INTRODUCTION

Colorectal cancer is the second leading cause of cancer death in both sexes in the United States [1]. Many factors are associated with the development of colorectal cancer, such as “unhealthy” diets [2-6], gut inflammation [7-10], and microbial dysbiosis [11-14].

Free fatty acid receptor 2 (FFAR2) has been reported as a tumor suppressor in colon cancer development. The current study investigated the effects of FFAR2 signaling on energy metabolism and gut microbiota profiling in a colorectal cancer mouse model (Apc\textsuperscript{Min/\textsuperscript{+}}). Ffar2 deficiency promoted colonic polyp development and enhanced fatty acid oxidation and bile acid metabolism. Gut microbiome sequencing analysis showed distinct clustering among wild-type, Apc\textsuperscript{Min/\textsuperscript{+}}, and Apc\textsuperscript{Min/\textsuperscript{+}}-Ffar2\textsuperscript{-/-} mice. The relative abundance of Flavobacteriaceae and Verrucomicrobiaceae was significantly increased in the Apc\textsuperscript{Min/\textsuperscript{+}}-Ffar2\textsuperscript{-/-} mice compared to the Apc\textsuperscript{Min/\textsuperscript{+}} mice. In addition, knocking-down FFAR2 in the human colon cancer cell lines (SW480 and HT29) resulted in increased expression of several key enzymes in fatty acid oxidation, such as carnitine palmitoyltransferase 2, acyl-CoA dehydrogenase, long-chain acyl-CoA dehydrogenase, C-2 to C-3 short chain, and hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit. Collectively, these results demonstrated that Ffar2 deficiency significantly altered profiles of fatty acid metabolites and gut microbiome, which might promote colorectal cancer development.

Key Words FFAR2, Apc\textsuperscript{Min/\textsuperscript{+}}, Colorectal cancer, Metabolomics, Gut microbiota
bacteria, we have demonstrated that the berries can modulate gut bacterial metabolites in colorectal cancer patients [26] and animals bearing colorectal cancer [27,28]. Interestingly, various components in black raspberries exerted different effects on gut microbiota [29]. Most importantly, we further showed that loss of Ffar2 significantly dampened the anti-colorectal cancer effects of black raspberries [22]. Accordingly, our previous results suggest that functional Ffar2 is vital for high-fiber foods to exert anti-colorectal cancer activities.

Our current study demonstrated that loss of Ffar2 promoted the colon adenomas development in the Apc<sup>Min/+</sup> mice. Besides, using a mass spectrometry-based metabolomic analysis, we determined the effects of Ffar2 deficiency on the metabolites. The 16S rRNA gene sequence-based microbial analysis was conducted to determine if loss of Ffar2 could change the gut bacterial composition. Lastly, we knockdown FFAR2 in the human colon cancer cell lines to determine its effects on the Expression of the key enzymes that are involved in energy metabolism.

**MATERIALS AND METHODS**

**Animals and cell lines**

All protocols followed institutional guidelines for animal care dictated by the Medical College of Wisconsin Animal Care and Use Committee (AUA2430). Breeding pairs of the WT and Apc<sup>Min/+</sup> mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Breeding pairs of the Ffar2 heterozygous (Ffar2<sup>+/−</sup>) mice were purchased from Deltagen, Inc. (San Mateo, CA, USA). Four-week-old WT, Apc<sup>Min/+</sup>, and Apc<sup>Min/+</sup>-Ffar2<sup>−/−</sup> mice were fed the synthetic diet AIN-76A from the American Institute of Nutrition (Dyets Inc., Bethlehem, PA, USA) for 8 weeks. Mice were euthanized by CO<sub>2</sub> asphyxiation. The number and the burden of polyps were determined. The colonic mucosa and plasma specimens were collected from a subgroup of the WT mice (n = 4), Apc<sup>Min/+</sup> mice (n = 5) and Apc<sup>Min/+</sup>-Ffar2<sup>−/−</sup> mice (n = 5) for metabolomic profiling. The cecal fecal specimens were collected from a subgroup of the WT mice (n = 5), Apc<sup>Min/+</sup> mice (n = 5), and Apc<sup>Min/+</sup>-Ffar2<sup>−/−</sup> mice (n = 5) for microbial analysis.

Human colorectal cancer cells HT29 and SW480 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) in April 2016 and were cultured as recommended by ATCC.

**Metabolomic profiling**

Specimen preparation and extraction, mass spectrometer platforms and setting, and data analysis were conducted by Metabolon, Inc. (Morrisville, NC, USA) [30-32] according to the previous description [26,27]. Briefly, samples were prepared using an automated MicroLab STAR® system (Reno, NV, USA). Homogenized mucosa samples were extracted using 5 μL of methanol per mg tissue, and the plasma samples were extracted using 5 μL of methanol per mL tissue. Samples were characterized using the ultra-high-performance-liquid chromatography/tandem mass spectrometry (UHPLC-MS/MS) in the negative ion mode, the UHPLC-MS/MS in the positive ion mode, and the gas chromatography-mass spectrometry (GC-MS) after sialylation. Chemical entities were identified by comparing them to the metabolomic library of purified standards based on chromatographic properties and mass spectra.

**DNA preparation and PCR amplification**

Cecal feces were collected from a subgroup of the WT mice (n = 5), Apc<sup>Min/+</sup> mice (n = 5), and Apc<sup>Min/+</sup>-Ffar2<sup>−/−</sup> mice (n = 5). The fecal DNA samples were isolated using the PowerSoil® DNA Isolation Kit (MO Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. The 515F-806R region of the 16S rRNA gene was amplified by PCR (94°C for 3 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 60 seconds, and 72°C for 90 seconds and a final extension at 72°C for 10 minutes, hold at 4°C) using primers 515F 5′-GTGCCAGCMGCCGCGTA-3′ and 806R 5′-barcode-GGACTACHVGGGTWTCTAAT-3′ [33]. PCR reactions were performed in triplicate with 25 μL of the reaction mixtures containing 10 μL of the five primers hot master mix (2200410; MO Bio Laboratories), 0.5 μL of each primer (10 μM) and 1 μL of the template DNA.

**Illumina MiSeq sequencing**

The PCR products were quantified by Picogreen (P11496; Thermo Fisher Scientific, Waltham, MA, USA). Two hundred and forty ng of the DNA was pooled for each sample and purified using UltraClean PCR Clean-Up kit (12500; MO Bio Laboratories) according to the manufacturer’s instructions. Sequencing was conducted using a paired-end, 2 × 250-bp cycle run on an Illumina MiSeq sequencing system and MiSeq Reagent Kit version 2 (500 Cycle) chemistry. Illumina BaseSpace’s 16s Metagenomics App was used to analyze the results.

**Sequencing data analysis**

To provide an even level of coverage for clustering and statistical comparisons, raw taxonomic counts were subsampled to 13,995 sequences per sample and aggregated at phylum through genus levels using QIIME [34]. Differential abundance analysis comparing the WT, Apc<sup>Min/+</sup>, and Apc<sup>Min/+</sup>-Ffar2<sup>−/−</sup> groups utilized the negative binomial test [35] with P-value adjustment using the False Discovery Rate [36]. Adjusted P-values that were less than 0.05 were considered statistically significant. Hierarchical clustering was performed using Ward’s method with log-normalized proportional values in R.

**Immunoblotting analysis**

Protein lysates of the human colorectal cancer cell lines were used for immunoblotting analysis. FFAR2-shRNA constructs to knockdown FFAR2 were purchased from OriGene Tech.
nologies, Inc. (Rockville, MD, USA), as indicated previously [23]. Antibodies to carnitine palmitoyltransferase 2 (CTP2) (ab181114), acyl-CoA dehydrogenase, long-chain (ACADL) (ab152160), acyl-CoA dehydrogenase, C-2 to C-3 short chain (ACADS) (ab156571), and hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit (HADHA) (ab203114) were purchased from Abcam (Cambridge, MA, USA) and were used to identify their respective proteins. Antibody to β-actin (691001) was purchased from MP Biomedical (Santa Ana, CA, USA) and was used as a loading control.

**Statistical analysis**
Data were expressed as mean ± SEM. One-way ANOVA was employed in R version 2.14.2 [37] to identify statistically significant metabolite differences across genotypes. Standard statistical analyses are performed in ArrayStudio on log transformed data. A P-value < 0.05 was considered statistically significant.

**RESULTS**

**Ffar2 deficiency promoted the development of colonic polyps**

The WT, Apc\textsuperscript{Min/+}, and Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-} mice were given the AIN-76A diet for 8 weeks. Forty % (4/10) of the Apc\textsuperscript{Min/+} mice developed colonic polyps, whereas the Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-} mice have an 100% (8/8) incidence of colonic polyps development.

![Figure 1. Loss of Ffar2 promotes colon adenoma development.](image)

**Table 1. List of significantly changed metabolites involved in the fatty acid β-oxidation pathway**

| Metabolites                     | Biochemical pathways | Metabolites                     | Fold control |
|---------------------------------|----------------------|---------------------------------|--------------|
| Mucosa                          | Medium chain fatty acid | 5-dodecenoate (12:1n7)          | 0.48\textsuperscript{a} 0.4\textsuperscript{a} |
|                                 | Long chain fatty acid | Margarate (17:0)                | 0.35\textsuperscript{b} 0.39\textsuperscript{b} |
|                                 |                      | Eicosenoate (20:1)              | 0.25\textsuperscript{b} 0.29\textsuperscript{b} |
|                                 |                      | Erucate (22:1n9)                | 0.23\textsuperscript{b} 0.34\textsuperscript{b} |
|                                 | Polyunsaturated fatty acid (n3 and n6) | Docosadienoate (22:2n6)        | 0.34\textsuperscript{a} 0.44\textsuperscript{a} |
|                                 |                      | Dihomo-linoleate (20:2n6)      | 0.31\textsuperscript{a} 0.32\textsuperscript{a} |
|                                 |                      | Dihomo-linolenate (20:3n3 or n6) | 0.43\textsuperscript{a} 0.59\textsuperscript{a} |
|                                 |                      | Docosapentaenoate (n6 DPA; 22:5n6) | 0.46\textsuperscript{a} 0.51\textsuperscript{a} |
|                                 | Fatty acid metabolism (Acyl Carnitine) | Acetylcarnitine     | 1.57\textsuperscript{a} 1.83\textsuperscript{a} |
|                                 |                      | Decanoylcarnitine               | 1.44\textsuperscript{a} 2\textsuperscript{a} |
|                                 |                      | 3-hydroxybutyrylcarnitine       | 4.34\textsuperscript{a} 2.73\textsuperscript{a} |
|                                 |                      | Stearoylcarnitine               | 1.68\textsuperscript{a} 1.4\textsuperscript{a} |
|                                 |                      | Cis-4-decenoyl carnitine        | 1.94\textsuperscript{a} 1.69\textsuperscript{a} |
|                                 |                      | Laurycarnitine                  | 1.48\textsuperscript{a} 1.42\textsuperscript{a} |
|                                 |                      | Myristoylcarnitine              | 1.65\textsuperscript{a} 1.71\textsuperscript{a} |
|                                 |                      | Palmitoylcarnitine              | 1.6\textsuperscript{a} 1.84\textsuperscript{a} |
|                                 |                      | Stearoylcarnitine               | 1.92\textsuperscript{a} 1.5\textsuperscript{a} |
|                                 |                      | Myristoleoylcarnitine           | 1.54\textsuperscript{a} 1.62\textsuperscript{a} |
|                                 |                      | Suberoylcarnitine               | 3.5\textsuperscript{a} 3.08\textsuperscript{a} |
|                                 |                      | Adipoylcarnitine                | 3.5\textsuperscript{a} 2.51\textsuperscript{a} |
|                                 |                      | 3-hydroxybutyrate (BHBA)        | 5.46\textsuperscript{a} 3.53\textsuperscript{a} |

Fold change is calculated as the ratio of the Apc\textsuperscript{Min/+} (A) vs. WT, Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-} (AF) vs. WT, and Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-} vs. Apc\textsuperscript{Min/+}. Fold change that is labeled * or † presents significantly increased or significantly decreased, respectively. WT, wild-type. P < 0.05.
Ffar2 deficiency enhanced the long-chain fatty acid β-oxidation and bile acid metabolism

To determine the effects of Ffar2 deficiency on the metabolic profiles, we collected the colonic mucosa and plasma specimens and conducted a mass spectrometry-based nontargeted metabolomic analysis. Five hundred and sixteen plasma metabolites and 568 colonic mucosa metabolites were annotated. Of these, 128 plasma metabolites and 75 colonic mucosa metabolites were significantly changed across three genotypes. Similar metabolic alterations, including 59 plasma metabolites (Table S1) and 23 mucosa metabolites (Table S2), have been observed in both the ApcMin+ and ApcMin−Ffar2−/− mice compared to the WT mice. More importantly, Ffar2 deficiency further modulated 31 plasma metabolites (Table S3) and 28 mucosa metabolites (Table S4). Significantly changed metabolites were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to identify biochemical pathways.

Fatty acids are oxidized in the mitochondria to generate energy and intermediates for cell proliferation. We observed significantly decreased fatty acid levels, including the medium-chain fatty acids, long-chain fatty acids, and polyunsaturated fatty acids in both the ApcMin+ and ApcMin−Ffar2−/− mice (Table 1). Also, the production of acetylcarnitine, the end-product of fatty acid β-oxidation, was significantly increased in plasma of both the ApcMin+ and ApcMin−Ffar2−/− mice compared to the WT mice. More importantly, Ffar2 deficiency further modulated plasma fatty acids (Table 1). Carnitine-conjugated long-chain fatty acids, long-chain fatty acids, and polyunsaturated fatty acids in both the ApcMin+ and ApcMin−Ffar2−/− mice were markedly increased in the ApcMin+ and ApcMin−Ffar2−/− mice compared to the ApcMin+ mice (Table 1), suggesting the long-chain fatty acid β-oxidation were enhanced by the Ffar2 deficiency. Acetylcarnitine can be converted to acetyl-CoA, which enters into the citric acid cycle to generate 3-hydroxybutyrate (BHBA) through ketogenesis. We observed a significant accumulation of BHBA in plasma of the ApcMin+−Ffar2−/− mice (Table 1). These results indicate an increased mitochondrial activity and a higher demand for energy by cancer cells.

Primary bile acids are synthesized by cholesterol catabolism in the liver and subsequently conjugated [38]. In the intestine, intestinal bacteria could deconjugate a significant portion of the primary bile acids, and structurally modify them into the secondary bile acids, which have been shown to promote colon carcinogenesis [38]. We observed significantly increased levels of both the primary and secondary bile acids in colonic mucosa in the ApcMin−Ffar2−/− mice compared to the ApcMin+ mice, including cholate, chenodeoxycholate, deoxycholate, and taurodeoxycholate (Table 2). Deoxycholate has been demonstrated to promote colon carcinogenesis by 165.1% in the ApcMin+ mice [39]. Thus, our findings suggest that an increased deoxycholate level could directly contribute to the Ffar2 deficiency-promoted colon cancer development.

Ffar2 deficiency changed the expression of key enzymes in the fatty acid β-oxidation pathway

After observing significant levels of the carnitine-conjugated long-chain fatty acids in the ApcMin+−Ffar2−/− mice compared to ApcMin+, we further investigated if loss of Ffar2 could alter the expression of the key enzymes involved in the fatty acid β-oxidation pathway. We first determined the endogenous expression levels of FFAR2 in four human colon cancer cell lines, and found higher levels of FFAR2 expression in SW480 and HT29 cell lines [23]. Furthermore, we knocked-down Ffar2 using shRNA in SW480 and HT29 cells as previously [23]. We observed increased expression levels of several

| Table 2. List of significantly changed metabolites in the bile acid pathway | Metabolites | Fold control | A/WT | AF/WT |
|---|---|---|---|---|
| Mucosa | Primary bile acid metabolism | Cholate sulfate | 0.05a | 0.25a |
| Plasma | Secondary bile acid metabolism | Deoxycholate | 3.19a | 2.03a |
| Mucosa | Primary bile acid metabolism | Cholate | 1.33 | 4.49a |
| | | Cholate | 1.13 | 4.95a |
| | | Beta-muricholate | 0.86 | 2.68a |
| | Secondary bile acid metabolism | Deoxycholate | 3.02a | 7.65a |
| | | Taurodeoxycholate | 2.75 | 6.29a |
| | | 6-beta-hydroxycholate | 2.1a | 4.29a |
| | | 7-ketolithocholate | 5.92a | 11.94a |
| | | Hyocholate | 0.84 | 3.55a |
| | | 3-dehydrocholate | 1.11 | 3.1a |
| | | 7-ketodeoxycholate | 0.9 | 3.83a |

Fold change is calculated as the ratio of the ApcMin+ (A) vs. WT, ApcMin+−Ffar2−/− (AF) vs. WT, and ApcMin−Ffar2−/− vs. ApcMin+. Fold change that is labeled a or b presents significantly increased or significantly decreased, respectively. WT, wild-type. P < 0.05.
FFAR2 deficiency changed gut microbiota composition

Evidence has been accumulated to imply the interplay between gut dysbiosis and colorectal cancer [40]. In order to investigate the effects of Ffar2 deficiency on the gut microbiome, we performed 16S rRNA gene sequencing on the cecal microbialome, we performed 16S rRNA gene sequencing on the cecal microbialome we knock-downed FFAR2 in the SW480 and HT29 cells [23]. Using the FFAR2 knocked-down cells, we found that the expression levels of several key enzymes in the fatty acid oxidation pathway have been increased in the FFAR2-deficient cells, including CPT2, ACADL, ACADS, and HADHA.

CPT2 has been shown to be over-expressed in primary prostate cancer [44], and knocking-down of CPT2 inhibited the tumor growth in triple-negative breast cancer [45]. Thus, our findings on increased CPT2 expression in FFAR2-deficient cells could be one of the mechanisms responsible for loss of FFAR2-enhanced colon cancer development.

HADHA has also been reported to be decreased in breast cancer [46] and clear cell renal cell carcinoma [47]. However, we observed increased expression of HADHA in the FFAR2-deficient SW480 and HT29 cells. These results, combined with increased expression of CPT2, ACADL, and ACADS in the FFAR2-deficient cells, suggest an overall accelerated fatty acid oxidation, which may contribute to the development of colon cancer [22,24]. Our current study, by utilizing Apc\(^{Min/+}\) mice, demonstrated that Ffar2 deficiency promoted the development of colorectal polyps. All the Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice developed colorectal polyps compared to only 40% of the Apc\(^{Min/+}\) mice. The Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice developed increased tumor burden of colon polyps. In addition, we investigated if Ffar2 deficiency has effects on the metabolic profiles and the gut bacterial composition. Thirty-one plasma metabolites and 28 colonic mucosa metabolites were changed in the Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice compared to the Apc\(^{Min/+}\) mice. Analysis using KEGG data suggests that loss of Ffar2 enhances the long-chain fatty acid β-oxidation and the bile acid metabolism. Furthermore, Ffar2 deficiency markedly increased the abundance of Flavobacteriaceae and Verrucomicrobiaceae.

Previously we observed significantly decreased fatty acid levels in the colonic mucosa of Apc\(^{Min/+}\) mice [27]. Similarly, the current study detected reduced levels of 11 fatty acids, including medium-chain fatty acids, long-chain fatty acids, and polyunsaturated fatty acids, in the colonic mucosa of Apc\(^{Min/+}\) mice (Table S2). Six of these fatty acids were also significantly decreased in the colonic mucosa of Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice (Table 1). In addition, Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice showed a substantial accumulation of carnitine-conjugated long-chain fatty acids in both colonic mucosa and plasma specimens (Table 1), including stearoylcarnitine, laurlylcarnitine, myristoylcarnitine, and palmitoylcarnitine. Increased levels of these carnitine-conjugated long-chain fatty acids have been observed in tumor samples from biofilm-positive colorectal cancer patients [41], suggesting association among the increased fatty acid β-oxidation, loss of Ffar2, and gut microbiota.

Enhanced fatty acid β-oxidation has been reported in colon cancer patients [42,43]. Our study used human colon cancer cell lines to investigate if the functional FFAR2 could influence the key enzymes of the fatty acid oxidation pathway. Based on relatively higher expression levels of FFAR2 in the SW480 and HT29 cells compared to the Caco-2 and HCT116 cells, we knock-downed FFAR2 in the SW480 and HT29 cells [23]. Using the FFAR2 knocked-down cells, we found that the expression levels of several key enzymes in the fatty acid oxidation pathway have been increased in the FFAR2-deficient cells, including CPT2, ACADL, ACADS, and HADHA.

Figure 2. FFAR2 deficiency significantly increased the expression of key enzymes in the fatty acid β-oxidation pathway. Immunoblotting of CPT2, ACADL, HADHA in the SW480 (A) and HT29 (B) cells treated with either the vector or the FFAR2-shRNA to knockdown FFAR2. Numbers under each blot indicate the fold changes. FFAR2, free fatty acid receptor 2; ACADL, acyl-CoA dehydrogenase, long chain; ACADS, acyl-CoA dehydrogenase, C-2 to C-3 short chain; CPT2, carnitine palmitoyltransferase 2; HADHA, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit.

DISCUSSION

Our previous studies and those of other groups have shown that the expression of FFAR2 was decreased in adenocarcinoma tissues compared to normal tissues of patients with colorectal cancer [22,24]. Our current study, by utilizing Apc\(^{Min/+}\) mice, demonstrated that Ffar2 deficiency promoted the development of colorectal polyps. All the Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice developed colorectal polyps compared to only 40% of the Apc\(^{Min/+}\) mice. The Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice developed increased tumor burden of colon polyps. In addition, we investigated if Ffar2 deficiency has effects on the metabolic profiles and the gut bacterial composition. Thirty-one plasma metabolites and 28 colonic mucosa metabolites were changed in the Apc\(^{Min/+}\)-Ffar2\(^{-/-}\) mice compared to the Apc\(^{Min/+}\) mice. Analysis using KEGG data suggests that loss of Ffar2 enhances the long-chain fatty acid β-oxidation and the bile acid metabolism. Furthermore, Ffar2 deficiency markedly increased the abundance of Flavobacteriaceae and Verrucomicrobiaceae.

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Dysregulated FFAR2 Alters Metabolism and Gut Microbiota

A strong link between microbial dysbiosis and colon cancer has been intensively explored. However, due to the complexity of the gut microbiome, the underlying mechanisms remain unclear. Our current study demonstrated that loss of Ffar2 significantly changed the composition of microbiota in the Apc\textsuperscript{Min/+} mice. Decreased Bifidobacterium and has been observed in human colon cancer tissues [25]. Also, increased Peptostreptococcaceae has been positively associated with biofilm and an enhanced acetylated polyamines pathway in human colon cancer patients, which promote colon cancer development [41]. In our study, the profile of gut microbiome was found to be significantly changed in the polybearing mice (Apc\textsuperscript{Min/+} and Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-}) compared to WT mice, as revealed by the decreased abundance of Bifidobacterium and increased proportion of Peptostreptococcaceae. More importantly, the abundance of Flavobacteriaceae and Verrucomicrobiaceae was raised in the Apc\textsuperscript{Min/+} mice compared to WT mice and further increased in Apc\textsuperscript{Min/+}-Ffar2\textsuperscript{-/-} mice, which might contribute to Ffar2 deficiency-enhanced colon cancer development.

We previously reported that the cAMP-protein kinase A (PKA)-cAMP Response Element-Binding Protein (CREB) pathway, downstream of Ffar2, was activated, and this...
event led to overexpression of histone deacetylases in the Ffar2-deficient mice [23]. Mechanistically, H3K27me3 and H3K4me3 histone marks bind differentially to the promoter regions of inflammation suppressors as verified by ChIP-qPCR analysis. This results in decreased expression of these genes in the Ffar2-deficient mice, thereby promoting colon cancer [23]. We anticipate the changes of histone marks in enzymes regulating fatty acid oxidation, such as CPT2, ACADL, and HADHA, which warrants further investigations.

In summary, we validated Ffar2 as a tumor suppressor Ffar2 in colon carcinogenesis. To the best of our knowledge, this is the first study to link the biochemical metabolites and the gut microbiome profiling to the Ffar2 deficiency-promoted colon cancer development (Fig. 4). Enhanced fatty acid oxidation and bile acid metabolism, as well as the altered gut microbiome, could be, at least in part, constitute, the underlying mechanisms.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.15430/JCP.2021.26.1.32.

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