Human colon tumors have elevated levels of 15-lipoxygenase-1 (15-LO-1), suggesting that 15-LO-1 may play a role in the development of colorectal cancer. 15-LO-1 metabolites can up-regulate epidermal growth factor signaling pathways, which results in an increase in mitogenesis. However, metabolites of 15-LO-1 can serve as ligands for peroxisome proliferator-activated receptor γ (PPARγ), and activation of this receptor causes most colon cancer cell lines to undergo a differentiative response and reverse their malignant phenotype. Hence, the role 15-LO-1 plays in colon cancer is not clear. To clarify the role of 15-LO-1 in carcinogenesis, the effect of 15-LO-1 and its metabolites on epithelial growth factor signaling and PPARγ was investigated. In HCT-116 cells, exogenously added 15-LO-1 metabolites, 13-(S)-hydroxyoctadecadienoic acid, 13-(R)-hydroxyoctadecadienoic acid, and 13-(S)-hydroperoxyoctadecadienoic acid, up-regulated the MAPK signaling pathway, and an increase in PPARγ phosphorylation was observed. Furthermore, in stable overexpressing 15-LO-1 HCT-116 cells, which produce endogenous 15-LO-1 metabolites, an up-regulation in mitogen-activated protein kinase and PPARγ phosphorylation was observed. Incubation with a MAPK inhibitor ablated MAPK and PPARγ phosphorylation. The 15-LO-1 up-regulates MAPK activity and increases PPARγ phosphorylation, resulting in a down-regulation of PPARγ activity. Thus, 15-LO-1 metabolites may not only serve as ligands for PPARγ but can down-regulate PPARγ activity via the MAPK signaling pathway.

Lipoxygenases (LOs) are lipid-peroxidizing enzymes that are categorized according to their position of oxygenation of arachidonic acid (1). For example, 15-LOs oxygenate the substrate arachidonic acid at C-15. Two different human 15-LOs have been identified that differ in tissue distribution and substrate preference. 15-LO-1 is expressed in reticulocytes, eosinophils, macrophages, and skin (2). 15-LO-2 has limited tissue distribution, with mRNA detected in prostate, lung, skin, and cornea (3). In terms of enzymatic characteristics, 15-LO-1 preferentially metabolizes linoleic acid primarily to 13-(S)-HODE but also metabolizes arachidonic acid to 15-(S)-HETE. 15-LO-2, on the other hand, converts arachidonic acid to 15-(S)-HETE and metabolizes linoleic acid poorly (4). In human colorectal carcinoma Caco-2 cells, sodium butyrate induces the expression of reticulocyte 15-LO-1, and these cells undergo differentiation and apoptosis (5). This study provided the first evidence that 15-LO-1 is clearly expressed in human colorectal carcinoma cells, and it was subsequently shown that the 15-LO-1 is uniquely regulated by histone acetylation (6). Furthermore, human colon tumors have elevated levels of 15-LO-1 compared with the normal adjacent tissue (7). The increased expression in tumors and regulation being linked to histone acetylation suggests a possible role for 15-LO-1 in tumor development.

Human colon carcinomas express peroxisome proliferator-activated receptor γ (PPARγ), a member of the nuclear receptor superfamily involved in fat cell differentiation and glucose homeostasis (8). Ligand activation of this receptor causes most, but not all, colon cancer cell lines to undergo a differentiative response and reverse their malignant phenotype (9). PPARγ regulates differentiation and/or cell growth in a large and increasing number of cell types (9–15). Furthermore, colon cancer in humans is associated with loss-of-function mutations in PPARγ (16). All this taken together suggests that PPARγ could be a tumor suppressor. Recently, metabolites of 15-LO-1 have been reported to serve as ligands for PPARγ. Metabolites of 15-LO-1 show binding activity in a reporter system for PPARγ ligand binding (17). 13-(S)-HODE, 13-(S)-HpODE, and 15-(S)-HETE all show binding activity for PPARγ, but at relatively high concentrations (17). This is supported by a recent study done in macrophages, where PPARγ activation has been shown in 15-LO-1 transfected macrophages using linoleic and arachidonic acid as substrates (18). These data suggest that endogenous 15-LO-1 and its metabolites are ligands for PPARγ. Thus, based on these results, one could conclude that 15-LO-1 may have anti-tumorigenic activity. However, this seems to be in conflict with the finding that 15-LO-1 expression is elevated in tumors versus normal tissue (7).

15-LO-1 metabolites stimulate epidermal growth factor (EGF)-dependent cell growth in Syrian hamster embryo cells. The metabolites 13-(S)-HpODE and 13-(S)-HODE enhance EGF-induced mitogenesis (19). In this system, EGF stimulated the metabolism of exogenous or endogenous linoleic acid to 13-(S)-HpODE/13-(S)-HODE, dependent on tyrosine kinase activity. The addition of tyrosine kinase inhibitors inhibited not only EGF-induced mitogenesis but also the formation of 15-LO-1 metabolites. Furthermore, the exogenous addition of 13-(S)-HpODE or 13-(S)-HODE, but not 15-(S)-HETE, in combi-
nation with EGF to Syrian hamster embryo cells inhibited the dephosphorylation of the EGF receptor, thereby up-regulating the EGF cascade and potentiating the mitogenic response (20). The 15-LO-1 linoleic acid metabolites, 13-(S)-HpODE and 13-(S)-HODE, up-regulated EGF-dependent cell proliferation and enhanced MAPK activity, but the 15-LO-2 arachidonic acid metabolite, 15-(S)-HETE, was not active.

In this study, we found that in HCT-116 cells overexpression of 15-LO-1 down-regulates PPARγ via the MAPK signaling pathway. The 15-LO-1 metabolite 13-HODE up-regulates MAPK activity and PPARγ phosphorylation, resulting in a down-regulation of PPARγ activity. Thus, 15-LO-1 metabolites may not only serve as ligands for PPARγ but can affect PPARγ activity via the MAPK signaling pathway. This is a novel and potentially important mechanism involving 15-LO-1 and PPARγ.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The human colorectal cell line HCT-116 was obtained from the American Type Culture Collection (ATCC). HCT-116 cells were cultivated in McCoy’s 5A medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Summit), and gentamicin (1 mg/100 ml; Life Technologies). 15-LO-1 constructs in pcDNA 3.1 vector were transfected into HCT-116 cells via LipofectAMINE (Life Technologies). 15-LO-1 constructs in pcDNA 3.1 vector were transfected into HCT-116 cells via LipofectAMINE (Life Technologies). 15-LO-1 constructs were transfected into HCT-116 cells via LipofectAMINE (Life Technologies).

**Western Analysis**—For Western analysis, cell lysates demonstrated phosphorylated MAPK (A) or PPARγ expression (B) in HCT-116 cells after treatment with EGF (10 ng/ml) in the presence or absence of 13-(S)-HODE (5 μM) for the indicated times. The data shown represent one of three separate experiments with similar results. 40 or 80 μg of total protein was loaded per lane for MAPK or PPARγ, respectively. 10 μg of PPARγ standard was used. Phosphorylation was measured by densitometry and normalized to actin. The values are reported in the brackets above and below the gels.

**Immunoblot Analysis**—For Western analysis, cell lysates demonstrated phosphorylated MAPK (A) or PPARγ expression (B) in HCT-116 cells after treatment with EGF (10 ng/ml) in the presence or absence of 13-(S)-HODE (5 μM) for the indicated times. The data shown represent one of three separate experiments with similar results. 40 or 80 μg of total protein was loaded per lane for MAPK or PPARγ, respectively. 10 μg of PPARγ standard was used. Phosphorylation was measured by densitometry and normalized to actin. The values are reported in the brackets above and below the gels.

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peroxidase-linked secondary antibody (Amersham Pharmacia Biotech) for PPARγ, or anti-goat IgG horseradish peroxidase-linked secondary antibody (Santa Cruz Biotechnology) for actin, respectively. After reacting by chemiluminescence (Amersham Pharmacia Biotech ECL detection system), bands were detected by exposure to Hyperfilm-MP (Amersham Pharmacia Biotech).

Analysis of Arachidonic Acid and Linoleic Acid Metabolites in Intact Cells—HCT-116 or 15-LO-1 clone cells cultured in 150-cm² dishes at each condition were washed with serum-free medium twice. 10 ml of PBS supplemented with 10 μM CaCl₂ was then added to each plate, the appropriate treatments were added, and each plate was incubated for 15 min at 37 °C. NDGA was used at a concentration of 10 μM. Each plate was then reacted with 1[^1]H]arachidonic acid (3 μCi, 30 μμL) (PerkinElmer Life Sciences) or [14C]linoleic acid (3 μCi, 30 μμL) (PerkinElmer Life Sciences) for 1 h at 37 °C. The media were collected, and each plate was washed with 2 ml of MeOH and 2 ml of 1% acetic acid. The cells were scraped into this wash and then collected and added to the appropriate tube containing the media previously collected. The total collected medium was then acidified with acetic acid to pH 3 and applied to a C18-PrepSep solid phase extraction column (Waters) pretreated with methanol. The samples were washed with acidified water, eluted with methanol, evaporated to dryness, and reconstituted with high pressure liquid chromatography (HPLC) solvent.

High Pressure Liquid Chromatography—Reverse-phase HPLC analysis was performed using an Ultrasphere ODS column (5 μm, 4.6 × 250 mm; Beckman). The solvent system consisted of a methanol/water gradient at a flow rate of 1.1 ml/min as previously described (24). Radioactivity was monitored using a Flow Scintillation Analyzer (Packard) with EcoLume (ICN Biochemicals) as the liquid scintillation mixture. Authentic standards of 13-(S)-HODE and 15-(S)-HETE (Cayman Chemical) were used.

Analysis of Densitometry Measurements—Autoradiograms from Western blots were scanned using a Umax™ Powerlook IIITM scanner equipped with a transparency adapter and scanning software. Bands were quantitated using Scion Image™ beta version 4.0.2. Western blot values were first corrected using their corresponding actin levels. Values shown are -fold increases versus vehicle or 0 h as described in the figure legends.

RESULTS

Endogenous 15-LO-1 and PPARγ Expression in HCT-116 Cells—HCT-116 cells, a human colorectal cancer cell line, were used for the experimental studies to follow. Basal levels of 15-LO-1 and PPARγ expression were first confirmed by Western analysis (Fig. 1). HCT-116 cells lack endogenous 15-LO-1 expression, while PPARγ1 is detectable. Differentiated 3T3-L1 cells, which express both PPARγ1 and PPARγ2 isoforms, were used as a positive control for PPARγ (25). Zhu et al. (26) demonstrated that these two isoforms are derived from a single PPARγ gene by alternative promoter usage and RNA splicing. However, thus far, no functional difference has been found between the two isoforms. It has been established that human colorectal cells express only the PPARγ1 isoform (9, 27). From this point on, we will refer to PPARγ1 as PPARγ, since all of the subsequent experiments are done in HCT-116 human colorectal cells.

Effect of 13-(S)-HODE on MAPK and PPARγ Phosphorylation—Following serum deprivation, HCT-116 cells were pre-treated with 5 μM 13-(S)-HODE for 45 min prior to treatment with EGF (10 ng/ml). The effect of 13-(S)-HODE on MAPK and PPARγ phosphorylation after EGF stimulation was examined by Western analysis at the indicated time points (Fig. 2). Using a phosphospecific MAPK antibody, an increase in MAPK phosphorylation was observed in cells treated with 13-(S)-HODE compared with cells treated with EGF alone (Fig. 2A). An ~4-fold increase in MAPK phosphorylation could be detected within 5 min after treatment, while a 2-fold increase was noted at later times. Total MAPK expression levels were also examined by antibodies to ERK-1 and ERK-2 for this experiment and all subsequent experiments measuring phosphorylated MAPK blots. In all cases, total MAPK levels did not change, and thus only the level of phosphorylated MAPK was altered by treatment with 13-(S)-HODE or the treatment indicated for each experiment (data not shown). The densities of the phosphorylated proteins (ERK-1/2 and PPARγ) were measured, nor-
malized to actin, and are reported in the brackets below the blots. The effect of 13-(S)-HODE on PPARγ phosphorylation after EGF stimulation was also examined by Western analysis at the indicated time points (Fig. 2B). A similar increase in PPARγ phosphorylation was observed in 13-(S)-HODE-treated cells over cells treated with only EGF (Fig. 2B). The upper band of the doublet observed from samples is the phosphorylated form of PPARγ, while the lower band of the doublet is the unphosphorylated form. The addition of 13-(S)-HODE increased PPARγ phosphorylation by ~4-fold at 5 and 15 min and ~8-fold at 30 min. Similar results of increased phosphorylation of MAPK and PPARγ with 13-(S)-HODE were also obtained when serum was substituted for EGF stimulation to activate the MAPK pathway, which suggests these findings are not restricted to EGF signaling (data not shown). Thus, the 15-LO-1 metabolite, 13-(S)-HODE, causes an increase in both MAPK and PPARγ phosphorylation in serum- or EGF-stimulated cells.

13-(S)-HODE Dose Response in MAPK and PPARγ Phosphorylation—In order to examine whether the response was dependent on the concentration of 13-(S)-HODE, we tested different 13-(S)-HODE concentrations on MAPK phosphorylation. Following serum deprivation, HCT-116 cells were pretreated with varying concentrations of 13-(S)-HODE, ranging from 0.1 to 50 μM, for 45 min prior to treatment with EGF (10 ng/ml). The effect of 13-(S)-HODE on MAPK phosphorylation after EGF stimulation was examined by Western analysis (Fig. 3). Using a phosphospecific MAPK antibody, an increase in MAPK phosphorylation was observed in cells treated with 13-(S)-HODE over the cells treated with EGF alone (Fig. 3A). An increase in MAPK phosphorylation was observed at concentrations as low as 0.1 μM, but the strongest increase in MAPK phosphorylation was detected at 5 μM 13-(S)-HODE. Total MAPK expression levels were also examined using antibodies to ERK-1 and ERK-2. Fig. 3B reports the normalized data for 13-(S)-HODE stimulation of EGF-dependent MAPK phosphorylation. Thus, 5 μM 13-(S)-HODE seems to be an optimal concentration for increases in MAPK phosphorylation in EGF-stimulated cells. At higher concentrations, a diminished response was observed.

15-LO-1 Overexpression and Activity—To determine if endogenous 15-LO-1 metabolites could affect MAPK and PPARγ phosphorylation, we prepared 15-LO-1-overexpressing cells. HCT-116 cells were transfected with either vector alone or the 15-LO-1 cDNA. Individual clones were isolated and tested for 15-LO-1 expression by Western analysis. Two separate clones, clones 20 and 22, were found to express 15-LO-1 abundantly (Fig. 4). Vector-transfected cells expressed no 15-LO-1, similar to the parent HCT-116 cells (Fig. 4). Actin was used as a control for the amount of protein loaded. To determine if these clones expressing 15-LO-1 were metabolically active, intact cells were reacted with radiolabeled linoleic acid (30 μM), and 15-LO-1 activity was examined by HPLC analysis of the metabolites (Fig. 5). Clone 20 produced 13-(S)-HODE, the main metabolite, with a retention time of about 64 min (Fig. 5). This 15-LO-1 activity was inhibited by nordihydroguaiaretic acid, a lipoxigenase inhibitor, consistent with a 15-LO-1 activity. Similar results were obtained for clone 22 (data not shown). In contrast, vector-transfected cells and HCT-116 cells, when reacted with radiolabeled linoleic acid, produced no 15-LO-1 metabolites (Fig. 5). Hence, we have established two catalytically active 15-LO-1 overexpressing cell lines.

MAPK and PPARγ Phosphorylation in 15-LO-1 HCT-116 Cells—The exogenous addition of 13-(S)-HODE to EGF-stimulated HCT-116 cells increased both MAPK and PPARγ phosphorylation. Here we investigate whether endogenous 13-(S)-HODE will have the same effect by utilizing 15-LO-1-overexpressing cells. Following serum starvation, clone 20 or 22 was pretreated with 30 μM linoleic acid for 45 min prior to treatment with EGF (10 ng/ml). MAPK and PPARγ phosphorylation after EGF stimulation was examined by Western analysis at the indicated time points (Fig. 6). Actin expression was measured and used to normalize the data. The normalized density measurements are reported in brackets. A greater than 2-fold increase in MAPK phosphorylation was observed in cells treated with linoleic acid over the cells treated with EGF alone (Fig. 6A). An increase in MAPK phosphorylation was noted in both clones 20 and 22 (Fig. 6A, data not shown). In contrast, the addition of linoleic acid to vector-transfected cells that do not express 15-LO-1 did not increase MAPK phosphorylation (Fig. 6A). Thus, the increase in MAPK phosphorylation is dependent on the 15-LO-1 activity.

An increase in PPARγ phosphorylation was also observed in linoleic acid-treated cells over cells stimulated with only EGF (Fig. 6B). With either clone 20 or 22, the addition of the 15-LO-1 substrate increased PPARγ phosphorylation but was less than 2-fold. In contrast, the incubation of vector-transfected cells with linoleic acid did not increase PPARγ phosphoryla-
tion. Thus, endogenously produced 13-(S)-HODE causes an increase in both MAPK and PPAR\(\gamma\) phosphorylation similar to that observed by the addition of exogenous 13-(S)-HODE.

**MAPK Phosphorylation of PPAR\(\gamma\)**—To demonstrate that the phosphorylation of PPAR\(\gamma\) is dependent on MAPK activity, the effect of PD98059 on PPAR\(\gamma\) phosphorylation was examined. PD98059 is a specific inhibitor of MEK (28). Following serum deprivation, HCT-116 cells were pretreated with 5 \(\mu M\) 13-(S)-HODE for 45 min in the presence or absence of PD98059 (50 \(\mu M\)) prior to treatment with EGF (10 ng/ml). MAPK and PPAR\(\gamma\) phosphorylation after EGF stimulation was examined by Western analysis at the indicated time points (Fig. 7). An increase in MAPK and PPAR\(\gamma\) phosphorylation was observed in cells treated with 13-(S)-HODE over the cells treated with EGF alone. However, in the presence of the MEK inhibitor, PD98059, MAPK phosphorylation was abolished. This is consistent with inhibition of MEK activity. Likewise, PPAR\(\gamma\) phosphorylation was ablated (Fig. 7B). Total MAPK levels did not change; only the levels of phosphorylated MAPK were altered by treatment with 13-(S)-HODE (data not shown). Normalization of the phosphorylation density measurements was done, and the values are reported in the brackets in Fig. 7. These results are consistent with the hypothesis that phosphorylation of PPAR\(\gamma\) observed upon treatment with 13-(S)-HODE in EGF-stimulated cells is dependent on MAPK activity and is occurring through the MAPK signaling pathway.

**Effect of Other 15-LO Metabolites**—To examine whether the effects we observed were specific to 13-(S)-HODE, we also tested the effect of 13-(S)-HpODE, the precursor of 13-(S)-HODE, 13-(R)-HODE, and 15-(S)-HETE, on MAPK and PPAR\(\gamma\) phosphorylation. Following serum deprivation, HCT-116 cells were pretreated with 5 \(\mu M\) 13-(R)-HODE, 10 \(\mu M\) 13-(S)-HpODE, or 10 \(\mu M\) 15-(S)-HETE for 45 min prior to treatment with EGF (10 ng/ml). The effect of 13-(R)-HODE and 13-(S)-HpODE were similar to that of 13-(S)-HODE on MAPK and PPAR\(\gamma\) phosphorylation after EGF stimulation at both time points tested (Fig. 8). An increase, between 2–4-fold, in MAPK or PPAR\(\gamma\) phosphorylation was observed in cells treated with 13-(R)-HODE or 13-(S)-HpODE over cells treated with EGF alone. Interestingly, in contrast, 15-(S)-HETE (from 1 to 10 \(\mu M\)) appeared to reduce the MAPK or PPAR\(\gamma\) phosphorylation after EGF stimulation at the time points tested (Fig. 9). Thus, the increase in MAPK-dependent phosphorylation appears to be restricted to linoleic acid metabolites.

**DISCUSSION**

Two metabolites of linoleic acid, 13-HODE and 9-HODE, and the metabolite of arachidonic acid, 15-HETE, formed by 15-
Lipoxygenase have been shown to bind and activate PPAR\(\gamma\) in vitro. This finding suggests that 15-HETE, 13-HODE, and 9-HODE may function as endogenous ligands for PPAR\(\gamma\). However, these findings were observed with high concentrations of the metabolites (30–100\(\mu\)M). Although many naturally occurring fatty acids and their metabolites can activate PPAR\(\gamma\), they bind with relatively low affinities and must be added to cells at high concentrations to stimulate transcription. It has therefore been difficult to establish the physiological relevance of any of these lipid substances as regulators of PPAR\(\gamma\) in vivo.

In this study, we have found that 13-HODE at 1–10\(\mu\)M can down-regulate PPAR\(\gamma\) activity by increasing phosphorylation mediated via the MAPK signaling pathway. The addition of exogenous 13-(S)-HODE, at lower concentrations than used to observe binding to PPAR\(\gamma\), up-regulates both the EGF- and serum-dependent MAPK pathway and subsequently PPAR\(\gamma\) phosphorylation. The addition of a MEK inhibitor results in the inhibition of MAPK activity and subsequently PPAR\(\gamma\) phosphorylation, which supports the conclusion that PPAR\(\gamma\) phosphorylation is mediated by MAPK as reported in fat cells (29). This phenomenon is specific for linoleic acid metabolites as 13-(S)-HODE, 13-(R)-HODE, and 13-(S)-HpODE all have the same effect, while 15-(S)-HETE, an arachidonic acid metabolite, was inactive and in fact appeared to inhibit phosphorylation.

To test the effects of endogenous 15-LO-1 metabolites, we constructed stable 15-LO-1 overexpressing cells. By treating these cells with linoleic acid, a substrate for 15-LO-1, we could examine if endogenous 15-LO-1 metabolites would increase...
15-LO-1 Down-regulates PPARγ Activity

MAPK and PPARγ phosphorylation. Interestingly, endogenous metabolites had the same effect as addition of exogenous 13-(S)-HODE, 13-(R)-HODE, or 13-(S)-HpODE. In HCT-116 cells, endogenous 15-LO-1 metabolites up-regulate MAPK activity and hence increase PPARγ phosphorylation. It has been well documented in the literature that phosphorylation of PPARγ results in a decrease in transcriptional activity (29–32). Since phosphorylation of PPARγ inhibits its transcriptional activity, 15-LO-1 metabolites down-regulate PPARγ activity via the MAPK signaling pathway.

MAPK, a central regulator of cell growth, can modify PPARγ in a way that significantly reduces PPARγ transcriptional activity (30, 33). The phosphorylation of a key residue, Ser82 on PPARγ, results in a decrease in transcriptional activity (29). MAPK may be particularly suitable for this purpose because, among the signal transduction machinery linked to the cell cycle, MAPK can enter the nucleus to modify transcription factors (34). It is interesting to note that MAPK has been implicated in the phosphorylation of another nuclear receptor, the estrogen receptor, although this correlates with an increase in transcriptional activity (35, 36).

In this study, 13-(S)-HODE, 13-(R)-HODE, and 13-(S)-HpODE all had similar effects resulting in the down-regulation of PPARγ activity. Interestingly, however, 15-(S)-HETE appeared to inhibit MAPK or PPARγ phosphorylation. 15-(S)-HETE is the main arachidonic acid metabolite formed by 15-LO-2 activity. 15-LO-2 metabolizes arachidonic acid to 15-(S)-HETE but metabolizes linoleic acid poorly (4). This is in contrast to 15-LO-1, which preferentially metabolizes linoleic acid primarily to 13-(S)-HODE. This result is particularly intriguing given the fact that 15-LO-2 has been found to be expressed higher in normal prostate tissue than in adjacent tumor tissue (3). This is in contrast to 15-LO-1, which has been found to be more highly expressed in tumor than normal colon tissue (7) and in prostate adenocarcinoma compared with normal tissue (37). This suggests that there may be different roles for 15-LO-1 and 15-LO-2. 15-LO-1 may be involved in the tumorigenic process, while 15-LO-2 may be involved in normal tissue function. The role of 15-LO-1 versus 15-LO-2 will need to be further investigated to clarify their functions.

The fact that loss-of-function mutations in PPARγ are associated with colon cancer suggests that activation of this receptor might have an anti-cancer effect in this disease (16). Ligand activation of PPARγ stimulates a dramatic differentiation response in one type of solid tumor, liposarcoma, in human patients (38). The fact that 15-LO-1 metabolites can shut down PPARγ via the MAPK signaling pathway essentially has the same net effect as a loss-of-function mutation in PPARγ. 15-LO-1 metabolites cause a down-regulation of PPARγ activity, and this suggests a pro-tumorigenic role in colorectal carcinogenesis.

The interaction between 15-LO-1 and PPARγ signaling can occur at several levels, and the balance between the two opposite biological effects of the metabolites could be an important regulator of PPARγ transcriptional activity in colorectal cells (Fig. 10). The 15-LO-1 metabolites of linoleic acid and arachidonic acid are ligands for PPARγ, but this response is observed at relatively high concentrations, and most of these studies employed exogenous metabolites (17). In contrast, the 15-LO-1 linoleic acid metabolites, 13-(S)-HODE, 13-(R)-HODE, and 13-(S)-HpODE, enhance growth factor-dependent MAPK activity, which down-regulates PPARγ by increasing phosphorylation. This effect was observed at lower concentrations of the 15-LO-1 metabolites and from endogenously generated metabolites. Thus, a balance between these two opposing effects could determine the role 15-LO-1 plays in the development of colorectal cancer. The down-regulation of PPARγ by linoleic acid metabolites may provide the rationale to explain why 15-LO-1 is up-regulated in colon cancer.

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