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Review

Application of the Apc$^{Min/+}$ mouse model for studying inflammation-associated intestinal tumor

Lu Wang, Qingzhu Zhang *

Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, Jinan, China

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ABSTRACT

Chronic inflammatory diseases of the intestinal tract have been known to increase risk of developing a form of colorectal cancer known as inflammation-associated cancer. The roles of inflammation in tumor formation and development in Apc$^{Min/+}$ mice have been broadly corroborated. The Apc$^{Min/+}$ mouse model contains a point mutation in the adenomatous polyposis coli (Apc) gene and only develops intestinal precancerous lesions, the benign adenomas. Thus, it provides an excellent *in vivo* system to investigate the molecular events involved in the inflammatory process which may contribute to multistep tumorigenesis and carcinogenesis. Recent investigations that employ this model studied the effects of gene alterations, intestinal microorganisms, drugs, diet, exercise and sleep on the inflammatory process and tumor development, and revealed the mechanisms involved in the formation, promotion and carcinogenesis of adenomas with the background of inflammation. Herein, we focus our review on the application of the Apc$^{Min/+}$ mouse model for studying inflammation-associated intestinal tumor and find that anti-inflammation is a possible strategy in combating intestinal tumor, but sometimes anti-inflammation cannot help reduce tumor burden. Moreover, various inflammation-related genes are involved in different mechanistic stages of tumor in Apc$^{Min/+}$ mice and intricate regulatory effects of inflammation exist in the whole progression of intestinal tumor.

1. Introduction

In human subjects, the high incidence of intestinal adenocarcinoma in individuals with inflammatory bowel diseases (IBDs) shows a close correlation between chronic inflammation and oncogenesis [1]. Patients suffering inflammation are at greater risk of developing colorectal cancer (CRC) than the general population. The risk of CRC was linked to both the degrees of inflammation and the disease duration in IBDs [2]. The roles of inflammation in tumor formation and development have been broadly corroborated. Besides, more and more evidence for the effects of inflammation on intestinal adenoma formation and development in the Apc$^{Min/+}$ mouse model has been found. C57BL/6J Apc$^{Min/+}$ mouse spontaneously develops pretumoric adenoma into the intestinal mucosa. Importantly, inflammation has shown a tendency to increase tumorigenesis in Apc$^{Min/+}$ mouse model. Therefore, it is necessary and possible to investigate the mechanisms of intestinal tumor development with the background of inflammation by using this model. Recently, the Apc$^{Min/+}$ mouse model is used to study the effects of genetics, intestinal microorganisms, anti-inflammatory drugs, diet and exercise on the progress of inflammation-associated intestinal tumor and to investigate the molecular events involved in the inflammatory process which may contribute to multistep tumorigenesis in Apc$^{Min/+}$ background.

The C57BL/6J Apc$^{Min/+}$ mouse carries a heterozygous point mutation at codon 850 of the adenomatous polyposis coli (Apc) gene, resulting in a truncated protein lacking C-terminal domains [3]. The homozygous embryos die *in utero* of a developmental defect in epiblast cells [4]. The Apc$^{Min/+}$ mouse model develops a large number of polyps in small bowel and few polyps in the colon and rectum [3,5,6]. Apc gene mutations are responsible for the familial adenomatous polyposis (FAP) and individuals with FAP carry a 100% risk of CRC [3,7]. For sporadic CRC, more than 80% of this kind of CRC carries Apc mutation [8]. Thus, mutations of Apc gene initiate the majority of CRC and Apc gene plays an important role in the pathogenesis of intestinal tumor [9]. Apc tumor suppressor gene is a negative regulator of Wnt/β-catenin signal pathway and the activation of Wnt signaling directly contributes to CRC. The Apc$^{Min/+}$ mouse model provides an *in vivo* system to

* Corresponding author at: School of Pharmaceutical Sciences, Shandong University, 44 West Culture Road, 250012, Jinan, Shandong, PR China.
E-mail address: zhangqzh@sdu.edu.cn (Q. Zhang).

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model FAP and to study the tumorigenesis, development, prevention and treatment of intestinal tumor.

However, intestinal adenomas in $Apc^{Min/+}$ mice only occasionally progress to invasive adenocarcinoma [3]. In most cases, $Apc^{Min/+}$ mice primarily develop to benign polyps and there is no metastasis, which is one of the main limitations of the $Apc^{Min/+}$ mouse model in the research of CRC [10]. Apc mutation is an initiating factor and an early causative event in CRC and a number of other factors play critical roles during the process from adenoma to carcinoma. Thus, Apc deficiency is not an obligate step in the development of intestinal lesions, and complex elements, such as underlying genetics and diet, are also involved in the transition from adenoma to carcinoma [3,10]. Herein, we will focus our review on the molecular events involved in the inflammatory process which may contribute to multistep tumorigenesis in $Apc^{Min/+}$ background and reveal the mechanisms of the incidence and development of intestinal inflammation-associated tumor.

2. Regulatory effects of genes on inflammation-associated tumor in $Apc^{Min/+}$ mice

Both the adenoma-carcinoma and the inflammation-carcinogenesis processes are characterized by inflammatory disorders. Inflammation regulating factors and effector cells play important roles in tumorigenesis. However, not all individuals with chronic inflammation in the intestinal tract develop dysplasia or tumor. The impact of multiple genetic factors likely contributes to the ultimate phenotype. In $Apc^{Min/+}$ mouse, a network of 45 genes/proteins, which are associated with inflammation and up-regulated in colon tumors compared to adjacent non-tumor tissues, is identified [11]. Based on $Apc^{Min/+}$ mouse model, the $Apc^{Min/+}$ double mutant mouse, which carries heterozygous Apc and a second genetic alteration, allows us to uncover the impact of genetic events on intestinal tumorigenesis under the background of Apc mutation. Mutations of inflammation-related genes include those for cytokines, chemokines, adhesion molecules, receptors, and inflammatory enzymes. To test these concepts, $Apc^{Min/+}$ mice are bred into a background of mice carrying inflammation and immune-related genetic alterations to determine whether the potent inflammatory response would augment dysplasia, and, potentially, the development of adenocarcinoma. Introduction of a second gene alteration into $Apc^{Min/+}$ mouse allows us to uncover whether the particular gene has any impact on intestinal tumorigenesis and furthermore to search the new targets for the prevention and treatment of CRC.

2.1. Inflammation-related genes

Accumulating evidences indicate that inflammatory cells in the intestinal tumor microenvironment secrete a variety of pro-inflammatory molecules and growth factors that can stimulate tumor cell proliferation, survival, migration, apoptosis and angiogenesis [12]. The products of inflammatory cells can promote the progression of intestinal tumor. They include overproduction of reactive oxygen and nitrogen, up-regulation of productions and enzymes of arachidonic acid biosynthesis pathway, and intestinal immune system dysfunction and so on [13]. Recent reports have shown that most of the inflammatory factors are up-regulated in the intestinal tumors of the $Apc^{Min/+}$ mice. They separately play important roles in promoting inflammatory response and inflammation-related tumorigenesis. In Apc compound mutant mice carrying both Apc and other gene deficiency, including Interleukin (IL)-17A, IL-17F, COX-2, monocyte chemotactic protein (MCP)-1, Macrophage migration inhibitory factor (MIF), myeloid differentiation factor 88 (MyD88) or Interferon-gamma (IFN-γ), the growth of intestinal tumor was inhibited [14–20]. Deficiency for those inflammatory factors could inhibit inflammatory response, protect the body against inflammatory injury and suppress the development of intestinal tumor in different pathways. Correspondingly, $Apc^{Min/+}Ikk\beta(EE)^{IEC}$ mice expressing constitutively active IκB kinaseβ (IKKβ) in intestinal epithelial cells (IECs) showed early lesions and visible tumors in the intestinal tract and their survival was severely compromised. Hence, persistent NF-κB activation in IECs promotes intestinal tumor progress in $Apc^{Min/+}$ mice [21]. Besides, $Apc^{Min/+}$ mice that express human IL-8 could increase mobilization of immature myeloid cells (IMCs), which exacerbates intestinal inflammation and accelerates carcinogenesis [22]. $Apc^{Min/+}$ mice carrying a 10-fold increase in circulating IL-6 levels exhibited an 89% increase in intestinal adenomas [23]. In $Apc^{Min/+}Sigirr^{-/-}$ mice, the intestinal tumors were deteriorated [24]. The Single immunoglobulin interlukin-1 receptor-related (Sigirr) molecule acts as a suppressor of intestinal inflammation and intestinal tumor. Sigirr molecule is a negative regulator of toll-like receptors and IL-1 receptors (TLR and IL-1R) signaling. IL signaling pathway including IL-1R signaling mediates inflammatory effects and plays a proinflammatory role, contributing to intestinal tumorigenesis. Therefore, overexpressions of inflammatory factors mediate inflammatory effects, enhance inflammatory injury and promote intestinal tumor development. Inhibition of inflammatory signaling will contribute to therapy for intestinal tumor. However, genetic ablation of TNF-α demonstrates no detectable suppressive effect on inflammation-related colon tumorigenesis [25]. Therefore, we can conclude that anti-inflammation is a possible strategy in combating tumorigenesis in $Apc^{Min/+}$ mice, but intricate inflammatory responses participate in the inflammation-related tumorigenesis.

Besides, some inflammatory factors show both proneoplastic and antineoplastic activities and the regulatory mechanism of them is extremely complex [26]. For example, iNOS induces the synthesis of NO, which works for the DNA damage and inhibits DNA repair, resulting in tumorigenesis [27]. However, when the expression of iNOS was reduced in epithelial cells of Apc^{Min+} mice, more intestinal adenomas were found in $Apc^{Min+}iNOS^{-/-}$ littermates [28]. Thus, iNOS plays both protective and antineoplastic roles. The mechanism of the protective effects of iNOS remains still unknown. Besides, type I IFN signaling, which has been proved to have an antiproliferative effect on several tumor types and cell types, showed a novel role in promoting aggressive fibromatosis. Furthermore, blocking type I IFN signaling had no any influence on the gastrointestinal tumors in Apc^{Min+} mice [29]. Therefore, type I IFN is also a less than optimal therapeutic approach. Therefore, they may be not the ideal therapeutic targets to prevent and treat intestinal tumor following Apc loss.

As shown in Fig. 1, in $Apc^{Min/+}$ mice, genetic deficiency in the pro-inflammatory factors could inhibit the development of intestinal tumor [14–20]. Correspondingly, over-expression of pro-inflammatory factors could aggravate intestinal tumor [21–24]. However, some genetic ablations demonstrate no detectable suppressive effect on inflammation-related colon tumorigenesis, such as the genetic ablation of TNF-α [25] and genetic ablations show both proneoplastic and antineoplastic activities [28,29]. Therefore, we can conclude that anti-inflammation is a possible strategy in combating tumorigenesis in $Apc^{Min/+}$ mice, but anti-inflammation is not the necessary measures in reducing intestinal tumor and intricate inflammatory responses participate in the intestinal tumorigenesis.

2.2. Immune-related genes

Moreover, the body can utilize the immune system to recognize and eliminate tumor cells. In response to immunological surveillance, tumor cells can escape from immunological surveillance and