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β-Catenin-accumulated crypts in the colonic mucosa of juvenile $Apc^{Min/+}$ mice

Kazuya Hata[a,b], Takuji Tanaka[c,*], Hiroyuki Kohno[c], Rikako Suzuki[c], Sheng Hong Qiang[b], Yasuhiro Yamada[b], Takeshi Oyama[b], Toshiya Kuno[b], Yoshinobu Hirose[b], Akira Hara[b], Hideki Mori[b]

[a]*BMR Laboratories, Sunplanet Co., Ltd, 4388 Hagiwara, Kamiishidu, Yourou, Gifu 503-1602, Japan*
[b]*Department of Tumor Pathology, Gifu University School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan*
[c]*Department of Oncologic Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan*

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Abstract

Although $Apc^{Min/+}$ mice are widely used for an animal model of human familial adenomatous polyposis (FAP), a majority of intestinal polyps locate in the small intestine. We recently reported that numerous β-catenin-accumulated crypts (BCAC), which are reliable precursor lesions for colonic adenocarcinoma, develop in the large bowel of aged $Apc^{Min/+}$ mice. In this study, we determined the presence and location of BCAC in the large intestine of juvenile $Apc^{Min/+}$ mice (3 and 5 weeks of age). Surprisingly, BCAC were noted in the colon of even $Apc^{Min/+}$ mice of both ages, and mainly located in the distal and middle segments of the colon. Also, a few microadenomas were detected in $Apc^{Min/+}$ mice of 5-week old. Our results may indicate need of further investigation of the colorectal mucosa of $Apc^{Min/+}$ mice for examining colorectal carcinogenesis using $Apc^{Min/+}$ mice.

Keywords: β-Catenin-accumulated crypts; C57BL/6J-$Apc^{Min/+}$; Juvenile mice

1. Introduction

Aberrant crypt foci (ACF) and BCAC are widely used as markers for evaluating colorectal carcinogenic risk in rodents [1,2] and humans [3]. ACF proposed by Bird [1,2], are morphologically distinguished from their surrounding crypts on colonic mucosa stained with methylene blue [1]. While they are considered as putative precursor lesions for colonic adenocarcinoma and frequently used for preclinical cancer chemoprevention studies [4,5], we have recently proposed that β-catenin-accumulated crypts (BCAC) rather than ACF are reliable precancerous lesions for colonic adenocarcinoma [6–8].

Mutant mouse lineage being predisposed to $Apc^{Min/+}$ is regarded as one of the models for colorectal tumorigenesis [9]. Originally, this lineage was established from an ethylnitrosourea-treated

* Corresponding author. Tel.: +81 76 286 2211x3611; fax: +81 76 286 6926.
E-mail address: takutt@kanazawa-med.ac.jp (T. Tanaka).

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C57BL/6J male mouse, and its phenotype is a fully penetrate autosomal dominant trait. The dominant mutation is known to be located in Apc, the mouse homologue of the human *APC* gene, resulting in truncation of the gene product at amino acid 850 [10]. Although homozygous $Apc^{Min/Min}$ mice die as embryos [11], $Apc^{Min/+}$ mice develop multiple intestinal tumors in the small intestine. However, unlike in human familial adenomatous polyposis (FAP), colonic neoplasms are rarely detected in the large intestine. We previously documented that numerous BCAC, which could be useful for detecting the modifying effects of xenobiotics in colon carcinogenesis in mice and rats [6,12], develop in the colorectal mucosa of adult $Apc^{Min/+}$ mice aged over 20 weeks [13]. The presence of spontaneous ACF [14] or dysplastic ACF (ACF_{Min}) [15] are also known in $Apc^{Min/+}$ mice, but their frequency is low.

Since certain hit(s) on the BCAC may lead to tumor development in $Apc^{Min/+}$ mice [16], we, in the present study, turned attention to the presence of BCAC in the large bowel of juvenile $Apc^{Min/+}$ mice and determined where BCAC develop.

2. Materials and methods

2.1. Animals

The mice were bred at our laboratory, from inbred mice originally purchased from The Jackson Laboratory (Bar Harbor, ME). The $Apc^{Min/+}$ pedigree was maintained by mating $Apc^{+/+}$ females with $Apc^{Min/+}$ males, and procedures to secure inbreeding were followed. The $Apc^{Min/+}$ mice were identified by allele-specific PCR on DNA isolated from tail. All mice used for the experiment were maintained in the well-controlled room with a high-efficiency particulate air (HEPA) filter, a 12 h lighting (7:00–19:00), 25 ± 2 °C room temperature, and $55 \pm 15\%$ humidity. Mice (5 mice/cage) were housed in polycarbonate cages measuring W225 × D338 × H140 mm (Japan CLEA, Inc., Tokyo, Japan) with the floor covered with a sheet of roll paper (Japan SLC). Water and diet were given ad libitum. The mice were given a basal diet, MF (Oriental Yeast Co., Ltd, Tokyo, Japan), during gestation and until 5 weeks of age. We fully complied with the 'Guidelines Concerning Experimental Animals' issued by the Japanese Association for Laboratory Animal Science and exercised due consideration so as not to cause any ethical problem.

2.2. Experimental procedure

A total of 54 mice were used in this study: $Apc^{Min/+}$ mice of 3 weeks of age (7 females and 8 males) or 5 weeks of age (7 females and 6 males)) and $Apc^{+/+}$ mice of 3 weeks of age (8 females and 6 males) or 5 weeks of age (5 females and 7 males). The $Apc^{Min/+}$ mice were autopsied at 3-week and 5-week of age respectively for measurement of the large intestines as well as detection of ACF and BCAC in the large intestinal mucosa. At sacrifice the large bowels were removed, flushed with saline, fixed flat in 10% buffered formalin for 24 h at room temperature, and then processed for histopathological evaluation by routine procedures [17]. To identify aberrant classical ACF [1], mucosal surface of the colons were stained with methylene blue. In brief, fixed colons were placed in 0.5% solution of methylene blue in distilled water for 30 s. They were then placed mucosal side up on a microscope slide and ACF were counted under a light microscope at a magnification of ×40.

2.3. Tissue preparation

To identify intramucosal lesions ACF and BCAC, colon was divided into three (distal, middle, and proximal) segments and embedded in paraffin. A total of 162 segments were examined by using an en face preparation and 3–5-μm thick serial sections were made [6,7]. For each case, two serial sections were used to analyze the intramucosal lesions.

2.4. Histopathology and immunohistochemistry

Two serial sections were subjected to hematoxylin and eosin (H and E) staining for histopathology and β-catenin immunohistochemistry for enumeration of BCAC [12]. Immunohistochemistry for β-catenin was performed on 4-μm-thick paraffin-embedded sections from all segments of the colons, using the labeled streptavidin-biotin method (LSAB KIT; DAKO, Glostrup, Denmark) with microwave