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The inflammatory microenvironment that promotes gastrointestinal cancer development and invasion

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ABSTRACT

Accumulating evidence has indicated that the inflammatory response is important for tumor promotion. However, the mechanisms underlying the induction of the inflammatory response in cancer tissues and how it promotes tumorigenesis remain poorly understood. We constructed several mouse models that develop inflammation-associated gastric and intestinal tumors and examined the *in vivo* mechanisms of tumorigenesis. Of note, the activation of cyclooxygenase-2 (COX-2)/prostaglandin E_2 (PGE_2) pathway and Toll-like receptor (TLR)/MyD88 signaling cooperatively induced the generation of an inflammatory microenvironment, which is required for early-stage tumorigenesis. The inflammatory response in the stroma induces TNF-α signaling in tumor cells, and the NOX1/ROS signaling pathway is activated downstream. In addition, the inflammatory pathway induces the expression of TLR2 in tumor epithelial cells. Both the NOX1/ROS and TLR2 pathways in tumor cells contribute to the acquisition and maintenance of stemness, which is an important tumor-promoting mechanism stimulated by inflammation. We also found that inflammation promotes malignant processes, like submucosal invasion, of TGF-β signaling-suppressed tumor cells through the activation of MMP2 protease. Moreover, we showed that mutant p53 induces innate immune and inflammatory signaling in the tumor stroma by a gain-of-function mechanism of mutant p53, which may explain the "cancer-induced inflammation" mechanism. These results indicate that the regulation of the inflammatory microenvironment via the inhibition of the COX-2/PGE_2 and TLR/MyD88 pathways in combination will be an effective preventive or therapeutic strategy against gastrointestinal cancer development and malignant progression, especially those carrying p53 gain-of-function mutations.

1. Introduction

It has been established that inflammatory responses play a tumor-promoting role in gastrointestinal cancer development. Epidemiological studies have shown that the regular use of non-steroidal anti-inflammatory drugs (NSAIDs), including aspirin, reduces the risk of gastrointestinal cancer (Thun et al., 1991, 2012). NSAIDs suppress the enzymatic activity of cyclooxygenase (COX)-1 and COX-2, rate-limiting enzymes for prostaglandin (PG) biosynthesis. We previously showed using genetic mouse models that COX-2 and its downstream product PGE_2 play an essential role in tumor development in the intestine and stomach (Oshima et al., 1996, 2004; Sonoshita et al., 2001). In the inflammatory microenvironment generated in tumor stroma, the infiltration of tumor-associated macrophages (TAMs), which express cytokines, chemokines, growth factors and proteases, can be seen (Pollard, 2009). TAMs support not only tumor cell migration and proliferation but also metastasis through chemokine CCL2 signaling (Kitamura et al., 2015). It has

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also been shown that the transcription factors NF-κB and Stat3 are activated in both inflamed tissues and tumors (He and Karin, 2011), and these transcription factors as well as the COX-2/PGE_2 pathway link inflammation and cancer.

To examine the mechanism of inflammation in gastrointestinal tumorigenesis in greater detail, we have constructed genetically engineered mouse models that develop tumors in the stomach (*Gan* mice) or intestine ($Apc^{\Delta 716}$ mice) and examined how the inflammatory microenvironment is generated in tumor tissues and how such a microenvironment promotes tumor development and malignant progression. In this chapter, we will summarize what we have learned from these mouse models.

2. COX-2/PGE_2 inflammatory pathway in intestinal tumorigenesis

$Apc^{\Delta 716}$ mice that carry a truncation mutation in the *Apc* gene develop intestinal tumors caused by canonical Wnt signaling activation in epithelial cells (Oshima et al., 1995). In the intestinal tumors of $Apc^{\Delta 716}$ mice, the COX-2 expression is induced in stromal fibroblasts but not in tumor cells, which triggers the generation of an inflammatory microenvironment via the biosynthesis of PGE_2 (Sonoshita et al., 2002). Importantly, the deletion of the COX-2 gene or pharmacological inhibition of COX-2 in $Apc^{\Delta 716}$ mice resulted in a significant decrease in polyp numbers, indicating that a COX-2-dependent inflammatory microenvironment is critical for tumor development (Oshima et al., 1996, 2001). The tumor-promoting mechanism of PGE_2 remains poorly understood, but it has been reported that PGE_2 induces the cancer stem cell expansion by activating NF-κB, via signaling through its receptor EP4 (Wang et al., 2015). Based on these results, COX-2 selective inhibitors were expected to be an effective chemopreventive agent for colon cancer, and indeed, the treatment of familial adenomatous polyposis patients with celecoxib, a COX-2 inhibitor, suppressed the development of intestinal polyposis (Steinbach et al., 2000). However, COX-2 inhibitors have not been widely used for cancer prevention because of potential serious side effects on the circulation system in certain populations. Thus, other target molecules, like the PGE_2 receptor, are now thought to be potential appropriate targets for colon cancer prevention.

3. COX-2/PGE_2 inflammatory pathway in gastric tumorigenesis

We first constructed a gastritis mouse model, *K19-C2mE* mice, via the transgenic expression of COX-2 and mPGES-1, an inducible PGE converting enzyme, in gastric mucosa. *K19-C2mE* mice develop inflammation-associated mucosal hyperplasia in the stomach caused by increased levels of PGE_2 (Oshima et al., 2004). Notably, the additional activation of canonical Wnt signaling in *K19-C2mE* stomach epithelial cells by the transgenic induction of *Wnt1* caused the development of glandular-type tumors (Oshima et al., 2006, 2009). These genetic results indicate that the cooperation of oncogenic Wnt activation and a COX-2-induced inflammatory response induces gastric tumorigenesis. We named this gastric tumor model "*Gan* (gastric neoplasia) mice". Later, the Cancer Genome Atlas (TCGA) network reported the results of a genome research analysis of human gastric cancers (The Cancer Genome Atlas Research Network, 2014). Using the TCGA database for gastric cancer, we examined the activated signaling pathways and found that the Wnt signaling, COX-2, and NF-κB inflammatory pathways are activated simultaneously in intestinal-type gastric cancers (Echizen et al., 2016). Accordingly, *Gan* mice recapitulate intestinal-type human gastric cancer development, from the molecular mechanisms to the histological phenotypes.

We next examined the expression profiles of the gastritis and gastric tumors that developed in *K19-C2mE* mice and *Gan* mice, respectively. On a microarray analysis, we found that a tumor-suppressor microRNA, miR-7a, was significantly downregulated by inflammatory responses (Kong et al., 2012). MiR-7a suppresses the expression of EGFR, thus its downregulation may support tumor cell proliferation via the activation of EGFR signaling (Kefas et al., 2008). By further RNA sequencing of gastritis and tumor tissues, we found that the expression profiles of gastric tumors were similar to those of gastritis (Echizen et al., 2016) (Fig. 1A). Of note, stem cell-related genes, such as *Cd44*, *Sox2*, *Noxo1*, *Sox9*, and *Prom1* were upregulated in both gastritis and gastric tumors, while the expression of differentiation-related genes was downregulated, suggesting that these changes in the expression of tumors were induced by an inflammation-dependent mechanism (Echizen et al., 2016) (Fig. 1B). Accordingly, the COX-2/PGE_2-induced inflammatory microenvironment may promote tumorigenesis by maintaining the tumor cells in an undifferentiated state.

4. TNF-α signaling in gastric tumorigenesis

Although TNF-α was isolated as a "tumor necrosis" factor, mouse genetic studies have indicated that TNF-α plays a tumor-promoting role in several solid tumors (Balkwill, 2009). In addition, a polymorphism of the TNF-α gene was linked to the gastric cancer incidence (El-Omar et al., 2002). In the *Gan* mouse gastric tumors, inflammatory cytokine levels were elevated because of constitutive activation of COX-2/PGE_2 pathway. To examine the role of TNF-α in gastric tumorigenesis, the TNF-α gene was deleted in *Gan* mice by crossing with $Tnf-/-$ mice, which resulted in significant suppression of gastric tumorigenesis (Oshima et al., 2014). Notably, the transplantation of TNF-α-wild-type bone marrow to $Tnf-/-$ *Gan* mice caused gastric tumor development, indicating that the TNF-α expression in the infiltrated bone marrow-derived cells in tumor stroma is important for tumorigenesis.

A microarray analysis using TNF-α wild-type and knockout *Gan* mouse tumors revealed that stem cell-related genes, such as *Cd44*, *Noxo1*, *Prom1*, and *EphB3*, were upregulated in tumor tissues in a TNF-α-dependent mechanism (Fig. 2). These genes overlap with the upregulated genes in *K19-C2mE* gastritis. We therefore performed functional screening for TNF-α-dependent genes using gastric cancer cell lines and found that the inhibition of *Noxo1* expression significantly suppressed soft-agar colony formation of all examined cell lines.

Noxo1 is a component of the NADPH oxidase 1 (NOX1) complex, which produces reactive oxygen species (ROS). It has been shown that NOX1 complex is important for tumorigenesis through the suppression of protein tyrosine phosphatases, which results in