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Dysregulated Free Fatty Acid Receptor 2 Exacerbates Colonic Adenoma Formation in *Apc*$^{Min/+}$ Mice: Relation to Metabolism and Gut Microbiota Composition

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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*$^{Min/+}$). *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*$^{Min/+}$, and *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice. The relative abundance of *Flavobacteriaceae* and *Verrucomicrobiaceae* was significantly increased in the *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice compared to the *Apc*$^{Min/+}$ 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$^{Min/+}$, Colorectal cancer, Metabolomics, Gut microbiota

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*, also named *GPR43*) is a member of the G protein-coupled receptor family and is expressed on the adipose tissue, leukocytes [15], and colon [16,17]. *FFAR2* receptor can be activated by the short-chain fatty acids (SCFAs), such as acetate, butyrate, and propionate, which are produced during fermentation of the undigested carbohydrates and dietary fiber by gut microbiota [18]. *FFAR2* signaling has been reported as a negative regulator of inflammation. For example, dextran sodium sulfate (DSS)-induced ulcerative colitis, a strong risk factor for colorectal cancer in humans, was enhanced in the *Ffar2* deficient (*Ffar2*$^{-/-}$) mice compared to the wild-type (WT) mice [19-21]. In addition, a potential tumor-suppressive role of *Ffar2* in colon cancer has been reported by our group [22,23] and others [24,25]. The decreased *FFAR2* expression in colorectal adenocarcinoma tissues compared to the normal tissues has been observed in human patients [22,24]. However, whether loss of FFAR expression is associated with altered biochemical metabolites and microbial dysbiosis in colorectal cancer is unknown. Using black raspberries containing plenty of soluble fibers that can be metabolized into SCFAs by gut

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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 $Apc^{Min/+}$ 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 knocked-down *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 $Apc^{Min/+}$ mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Breeding pairs of the *Ffar2* heterozygous ($Ffar2^{+/-}$) mice were purchased from Deltagen, Inc. (San Mateo, CA, USA). Four-week-old WT, $Apc^{Min/+}$, and $Apc^{Min/+}$-$Ffar2^{-/-}$ 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_2 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), $Apc^{Min/+}$ mice (n = 5) and $Apc^{Min/+}$-$Ffar2^{-/-}$ mice (n = 5) for metabolomic profiling. The cecal fecal specimens were collected from a subgroup of the WT mice (n = 5), $Apc^{Min/+}$ mice (n = 5), and $Apc^{Min/+}$-$Ffar2^{-/-}$ 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), $Apc^{Min/+}$ mice (n = 5), and $Apc^{Min/+}$-$Ffar2^{-/-}$ 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'-GTGCCAGCMGCCGCGGTAA-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, $Apc^{Min/+}$, and $Apc^{Min/+}$-$Ffar2^{-/-}$ 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*$^{Min/+}$, and *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice were given the AIN-76A diet for 8 weeks. Forty % (4/10) of the *Apc*$^{Min/+}$ mice developed colonic polyps, whereas the *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice have an 100% (8/8) incidence of colonic polyps development

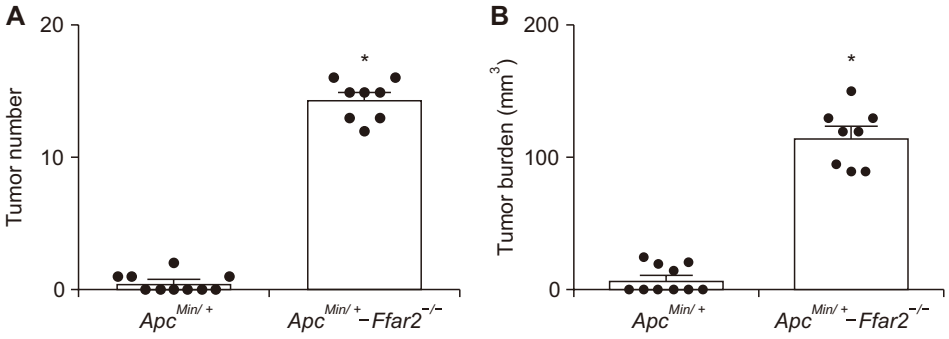

Figure 1. Loss of *Ffar2* promotes colon adenoma development. Colonic polyp number (A) and burden (B) from 12-14 weeks old, the *Apc*$^{Min/+}$ and *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice were measured. *Ffar2*, free fatty acid receptor 2. *P < 0.05.

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

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

Fold change is calculated as the ratio of the *Apc*$^{Min/+}$ (A) vs. WT, *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ (AF) vs. WT, and *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ vs. *Apc*$^{Min/+}$. Fold change that is labeled [a] or [b] presents significantly increased or significantly decreased, respectively. WT, wild-type. P < 0.05.

with an increased number (Fig. 1A) and burden (Fig. 1B) of colonic polyps, which were consistent with our previous studies with an increase Ki-67 staining as a proliferation marker and H&E staining [22].

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 $Apc^{Min/+}$ and $Apc^{Min/+}$-$Ffar2^{-/-}$ 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 $Apc^{Min/+}$ and $Apc^{Min/+}$-$Ffar2^{-/-}$ mice (Table 1). Also, the production of acetylcarnitine, the end-product of fatty acid β-oxidation, was significantly increased in plasma of both the $Apc^{Min/+}$ and $Apc^{Min/+}$-$Ffar2^{-/-}$ mice (Table 2). Carnitine-conjugated long-chain fatty acids were markedly increased in the $Apc^{Min/+}$-$Ffar2^{-/-}$ mice compared to $Apc^{Min/+}$ mice (Table 1), suggesting the long-chain fatty acid β-oxidation were enhanced by the *Ffar2* deficiency. Acetylcarnithine 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 $Apc^{Min/+}$-$Ffar2^{-/-}$ 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 $Apc^{Min/+}$-$Ffar2^{-/-}$ mice compared to the $Apc^{Min/+}$ mice, including cholate, chenodeoxycholate, deoxycholate, and taurodeoxycholate (Table 2). Deoxycholate has been demonstrated to promote colon carcinogenesis by 165.1% in the $Apc^{Min/+}$ 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 $Apc^{Min/+}$-$Ffar2^{-/-}$ mice compared to $Apc^{Min/+}$, 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	Biochemical pathways	Metabolites	Fold control		
			A/WT	AF/WT	
Mucosa	Primary bile acid metabolism	Cholate sulfate	0.05[b]	0.25[b]	
Plasma	Secondary bile acid metabolism	Deoxycholate	3.19[a]	2.03[a]	
			A/WT	AF/WT	AF/A
Mucosa	Primary bile acid metabolism	Cholate	1.33	4.49[a]	3.38[a]
		Chenodeoxycholate	1.13	4.95[a]	4.37[a]
		Beta-muricholate	0.86	2.68[a]	3.11[a]
	Secondary bile acid metabolism	Deoxycholate	3.02[a]	7.65[a]	2.53[a]
		Taurodeoxycholate	2.75	6.29[a]	2.28[a]
		6-beta-hydroxylithocholate	2.1[a]	4.29[a]	2.05[a]
		7-ketolithocholate	5.92[a]	11.94[a]	2.02[a]
		Hyocholate	0.84	3.55[a]	4.23[a]
		3-dehydrocholate	1.11	3.1[a]	2.8[a]
		7-ketodeoxycholate	0.9	3.83[a]	4.24[a]

Fold change is calculated as the ratio of the $Apc^{Min/+}$ (A) vs. WT, $Apc^{Min/+}$-$Ffar2^{-/-}$ (AF) vs. WT, and $Apc^{Min/+}$-$Ffar2^{-/-}$ vs. $Apc^{Min/+}$. Fold change that is labeled [a] or [b] presents significantly increased or significantly decreased, respectively. WT, wild-type. $P < 0.05$.

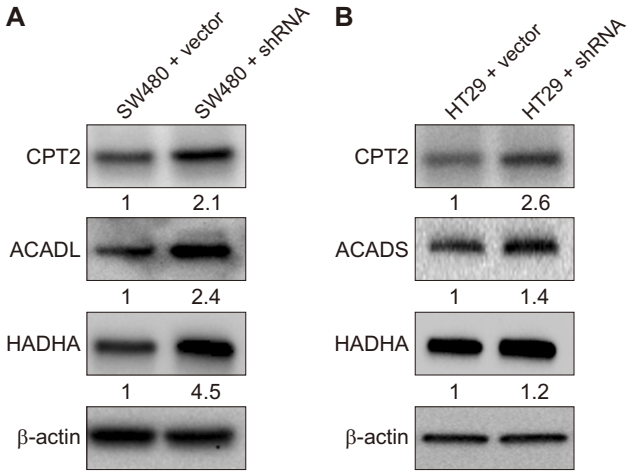

Figure 2. *FFAR2* deficiency significantly increased the expression of key enzymes in the fatty acid β-oxidation pathway. Immunoblotting of CPT2, ACADS/L and HADHA in the SW480 (A) and HT29 (B) cells treated with either the vector or the *FFAR2*-shRNA to knockdown *FFAR2*. Numbers under each blot indicate the fold differences. 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.

key enzymes in the fatty acid β-oxidation pathway, including CPT2, ACADL, ACADS, and HADHA (Fig. 2A and 2B). This finding suggests the enhanced fatty acid β-oxidation in the *FFAR2*-deficient colon cancer cells.

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 fecal samples. Principal coordinate analyses showed clustering according to genotypes (Fig. 3A). The relative abundance of bacteria at the family level (Fig. 3B) showed increased levels of *Porphyromonadaceae*, *Sphingobacteriaceae*, *Deferribacteraceae*, *Flavobacteriaceae*, and *Verrucomicrobiaceae* and decreased levels of *Ruminococcaceae* and *Bifidobacteriaceae* in both the $Apc^{Min/+}$ mice and $Apc^{Min/+}$-$Ffar2^{-/-}$ mice compared to the WT mice. In particular, *Flavobacteriaceae* and *Verrucomicrobiaceae* were further increased in the $Apc^{Min/+}$-$Ffar2^{-/-}$ mice compared to the $Apc^{Min/+}$ mice, which might contribute to the *Ffar2* deficiency-promoted colon cancer development (Fig. 3C).

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 colonic polyps. All the $Apc^{Min/+}$-$Ffar2^{-/-}$ mice developed colonic polyps compared to only 40% of the $Apc^{Min/+}$ mice. The $Apc^{Min/+}$-$Ffar2^{-/-}$ mice developed increased tumor burden of colonic 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, laurylcarnitine, 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.

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

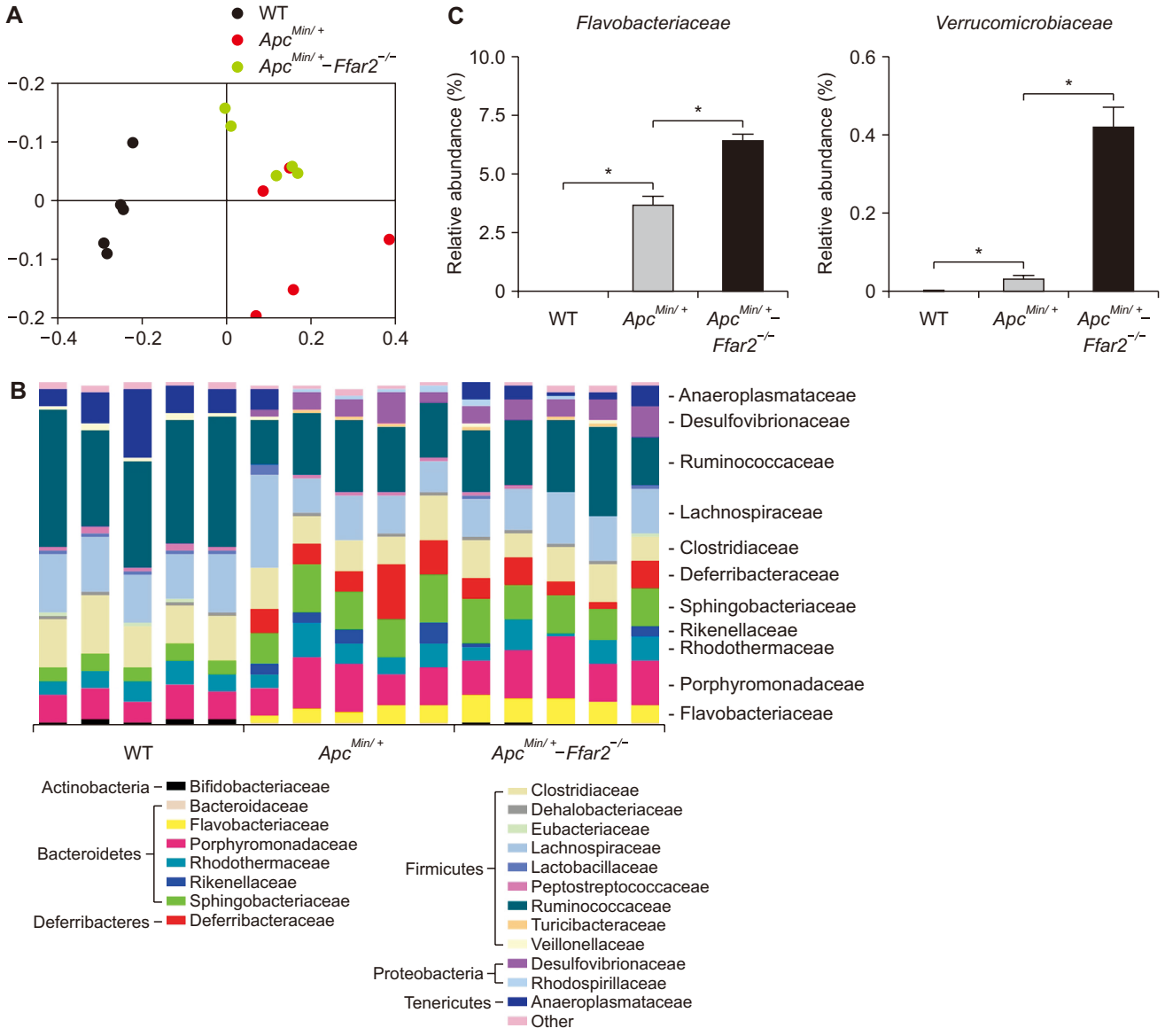

Figure 3. The *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice show a structurally different microbiota compared to the *Apc*$^{Min/+}$ mice. (A) Assessment of the structure of microbial communities by principal coordinate analyses. Plots were presented for gut bacteria sequenced at species levels. (B) Relative abundance of bacteria presented at the family level. (C) Relative abundance of *Flavobacteriaceae* and *Verrucomicrobiaceae* were significantly increased in the *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice compared to the *Apc*$^{Min/+}$ mice. FFAR2, free fatty acid receptor 2; WT, wild-type. *$P < 0.05$.

FFAR2 deficiency-promoted colon cancer development.

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*$^{Min/+}$ 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 polyp-bearing mice (*Apc*$^{Min/+}$ and *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ mice) 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*$^{Min/+}$ mice compared to WT mice and further increased in *Apc*$^{Min/+}$-*Ffar2*$^{-/-}$ 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

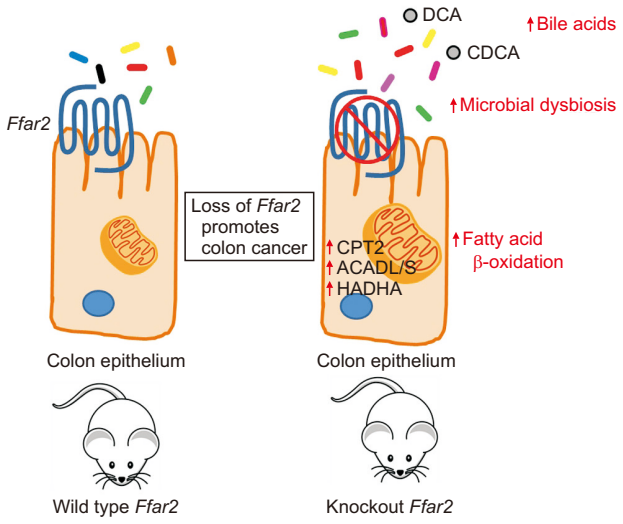

Figure 4. Dysregulated free fatty acid receptor 2 exacerbates colonic adenoma formation in *Apc*$^{Min/+}$ mice by altering metabolism and gut microbiota composition. DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; ACADL, acyl-CoA dehydrogenase, long chain; ACADS, acyl-CoA dehydrogenase, C-2 to C-3 short chain; CPT2, carnitine palmitoyltransferase 2; HADHA, hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase, alpha subunit.

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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