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Interplay between APC and ALDH1B1 in a newly developed mouse model of colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is a leading cause of cancer mortality worldwide. Mutations in the adenomatous polyposis coli (*APC*) gene are pivotal in colorectal tumorigenesis.

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Recently, we demonstrated that aldehyde dehydrogenase 1B1 (*ALDH1B1)* knockdown dramatically reduced colon tumor growth in a mouse xenograft model. The purpose of the present preliminary study is to examine the effect of loss of ALDH1B1 in CRC development in an inducible colon-specific *Apc* mouse model.

Methods: $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice develop uni-allelic inactivation of *Apc* specifically in colon epithelial cells following tamoxifen treatment. $Aldh1b1^{-/-}$ KO mice were crossed with $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice. Six-month-old male $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$, and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice were treated with tamoxifen (50 mg/kg, i.p.) for three consecutive days. $Apc^{W/F}/Aldh1b1^{-/-}$ and $Apc^{W/F}/Aldh1b1^{+/+}$ mice were treated with corn oil (i.e., tamoxifen vehicle control) for three consecutive days. Eighteen days later, mice were sacrificed and their colons examined microscopically, macroscopically and histologically for the presence of adenoma.

Results: All $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice treated with tamoxifen developed colorectal adenoma. The $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice showed a significant decrease in the total volume of all ileal and colonic adenomas, and decreased incidence of large colonic adenoma compared to $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice. Immunohistochemical analysis of p53 and β-catenin showed a trend toward decreased expression score in colonic adenomas of $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice.

Conclusion: The present preliminary study suggests that deletion of ALDH1B1 may protect against the full development of colorectal cancer. Further mechanistic studies are required to elucidate how ALDH1B1 contributes for colorectal cancer.

Keywords

Colorectal cancer; Adenomatous Polyposis Coli; Aldehyde dehydrogenase 1B1; Mouse models; Beta-catenin

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in the USA [1]. Approximately 150,000 U.S. residents are diagnosed with CRC annually, and one-third of CRC patients die from the disease [1]. The pathogenic and etiological factors underlying the development of CRC are complex and still not clearly understood [2]. The development of CRC has been shown to be influenced by diet, lifestyle, environmental factors, and acquired somatic mutations [3, 4]. CRC arises by accumulation of multiple genetic and epigenetic alterations associated with genes regulating cell growth and differentiation [5]. Such constant alterations push the colonic epithelium to progress from normal to dysplastic, then adenomatous, and finally to adenocarcinoma [6], with metastasis to other sites [7]. Inflammatory mediators, such as cytokines and chemokines, may serve as tumor growth and survival factors, as well as promoting angiogenesis and suppressing immune-mediated tumor elimination [8–10]. Colonic tumors exhibit activation of multiple transcription factors, including nuclear factor-κB (NFκB), a master regulator of inflammatory pathways [11, 12], and signal transducer and activator of transcription 3 (STAT3) [9].

Mutations in the adenomatous polyposis coli (*APC*) gene are responsible for the familial adenomatous polyposis coli syndrome (FAP), a condition characterized by development of colorectal adenomas [13]. Germline-inactivating *APC* mutations have been identified in FAP patients, and inactivation of both *APC* alleles has been observed in greater than 80% of sporadic colorectal adenomas [14]. These results suggest that APC is a tumor suppressor, and acts as a central gatekeeper protein in colorectal tumorigenesis. One proposed gatekeeper mechanism revolves around APC regulation of β-catenin (a dual function cell-cell adhesion and gene transcription protein) and activation of Wnt signaling [15, 16]. Multiple mouse models carrying germline *Apc* mutations have been developed [15]. The multiple intestinal neoplasia (MIN) mouse model ($Apc^{Min/+}$) recapitulates some of the features observed in FAP patients; however, these mice develop polyps and adenomas in the small intestine [17, 18]. Recently, colon-specific deletion of *Apc* has been achieved through the use of the colon-specific *Cre* construct, *CDX2P-CreERT2* [19]. These mice carry a *CDX2-NLS Cre* recombinase transgene and a floxed *Apc* allele that, upon tamoxifen treatment, produces a colon-specific APC knockout animal. This model develops tumors that are predominantly located in the distal colon and rectum, forms pedunculated-type tumors similar to human colorectal cancers, and exhibits biallelic *Apc* inactivation, β-catenin dysregulation, and global DNA hypomethylation [19].

Aldehyde dehydrogenase (ALDH) catalytic activity has been identified as a biomarker for many cancers and cancer stem cells [20]. The expression of ALDH1B1 in normal colon tissue is limited to the bottom of the intestinal crypts where the stem cell resides [21]. In cancerous colon tissue, there is a drastic increase of ALDH1B1 expression [22]. Importantly, we have demonstrated that ALDH1B1 knockdown dramatically reduces tumor growth in a xenograft model [23]. In order to better understand the role of ALDH1B1 in colorectal carcinogenesis, we crossed the $Aldh1b1^{-/-}$ (KO) mouse line with the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mouse line. Our hypothesis is that ALDH1B1 plays a role in CRC development. As such, it is anticipated that deletion of ALDH1B1 will reduce CRC development in the colon-specific APC knockout animal.

Materials and Methods

Animals

Global $Aldh1b1^{-/-}$ mice were developed and originally characterized by our laboratory [23]. $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice carrying a *CDX2P-NLS Cre* recombinase transgene and a *loxP*-targeted Apc allele were provided by Dr. Yatrik M. Shah (University of Michigan, Ann Arbor, USA). For tumor studies, $Aldh1b1^{-/-}$ mice were bred with $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice to generate four genotype groups: $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$, $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$, $Apc^{W/F}/Aldh1b1^{-/-}$, and $Apc^{W/F}/Aldh1b1^{+/+}$ mutant mice. All animal procedures were reviewed and approved by the Yale University's Institutional Animal Care & Use Committee (IACUC).

Adenoma induction and macroscopic evaluation

A total of thirteen 6 mo-old, male mice were examined in this study. For adenoma induction, mice were treated with tamoxifen or an equivalent volume of tamoxifen vehicle

(corn oil) as shown in Fig. 1. Specifically, tamoxifen (TAM, 50 mg/kg body weight; i.p.) was administered i.p. to $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ (n=3) and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ (n=3) mice whereas 5 ml/kg corn oil (CON) was administered i.p. to $Apc^{W/F}/Aldh1b1^{-/-}$ (n=3), and $Apc^{W/F}/Aldh1b1^{+/+}$ (n=4) mice. Tamoxifen or CON were administered on the first three consecutive days (days 1, 2 and 3) of the experiment. Mice were individually housed at room temperature under 12 h light/12 h dark cycle with *ad libitum* access to water and food (regular chow diet). The health of the mice was monitored closely for any deterioration, including rectal bleeding, rectal prolapse, anemia, and weight loss (>15%). On day 18, the mice were anesthetized using 5% isoflurane, exsanguinated by collecting blood from posterior vena cava and performing whole body perfusion with room temperature normal saline. The small and large intestines were then carefully removed from each mouse and flushed with normal saline. The jejunum, ileum, and colon of each mouse were surgically separated and opened longitudinally, and examined under a stereomicroscope (Nikon, C-DS, Melville, NY) for adenoma by an observer blinded to the animal genotype. The number and size (length, width) of adenomas were measured in each intestinal segment. Visually observable lesions were termed macroscopic adenomas. Later, lesions visualized in the longitudinally opened colon and ileum tissue by stereomicroscope were termed microscopic adenomas. The volume of each adenoma was quantified from its length and width as described previously [24].

Histology and immunohistochemical staining

Following macroscopic examination, each intestinal tissue segment was carefully rolled-up and placed in 10% neutral buffered formalin (NBF) for fixing. After 24–48 hr, the tissues were transferred to 70% ethanol and processed for hematoxylin & eosin (H&E) and immunohistochemical (IHC) staining by the Yale Histology Core Laboratory. All tissues were fixed in phosphate-buffered 10% formalin (Fisher Scientific, Pittsburgh, PA) and embedded in paraffin. Multiple sections were cut and mounted on glass slides (Superfrost™/Plus, Fisher Scientific, Hampton, NH) and stored at 4°C. Tissue sections were subjected to H&E staining using routine techniques. Immunohistochemical staining was performed according to a standard procedure [25]. Briefly, paraffin-embedded tissue sections (5 μm) were deparaffinized and rehydrated. The section underwent heat-induced antigen retrieval using 0.01 M citrate buffer (pH 6.6) for 30 min at 95°C, was then cooled to room temperature, and subsequently rinsed and placed in Tris Buffered Saline (50 mM Tris-Cl, 150 mM NaCl; pH 7.6) containing Tween 20 (0.1%) (TBS-T). After incubation with 3% hydrogen peroxide, sections were incubated with β-catenin mouse monoclonal antibody (Biocare Medical, Pacheco, CA) or p53 mouse monoclonal antibody (Thermo Scientific, Waltham, MA) at a dilution of 1:100 dilution in Protein Blocker (Open Biosystems, Huntsville, AL) for 60 min at room temperature. Horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Biocare Medical, Pacheco, CA) was used at a 1:50 dilution for 10–20 min at room temperature. Slides were incubated with diaminobenzidine (Open Biosystems, Huntsville, AL) for 10 min, followed by counterstaining with hematoxylin, dehydration, clearing and mounting using mounting media. Microscopic identification and enumeration, and quantification of the staining of the adenomas were performed by a trained histologist blinded to the mouse genotypes. To quantify the IHC signal, the relative staining intensity (**I**) was assigned a value from

0–3 as follows: no signal (0), weak signal (1), moderate signal (2), or intense signal (3). The staining extensiveness (**E**) was calculated as the percent of positively-stained cells (1–100%). The overall IHC expression score (**S**) was calculated as the product of **I** and **E**, yielding a number between 0 and 300 for each adenoma.

Statistical analyses

Differences between the body weight and changes in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice were determined by two way ANOVA with *post-hoc* Tukey's or Sidak's multiple comparison test and two-tailed Mann-Whitney test (respectively) using the GraphPad Prism 7 software for Windows (GraphPad Software Inc., La Jolla CA, USA). The differences were considered significant when the P value was less than 0.05.

Results

Effect of genotype on small and large intestinal adenomas

During the course of this study, no significant difference in the body weight change was observed in any of the four experimental groups (Fig. 2). None of the vehicle-treated $Apc^{W/F}/Aldh1b1^{-/-}$ (Fig. 3A) or $Apc^{W/F}/Aldh1b1^{+/+}$ (Fig. 3B) mice developed macroscopic- or microscopic size intestinal adenomas. In the two TAM-treated groups ($Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$, $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$), adenomas were not evident at the macroscopic level in either the jejunum or ileum. Similarly, no adenomas were evident in the jejunum at the microscopic level as well. By contrast, ileal adenomas were observed microscopically in all (three out of three) $Apc^{W/F}Cdx2^{ERT2-Cre}/Aldh1b1^{-/-}$ mice and two out of three $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice (Fig. 4A). All of the ileal adenomas in these two groups of mice were in the range 0.9–1.0 mm in diameter (Fig. 4B). The average and total volume of ileal adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice (0.8 mm^3 and 5.5 mm^3 (respectively)) were significantly lower (P<0.001) than that from the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice (2.9 mm^3 and 17.8 mm^3 (respectively)) (Fig. 4C). Adenomas were visualized at the microscopically (1.0–1.6 mm)- (n=3) and macroscopically- (n=10) ranging in size from 2.3–4.4 mm in the colons of two of the three $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice and all three of the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice had both microscopically (0.5–1.0)- (n=3) and macroscopically- (n=13) observable adenomas ranging from 2.5–12.5 mm. The colon adenoma multiplicity (i.e., total number of adenomas per mouse in the colon) was similar between $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice (Fig. 4D). Interestingly, it was noted that $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice developed significantly lower (P<0.001) number of macro adenomas (i.e., ≥ 2mm) compared to $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$mice (Fig. 4E). However, there was no significant difference in the number of micro adenomas (i.e., ≤2mm) in these two groups of mice. The average and total volume of all macro adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice (40.3 and 403.7 mm^3 (respectively)) were significantly lower (P<0.001) than that in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice where average and total volume was 314.7 mm^3 and 4,091 mm^3 respectively (Fig. 4F). In contrast, there was no significant difference in the average and total volume of micro adenomas in these two groups of mice.

Occurrence of histologically-identifiable adenomas in the small and large intestines

In order to examine the effect of genotypic variations of *Apc* and *Aldh1b1* genes, abnormal histologic lesions were induced in all experimental mice. Representative images of H&E-stained adenomas in the ileum and colon are shown in Figs. 5A and 5D, respectively. Colonic lesions in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{-/-}$ showed highly pleomorphic nuclei but did not appear to invade through the basement membrane and did not include any mucous secreting cells which represents adenoma characteristics. Mice from the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ possess similar tumors. Eighteen days into the experimental protocol, adenomas were present in the colon (but not in the ileum) of $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice (Table 1A). In contrast, $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice developed adenomas in both the colon and ileum. As shown in the table 1A, the numbers of adenomas in the colon were equally distributed between two groups. The adenomas identified in these experiments were remarkably similar histologically to those described previously [19, 26].

Expression of p53 and β-catenin in ileum and colon adenomas

To further characterize adenomas, the content of p53 and β-catenin expression, was examined using immunohistochemistry (IHC) approach. Representative images of p53 IHC staining are provided in Figs. 5B and 5E. As noted, ileal adenomas (5B) were present only in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice. The p53 expression in these adenomas was variable, with the total score (272) ranging from 2 – 270 (Table 1B). In the colon adenomas (5E) of $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice, p53 expression tended to be lower (total score (2) range 0–2) than the levels observed in the ileal adenomas (total score (272) range 2–270) (Table 1B). The p53 expression (total score (110)) in colon adenomas of $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice ranging from 5–105. Representative images of the β-catenin IHC staining are shown in Figs. 5C and 5F. The presence of ileal adenomas (5C) was found only in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$, with the total expression score of 1260, ranging from 260–730. Colonic expression of β-catenin (5F) was found in both group of mice. The total expression score in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice was 675 and ranged from 5–670. However, the expression in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice group showed a trend towards increased expression score of 1220, that ranged from 200–780 (Table 1C). Although it did not reach statistical significance, the total β-catenin expression score in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice group was lower than those from $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ group. Notably, the average score of β-catenin expression was higher than p53 in adenomas of both groups.

Discussion

Colonic ALDH1B1 activity is normally confined to the stem cell compartment found at the colonic crypt base; increased expression of ALDH1B1 was found in human colon cancer specimens [22]. Furthermore, ALDH1B1 knockdown caused dramatic reduction of colon tumor growth in xenograft models [23]. Based on this strong association towards colon tumor pathology, we hypothesized and investigated the decisive function of ALDH1B1 using APC model of colorectal carcinogenesis.

Since, normal expression of the *APC* gene was found to be absent in 80–90% of colon carcinomas, several mouse models were created to understand the functional role of *Apc* gene towards colon carcinogenesis [27]. Human colorectal tumorigenesis was accurately recapitulated in the mouse by targeted colon-specific deletion of *Apc* using the *CDX2P-CreERT2* gene [19]. Specifically, Cre targeting of the $Apc^{+/loxP}$ allele with tamoxifen treatment in these mice led to loss of APC expression that was restricted to the distal small intestine, cecum, colon and rectum [19].

We examined the effects of functional loss of *Aldh1b1* gene on colorectal carcinogenesis by cross breeding $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice strain with the ALDH1B1 knockout (i.e., $Aldh1b1^{-/-}$) mice. All of the resultant $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mice developed micro adenoma in the ileum and both micro and macro adenomas in the colon, and had equivalent numbers of adenomas. The distribution of the adenomas in the small and large intestine were restricted to distal region. Our results are similar to the observation of Hinoi and colleagues [19], who showed the distribution of intestinal tumors of the CDX*2P-NLS Cre;* $Apc^{+/loxP}$ *(CPC;Apc)* mice to the distal region of the intestines. These data suggest that differential expression of Cdx2 promoter or regulated transgenes may play a primary role in regional distribution.

The newly created $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mice showed ~100-fold lower colonic volume (macro adenomas) than $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Adh1b1^{+/+}$ mice. Whereas, the volume of ileal microadenoma was ~3 fold lower than in $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ mice. The overall observation supports the notion that ALDH1B1 expression strongly influences colon carcinogenesis in the $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mouse. Our studies did not demonstrate an impact of ALDH1B1 expression on adenoma multiplicity between $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mice. This may have been a result of the small number of animals in each group.

p53, a potent tumor suppressor, is genetically altered in over 80% of colorectal tumors [28]. Mutations in p53 are found in 50 – 60% of human colorectal carcinomas; however, these mutations are only observed in less than 10% of colorectal adenomas [29]. We found p53 expression tend to be higher in colonic adenomas of $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ than $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mice. One study reported moderately elevated p53 expression in neoplastic cells of colonic tumors of *CPC;Apc* mice and absence of *Trp53* missense mutations in colonic adenomas [19]. Similarly, p53 mutations have previously been observed to occur at a low frequency (15–30%) in mouse colonic adenomatous polyps [30]. The present study suggests that p53 expression does not play a role in the formation of the adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice nor in changes in colon tumorigenesis occurring in mice devoid of ALDH1B1.

Beta-Catenin has been observed to be upregulated in all adenomas arising on a mutant APC background [31]. A close association between ALDH1B1 and activation of Wnt/β-catenin signaling was established previously in colon cancer cells [23]. In the present study, we observed a trend towards increased expression of β-catenin in colonic adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ mice. In human colorectal cancer, expression of β-catenin was closely correlated with the degree of tumor differentiation [32], suggesting that

activation of β-catenin plays a significant role in cancer progression. Furthermore, in human patients with stage II or III colorectal cancer, Wnt1 expression was strongly correlated with high expression of nuclear β-catenin [33]. Curiously, the expression of Wnt/β-catenin, Notch and PI3K/Akt are all down-regulated in cultured human colon cancer cells following shRNA knockdown of *ALDH1B1* expression [23].

APC mutations contributes approximately 85% of sporadic and inherited forms of human colorectal cancer cases which was reviewed extensively [34]. In addition to the contributing role of *APC* gene, activating missense mutations in the β-catenin gene (*CTNNB1*) [35] and/or human homologue of mouse conductin (*Axin1* and *−2*) truncating mutations also associated with the development of colorectal tumors in humans [36, 37]. Binding elements for the Wnt/β-catenin signaling transcription factor T-cell factor/lymphoid enhancing factor have been identified in the human *ALDH1B1* gene promoter [38]. Singh and colleagues hypothesized [23] that increased expression of ALDH1B1 in colon cancer may lead to increased levels of retinoic acid and activation of PPARβ/δ, thereby increasing expression of pro-survival genes through the PI3K/Akt pathway [39]. Loss-of-function of *Apc* mutation induces β-catenin signaling that was attributed for the colorectal cancer progression. The activation of Wnt/β-catenin signaling elevates the expression of ALDH1B1 as demonstrated in colon cancer cell lines [23]. Since Wnt/β-catenin signaling helps in progression of tumorigenesis, the collective effect of functional loss of *Apc* and expression of *Aldh1b1* sophisticates to generate more aggressive colon cancer outcomes. The colon-specific inducible $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mutant lines that predominantly develop adenomas similar to human CRCs help to gain a better insight for the collective response. $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$ mice showed dramatic decrease in the β-catenin expression even with loss of *Apc* gene function.

In addition, these $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$mice showed a significant decrease in total adenoma volume in the small intestine when compared to $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{+/+}$. The decrease in ileal adenoma volume might correspond to the activity of the *Cdx2* promoter, which was involved in deletion of *Apc*. Normally, the human caudal type homeo box 2 transcription factor, CDX2, is important in the anterior to posterior patterning of the intestinal epithelium and in defining patterns of proliferation and differentiation along the crypt-villus axis [37]. Its expression is stronger in the colon than in the ileum. Further investigations are needed to identify the effect of CDX2 on the expression of ALDH1B1.

For the first time we demonstrate the effect of loss-of-function of *Apc* and absence of *Aldh1b1,* which reduces colon adenoma and in turn delays the tumorigenesis and progression of cancer. This study indicates that ALDH1B1 functions as a key player in the development of *Apc* associated colon cancer, making it a potential target for anticancer therapy. In summary, our findings are consistent with a modulatory role of ALDH1B1 in colorectal cancer development. Our newly created $Apc^{W/F}Cdx2^{ERT2\text{-}cre}/Aldh1b1^{-/-}$mouse may be valuable for studying the role of tumor suppressor genes or proto-oncogenes in colon tumorigenesis *in vivo*.

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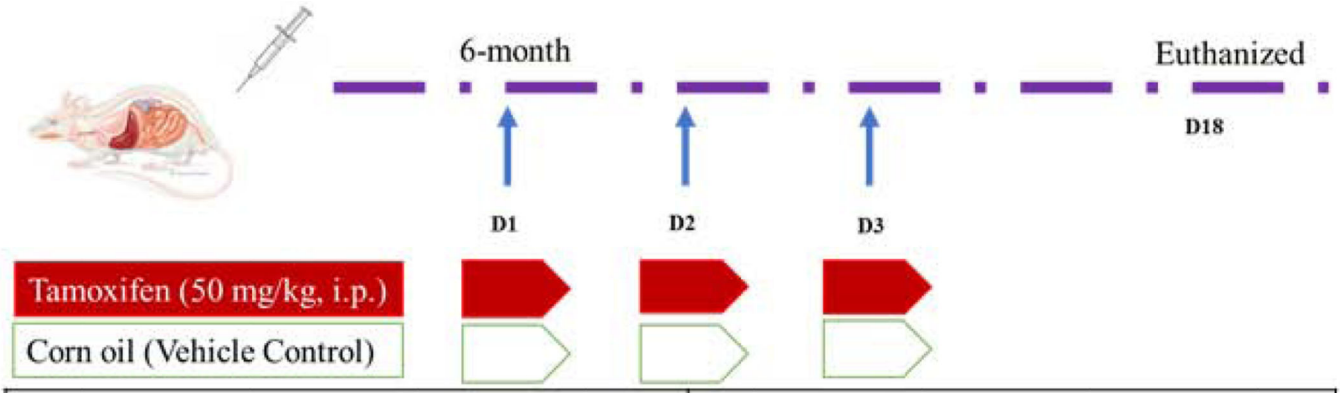

Genotype	Treatment
$Apc^{W/F}/Aldh1b1^{+/+}$	Corn Oil (CON)
$Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$	Tamoxifen (50 mg/kg/i.p.) (TAM)
$Apc^{W/F}/Aldh1b1^{-/-}$	Corn Oil (CON)
$Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$	Tamoxifen (50 mg/kg/i.p.) (TAM)

Figure 1.

Schematic representation of the animal study design. Tamoxifen (TAM, 50 mg/kg, i.p.) or corn oil (CON, 5 ml/kg, i.p.) was administered to mice on three consecutive days, i.e., days 1, 2, 3. Mice were euthanized on day 18 and their intestines were removed for morphological, histological and immunohistochemical evaluation.

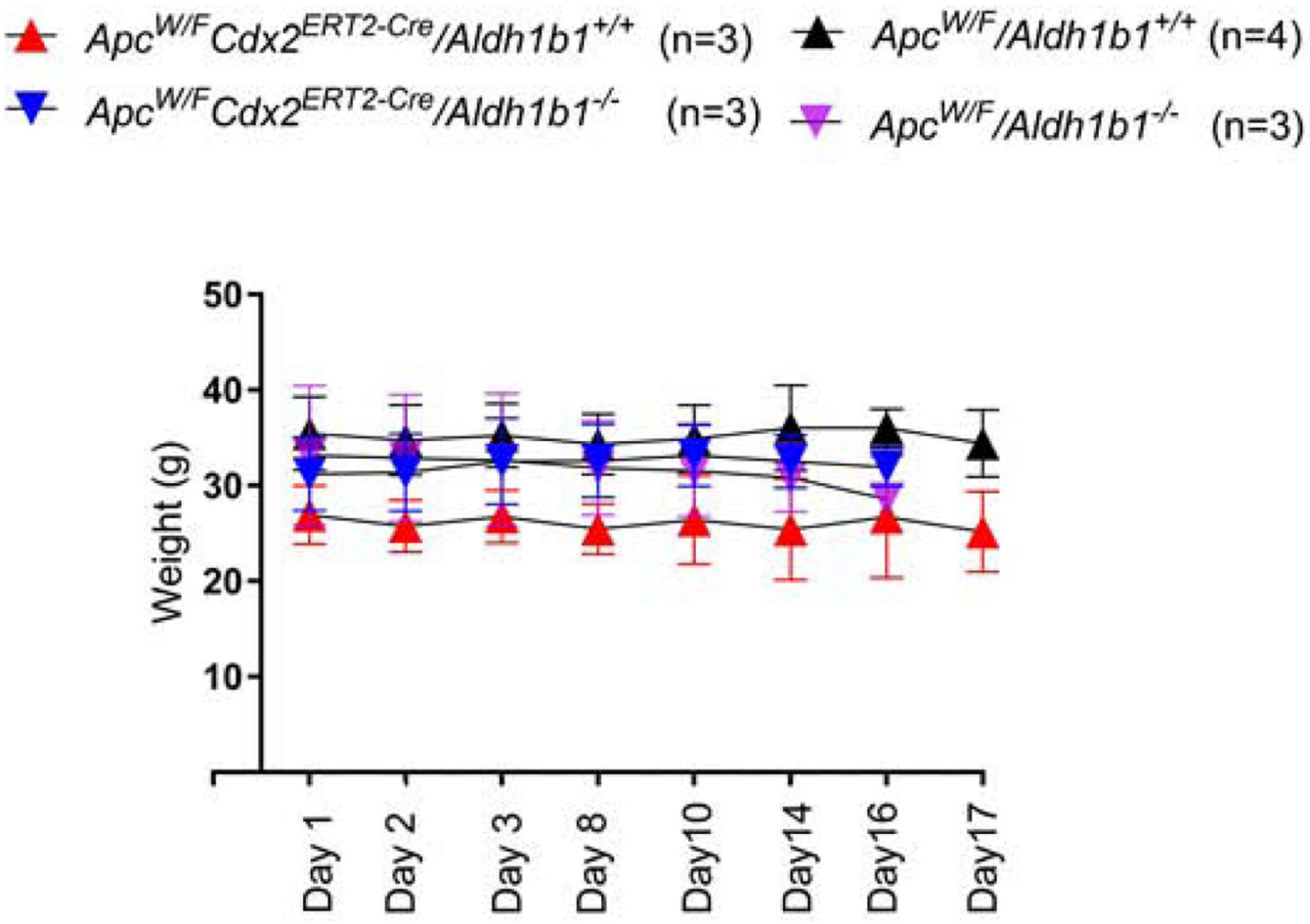

Figure 2.

Mouse genotype and body weights. The weight of each mouse was monitored daily for the duration of the experimental protocol. Data are presented as the mean ± standard deviation from n=3–4 mice.

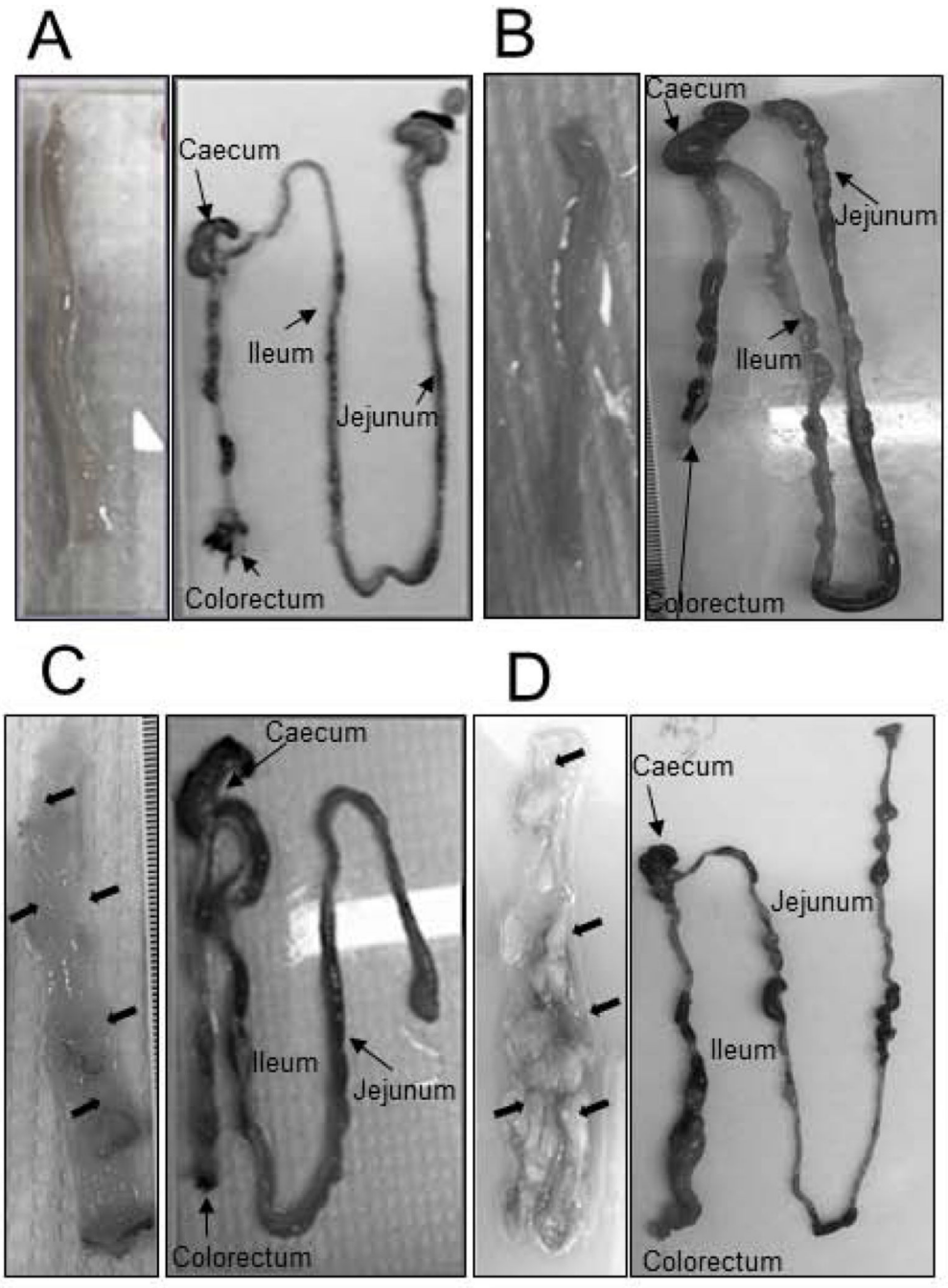

Figure 3.

Macroscopic evaluation of gastrointestinal tissues. Representative images of tissues from $Apc^{W/F}/Aldh1b1^{-/-}$ (**A**), $Apc^{W/F}/Aldh1b1^{+/+}$ (**B**), $Apc^{W/F}Cdx2^{ERT2-Cre}/Aldh1b1^{-/-}$ (**C**) and $Apc^{W/F}Cdx2^{ERT2-Cre}/Aldh1b1^{+/+}$ (**D**) mice on day 18 of the experimental protocol are shown. Right panels show a low power magnification of the small and large intestine, with jejunum, ileum, caecum and colorectal regions highlighted. Left panels show a higher magnification (A 100X, B 100X, C 400X, D 400X) of the luminal surface of the colon. Macroscopically-observable growths within the colon are indicated by arrows.

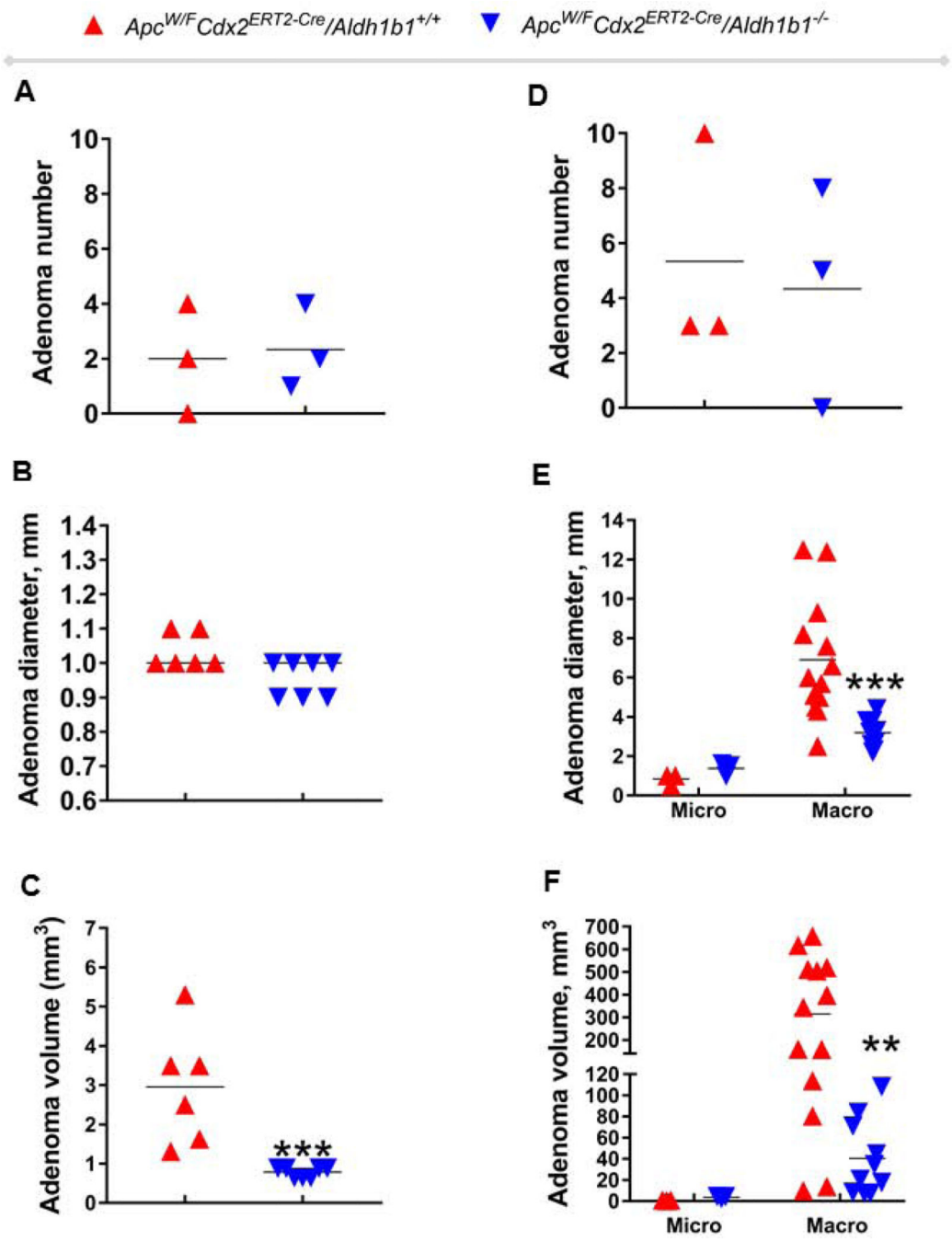

Figure 4.

Effect of ALDH1B1 expression on characteristics of ileal and colonic adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}$ mice. The total number of ileal adenomas (multiplicity) (**A**), number of ileal adenomas with diameter mm (**B**), total ileal adenomas volume (**C**), total number of colon adenomas (multiplicity) (**D**), number of colon adenomas with diameter mm (**E**) and total colon adenoma volume (**F**) in individual $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice (red symbols) and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ mice (blue symbols) are shown. * $P < 0.05$, *** $P < 0.001$, two-tailed Mann-Whitney test or two-way ANOVA with Sidak's multiple

comparison test, compared to $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice. The horizontal line represents the mean.

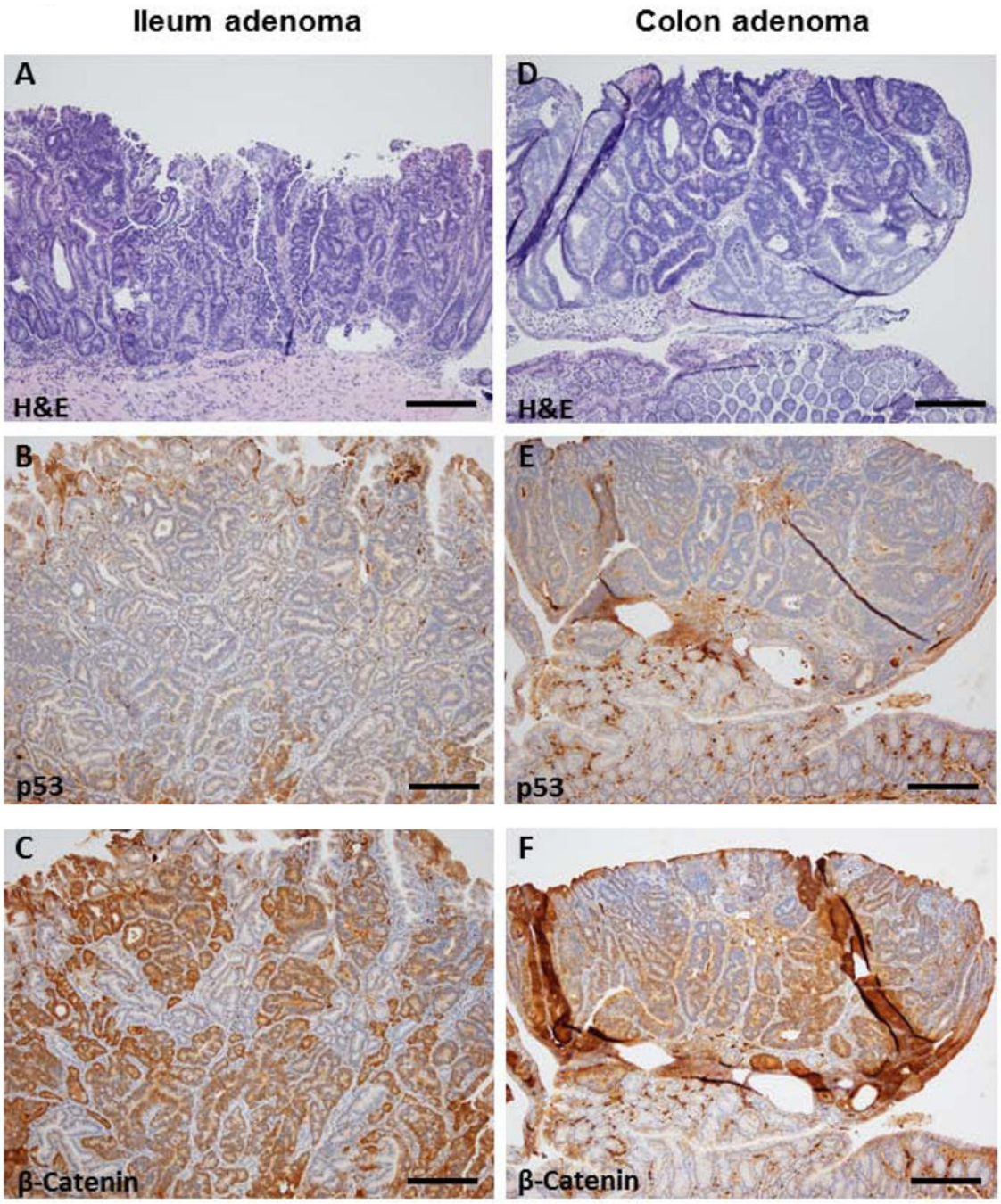

Figure 5.

Histological analysis of intestinal adenomas in $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{-/-}$ and $Apc^{W/F}Cdx2^{ERT2\text{-}Cre}/Aldh1b1^{+/+}$ mice. Formalin-fixed sections of ileum (**A**, **B**, **C**) or colon (**D**, **E**, **F**) were subjected to H&E staining (**A**, **D**) or immunohistochemical staining for p53 (**B**, **E**) or β-catenin (**C**, **F**). Black bar = 200 microns.

Table 1.

Microscopic Analysis of the Adenomas in Tamoxifen-Treated Mice

Genotype	Mouse ID	Ileum		Colon	
		# lesions [a]	*Total score* [b]	*# lesions* [a]	*Total score* [b]
1A. Histologic adenoma quantification					
Apc$^{W/F}$*Cdx2*$^{ERT2\text{-}Cre}$/*Adh1b1*$^{+/+}$	AA01	0		3	
	AA04	0		1	
	AA09	0		1	
Apc $^{W/F}$ *Cdx2* $^{ERT2\text{-}Cre}$ /*Aldh1b1* $^{-/-}$	AA02	4		1	
	AA05	2		3	
	AA07	1		0	
1B. p53 IHC quantification					
Apc $^{W/F}$ *Cdx2* $^{ERT2\text{-}Cre}$ /*Adh1b1* $^{+/+}$	AA01	0	0	3	105
	AA04	0	0	1	0
	AA09	0	0	1	5
Apc $^{W/F}$ *Cdx2* $^{ERT2\text{-}Cre}$ /*Aldh1b1* $^{-/-}$	AA02	4	16	1	0
	AA05	2	2	3	2
	AA07	1	270	0	0
1C. β-catenin IHC Quantification					
Apc $^{W/F}$ *Cdx2* $^{ERT2\text{-}Cre}$ /*Adh1b1* $^{+/+}$	AA01	0	0	3	780
	AA04	0	0	1	200
	AA09	0	0	1	240
Apc $^{W/F}$ *Cdx2* $^{ERT2\text{-}Cre}$ /*Aldh1b1* $^{-/-}$	AA02	4	730	1	5
	AA05	2	260	3	670
	AA07	1	270	0	0

[a]The number of histologically-identifiable adenomas in ileum or colon

[b]Total score = (percentage of cells that label positive the immunofluorescent antibody) × (relative intensity of IHC fluorescence signal)