Molecular Pathways: Dietary Regulation of Stemness and Tumor Initiation by the PPAR-δ Pathway

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Abstract

Peroxisome proliferator-activated receptor delta (PPAR-δ) is a nuclear receptor transcription factor that regulates gene expression during development and disease states, such as cancer. However, the precise role of PPAR-δ during tumorigenesis is not well understood. Recent data suggest that PPAR-δ may have context-specific oncogenic and tumor-suppressive roles depending on the tissue, cell-type, or diet-induced physiology in question. For example, in the intestine, pro-obesity diets, such as a high-fat diet (HFD), are associated with increased colorectal cancer incidence. Interestingly, many of the effects of an HFD in the stem and progenitor cell compartment are driven by a robust PPAR-δ program and contribute to the early steps of intestinal tumorigenesis. Importantly, the PPAR-δ pathway or its downstream mediators may serve as therapeutic intervention points or biomarkers in colon cancer that arise in patients who are obese. Although potent PPAR-δ agonists and antagonists exist, their clinical utility may be enhanced by uncovering how PPAR-δ mediates tumorigenesis in diverse tissues and cell types as well as in response to diet.

Background

Peroxisome proliferator-activated receptor delta (PPAR-δ) belongs to the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptor transcription factors that is composed of three members (alpha, beta/delta, and gamma; ref. 1). PPARs are critical regulators of metabolism and exhibit tissue and cell type–specific expression patterns and functions that can be altered by physiologic cues. Although PPAR isoforms have highly homologous structures and overlapping functions, distinct selective ligands have been identified to activate each family member. PPAR-α is the first member of the family that was cloned and characterized in mouse liver, where it regulates lipid homeostasis and reduces triacylglycerol levels. PPAR-γ has been extensively studied in adipose tissue, where it controls adipogenesis and lipid metabolism and enhances glucose metabolism (1). PPAR-δ is highly expressed in the intestinal crypts, keratinocytes, and oxidative skeletal muscle fibers (2) and is a master regulator of lipid metabolism (3). For example, PPAR-δ–mediated metabolic regulation protects against diet-induced obesity and metabolic syndrome, and it enhances skeletal muscle endurance and function (4, 5). Furthermore, dietary interventions, such as fasting (3), and inflammatory cytokines, such as TNFα and IFNγ (6), augment PPAR-δ signaling. Thus, PPAR-δ couples diverse dietary, nutritional, or physiologic inputs with organismal metabolism (7–9).

Although long-chain fatty acids and their derivatives were initially recognized as endogenous activators of the PPAR-δ pathway, the actual nature of the physiologic ligands of PPAR-δ remains elusive. In the past decade, in addition to fatty acids, derivatives of arachidonic acid (10), lipoperoxidation products (11), and all-trans retinoic acid (12) have been proposed to activate the PPAR-δ pathway. Although a general consensus is lacking, two major models have been postulated regarding how nuclear PPAR-δ senses its ligand(s). In the first model, it is proposed that the ligands are delivered from the cytoplasm to the nucleus by fatty acid–binding proteins (FABP; refs. 13, 14), and in the second model, it is suggested that the ligands are products of active nuclear lipid metabolism (15). Importantly, several very potent and selective small-molecule PPAR-δ agonists, including GW501516, GW0742 (16), and MBX-8025 (17), have been developed and widely used experimentally to decipher the biological significance of PPAR-δ–mediated transcriptional activity (Fig. 1).

PPAR-δ controls transcription both directly and indirectly (1). Direct regulation involves partnering with retinoid X receptor and binding to peroxisome proliferator response elements in the enhancers and promoters of its target genes (Fig. 1). Furthermore, PPAR-δ–mediated transcriptional activation relies on ligand binding and concomitant epigenetic mechanisms that include displacement of corepressors and recruitment of coactivator-associated chromatin remodeling factors, which orchestrate the framework necessary for transcriptional initiation (18). Over the past decade, PPAR-δ or its direct transcriptional targets have been demonstrated to bear on diverse cellular functions, such as stem cell maintenance, cellular differentiation, metabolism, and inflammation.
PPAR-δ also regulates many genes indirectly by affecting the activity of other transcription factors through physical interaction or through signal transduction pathways that affect the function of other transcription factors (Fig. 1). For example, PPAR-δ is found to interfere with the NF-κB pathway (an important pathway in controlling inflammatory gene expression) and attenuate inflammation (19). Moreover, PPAR-δ cooperates with β-catenin (a key transcription factor for cell fate determination) in bone turnover and intestinal tumorigenesis (7, 20).

Gene expression analysis studies in combination with chromatin immunoprecipitation sequencing have identified direct and indirect PPAR-δ–regulated genes (21, 22). Well-documented direct targets of PPAR-δ include genes involved in fatty acid oxidation and lipid metabolism, such as Cpt1a, Fabp1, and Pdk4, whereas proinflammatory cytokines, such as TNFα and IL6, are regulated indirectly. Overall, PPAR-δ controls the expression of a large set of genes through different mechanisms that are influenced by PPAR-δ expression levels, interaction with additional transcription factors, as well as the abundance and nature of its ligands. These different levels of control confer versatility to PPAR-δ–mediated gene regulation in myriad tissues and cell types during development and disease states such as cancer.

**PPAR-δ in development and cancer**

Several developmental abnormalities have been reported in PPAR-δ–null mice, including reduced gestational and postnatal body weight as well as abnormalities in multiple tissues such as adipose tissue, skin, and intestine (23, 24). In adipose tissue, PPAR-δ deficiency leads to a reduction in adipocyte numbers and PPAR-γ expression levels (as noted, PPAR-γ is the PPAR family member that controls adipogenesis; ref. 23). Although PPAR-δ is not directly implicated in the regulation of adipogenesis, it contributes to the metabolic response of adipose tissue to dietary fatty acids (8, 23). Although PPAR-δ is expendable for homeostatic function in the skin, it plays a critical role in skin repair after injuries that disrupt skin integrity. Also, it is understood that
agonist-activated PPAR-δ hampers cellular proliferation and induces keratinocyte differentiation (24, 25). Thus, these findings are consistent with the notion that PPAR-δ signaling may possess antitumorogenic roles in the skin by preventing the growth and promoting the differentiation of (pre-) malignant cells through antagonizing the expression of proteins known to contribute to keratinocyte hyperproliferation and skin tumorigenesis (24–27). However, these antitumorogenic effects of PPAR-δ signaling in the skin may be context specific, as they are observed in only some models of skin cancer (28, 29).

In the intestine, PPAR-δ performs critical regulatory functions and modulates intestinal physiology in multiple cell types, yet the underlying mechanisms are poorly understood. For instance, PPAR-δ was identified as a crucial factor in Paneth cell differentiation, thus implicating it as a significant player in intestinal homeostasis (30). Paneth cells not only possess antimicrobial functions but also constitute a niche component for intestinal stem cells (ISC) that reside at the bottom of the crypt, a location where PPAR-δ expression is the highest in the intestine (30, 31). By influencing Paneth cell differentiation, PPAR-δ regulates a significant support cell of the ISCs, which drives the rapid renewal of the intestinal epithelium. In addition, PPAR-δ contributes to intestinal physiology through the control of lipid absorption, cholesterol trafficking, and enteroendocrine cell function in more differentiated or specialized intestinal cell types (32–34).

Initial reports on the role of PPAR-δ in intestinal tumorigenesis suggested that PPAR-δ is a direct transcriptional target of the Wnt/β-catenin pathway, a pathway that governs intestinal maintenance and tumorigenesis. Aneuploid polypoid cell (APC) orifice, a model of the destruction of cytoplasmic β-catenin in the absence of an upstream Wnt activation signal to tightly control β-catenin nuclear localization and activity. APC inactivation leads to strong activation of the β-catenin pathway and is the most common initiating oncogenic event of intestinal dysplasia (35). Although early studies demonstrated induction of PPAR-δ in intestinal tumors that were formed upon loss of APC or by chemical mutagenesis, subsequent reports in both rodents and humans failed to establish an unequivocal induction of PPAR-δ expression or activity in intestinal cancers (36). Several studies have used either whole-body or intestinal-specific genetic ablation of PPAR-δ to delineate the precise function of PPAR-δ in tumor-prone Apcmin/+ mice (mice with nonsense mutation in Apc gene that results in a truncated protein) or chemically induced models of intestinal tumorigenesis. Whereas some studies found a protumorigenic role for PPAR-δ, others posited an antitumorigenic function. Conflicting results from these loss-of-function studies have raised important questions regarding the role of PPAR-δ in intestinal tumorigenesis (23, 37–39). In contrast, however, gain-of-function studies that used the PPAR-δ agonist GW501516 have more consistently demonstrated that PPAR-δ activation mediates a protumorigenic phenotype in the intestine (40).

These conflicting findings regarding PPAR-δ necessity in intestinal tumorigenesis may be due to different genetic strategies used to disrupt PPAR-δ function. Studies that supported an antitumorigenic role for PPAR-δ in Apcmin/+ mice use germline disruption of PPAR-δ exon 8 (37, 38). Whereas ablation of PPAR-δ by disrupting exon 4 failed to decrease tumor incidence in Apcmin/+ mice but led to a reduction in size of the largest intestinal adenomas (23), deletion of PPAR-δ exon 4 and part of exon 5 significantly diminished tumor numbers in Apcmin/+ mice, and exon 4 deletion reduced dysplastic lesions in Apcmin/+ mice exposed to chemical injury (39, 41). Some of the discrepancy likely arises from the fact that disruption of exon 8 (the last PPAR-δ exon) may produce a hypomorphic protein, while disruption of exon 4 (an essential portion of the PPAR-δ DNA-binding domain) ablates PPAR-δ activity (42). Finally, it is important to point out that these studies used germline PPAR-δ mutants. More recently, intestine-specific PPAR-δ loss (targeting exon 4) decreased the numbers of dysplastic lesions in response to chemical mutagenesis (42). Another possible confounder in these studies is the use of different genetic backgrounds, which is known to influence tumor susceptibility in Apcmin/+ mice and in chemical mutagenesis models (1).

Dietary regulation of stemness and tumor initiation

As discussed above, accumulating evidence underscores the importance of the PPAR-δ pathway in health and disease. However, little is known about how PPAR-δ contributes to the regulation of stemness and tumor initiation in response to diet-induced physiologies. Diet is a modifiable lifestyle factor that has a profound impact on mammalian physiology, health, and disease, including cancer. Long-lived mammalian tissue–specific stem cells play a key role in how tissues adapt to diverse diet-induced physiologic states. Such stem cells dynamically remodel tissue composition in response to physiologic cues by altering the balance between self-renewal and differentiation divisions (43). Factors that increase somatic stem cell self-renewal division rate are implicated to elevate cancer risk (44, 45). This suggests that dietary interventions that boost stem cell self-renewal increase the potential pool of cells that can undergo mutagenesis and give rise to cancer.

Consistent with this notion, we recently demonstrated that a pro-obesity, high-fat diet (HFD)–activated PPAR-δ program augments the numbers, proliferation, and function of ISCs in the mouse intestine, providing a possible explanation to the long-sought link between diet-induced obesity and cancer (7, 46). In addition, intestinal adaptations to a long-term HFD not only enabled ISCs to acquire niche independence (i.e., initiate mini intestines in culture without need of their Paneth niche) but also allowed non–stem cell progenitors to obtain stem cell attributes. These changes effectively augmented the number of cells within the intestine that possessed stemness and that could initiate tumors upon loss of the APC tumor suppressor gene (7). Moreover, enforced PPAR-δ activation mimicked many of these effects of an HFD. As mentioned, there are differing opinions on the role of PPAR-δ in intestinal tumor initiation. However, these findings demonstrate that PPAR-δ activation in ISCs and progenitors has protumorigenic effects in the intestine and raises the possibility that PPAR-δ inhibition in the setting of an HFD may dampen tumor initiation, progression, or both.

An important question is whether an HFD has similar stem cell–enhancing effects in other tissues. Interestingly, in contrast to the enhanced stemness observed in the intestine, an HFD decreases neural progenitor cells proliferation and hippocampal neurogenesis by increasing lipid peroxidation and reducing brain-derived neurotrophic factor (BDNF) levels in the hippocampus (47). On the other hand, an HFD alters the differentiation potential of bone marrow–derived mesenchymal stem cells, subcutaneous adipose–derived stem cells, and infrapatellar fat pad–derived stem cells. Notably, the fatty acid constituents of the diet, in part, mediated this effect, implicating a possible role for PPAR-δ signaling (48). Also, the impact of an HFD in hematopoiesis is context dependent. A recent study demonstrated that a maternal HFD compromises the

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expansion of the fetal hematopoietic stem/progenitor cell (HSPC) pool and hematopoietic lineage specification. Conversely, adult hematopoiesis is augmented in response to HFD-induced obesity (49, 50). The fact that PPAR-δ—activated fatty acid oxidation drives hematopoietic stem cell self-renewal (51) suggests that HFD-mediated regulation of hematopoiesis may involve PPAR-δ function. Nonetheless, whether HFD or PPAR-δ is linked to tumor initiation in these tissues warrants further investigation.

**Clinical–Translational Advances**

Despite the fact that preclinical studies have provided evidence that PPAR-δ agonists possess therapeutic value in the treatment of several metabolic diseases, no FDA-approved agonists for PPAR-δ currently exist (5, 9). This is in contrast to PPAR-α and PPAR-γ, which have FDA-approved agonists that are standard of care for metabolic disorders, such as hyperlipidemia and diabetes. Although clinical trials have revealed that short-term administration of the PPAR-δ agonists GW501516 (NCT00841217; ref. 52) and MBX-8025 (NCT00701883; ref. 53) had beneficial effects in patients with metabolic syndrome, preclinical long-term studies in mice have indicated that PPAR-δ agonists might have protumorigenic effects, thus dampening enthusiasm for these compounds in human trials.

Several studies have suggested that PPAR-δ expression has prognostic value for cancer patients, including those with colorectal cancer (54, 55). However, there are inconsistencies in the reported PPAR-δ expression levels of different human tumors compared with normal tissue as well as how PPAR-δ expression correlates with prognosis. For instance, a study that examined 52 patients with colorectal cancer found that PPAR-δ and COX-2 expression in tumors negatively correlated with patient survival (54). However, another study with 141 patients with colorectal cancer revealed that higher expression of PPAR-δ in primary colorectal tumors was associated with lower frequency of Ki67 (a cellular marker for proliferation), a higher frequency of stage I cases, a lower frequency of late-stage cases, and a lower rate of lymph node metastasis (55). More studies are required to precisely ascertain the molecular mechanisms that PPAR-δ employs during cancer initiation and progression and how they affect patient prognosis.

PPAR-δ polymorphisms have been associated with physical performance (56), diabetes (57), obesity (58), and cancer (59, 60). A recent study focusing on patients with colorectal cancer identified seven novel variants among 22 inherited or acquired PPARδ variants. Interestingly, four recurrent variants were detected in or adjacent to exon 4, which encodes an essential portion of the PPAR-δ DNA-binding domain, suggesting that these variants might have functional significance (60). Although these variants may have clinical importance, the effect of PPAR-δ polymorphisms on gene expression is still poorly understood.

Our recent data indicate that HFD-activated PPAR-δ engages a specific β-catenin program that involves expression of genes implicated in intestinal tumorigenesis, such as Jag1 and Bmp4, in ISC and progenitors (7). Future studies need to address whether increased expression of Jag1 or Bmp4 has any prognostic or correlative value in colorectal cancer that arises in patients who are obese. Furthermore, it will be important to determine the functions of PPAR-δ in the setting of diverse tissues, cell types, diets, and risk factors that are associated with cancer in humans. The fact that PPAR-δ links dietary regulation of stemness to tumor initiation in the intestine raises the possibility of a new therapeutic approach in the treatment of intestinal cancers in obesity (7), such as exploiting possible PPAR-δ dependencies in tumors that arise in an HFD or obesity. Uncovering the cellular and molecular mechanisms by which PPAR-δ drives its effects may lead to new therapeutic insights, especially in those cancers with high PPAR-δ activity.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: S. Beyaz

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**References**


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12. Schug TT, Berry DC, Shaw NS, Harris SN, Noy N. Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors. Cell 2007;129:723–33.


44. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 2015;347:78–81.


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