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Review

Dual gene deficient models of $Apc^{Min/+}$ mouse in assessing molecular mechanisms of intestinal carcinogenesis

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ABSTRACT

The $Apc^{Min/+}$ mouse, carrying an inactivated allele of the adenomatous polyposis coli (Apc) gene, is a widely used animal model of human colorectal tumorigenesis. While crossed with other gene knockout or knock-in mice, these mice possess advantages in investigation of human intestinal tumorigenesis. Intestinal tumor pathogenesis involves multiple gene alterations; thus, various double gene deficiency models could provide novel insights into molecular mechanisms of tumor biology, as well as gene-gene interactions involved in intestinal tumor development and assessment of novel strategies for preventing and treating intestinal cancer. This review discusses approximately 100 double gene deficient mice and their associated intestinal tumor development and progression phenotypes. The dual gene knockouts based on the Apc mutation background consist of inflammation and immune-related, cell cycle-related, Wnt/β-catenin signaling-related, tumor growth factor (TGF)-signaling-related, drug metabolism-related, and transcription factor genes, as well as some oncogenes and tumor suppressors. Future studies should focus on conditional or inducible dual or multiple mouse gene knockout models to investigate the molecular mechanisms underlying intestinal tumor development, as well as potential drug targets.

1. Introduction

Colorectal cancer (CRC) remains one of the most significant health problems in the world and accounts for the third most commonly diagnosed cancer in men and the second in women globally in 2012 [1]. CRC risk factors include an older male [2], consumption of excessive red or processed meat, fat or alcohol, obesity, tobacco smoking, physical inactivity, and pre-cancer conditions, such as chronic bowel inflammation or disease [3–7]. Genetic factors account for small fraction in developing CRC [2]; for example, hereditary nonpolyposis-related CRC comprises approximately 3% of all CRC cases [2], while Gardner syndrome and familial adenomatous polyposis (FAP) comprise 1% of all CRC cases [8–10]. Colorectal carcinogenesis, like most other human cancers, involves multiple gene alterations [2,3]. Thus, animal models with different gene knockout or knock-in are frequently used to investigate molecular mechanisms underlying intestinal carcinogenesis, tumor biology, and the impact of specific molecular events on colon biology. Most importantly, animal models are also frequently used to assess novel strategies for cancer prevention and treatment [11,12], while other animal models used in CRC research include spontaneous tumor development, inducible tumor, transplant intestinal tumor, and transgenic animal models [13].

Among these models, the $Apc^{Min/+}$ mouse model was the first and most frequently used to investigate intestinal tumorigenesis [14,15]. The adenomatous polyposis coli (Apc) mutant mice were generated by random Apc mutations to induce Min (multiple intestinal neoplasia). These mice carry a truncated mutation at Apc codon 850. The Min mouse can develop hundreds of polyps in the small intestine in addition to colon tumors [14]. Clinically, Apc mutations occur in approximately 60% of CRC patients and loss of Apc functions due to Apc deletion or mutation occur in 80% of sporadic CRCs and 100% of familial adenomatous polyposis (FAP) [16–18]. However, development of small intestinal tumors in $Apc^{Min/+}$ mice, as well as this being a single gene deficiency mouse model, limit our capacity to study colorectal tumorigenesis [19–21]; thus, many researchers have bred $Apc^{Min/+}$ mice with another gene deficient mice to generate offspring that have double gene defects. This novel strategy allows investigation of the role of gene-gene interactions in CRC pathogenesis. Thus, in this review, we discuss approximately 100 types of double gene deficient mice and their advantages and disadvantages for understanding the roles of these genes in intestinal tumor development.

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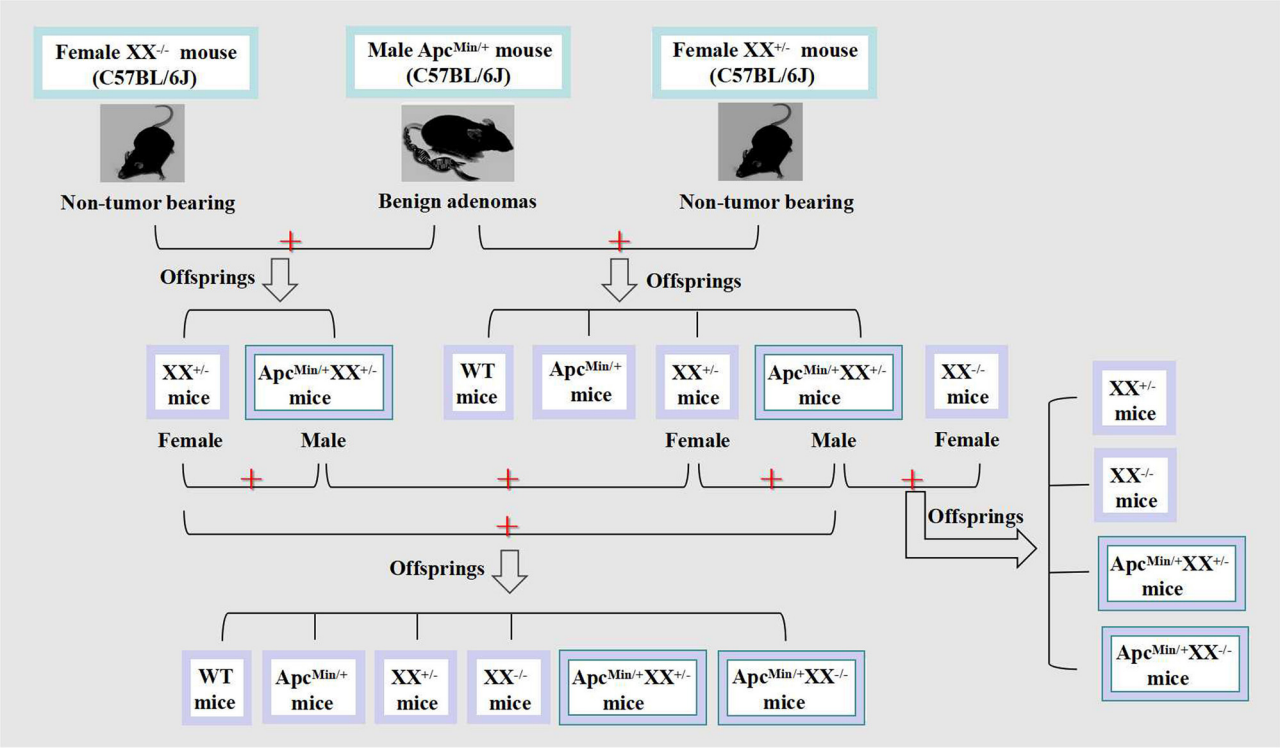

Fig. 1. Generation of dual gene knockout or knock-in animal models in $Apc^{Min/+}$ mouse. To produce a dual gene knockout or knock-in models suing $Apc^{Min/+}$ mice, male $Apc^{Min/+}$ mice are to mate with some gene knockout or knock-in female mice $XX^{+/-}$ or $XX^{-/-}$ mice on the C57BL/6 background to generate the offspring. The offspring mice, including wild type (WT) mice, single gene mutant mice, and double gene deficient mice, are screened by genotyping to generate the dual gene deficient mouse model, $Apc^{Min/+}XX^{+/-}$ and $Apc^{Min/+}XX^{-/-}$ mouse model. "+" indicates mating.

2. Establishment of dual gene deficient mouse models using $Apc^{Min/+}$ mice to understand intestinal carcinogenesis

C57BL/6 mice carrying the Apc mutation at codon 850 develop small intestinal polyps. However, dual gene deficient female $Apc^{Min/+}$ mice have fertility impairment; thus, male $Apc^{Min/+}$ mice are commonly bred to gene defective female transgenic mice to produce offspring, which will generate wild type, single gene mutant, and double gene deficient mice [18,19]. Fig. 1 summarizes the procedures and data from dual gene deficient mouse breeding. All mice are bred and maintained in a pathogen-free animal facility and gene deficiency is assessed through PCR genotyping of mouse-tail biopsies [20–22]. The following sections will discuss each dual gene deficient mouse model to better understand the role of specific genes in intestinal tumorigenesis and their importance for assessing agents that can prevent and treat CRC.

2.1. Dual gene knockout models involved in inflammation and immune-related pathogenesis

Chronic inflammation is an important risk factor in the development of gastrointestinal tumors [6,7]. The effects of inflammation and immune-related gene mutations on intestinal tumor progression have been studied using the double gene deficient mouse models on the $Apc^{Min/+}$ background. Deficiency in inflammatory factors, such as interleukin (IL)-17 A, IL-17 F, cyclooxygenase-2 (COX-2), monocyte chemoattractant protein 1 (MCP-1), macrophage migration inhibitory factor (MIF), or myeloid differentiation primary response 88 (MyD88), can alter or block intestinal inflammation, leading to inhibition of intestinal tumor development and progression [23–28]. In contrast, overexpression of other inflammatory factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), IL-8, or IL-6, promotes intestinal tumor growth in $Apc^{Min/+}$ mice [29–32]. Furthermore, intestinal inflammation induced by genetic aberrations can also promote intestinal tumor development and progression. For example, deficiency of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in $Apc^{Min/+}$ mice can induce expression of COX-2 and other inflammatory factors, therefore promoting progression of intestinal tumors [33]. In addition, glutathione S-transferase Pi (Gstp)-null mice carrying the Apc^{Min} mutation showed up-regulated expression of IL-6, IL-4, interferon gamma (IFN-γ), and inducible nitric oxide synthase (iNOS) in colon tissues, and the incidence and multiplicity of colon tumors were increased in $Apc^{Min/+}GSTP1/p2^{-/-}$ mice compared to $Apc^{Min/+}$ mice [34]. Another study reported that the number of intestinal polyps and intestinal inflammation in $Apc^{Min/+}Tpl2^{-/-}$ mice were up-regulated compared to $Apc^{Min/+}$ mice [35]. Also, the intestinal inflammation and tumor burden in $Apc^{Min/+}$Faslpr mice were significantly increased compared to $Apc^{Min/+}$ mice [36]. Therefore, chronic inflammation can trigger cellular events in the intestine and promote malignant transformation of normal intestinal epithelial cells. In contrast, these dual gene manipulations in the Apc^{Min} mice confirmed the role of these related genes in inflammation.

However, inhibiting inflammatory reactions may not always efficiently suppress growth of intestinal tumors in $Apc^{Min/+}$ mice. For instance, knockout of tumor necrosis factor (TNF)-α in $Apc^{Min/+}$ mice had no inhibitory effect on intestinal tumor progression [37], but knockout of iNOS, IFN-γ, or IFN-γR1 in $Apc^{Min/+}$ mice enhanced growth of intestinal tumors [38–40]. Indeed, iNOS deficiency induced in *in vitro* assays also inhibited proliferation of intestinal tumor cells [41]. Moreover, cysteinyl leukotriene and its receptor 1 (CysLTR1) regulate leukotriene and 5-lipoxygenase production in humans, and the $CysLTR1^{-/-}Apc^{Min/+}$ mouse model showed that CysLTR1 deficiency reduces the intestinal inflammatory response and tumor formation in female mice, but with no effect on male mice [42], for a reason that remains unknown yet.

The host immune response against carcinogenesis can be mediated