REVIEW

The role of PGE$_2$-associated inflammatory responses in gastric cancer development

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Abstract Accumulating evidence indicates that inflammation plays a critical role in cancer development. Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme for prostanoid biosynthesis, including prostaglandin E$_2$ (PGE$_2$), and plays a key role in both inflammation and cancer. It has been demonstrated that inhibition of COX-2 and PGE$_2$ receptor signaling results in the suppression of tumor development in a variety of animal models. However, the molecular mechanisms underlying COX-2/PGE$_2$-associated inflammation in carcinogenesis have not yet been fully elucidated. In order to study the role of PGE$_2$-associated inflammatory responses in tumorigenesis, it is important to use in vivo mouse models that recapitulate human cancer development from molecular mechanisms with construction of tumor microenvironment. We have developed a gastritis model (K19-C2mE mice) in which an inflammatory microenvironment is constructed in the stomach via induction of the COX-2/PGE$_2$ pathway. We also developed a gastric cancer mouse model (Gan mice) in which the mice develop inflammation-associated gastric tumors via activation of both the COX-2/PGE$_2$ pathway and Wnt signaling. Expression analyses using these in vivo models have revealed novel mechanisms of the inflammatory responses underlying gastric cancer development. PGE$_2$-associated inflammatory responses activate epidermal growth factor receptor (EGFR) signaling through the induction of EGFR ligands and ADAMs that release EGFR ligands from the cell membrane. In Gan mice, a combination treatment with EGFR and COX-2 inhibitors significantly suppresses gastric tumorigenesis. Moreover, PGE$_2$-associated inflammation downregulates tumor suppressor microRNA, miR-7, in gastric cancer cells, which suppresses epithelial differentiation. These results indicate that PGE$_2$-associated inflammatory responses promote in vivo gastric tumorigenesis via several different molecular mechanisms.

Keywords Gastric cancer · Inflammation · COX-2 · PGE$_2$ · EGFR · MicroRNA

Introduction

It has been shown that approximately 15–20 % of malignant cancers are associated with chronic infection [1, 2]. For example, infection with *Helicobacter pylori*, hepatitis C virus, and human papilloma virus is closely associated with the development of gastric cancer, hepatocellular carcinoma, and cervical cancer, respectively. On the other hand, it has also been reported that tobacco smoke and obesity contribute to tumor development through induction of inflammatory responses in the lungs and liver, respectively [3, 4]. These results, together with those of recent genetic studies, indicate that inflammatory responses play important roles in cancer development regardless of whether they are caused by infectious or noninfectious stimuli [5–7]. Among the various inflammatory networks involved in tumor microenvironment, the cyclooxygenase-2 (COX-2)/prostaglandin E$_2$ (PGE$_2$) pathway was first identified as a key player in tumorigenesis [8, 9]. COX-2 is an inducible rate-limiting enzyme for prostaglandin biosynthesis and has been shown to play an essential role in inflammatory responses. PGE$_2$ is a downstream product of COX-2, and the level of PGE$_2$ increases significantly in tumor tissues, suggesting that PGE$_2$ plays a role in

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tumorigenesis. Although almost 20 years have passed since the discovery of the role of COX-2 in cancer development, the mechanisms underlying the role of the COX-2/PGE$_2$ pathway in tumorigenesis have not yet been fully elucidated. It was recently shown that COX-2 expression and PGE$_2$ production are induced in mesenchymal stem cells infiltrated in cancer tissues, which leads to the expression of inflammatory cytokines and the induction of epithelial mesenchymal transition through the construction of niches for tumor-initiating cells [10]. However, it remains unclear how the COX-2/PGE$_2$ pathway promotes in vivo tumorigenesis. To understand the role of PGE$_2$-induced inflammatory responses in cancer development, it is important to construct animal models in which the animals develop cancer caused by oncogenic activation in epithelial cells together with PGE$_2$-associated host responses like human cancers. We developed a gastric cancer mouse model (Gan mice for gastric neoplasia mice) in which activation of both Wnt signaling and the COX-2/PGE$_2$ pathway in the stomach is achieved by the transgenic expression of *Wnt1*, *Ptgs2*, and *Ptges*, which encode Wnt1, COX-2, and mPGES-1, respectively [11, 12]. By conducting expression analyses of mRNA and microRNA (miRNA) using murine gastritis tissues and Gan mouse gastric tumors, we identified novel molecular pathways that are activated or suppressed in PGE$_2$-associated inflammatory microenvironment and thereby contribute to gastric cancer development. In the first part of this article, we review the role of the COX-2/PGE$_2$ pathway in gastrointestinal cancer development and the construction of the Gan mouse model. Then, we discuss the novel mechanisms underlying inflammation in gastric tumorigenesis, which we found using the Gan mouse model system.

The COX-2/PGE$_2$ pathway in gastrointestinal tumor development

Approximately 20 years ago, epidemiological studies reported that the regular use of non-steroidal anti-inflammatory drugs (NSAIDs) lowers the mortality rate of patients with gastrointestinal cancer [13, 14]. The target molecules of NSAIDs are COX-1 and COX-2, which are rate-limiting enzymes for prostaglandin biosynthesis. COX-1 is constitutively expressed and plays a physiological house-keeping role, while COX-2 expression is induced in both inflammation and cancer. It has also been reported that treatment of patients with familial adenomatous polyposis (FAP) using NSAIDs results in significant decreases in the number and size of colon polyps [15]. These results strongly suggested that COX-2 plays an important role in intestinal tumorigenesis. Moreover, a large number of animal experiments have confirmed that NSAID treatment suppresses chemical carcinogen-induced colon tumor development, indicating that induction of the COX pathway is required for tumor development [16]. Mouse genetic studies clearly demonstrate the role of COX-2 in intestinal tumorigenesis. Disruption of the *Ptgs2* gene in $Apc^{\Delta716}$ and Apc^{Min} mice, mouse models for FAP, significantly suppresses the development of intestinal polyposis, thus indicating an essential role for COX-2 in tumor development [17, 18]. Interestingly, disruption of *Ptgs1* (COX-1 gene) also suppresses intestinal polyposis in Apc^{Min} mice. It is therefore possible that the COX-1-derived basal level of PGE$_2$ is also involved in tumor formation before COX-2 expression is induced [16] (Fig. 1).
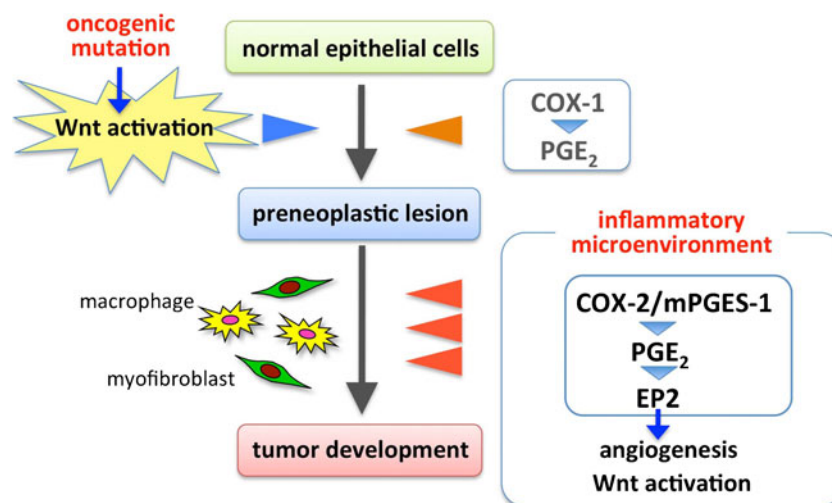

Fig. 1 Schematic diagram showing the role of PGE$_2$ in tumorigenesis. Wnt signaling activation triggers initiation of tumorigenesis. Expression of COX-2 and mPGES-1 is induced in microadenomas, which results in an increase of PGE$_2$ level. Activated COX-2/PGE$_2$ pathway contributes to tumor development through a variety of mechanisms, including induction of angiogenesis and Wnt activation, or construction of inflammatory microenvironment as indicated in Figs. 4 and 5. COX-1-derived PGE$_2$ may play a role in tumorigenesis at the early stage of tumorigenesis when COX-2 expression is not yet induced

Microsomal PGE synthease-1 (mPGES-1) is an inducible PGE-converting enzyme that functionally couples with COX-2 for PGE$_2$ biosynthesis, and the expression of mPGES-1 is induced in cancer tissues together with that of COX-2 [19, 20]. Accordingly, the PGE$_2$ level is increased in cancer tissues due to the expression of both COX-2 and mPGES-1. Importantly, a disruption of the *Ptges* gene encoding mPGES-1 in *Apc*$^{\Delta 14}$ mice, another FAP model, and in a chemical carcinogen-treated colon cancer mouse model results in significant decreases in the PGE$_2$ level in the intestinal mucosa, which causes further suppression of intestinal tumor development [21, 22]. There are four G-protein coupled receptors for PGE$_2$, namely, EP1, EP2, EP3, and EP4. Signaling through EP2 and EP4 increases intracellular cyclic AMP and has been shown to be important for tumor development. Disruption of the *Ptger2* gene encoding EP2 in *Apc*$^{\Delta 716}$ mice causes significant suppression of intestinal polyp development, whereas disruption of *Ptger1* and *Ptger3* does not affect the tumor phenotype [23]. EP2 signaling accelerates angiogenesis in intestinal polyps through the induction of the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor expression [24]. Moreover, EP2 signaling has been shown to activate Wnt signaling in colon cancer cells through the activation of the PI3K/Akt pathway and the direct association between G protein and Axin, thus resulting in the stabilization of β-catenin [25]. These results indicate that the COX-2/mPGES-1/PGE$_2$/EP2 pathway is important for tumor development

through the involvement of several different mechanisms (Fig. 1). Although several studies have identified possible mechanisms underlying the COX-2/PGE$_2$ pathway in cancer development [8, 9], the key function of the COX-2/PGE$_2$ pathway in in vivo cancer development has not yet been fully elucidated.

The expression of proinflammatory cytokines, including TNF-α and IL-6, is induced in the inflammatory microenvironment of cancer, and mouse genetic studies have shown that these cytokines also promote tumorigenesis. TNF-α was originally recognized as a tumor necrosis factor; however, it is now established that TNF-α exhibits important tumor-promoting functions [26]. Inhibition of TNF-α signaling by disruption of TNF-α or its receptor gene results in the suppression of skin carcinogenesis and inflammation-associated colon cancer development [27–29]. TNF-α activates transcription factor NF-κB, which further induces the expression of inflammatory factors, including COX-2, IL-6, IL-8, and TNF-α itself, forming an inflammatory network in tumor microenvironment [30] (Fig. 2). Genetic inhibition of NF-κB in colitis-associated colon cancer model mice results in significant suppression of tumor development [31]. On the other hand, IL-6 expression is induced in colon cancer [32]. IL-6 signaling activates Stat3 through the gp130 receptor. Notably, disruption of IL-6 or Stat3 genes in colitis-associated colon cancer model mice results in significant suppression of colonic tumors [33, 34]. Taken together, these results indicate that the COX-2/PGE$_2$, TNF-α/NF-κB, and

Fig. 2 Schematic diagram showing inflammatory network in tumor microenvironment. Induction of COX-2/PGE$_2$ pathway is responsible for the construction of an inflammatory microenvironment where macrophages are recruited. NF-κB is activated in tumor-associated macrophages, resulting in the induction of inflammatory factors including COX-2, TNF-α, and IL-6. TNF-α and IL-6 activate the transcription factors NF-κB and Stat3, respectively, forming an inflammatory network in the tumor microenvironment. Such network plays a critical role in tumor promotion
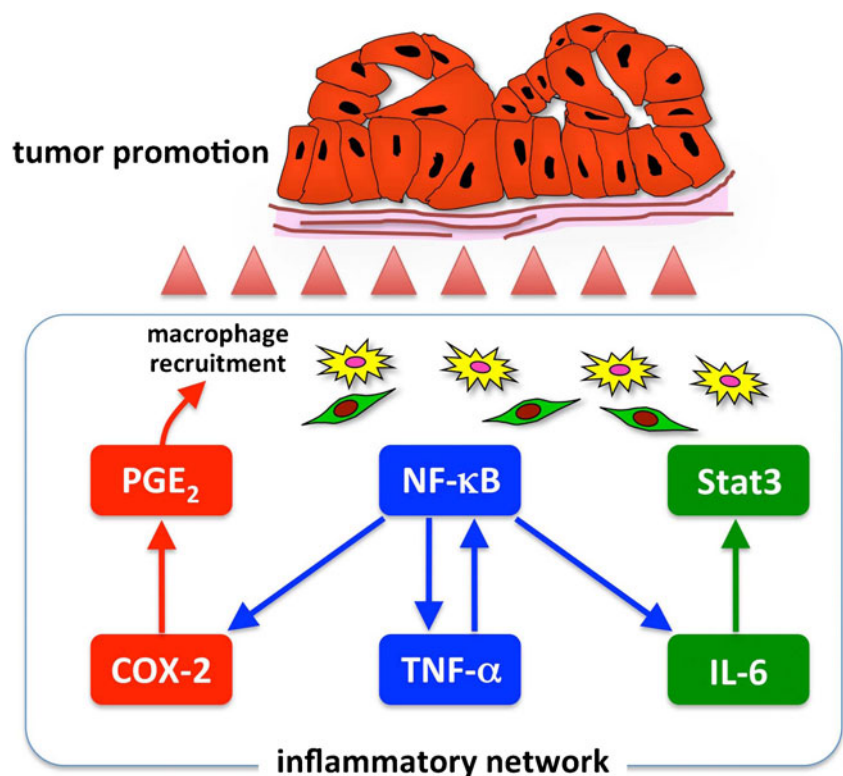
IL-6/Stat3 pathways construct the inflammatory tumor microenvironment required for the promotion of tumorigenesis [30] (Fig. 2).

Inflammation-associated gastric cancer mouse model

Preneoplastic changes induced by oncogenic Wnt signaling

Based on the results of the role of the COX-2/PGE$_2$ pathway in cancer development, we developed a PGE$_2$-associated gastritis and gastric cancer mouse models as follows: Canonical Wnt signaling (Wnt/β-catenin signaling) is important for the undifferentiated status of epithelial cells and thus induces tumorigenesis [35, 36]. Mutations in APC or β-catenin genes result in constitutive activation of canonical Wnt signaling, which causes tumor development in the entire intestinal tract [37, 38]. Nuclear or cytoplasmic accumulation of β-catenin, a hallmark of Wnt signaling activation, is detected in 30–50 % of gastric cancer tissues [39–42]. Moreover, mutations in the β-catenin gene have been found in a subpopulation of gastric cancers [39, 40, 43, 44]. These results indicate that Wnt signaling activation is an important oncogenic pathway for gastric cancer development.

To examine the role of Wnt activation in gastric tumorigenesis, we created K19-Wnt1 transgenic mice that express Wnt1, a ligand for canonical Wnt signaling, using the K19 gene promoter that is transcriptionally active in gastric epithelial cells [42]. Although K19-Wnt1 mice do not develop gastric tumors, they do develop small aberrant cryptic foci on the surface of the gastric mucosa (Fig. 3a). Histologically, these lesions consist of dysplastic epithelial cells with increased cell proliferation and cytoplasmic β-catenin accumulation and are thus diagnosed as preneoplastic lesions [11, 12]. Importantly, macrophages infiltrate the stroma of preneoplastic lesions, while tissue macrophages are rarely detected in normal gastric mucosa (Fig. 3a). These results suggest that inflammatory microenvironments consisting of macrophages are constructed in the early stage of tumorigenesis and may be required for tumorous changes in Wnt-activated epithelial cells. To support this hypothesis, we demonstrated that macrophage-derived TNF-α promotes Wnt signaling activity in epithelial cells, which contributes to the development of preneoplastic lesions in K19-Wnt1 mice [45].

Gastritis induced by the inflammatory COX-2/PGE$_2$ pathway

On the other hand, the COX-2/PGE$_2$ pathway is widely induced in gastric cancer, as found in colon cancer tissues [46]. H. pylori infection is tightly associated with gastric cancer, and the levels of the COX-2 expression and PGE$_2$ production are related to the status of H. pylori infection [47, 48]. Accordingly, it is possible that the induction of the COX-2/PGE$_2$ pathway is an important tumor-promoting mechanism of H. pylori infection. To examine the role of the COX-2/PGE$_2$ pathway in gastric tumorigenesis, we created another group of transgenic mice, K19-C2mE mice, that expresses Ptgs2 and Ptges encoding COX-2 and mPGES-1, respectively, in the gastric mucosa [49]. K19-C2mE mice develop gastric hyperplasia and mucous cell metaplasia, which are histologically similar to spasmolytic polypeptide/trefoil factor 2 (TFF2)-expressing metaplasia (SPEM) (Fig. 3b) [49, 50]. SPEM is associated with human gastric cancers [51, 52], thus suggesting that SPEM is a possible precursor of gastric cancer [53]. SPEM development has also been observed to occur in the stomachs of several different gastric tumor model mice, including Helicobacter-infected mice, gastrin gene knockout mice, and gp130 receptor-active mutant mice [54–56], suggesting that the COX-2/PGE$_2$ pathway is responsible for SPEM formation in human gastric cancer as well as in these mouse models.

Hyperplastic lesions in K19-C2mE mice are associated with submucosal infiltration of lymphocytes, granulocytes, and mononuclear cells. Moreover, macrophage accumulation is also observed in the gastric mucosa (Fig. 3b). Notably, α-smooth muscle-expressing myofibroblasts are detected in K19-C2mE mouse inflamed gastric mucosa, and approximately 10 % of myofibroblasts is derived from bone marrow [57]. Myofibroblasts are also important components of the tumor microenvironment, together with tumor-associated macrophages. Accordingly, it is possible that induction of the COX-2/PGE$_2$ pathway is responsible for the construction of inflammatory microenvironment consisting of macrophages and myofibroblasts. Importantly, disruption of the TNF-α gene in K19-C2mE mice results in suppression of gastritis and SPEM development, while IL-1 receptor or Rag2 gene disruption does not affect these phenotypes [50]. Accordingly, it is possible that TNF-α induced in the COX-2/PGE$_2$-associated inflammatory microenvironment plays a key role in SPEM formation.

Gastric cancer induced by Wnt and the COX-2/PGE$_2$ pathway

By crossing K19-Wnt1 and K19-C2mE transgenic mice, compound K19-Wnt1/C2mE transgenic mice were created that express Wnt1, Ptgs2, and Ptges simultaneously in the gastric epithelial cells, resulting in activation of Wnt signaling and the COX-2/PGE$_2$ pathway simultaneously in the stomach [42]. Importantly, K19-Wnt1/C2mE mice develop large gastric tumors at around 40 to 50 weeks of age with 100 % incidence (Fig. 3c). We thus named the K19-Wnt1/
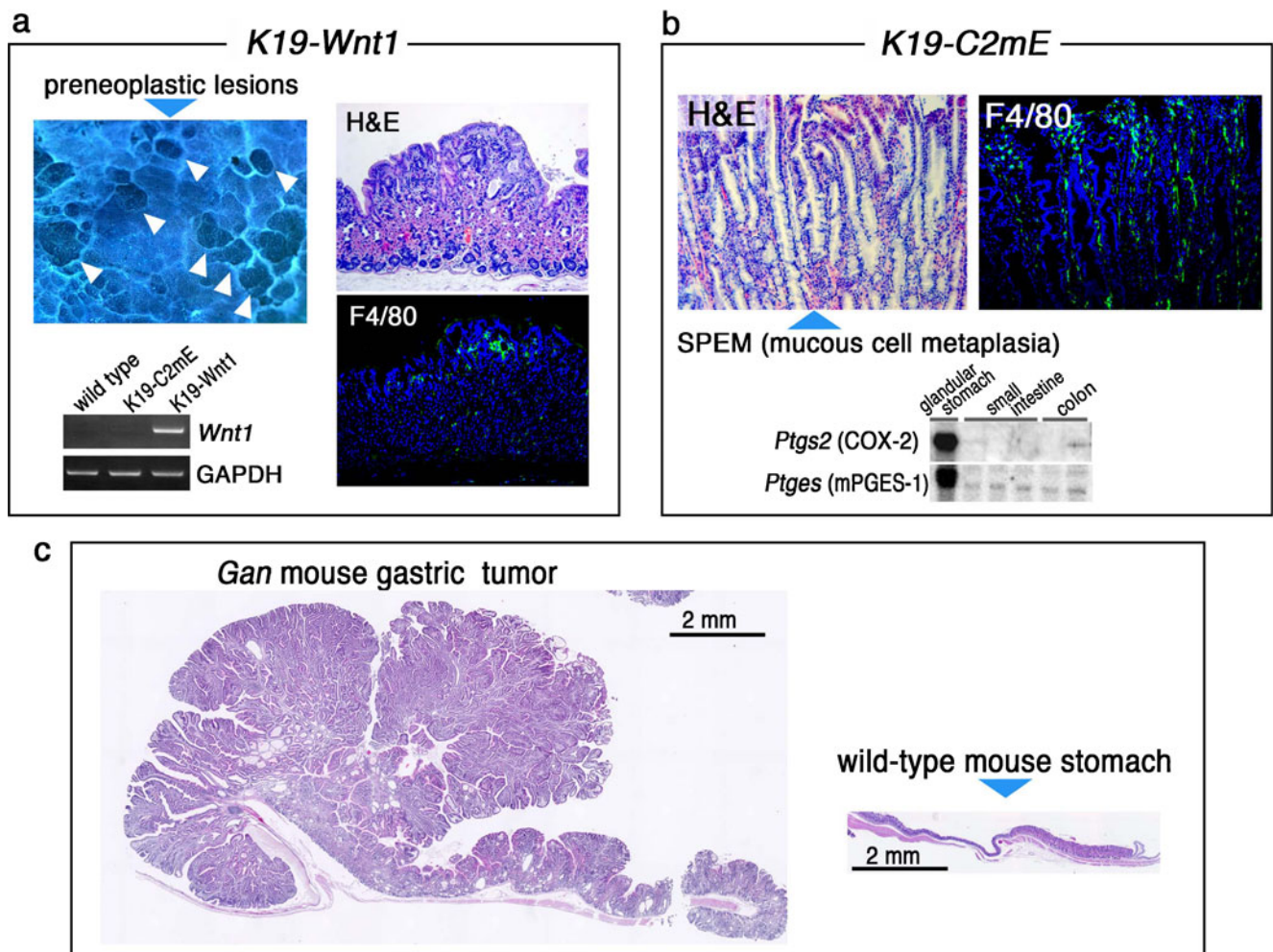
Fig. 3 Gastric phenotypes by Wnt activation, COX-2/PGE₂ induction, or both in combination. **a** *K19-Wnt1* mice expressing *Wnt1* in gastric epithelial cells develop preneoplastic lesions by Wnt activation (*left, right top*). Expression of *Wnt1* gene in *K19-Wnt1* mouse stomach was confirmed by RT-PCR (*left, bottom*). F4/80-positive macrophages are infiltrated in the stroma of preneoplastic lesion (*right, bottom*). **b** *K19-C2mE* mice express *Ptgs2* and *Ptges* in the stomach, resulting in the induction of the COX-2/PGE₂ pathway, and they develop SPEM lesion consisting of metaplastic mucous cells (*left*). F4/80-positive macrophages are accumulated in metaplastic and hyperplastic gastric mucosa (*right*). Expression of *Ptgs2* and *Ptges* in the glandular stomach was confirmed by Northern blotting (*bottom*). **c** *Gan* mice expressing *Wnt1*, *Ptgs2*, and *Ptges* in the stomach develop large gastric tumors (*left*) caused by the activation of both Wnt signaling and COX-2/PGE₂ pathway. Wild-type mouse stomach is shown as a control of the same magnification (*right*) (RT-PCR shown in **a** was reproduced from Oshima et al. [42] with permission from Elsevier, and Western blotting shown in **b** was reproduced from Oshima et al. [49] with permission from Nature Publishing)

C2mE mice as *Gan* mice, which stands for gastric neoplasia mice. Histologically, gastric tumors in *Gan* mice consist of dysplastic epithelial cells with an irregularly branching glandular structure, thus diagnosed as glandular-type gastric tumors. Moreover, the Ki-67 labeling index is significantly increased, indicating accelerated proliferation of tumor cells. Mucosal macrophage infiltration is also found in *Gan* mouse tumors, and the expression of proinflammatory cytokines and chemokines increases significantly. Accordingly, these results, taken together, indicate that Wnt signaling activation in the COX-2/PGE₂-associated inflammatory microenvironment cooperatively causes the development of gastric cancer. Importantly, the gene expression profiles of

Gan mouse tumors are similar to those found in human intestinal-type gastric cancer [58], thus indicating that *Gan* mice recapitulate the development of human intestinal-type gastric cancer from genetic changes and host inflammatory responses to tumor morphology and gene expression profiles.

In the inflamed tissues, reactive oxygen species (ROS) and nitric oxide are induced, which play a role in tumor development. In the gastric tumor tissues of *Gan* mice, ROS level is also increased [59]. However, we have recently demonstrated that the expression of CD44 variant form (CD44v) is significantly induced in gastritis of *K19-C2mE* mice [60], and CD44v plays a critical role in tumor

development in *Gan* mice through protection of tumor cells from oxidative stress [59]. Accordingly, it is possible that ROS plays a role in tumorigenesis, and at the same time protection from ROS leads to tumor promotion.

Interestingly, macrophage infiltration and inflammatory responses in gastric tumors are significantly suppressed in germ-free *Gan* mice, although the COX-2/PGE$_2$ pathway is still activated [61]. Moreover, the gastric tumor volume decreases significantly in germ-free *Gan* mice. In contrast, *Helicobacter* infection in the stomachs of germ-free *Gan* mice induces inflammatory responses and the development of large gastric tumors. These results indicate that increased PGE$_2$ signaling is not sufficient to induce inflammatory responses in the stomach; however, bacterial infection together with the COX-2/PGE$_2$ pathway is required for the construction of the inflammatory microenvironment. It has been shown that Toll-like receptor (TLR) signaling is important for intestinal epithelial homeostasis, and the suppression of TLR signaling suppresses regenerative proliferation in injured mucosa [62]. Moreover, a disruption of the *Myd88* gene encoding MyD88, an important adaptor molecule of the TLR signaling pathway, results in the suppression of intestinal polyposis in *Apc^{Min}* mice with a decreased expression level of COX-2 [63]. Accordingly, it is possible that innate immune signaling activated by bacterial infection and the TLR/MyD88 pathway is required for the construction of the PGE$_2$-associated tumor microenvironment. On the other hand, it has been shown that epithelial cell-derived PGE$_2$ suppresses inflammatory cytokine expression in the LPS-stimulated bone marrow-derived cells, which is important for protection from bacterial infection-associated colitis [64]. It is therefore possible that PGE$_2$ has distinct functions on inflammatory cytokine expression in immune tolerance and in cancer microenvironment, although the mechanism underlying such difference remains to be investigated.

It is therefore expected that complicated interactions between tumor cells and the inflammatory microenvironment in human gastric cancer are repeated in *Gan* mouse gastric tumor tissues. Accordingly, using the *Gan* mouse model, we are able to uncover the mechanisms underlying PGE$_2$-associated inflammatory responses in gastric tumorigenesis. For example, we have determined that gastric tumor cells educate and activate stromal fibroblasts and bone marrow-derived cells to be myofibroblasts, resulting in the induction of the VEGF expression and enhancement of angiogenesis in tumor tissues [57]. Moreover, we examined the gene expression profiles of mRNAs and microRNAs (miRNAs) in *K19-C2mE* gastritis and *Gan* mouse gastric tumor tissues and found several novel mechanisms of inflammation in tumor development, as described in the following section.

Activation of epidermal growth factor receptor signaling by PGE$_2$-associated inflammation

EGFR signaling is an important target for cancer prevention [65]. The genetic or pharmacological inhibition of EGFR signaling in *Apc^{Min}* mice results in significant suppression of intestinal polyposis [66–68], indicating that EGFR signaling is required for tumor cell proliferation even when Wnt signaling is constitutively activated. Importantly, the combination treatment of *Apc^{Min}* mice with EGFR inhibitors and NSAIDs or COX-2 inhibitors suppresses intestinal polyposis more effectively than simple treatment, thus suggesting a possible link between the COX-2/PGE$_2$ pathway and EGFR signaling [67, 68]. It has already been shown using in vitro experiments that the PGE$_2$ pathway transactivates EGFR signaling through the activation of cSrc or MMPs [69–71] or the induction of EGFR ligands and a disintegrin and metalloproteinases 17 (ADAM17), a shedding enzyme for EGFR ligands [72–74]. We thus examined the mechanisms underlying EGFR activation by the COX-2/PGE$_2$ pathway in in vivo tumors using *K19-C2mE* and *Gan* mice.

We compared the expression levels of all EGFR ligands, their receptors, and shedding enzymes for EGFR ligands in gastritis and gastric tumors with those in normal stomachs using microarray results [58]. Surprisingly, the expression of most EGFR ligands, including *Areg*, *Ereg*, *Hbegf*, and *Btc*, encoding amphiregulin, epiregulin, HB-EGF, and betacellulin, respectively, as well as *Erbb2* and *Erbb3* encoding Her2 and Her3, respectively, increased significantly in both *K19-C2mE* mouse gastritis and *Gan* mouse gastric cancer compared with that observed in wild-type mice [75] (Fig. 4a). ADAM family proteases activate EGFR signaling by shedding the ectodomain of EGFR ligands to release them from the cell membrane, and some ADAM family genes are induced in a variety of cancer tissues, suggesting that ADAMs play a role in the activation of EGFR signaling in cancer development [76]. Notably, the expression of *Adam8*, *Adam9*, *Adam10*, and *Adam28* is increased significantly in the stomach in *K19-C2mE* gastritis and *Gan* mouse tumors. Taken together, these results indicate that the expression of EGFR ligand members, their receptors, and ADAM family members is upregulated simultaneously in the PGE$_2$-associated inflammatory microenvironment, which causes activation of EGFR signaling in cancer cells. Consistently, EGFR is phosphorylated in the epithelial cells of *K19-C2mE* and *Gan* mouse stomachs, indicating the activation of EGFR, which is not found in the wild-type mouse gastric mucosa (Fig. 4b). Among the four PGE$_2$ receptors, the expression of EP4 is significantly increased in the stomachs of both *K19-C2mE* and *Gan* mice [77]. Notably, treatment of *Gan* mice with EP4 receptor inhibitors results in significant decreases in the EGFR ligand and

Fig. 4 Activation of EGFR signaling in COX-2/PGE$_2$ pathway-induced inflammatory microenvironment. **a** Expression of EGFR ligands and Adam family proteases is upregulated in both *K19-C2mE* mouse gastritis and *Gan* mouse gastric tumors. **b** Phosphorylated EGFR is detected by immunohistochemistry (*green*) in gastric epithelial cells of *K19-C2mE* mice and *Gan* mice, which is not found in the wild-type mouse stomach. Nuclei are stained with DAPI (*red*). **c** X ray CT photographs of a *Gan* mouse that was treated with EGFR inhibitor, ZD1839, and COX-2 inhibitor, celecoxib, in combination for 3 weeks. Note that the gastric tumor volume decreased dramatically by drug treatment. **d** Schematic diagram of EGFR activation in the COX-2/PGE$_2$ pathway-associated inflammatory microenvironment. Induction of EGFR ligands and Adams cooperatively activates EGFR signaling, which further activates PGE$_2$ signaling through downregulation of 15-PGDH by positive feedback mechanism (**a**, **b**, and **c** were reproduced from Oshima et al. [73] with permission from Wiley-Blackwell)
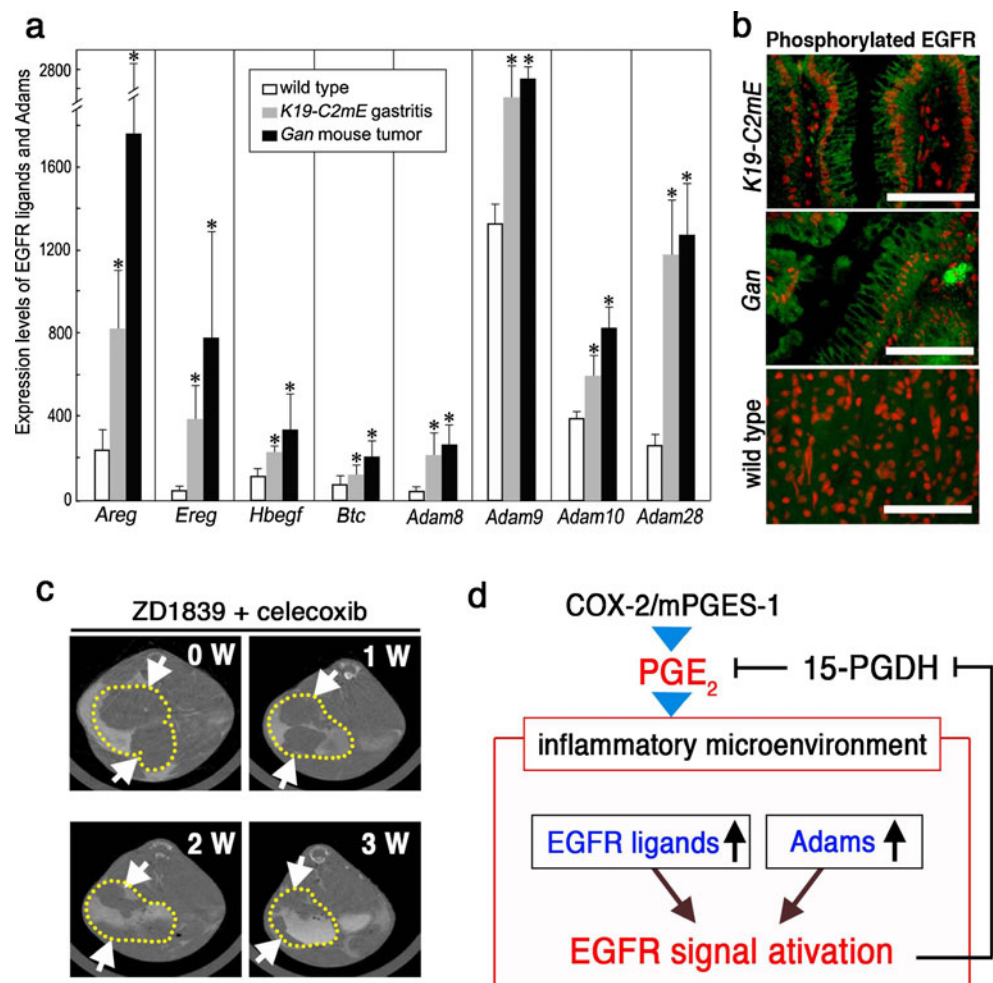
ADAM expression levels [75], thus indicating that PGE$_2$ signaling via the EP4 receptor is responsible for EGFR activation in gastric epithelial cells.

EGFR signaling activates the MAPK and PI3K/Akt pathways, which results in phosphorylation of Erk1/2 and Akt [78]. Notably, the phosphorylation levels of Erk1/2 and Akt in *Gan* mouse gastric tumors are decreased significantly by treatment of the mice with the COX-2 inhibitor celecoxib. Accordingly, it is possible that the COX-2/PGE$_2$ pathway is a major cause of activation of EGFR signaling in gastric cancer cells. On the other hand, EGFR signaling can activate the COX-2/PGE$_2$ pathway as follows: The cellular PGE$_2$ level is regulated by 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catalyzes and thus inactivates prostaglandins. Importantly, the expression of 15-PGDH is downregulated by EGFR signaling [79], resulting in the maintenance of an increased PGE$_2$ level. Moreover, disruption of the 15-PGDH gene in *Apc^{Min}* mice accelerates intestinal tumorigenesis [80]. Accordingly, the COX-2/PGE$_2$ pathway activates EGFR signaling through the induction of EGFR ligands and ADAM proteases, which in turn

downregulates 15-PGDH, resulting in an increased level of PGE$_2$ in the inflammatory microenvironment. Such PGE$_2$/EGFR signaling feedback loops therefore play an important role in gastric cancer development (Fig. 4d).

Treatment of *Gan* mice with either EGFR inhibitor, ZD1839, or celecoxib significantly reduces gastric tumor volume [75] (Fig. 4c). Importantly, combination treatment of *Gan* mice with ZD1839 and celecoxib results in the complete regression of gastric tumors, possibly through inhibition of individual pathways as well as feedback loops between PGE$_2$ and EGFR. These results suggest that the use of such combination treatment is an effective preventive strategy against gastric cancer development.

Downregulation of tumor suppressor microRNA, miR-7, by PGE$_2$-associated inflammation

miRNAs are single-strand, small, noncoding RNAs that regulate gene expression via post-transcriptional interference of target mRNAs [81, 82]. Accordingly, miRNAs can

function as oncogenes or tumor suppressors through the inhibition of tumor suppressor genes or of oncogene expression, respectively [83, 84]. Several mechanisms for dysregulation of the miRNA expression have been demonstrated, such as genetic or epigenetic alterations or transcriptional or post-transcriptional mechanisms [85]. Notably, it has also been shown that inflammation induces the expression of oncogenic miRNAs [86]. For example, miR-155 and miR-21 are oncogenic miRNAs that are induced by NF-κB, TLR, interferon-β, or the Stat3 pathway [87–89]. NF-κB and Stat3 are activated by TNF-α and IL-6, respectively, and are key factors in the network of tumor microenvironment [30] (Fig. 2).

Microarray analyses indicate that 50 microRNAs are upregulated (>2.0-fold) and 42 miRNAs are downregulated (<0.5-fold) in *Gan* mouse gastric tumors [90] (Fig. 5a). Among these miRNAs, 21 and 29 show the same upregulation and downregulation, respectively, in *K19-C2mE* mouse gastritis, thus indicating that expression changes in these miRNAs (21 up and 29 down) in gastric tumors are caused by COX-2/PGE$_2$ pathway-associated inflammatory responses. Notably, the oncogene miRNAs miR-21 and miR-155 [91] are upregulated both in *K19-C2mE* mouse gastritis and *Gan* gastric tumors, whereas the tumor suppressor miRNAs miR-145 and miR-7 [92, 93] are downregulated in both tissues. Accordingly, it is possible that
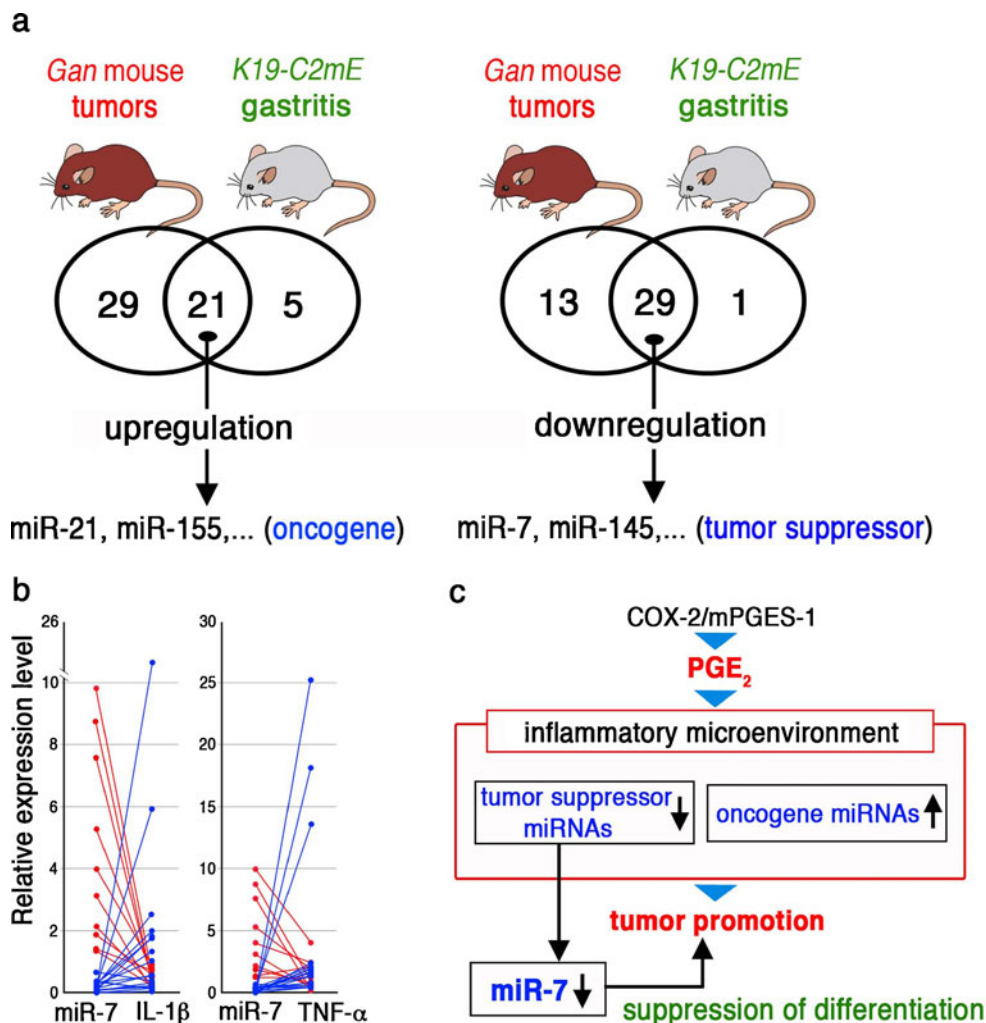

Fig. 5 Downregulation of tumor suppressor miRNA, miR-7, in COX-2/PGE$_2$ pathway-induced inflammatory microenvironment. **a** Venn diagrams of upregulated and downregulated miRNAs in *K19-C2mE* mouse gastritis and *Gan* mouse gastric tumors. Note that 21 and 29 miRNAs are upregulated and downregulated, respectively, both in gastritis and gastric tumors, indicating inflammation-dependent expression changes. Notably, oncogene miRNAs, miR-21 and miR-155, are upregulated, whereas tumor suppressor miRNAs, miR-7 and miR-145, are downregulated simultaneously both in gastritis and gastric tumors.

b Inverse relations between the expression levels of miR-7 and inflammatory cytokines, TNF-α and IL-1β, in human gastric cancer tissues: namely, cytokine high gastric cancers show lower miR-7 level, indicating that the severity of inflammatory responses is associated with miR-7 downregulation. **c** Schematic diagram of miR-7 downregulation in PGE$_2$-associated inflammatory microenvironment. Downregulation of miR-7 promotes gastric tumorigenesis possibly through suppression of epithelial differentiation (**a** and **b** were reproduced from Kong et al. [88] with permission from Nature Publishing)

inflammation promotes tumorigenesis by inducing oncogene miRNAs and suppressing tumor suppressor miRNAs simultaneously, possibly through different mechanisms.

It has been shown that miR-7 functions as a tumor suppressor in glioblastoma, breast cancer, and lung cancer [93–97]. The expression level of miR-7 is downregulated both in gastritis and gastric tumors, indicating that miR-7 is an inflammation-dependent tumor suppressor miRNA (Fig. 5a). Moreover, the miR-7 level is significantly decreased in *Helicobacter*-infected inflamed mouse gastric mucosa compared with control inflamed stomachs. In a normal stomach, the basal level of miR-7 is found in undifferentiated gastric epithelial cells; however, its expression level increases during the differentiation of epithelial cells. Accordingly, it is possible that miR-7 plays a role in cell differentiation.

In human gastric cancer tissues, miR-7 expression is downregulated in 65 % of cases. Moreover, the expression levels of miR-7 in human gastric cancers are inversely correlated with those of the inflammatory cytokines IL-1β and TNF-α (Fig. 5b). Namely, miR-7 expression is downregulated more significantly in high TNF-α or high IL-1β gastric cancers compared with low TNF-α or low IL-1β gastric cancers. Accordingly, it is possible that the severity of inflammatory responses is correlated with effective downregulation of miR-7.

Moreover, transfection of precursor miR-7 in gastric cancer cells results in decreased cell proliferation and suppression of soft agar colony formation. These results indicate that miR-7 functions as a tumor suppressor in gastric cancer cells as well. These results, taken together, indicate that miR-7 is an important factor that links inflammation and cancer development, namely, inflammation induces downregulation of the miR-7 expression, which may suppress the differentiation of epithelial cells and thus increase tumorigenicity (Fig. 5c). It has been shown that EGFR mRNA is a miR-7 target gene [93, 95]. Consistently, in gastric cancer cells, precursor miR-7 transfection causes downregulation of the EGFR expression. Moreover, p21-activated kinase 1, Raf1, and insulin-like growth factor I receptor have been identified as possible miR-7 target genes that are upregulated in cancer cells [94–96]. Using the expression profile data set [58], we identified novel miR-7 target genes, including *Lphn2*, *Basp1*, and *MafG*. It is possible that upregulation of these genes by miR-7 downregulation contributes to gastric tumorigenesis, although the role of these factors in cancer remains to be investigated in the future.

Conclusions

Accumulating evidence has established that inflammatory responses are important for cancer development. As discussed in this review article, in vivo model systems such as *K19-C2mE* and *Gan* model mice are important for investigating the role of inflammatory responses in cancer development because the tumor-promoting microenvironment consisting of residential fibroblasts and bone marrow-derived cells is constructed in tumor tissues. Using such a model system, we identified several novel mechanisms underlying PGE$_2$-associated inflammation in gastric tumorigenesis, i.e., EGFR activation through the induction of EGFR ligands and ADAM proteases and downregulation of tumor suppressor miRNAs, including miR-7. On the other hand, most malignant cancer cells that carry accumulated genetic alterations do not require expression of COX-2 or EGFR ligands in stromal cells because oncogenic mutations cause induction or activation of these endogenous pathways in the tumor cells. For example, *ras* mutation causes constitutive expression of COX-2 and activation of EGFR signaling pathway. It is thus possible that PGE$_2$-associated inflammatory responses are more important for cancer development during promotion stage rather than malignant progression stage. Accordingly, regulation of such PGE$_2$-associated inflammatory responses will be an effective preventive strategy against gastric cancer development.

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