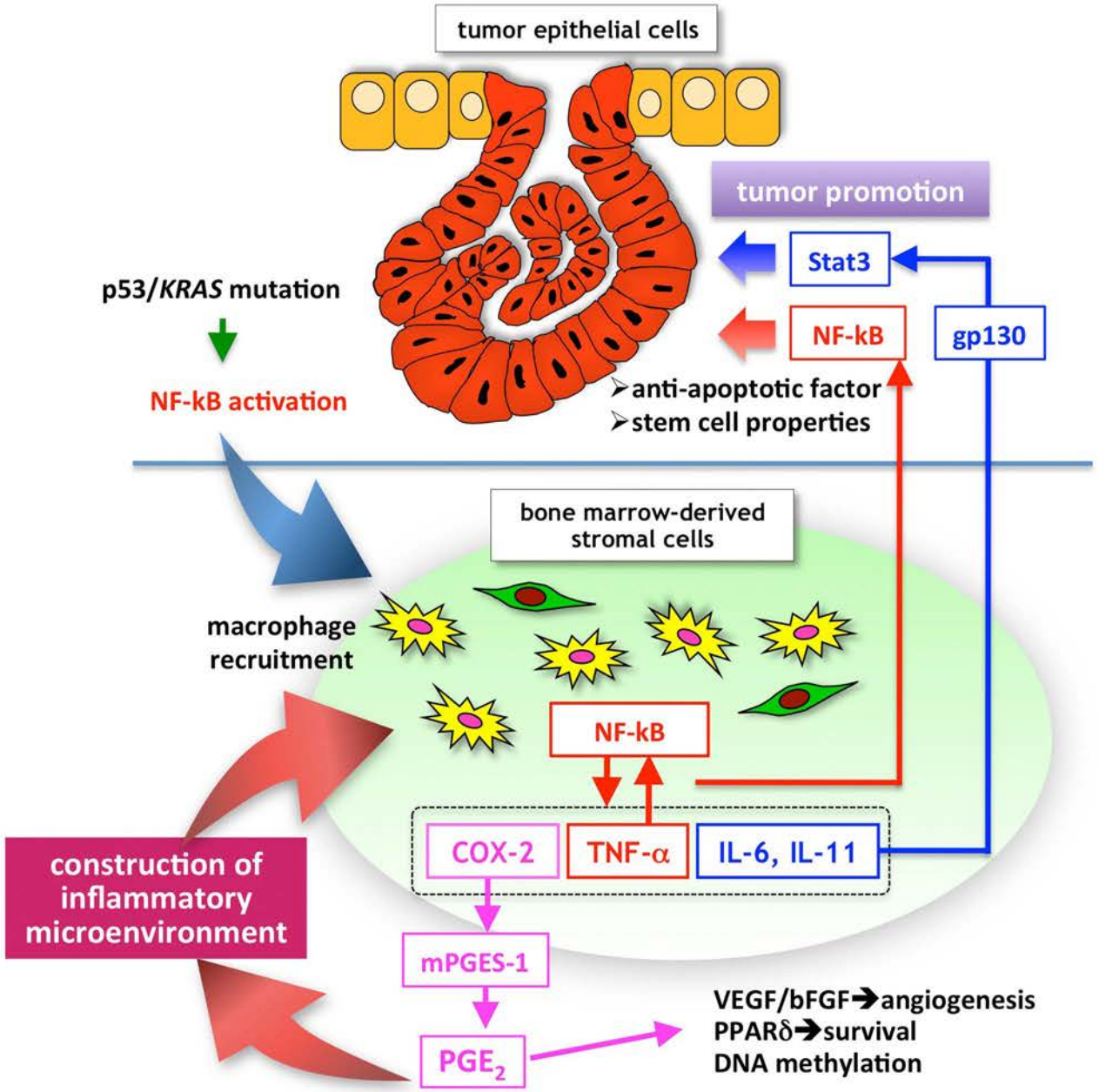

tumor epithelial cells
tumor promotion
Stat3
NF-kB
gp130
p53/*KRAS* mutation
NF-kB activation
➢anti-apoptotic factor
➢stem cell properties
bone marrow-derived stromal cells
macrophage recruitment
NF-kB
COX-2
TNF-α
IL-6, IL-11
construction of inflammatory microenvironment
mPGES-1
PGE2
VEGF/bFGF➔angiogenesis
PPARδ➔survival
DNA methylation

Figure 2

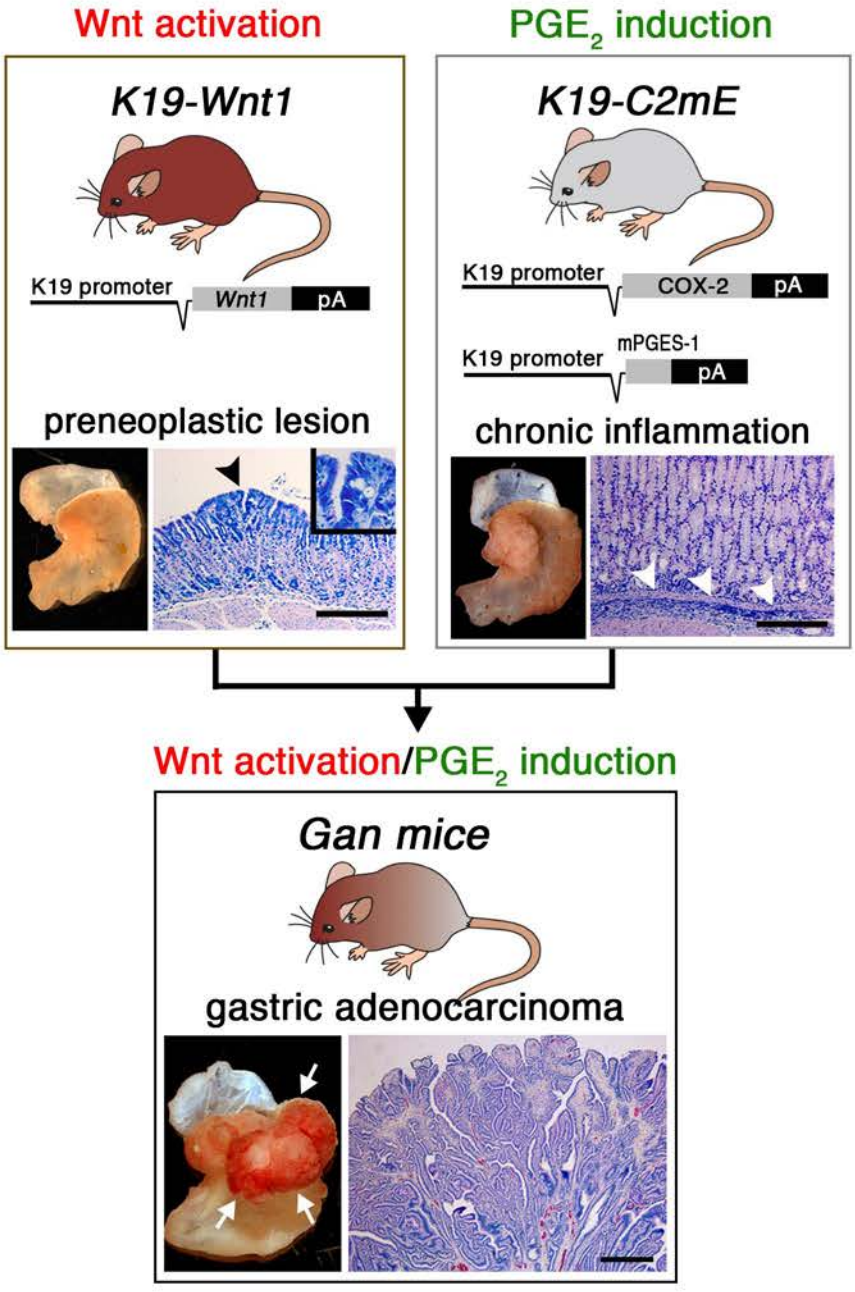

Wnt activation
PGE2 induction
K19-Wnt1
K19 promoter
Wnt1
pA
preneoplastic lesion
K19-C2mE
K19 promoter
COX-2
pA
K19 promoter
mPGES-1
pA
chronic inflammation
Wnt activation/PGE2 induction
Gan mice
gastric adenocarcinoma

Figure 3

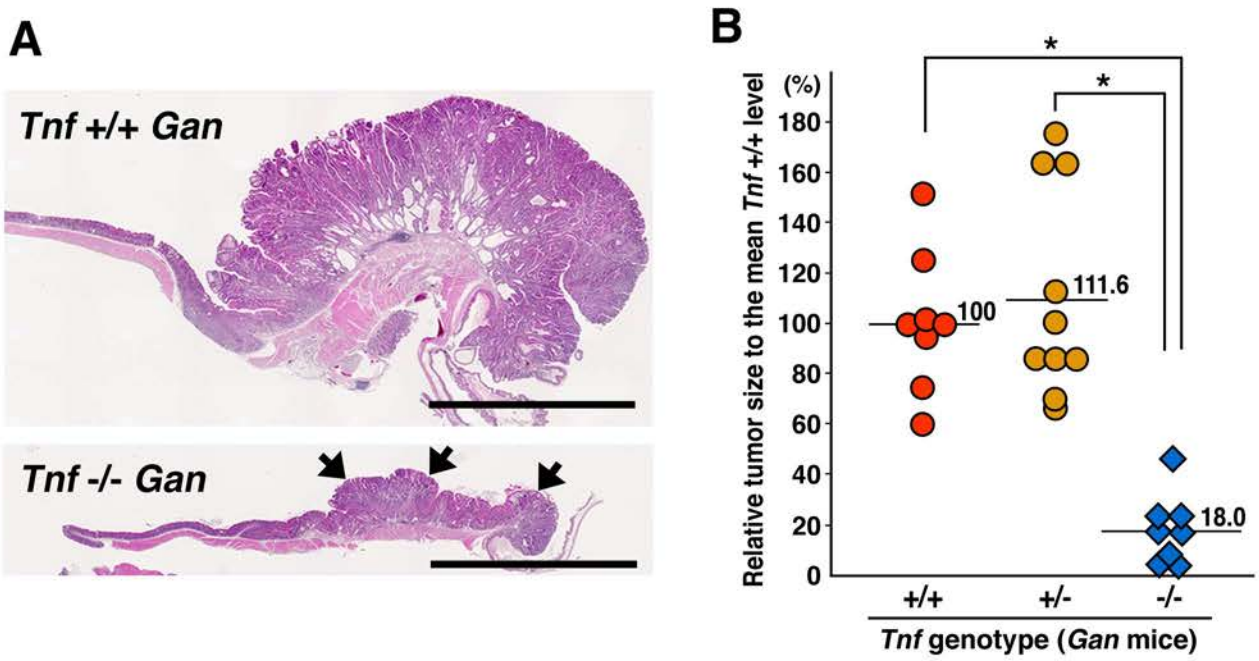
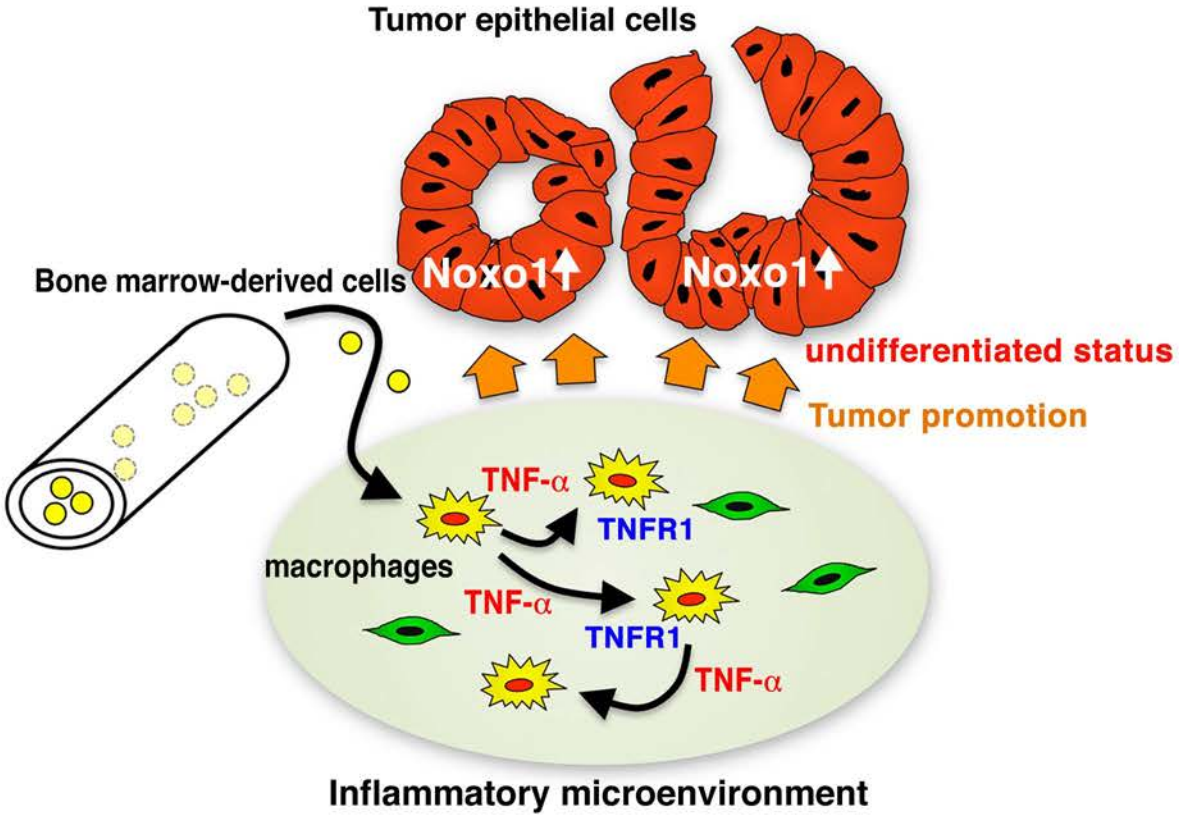

A
Tnf +/+ Gan
Tnf -/- Gan
B
Relative tumor size to the mean Tnf +/+ level
(%)
180
160
140
120
100
80
60
40
20
0
100
111.6
18.0
*
*
+/+
+/-
-/-
Tnf genotype (Gan mice)

Figure 4


Tumor epithelial cells
Noxo1
Noxo1
Bone marrow-derived cells
undifferentiated status
Tumor promotion
TNF-α
TNFR1
macrophages
TNF-α
TNFR1
TNF-α
Inflammatory microenvironment