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Targeting peroxisome proliferator -activated receptor-β/δ in colon cancer: How to aim?

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Abstract

Peroxisome proliferator-activated receptor-β/δ (PPARδ) is a ubiquitously expressed, ligand-activated transcriptional factor that performs diverse critical functions in normal cells (e.g., fatty acid metabolism, obesity, apoptosis, and inflammation). Various studies in humans have found that PPARδ is upregulated in primary colorectal cancers; however, these findings have been challenged by those of other reports. Similarly, various *in vitro* and *in vivo* mechanistic pre-clinical models have yielded data demonstrating that PPARδ promotes colonic tumorigenesis, but other models have yielded data that contradicts this notion. Definitive studies are therefore needed to establish the exact role of PPARδ in human colorectal tumorigenesis and to provide a theoretical basis for PPARδ therapeutic targeting.

Keywords

Peroxisome proliferator-activated receptor-delta; colonic tumorigenesis

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) have been identified as ligand-activated transcription factors and members of the nuclear hormone receptor superfamily since the early 1990s [1-3]. Members of the PPAR subfamily include three distinct genes: *PPARα*, *PPARγ*, and *PPARβ/δ* (*PPARδ*). PPARs as nuclear receptors to various molecules (e.g., lipids) act as master regulators of not only important cellular functions associated with fatty acid-metabolism [4] but also inflammation [5], cell differentiation, and tissue development [6, 7]. The critical functions of PPARs extend from functions affecting health to those impacting major human illnesses such as cardiovascular diseases [8] and cancer [2, 9]. PPARs' important role in tumorigenesis, coupled with the availability of molecules to modulate PPARs' activity, affords a major opportunity to use them in the development of anti-cancer drugs. However, PPARs' function in tumorigenesis, especially in the case of *PPARδ*, is complex and not yet fully defined [2, 9], a situation that represents a major challenge to efforts to develop molecular interventions that target PPARs in cancer.

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The aim of this review article is thus to examine the current literature on *PPARδ* role in colonic tumorigenesis and how to modulate this role to inhibit tumorigenesis. We have focused on colon cancer because it is one of the most common cancers in humans [10] and, more important, because the majority of studies of *PPARδ* role in tumorigenesis have been conducted on this cancer. Defining the role of *PPARδ* in tumorigenesis is essential for determining whether *PPARδ* inhibitors or *PPARδ* agonists should be developed to treat tumorigenesis. Answering this question is crucial to the field of *PPARδ* therapeutic targeting, which has so far been focused on developing *PPARδ* agonists for treatment of diseases other than cancer. For example, the *PPARδ* agonist GW-501516 is being evaluated in phase II clinical efficacy studies for the treatment of dyslipidemia [11]. However, in diseases other than cancer, such as dyslipidemia, evaluation of therapies for clinical efficacy usually requires only short-term testing. As tumorigenesis is usually a long process, long-term studies are needed to assess the effects of new treatments on the incidence of cancer.

2. *PPARδ* constitutive functions in normal cells

PPARδ is the most commonly expressed PPAR in normal cells [2] . Despite its ubiquitous expression, *PPARδ* role has not been fully appreciated; it has been the subject of only limited investigation, perhaps initially marginalized by the intense research focus on other isoforms. Various critical functions for *PPARδ* have since been identified in embryonic development, fatty acid metabolism, obesity, wound healing, apoptosis, and inflammation [12-20]. *PPARδ* influences transcription not only via the classic mechanism of ligand-binding activation but also via the direct repressive effects of competitively binding to the DNA response elements of other PPARs, especially *PPAR-γ*[21, 22]. Because of *PPARδ's* profound effects on normal cellular homeostasis, determining whether and how *PPARδ* is differentially expressed and/or activated in cancer cells is critical to developing effective molecular targeting for therapeutic applications.

3. Is *PPARβ/δ* expression differentially altered during human colonic tumorigenesis?

The 1999 initial study of PPAR*δ* role in colon cancer by He et al. showed that *PPARδ* mRNA was markedly upregulated in colorectal cancers compared with normal colorectal mucosa in a small sample of patients [23]. These findings were confirmed by Gupta et al. in a subsequent study that additionally showed localization of mRNA expression by *in situ* hybridization to be shifted from luminal epithelial cells in normal mucosa to a more diffuse distribution resembling that of dysplastic cells and co-localizing with cyclooxygenase-2 (*COX-2*) expression [24].

Nine follow-up studies examining *PPARδ* expression in colorectal tumors in humans have since been reported (Table 1). These studies varied in sample size (from 10 to 141 patients, median 33) and used either immunohistochemical analysis (IHC) (four of nine studies) or quantitative real-time polymerase chain reaction (qRT-PCR) (five studies). Protein immunoblotting (Western blotting) was also used with IHC in one study and with qRT-PCR in another. The sample was limited to patients with familial adenomatous polyposis in two of nine studies and to rectal cancer in two other studies, with the remaining five studies including both colon and rectal cancer patients. Several observations can be made about these studies as a whole:

1. Relative expression of *PPARδ* was higher in colorectal cancer than in normal mucosa in three of five studies that included colorectal cancer cases. Two of the three studies that showed consistent and significant *PPARδ* upregulation in colorectal cancer used IHC. Results of qRT-PCR measurements were, however,

mixed. One small study (n = 17) by Feilchenfeldt et al., which used qRT-PCR, showed more than two-fold upregulation of *PPARδ* in colorectal cancers in 4 (24%) patients and 50% decrease in only one patient [25]. Another study of 20 patients showed significant *PPARδ* upregulation in colorectal cancers [26]. A third study by Foreman et al., however, reported *PPARδ* downregulation in colorectal cancer on the basis of data from protein immunoblotting in colon cancer patients. The same study found no significant difference in *PPARδ* expression between normal cells and cancerous mucosa for the rectal cancer cases by either protein immunoblotting or qRT-PCR [27]. Furthermore, qRT-PCR measurements for the colon cancer cases showed no significant difference between normal and cancer mucosa for the non-log transformed data[27].

Technical differences between IHC and qRT-PCR techniques could potentially have produced the differences in results between the various studies, as has been proposed previously [9]. In this context, the IHC approach, although criticized for its antibody-limited specificity [9], has an advantage over protein immunoblotting and qRT-PCR in being able to specifically compare gene expression in the cancer versus normal cells. In contrast, other techniques (e.g., qRT-PCR) use cell mixtures in which cancer specimens are contaminated with normal cells, particularly the inflammatory and stromal cells in which cancer cells are often embedded. Additionally, in the study by Foreman et al., [27]the lack of difference between normal and cancer mucosa in the rectal cancer cases remains unexplained, and the difference between normal and cancer mucosa in colon cancer patients was observed mainly via immunoblotting protein studies, which are, like IHC, limited by problems with antibody specificity. Interestingly, in one of the IHC studies (Takayama et al.), *PPARδ* upregulation was noted to increase progressively from normal cells to adenoma to cancer [28]. In two other studies, *PPARδ* overexpression was associated with *COX-2* overexpression (as reported earlier by Gupta et al[24]) and with increased angiogenesis, upregulation of pro-metastatic genes vascular endothelial growth factor-a (VEGF-a) and C-X-C chemokine receptor type 4 (CXCR4), and increased risk of having liver metastases and shorter survival [29, 30]. Taken together, the findings from these studies show the progressive contribution of *PPARδ* upregulation to colonic tumorigenesis.

2. Despite the large size of the two rectal cancer studies by Yang et al. (86 and 141 subjects), the authors' reported conclusions were far from definitive [31, 32]. The initial 2006 study measured *PPARδ* by qRT-PCR and found that *PPARδ* levels were higher in cancer than in normal rectal mucosa in 56% of 86 cases studies [31]. However, based on recalculated *PPARδ* relative expression levels using additional methods to the commonly used delta delta CT method [33], the authors concluded that there were no significant differences in *PPARδ* expression between the normal and cancer rectal mucosa.

In the subsequent study that examined *PPARδ* expression using IHC and protein immunoblotting in 141 subjects, 64 subjects received radiation therapy prior to rectal cancer resection and the remaining 77 subjects received no neoadjuvant irradiation. In the non-irradiated group, the frequency of moderate or strong *PPARδ* expression increased from between 2% and 10% for the normal mucosa to 61% for the paired primary rectal cancers—a marked increase that was confirmed by immunoblotting. Nevertheless, the authors concluded that the study findings supported the characterization of *PPARδ* as a tumor suppressor. That conclusion was based on additional analyses and observations, including the lower frequency of moderate to high *PPARδ* expression in lymph node metastases than in primary tumors, the reduction of *PPARδ* upregulation in both primary tumors and lymph

node metastases with radiation therapy, and the improved prognosis associated with *PPARδ* upregulation. This proposed tumor-suppressive role, however, does not seem to fit with the expectation that tumor suppressor gene expression will decrease in primary tumors relative to expression in normal tissues, which is opposite to the study findings.

3. Of the two studies that reported on *PPARδ* in precancerous lesions in familial adenomatous polyposis patients, the study by Modica et al. [34] had a sample of only 10 patients and showed a statistically non-significant trend toward *PPARδ* downregulation in tumors ($P = 0.083$); the other study, however, reported an opposite trend but provided no details on sample size or statistical significance [35]. The two studies used different methodologies: qRT-PCR for the first and IHC for the second. The reason for the contrasting findings is difficult to determine on the basis of the available published information.

Although the data remain inconsistent and are thus far from definitive, the general trend seems to favor the notion that *PPARδ* is upregulated in primary colorectal cancers. However, more definitive studies are needed to establish the exact role of *PPARδ* in human colorectal tumorigenesis.

4. Impact of PPARβ/δ-altered activation on colonic tumorigenesis

He et al. initially identified *PPARδ* as a target gene of β-catenin/Tcf-4 pro-tumorigenic signaling through global gene expression profiling of human colon cancer cell lines [23]. Suppression of *PPARδ* contributes to the antitumorigenic effects of non-steroidal anti-inflammatory drugs (NSAIDs) in colon cancer cells and APC^{min} mouse models [23, 36-38]. *PPARδ* polymorphisms were shown to modulate NSAID chemopreventive effects on colorectal adenoma in a large case-control study [39]. However, studies by another group found that NSAIDs had no significant effects on *PPARδ* expression or activity and no regulatory role for β-catenin on *PPARδ* in either colon cancer cell lines or APC^{min} mouse models [27, 40]. These findings contrast with those of studies showing that prostaglandin E2 (PGE2), which is inhibited by NSAIDs, activates *PPARδ* to inhibit apoptosis in colon cancer cells *in vitro* and promote intestinal tumorigenesis in APC^{min} mouse models [41] . Furthermore, treatment of APC^{min} mice with a specific synthetic *PPARδ* ligand (GW501516) also strongly promotes intestinal tumorigenesis [42].

Various studies have examined the effects on colonic tumorigenesis of genetically manipulating *PPARδ* expression. *PPARδ* deletion has been found to suppress tumorigenesis profoundly in subcutaneous xenografts of human HCT-116 colon cancer cells [43]. A germline deletion of *PPARδ* exon 4 in APC^{min} mice produced high rates of embryonic lethality because of the important role played by *PPARδ* in placental development. The germline deletion reduced the number of large intestinal polyps (> 1 mm) than in the wild-type mice. The difference was below the threshold for statistical significance, however, likely because of the study's small sample size (only three knockout mice were studied) [17]. In a 2004 study by another group (Harman et al.), deletion of *PPARδ* exon 8 had no significant impact on embryonic survival but increased intestinal tumorigenesis when induced by either azoxymethane or APC^{min} mutation [44]. In a 2006 study by Wang et al., genetic deletion of exon 4 and part of exon 5 of *PPARδ* strongly suppressed intestinal tumorigenesis in APC^{min} mice [45]. The differences between these various models in relation to the effect on intestinal tumorigenesis have been attributed to variations in the genetic deletion strategies that target different *PPARδ* codons. This concept is supported by the observation that codon 8 deletion had none of the *PPARδ* effects on embryonic lethality that would be expected given *PPARδ* essential role in placental development [17, 20]. Because the results from germline *PPARδ* knockout studies in APC^{Min} mouse models are

contradictory, we studied the role of *PPARδ* in colonic tumorigenesis induced by targeting *PPARδ* knockout of exon-4 into the intestinal epithelial cells via the villin promoter-driven Cre recombinase expression to circumvent the problem of embryonic lethality with germline *PPARδ* knockout. Targeting *PPARδ* genetic disruption into the intestine profoundly inhibited colonic tumorigenesis, reducing tumor incidence by 98.5% in $PPAR\delta^{(-/-)}$ mice [46]. These findings strongly support the notion that *PPARδ* expression in intestinal epithelial cells contributes significantly to colonic tumorigenesis.

Various signaling pathways are potentially involved in *PPARδ* effects on colonic tumorigenesis. In addition to the proposed role for PPAR*δ* as a downstream target of the β-catenin/Tcf-4 signaling pathway (reviewed earlier), the other potential pathways include:

1. *COX-2*-derived PGE2 activates *PPARδ* via the phosphoinositide 3-kinase-Akt pathway [41, 45]. Ligand-dependent activation of *PPARδ*, on the other hand, enhances *COX-2* expression, PGE2 production, and PGE2 receptor (EP2) expression, suggesting a positive feedback loop between *COX-2* and *PPARδ* signaling [47, 48].
2. *KRAS* upregulates *PPARδ* via *COX-2* during cell transformation [49].
3. *15-lipoxygenase-1* downregulates *PPARδ* via its oxidative main product, 13-S-hydroxyoctadecadienoeic acid, to facilitate *PPAR-γ* binding to DNA response elements and thereby promote the transcription of pro-apoptotic genes [22, 36].
4. *PPARδ* is a critical downstream target for the pro-tumorigenic epidermal growth factor signaling to drive cancer cell growth [50, 51].
5. *PPARδ* activation promotes various events related to angiogenesis [52], including increasing VEGF expression during colonic tumorigenesis [45, 46].
6. *PPARδ* antagonizes *PPARγ* ability to induce colorectal carcinoma cell apoptosis by inhibiting *PPARγ* transcriptional activity [22] and downregulating survivin and caspase-3 [53].

5. Conclusion

A growing body of evidence indicates that *PPARδ* is likely to be differentially upregulated during colonic tumorigenesis and that this upregulation strongly promotes tumorigenesis. However, other published data suggest that *PPARδ* might perform an opposite role. Therefore, more studies are needed to resolve this controversy and to clearly define the role of *PPARδ* in colonic tumorigenesis, to ensure the safety of *PPARδ* agonists before they are developed clinically for various non-cancerous indications, and to determine whether *PPARδ* inhibitors or *PPARδ* agonists should be developed to treat tumorigenesis.

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Table 1
Human studies of *PPARδ* expression in colorectal tumorigenesis

Study reference number	Sample size	Method	Results
[32]	141 rectal cancer patients**	IHC and Western blotting	*PPARδ* expression was cytoplasmic by IHC and higher in primary tumors (61%) and lymph nodes (32%) than in normal mucosa (2-10%) ($P = 0.008$). Similarly, expression was higher in primary cancer and lymph node metastases than in normal mucosa ($P = 0.036$).
[27]	33 (19 colon and 14 rectal cancer patients)	Western blotting and qRTPCR	Colonic but not rectal adenocarcinomas had lower protein expression than paired normal mucosa. Normalized mRNA expression was similar between normal and tumor mucosa for both the colon and rectal cancers.
[34]	10 FAP patients	qRTPCR	*PPARδ* expression is lower in precancerous cells than in paired normal tissues ($P = 0.083$).
[29-30]	52 patients with colorectal cancer	IHC	PPARδ expression was upregulated in colorectal cancer. PPARδ expression was nuclear and cytoplasmic in 48% of colorectal cancer mucosa whereas the paired normal mucosa had focal nuclear staining.
[26]	20 colorectal cancer patients (4 with rectal cancer)	qRTPCR	Relative expression of *PPARδ* was significantly higher in cancer than in normal mucosa ($P = 0.001$).
[28]	32 (14 colon and 18 rectal)	IHC	*PPARδ* expression increased from normal to adenoma to colorectal cancer. Cytoplasmic expression was higher in cancer than in adenoma ($P < 0.0001$).
[31]	86 patients with rectal cancer	qRTPCR	Forty-eight patients (56%) had relative expression levels that were higher in cancer than normal; however, the “general level of PPARδ mRNA” was similar for the cancer and normal mucosa.
[35]	FAP patients (sample size not stated)	IHC	*PPARδ* expression levels in were higher in adenomas than in normal cells.
[25]	17 colorectal cancer patients	qRTPCR	Relative tumor/normal mucosa levels were higher than 2-folds in tumors in 24% of patients, similar in 70%, and lower in tumors than 0.5-fold in 6%.

*Reference numbers are as cited in the manuscript text

**Seventy-seven patients were treated with surgery and 64 with both surgery and radiation.

IHC: Immunohistochemistry; qRT-PCR: quantitative reverse transcription real time polymerase chain reaction; FAP: familial adenomatous polyposis.