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Establishing the role of PPARβ/δ in carcinogenesis

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Abstract

The role of the nuclear hormone receptor peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in carcinogenesis is controversial because conflicting studies indicate that PPARβ/δ inhibits and promotes tumorigenesis. This review focuses on recent studies on PPARβ/δ including: 1) the significance of increased or decreased PPARβ/δ expression in cancers, 2) a range of opposing mechanisms describing how PPARβ/δ agonists, antagonists and inverse agonists regulate tumorigenesis and/or whether there may be cell context-specific mechanisms, and 3) whether activating or inhibiting PPARβ/δ is feasible for cancer chemoprevention and/or therapy. Research questions that need to be addressed will be highlighted in order to establish whether PPARβ/δ can be effectively targeted for cancer chemoprevention.

Keywords

peroxisome proliferator-activated receptor-β/δ; cancer; chemoprevention; mechanisms; transcription factor

The complexity and controversial role of PPARβ/δ in carcinogenesis

There is great heterogeneity in the factors required for cancers to develop, grow and metastasize, from multiple mutations and genetic instability in critical genes, to alterations in “hallmark” signal transduction checkpoints, that collectively drive proliferation of genetically-altered cells into cancerous lesions [1, 2, 3]. This heterogeneity dictates that discovering new and improved approaches for cancer chemoprevention and treatment requires the targeting of pivotal gene products whose function directly drives cancer. Given these caveats, the focus of this review is on the nodal transcription factor, **PPARβ/δ** (see Glossary), which may have important regulatory effects on hallmark checkpoints. A number

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of recent reviews have summarized the controversial nature of PPARβ/δ in cancer [4, 5, 6, 7, 8]. Thus, the primary focus of this review is to critique recent studies that have influenced this field in the past five years.

To frame this review, it is important to briefly outline the first study to report a relationship between PPARβ/δ and cancer. This was based on observations made in a cohort of four human colon cancer tumors showing higher expression of PPARβ/δ as compared to control tissue [9]. The mechanism hypothesized to mediate increased PPARβ/δ expression was mutations of the *APC* gene, that encodes the protein adenomatous polyposis coli, in colon tumors led to increased **β-CATENIN/TCF4** signaling causing increased transcription of the *CCND1*, *MYC* and *PPARD* genes that collectively increased net proliferation of mutant cancer cells. This study led to a hypothesis that putatively explained how inhibitors of cyclooxygenase 2 (COX2), a key enzyme involved in the production of prostaglandins, prevented cancer: inhibition of COX2 decreased the production of endogenous PPARβ/δ agonists, causing increased expression of yet-to-be identified target genes that combined with expression of CYCLIN D and MYC causes net proliferation of cancerous cells. However, to date, both this putative APC-driven mechanism of PPARβ/δ regulation and the targeting of this receptor by inhibiting COX2 metabolites remain uncertain (reviewed in [4, 5, 6, 7, 8]).

Expression and regulation of PPARβ/δ in cancers

PPARβ/δ and colon cancer

Since the first claim that PPARβ/δ expression is increased in APC colon cancer due to increased β-CATENIN/TCF4 signaling and enhanced transcription of the *CCND1*, *MYC* and *PPARD* genes, several studies that contradict this hypothesis have emerged (reviewed in [4, 5, 10]). For example, human colorectal cancer cell lines with mutations in either *APC* or *CTNNB1* exhibit markedly increased expression of CYCLIN D1, but no change in PPARβ/δ expression, as compared to human colorectal cancer cell lines with wild-type *APC* and *CTNNB1* [11]. Further, similar observations were noted in mice with a mutant *Apc* gene, as expression of CYCLIN D1 is markedly increased in colon tumors from mutant APC mice, while expression of PPARβ/δ is actually decreased in tumors as compared to colon tissue in wild-type mice [12]. These results directly contradict the hypothesis that expression of PPARβ/δ is increased in colon cancer because mutant APC/β-CATENIN proteins cause increased expression of *CCND1*, but not *PPARD*. This is important to note because more recently, a comprehensive analysis indicates that lower expression of PPARβ/δ protein is found in numerous tumor types as compared to non-transformed tissue, including breast, colorectal, gliomas, liver, lung, melanoma, ovarian, pancreatic, prostate, skin and urothelial cancers [7, 13]. However, the *APC* genotype in these tumors was not directly examined, nor was the *APC* genotype correlated with PPARβ/δ protein expression during tumor progression. Furthermore, potential differences in the function of PPARβ/δ expressed in subpopulations of tumor cells, such as cancer stem cells, have not been analyzed, but represent a possible source of further conflicting observations.

Limitations in measuring PPARβ/δ expression levels

By contrast, higher expression of PPARβ/δ protein and/or *PPARD* mRNA has also been reported in other cancer besides colon, where mutations in critical oncogenic genes besides *APC* are more closely correlated with the mutation "signature" genotype required for carcinogenesis [2]. Given the fact that mutations in *APC* are primarily associated with colon cancer, this lack of concordance may not be surprising. Whether genes such as *TP53*, *KRAS*, *EGFR*, *PI3KCA*, *PTEN* and others influence PPARβ/δ expression and/or function has not been critically examined to date. Moreover, there are numerous genomic consortiums, notably The Cancer Genome Atlas Network (TCGA)[i] with thousands of cancer and normal tissue samples that have been examined for gene mutations, mRNA expression profiles and other measurements that provide a useful resource for comparison of PPARβ/δ expression in cancer. Interestingly, while the expression of *PPARD* mRNA is lower in some cancers as compared to normal tissue based on bioinformatics analysis of TCGA datasets[ii], there are also examples where expression of *PPARD* mRNA is higher or unchanged as compared to normal tissue in different cancer types. However, it is critical to note that there are limitations to the analysis of such expression data including: 1) the relative mRNA expression level is usually not confirmed using quantitative approaches (i.e. quantitative real-time polymerase chain reaction), 2) expression of mRNA does not always correlate with protein expression, 3) the subcellular distribution of the protein is unclear from simple mRNA analysis, and 4) the transcriptome databases are highly variable due to the presence of contaminating non-tumor cells (e.g. expression of PPARβ/δ can be higher in tumor associated macrophages (TAM) that influence tumorigenesis and immune function in the tumor microenvironment, compared to tumor cells). This illustrates the important need to quantitatively examine the expression of PPARβ/δ protein in cancer cells, including the nuclear and cytosolic distribution, and to determine whether APC/β-CATENIN/TCF4 signaling or other genes modulate expression and/or function of PPARβ/δ during progression of different cancers. For quantitative purposes, the use of immunohistochemistry has not proven to be reliable due to the lack of validated anti-PPARβ/δ antibodies (reviewed in [4, 5, 6, 7, 8]). These analyses should be performed using more quantitative approaches such as western blotting, which include positive controls with recombinant PPARβ/δ as a standard, and examination of the subcellular cytosolic and nuclear fractions in control and tumor tissues during the early and later stages of tumorigenesis.

Lessons from mouse models

Studies using *Ppard*-null mice to determine the requirement for PPARβ/δ in colon cancer have also led to conflicting results, as some show that lack of PPARβ/δ causes increased, decreased, or no change in colon tumor multiplicity, with and/or without ligand activation of PPARβ/δ (reviewed in [4, 5, 6, 7, 8]). Recent studies using colon-specific *Ppard*-null or transgenic mice that were treated with the colon cancer carcinogen azoxymethane either in combination with dextran sodium sulfate (DSS), or alone have also provided conflicting results. Colon-specific disruption of *PPARD* caused no change in colon tumor multiplicity

[i] http://cancergenome.nih.gov
[ii] https://tcga-data.nci.nih.gov/tcga/tcgaAnalyticalTools.jsp

following administration of azoxymethane and DSS [14]. By contrast, disruption of PPARβ/δ mitigated azoxymethane-induced colon tumor multiplicity, compared to controls [15]. Further, enhanced colon-specific expression of PPARβ/δ caused an increase in colon tumor multiplicity following administration of azoxymethane and DSS [16]. Consistent with the latter observation, over-expression of PPARβ/δ in colon caused a dose-dependent increase in azoxymethane-induced colon carcinogenesis in two different FVB/N mouse lines, and overcame the relative resistance to azoxymethane-induced colon carcinogenesis in C57BL/6 mice as compared to controls, [17]. While the findings from these four studies from two laboratories are difficult to reconcile, the lack of changes in tumor multiplicity in the one model [14] could reflect the requirement of PPARβ/δ to either inhibit or promote tumorigenesis by cells in the tumor microenvironment, such as tumor stromal cells, fibroblasts, macrophages, etc. Thus, it is somewhat counter-intuitive that over-expression of PPARβ/δ in the colon caused enhanced tumorigenesis, because recent evidence from three laboratories and database searches indicate that PPARβ/δ expression is highly constitutively expressed in this tissue, in both humans and mice [13, 18, 19]. It remains a possibility that unidentified endogenous PPARβ/δ agonists or antagonists exist that modulate these effects, that the gut microbiome influenced these study results, or that the phenotype is altered by disruption of a gene or genes by the recombinant transgene. Further studies are needed to examine these ideas.

The role of PPARβ/δ in human cancer cell lines

Similar to the recent studies examining the role of PPARβ/δ in mouse colon cancer models, examination of the role of this receptor in human cancer cell lines have also been difficult to interpret. Consistent with the notion that relatively higher expression of PPARβ/δ inhibits tumorigenesis, the growth and proliferative indices of a human colon cancer cell line and of ectopic xenografts in immune-compromised mice developing from a derivative of this cell line expressing an RNAi against PPARβ/δ, was markedly increased as compared to controls [20]. This effect may have been due to reduced differentiation and increased expression of vascular endothelial growth factor (VEGF) [20]. These experiments are consistent with studies showing that over-expression of PPARβ/δ and ligand activation of PPARβ/δ markedly inhibited ectopic xenografts using both estrogen receptor (ER)+ and ER− human breast cancer cell lines, in an immune-compromised mouse model [21]. These results suggest that expression of PPARβ/δ may inhibit hallmark cancer checkpoints, such prevention of sustained cell growth, by promoting terminal differentiation, and inhibiting angiogenesis. By contrast, knockdown of PPARβ/δ in both ER+ and ER− human breast cancer cells inhibited proliferation in vitro [22]. These opposing results are somewhat striking, since previous studies suggested that only ER+ breast cancer cells were sensitive to the growth stimulatory effects of a PPARβ/δ ligand [23]. This hypothesis is contradicted by studies showing that knocking down PPARβ/δ inhibits growth in both ER+ and ER− human breast cancer cells [22], and that inhibition of tumorigenicity is observed in ER+ and ER− human breast cancer cells when PPARβ/δ is over-expressed [21].

Differential and opposing role for PPARβ/δ in tumor progression

Despite attempts using new approaches to determine whether expression of PPARβ/δ promotes or inhibits cancer using both mouse and human models, this issue remains unclear.

This is of interest given the known expression patterns for PPARβ/δ in various tissues and in cancers, with the former typically exhibiting relatively high expression and the latter exhibiting relatively low expression [13, 18, 19, 24]. Moreover, there is also evidence in some models that PPARβ/δ is found primarily in the nucleus where it is constitutively active, as revealed by chromatin binding and both repression and activation of a number of target genes [25, 26]. These data are in line with the view that relatively high expression of PPARβ/δ has a functional role in normal tissues, and argues for an anti-tumorigenic role for this receptor. However, if an endogenous PPARβ/δ antagonist/inverse agonist does exist, this could also indicate a pro-tumorigenic role for PPARβ/δ. The relative activity and biological effects of PPARβ/δ following binding to agonists, antagonists and/or inverse agonists (Box 1), and whether the target gene(s) are tumor suppressor or oncogenic in nature, is another area that is related to relative expression levels.

Box 1

Regulation of transcription by PPARβ/δ

There are three modes of direct target gene regulation as shown by combining ChIP-seq transcriptome studies and analyses of the effects of siRNA and ligands [25, 26, 49]: 1) activation of target genes by PPARβ/δ-RXR by endogenous or synthetic agonists, repression in the absence of agonists and de-repression by siRNA or knockout of PPARβ/δ (canonical regulation), 2) agonist-insensitive repression of target genes by PPARβ/δ-RXR and de-repression by siRNA or knockout of PPARβ/δ), and 3) agonist- and antagonist-insensitive activation by PPARβ/δ-RXR and inhibition by siRNA or knockout. Additionally, DNA binding independent interaction of nuclear PPARβ/δ with other proteins, such as the p65 subunit of NFκB, causes down-regulation of p65-dependent pro-inflammatory genes (reviewed elsewhere [4, 5, 6, 7, 8]), and cytoplasmic PPARβ/δ can have the opposite effect by interacting with TAK-TAB-HSP27 [73]. It is unclear whether fatty acid agonists are always bound with nuclear PPARβ/δ (as suggested from crystallized LBD of PPARβ/δ), since expression of some target genes is increased only by exogenous agonists and other genes are not affected by pure antagonists. Thus, agonists and *Ppard* knockdown or disruption can have similar or opposite effects on individual genes dependent on the mode of regulation. Further, even regulation of the same gene is often cell type-dependent, presumably due to the formation of specific transcription complexes. Agonists may not have the same of effect on tumorigenesis as *Ppard* disruption or over-expression in transgenic mouse models.

Inverse PPARβ/δ agonists generally prevent expression of target genes with **PPREs** due to the PPARβ/δ-driven formation of a repressor complex that also blocks other bound and potentially activating transcription factors [49]. This effect is entirely different than the competitive inhibition resulting from PPARβ/δ antagonists. However, neither PPARβ/δ antagonists nor PPARβ/δ inverse agonists have yielded clear results in mouse tumor models (mainly due to bioavailability problems). Thus, the role of endogenous PPARβ/δ ligands and PPARβ/δ-repressed genes in tumorigenesis remains unknown.

The binding of PPARβ/δ to chromatin is not necessarily dependent on agonist-induced interactions with PPARβ/δ, but is likely influenced by other factors that modulate

chromatin binding [31, 32, 33]. This effect may be due to agonist-induced effects on protein stability. Lastly, the regulation of many PPARβ/δ target genes may be influenced because there are identical binding sites that are also recognized by PPARα and PPARγ (similar to *ANGPTL4*). This suggests that there could be potential compensatory effects by other PPAR subtypes in null mouse models of knockdown/knockout cells/cell lines.

Public expression databases provide a useful tool to identify possible associations of specific genes with the clinical course of cancers. Analyses of microarray databases indeed identified significant associations between expression of *PPARD* and its target gene ***ANGPTL4*** (encodes for angiopoietin-like 4 protein) with the relapse-free survival of cancer patients (Figure 1). Thus, *PPARD* and *ANGPTL4* expression is positively associated with early relapse of lung and gastric cancer. Consistent with the variable effect of PPARβ/δ manipulation in different animal models discussed above, a different picture emerged for other human tumors. Thus, PPARβ/δ was only weakly associated with serous ovarian carcinoma, and in breast cancer (all subtypes) high PPARβ/δ expression was linked to a more favorable prognosis. However, when breast cancer patients were dichotomized into ER + and ER− tumors, a weak but significant association with early relapse was found for the former ($p=0.015$; HR=0.78). Taken together, these clinical correlations clearly support a differential and probably opposing role for PPARβ/δ in tumor progression.

Modulating hallmarks and enabling characteristics of cancer by PPARβ/δ

While the expression of PPARβ/δ is required for this receptor to modulate cellular processes including cancer, an endogenous or exogenous agonist, antagonist or inverse agonist is also necessary to activate or inhibit this transcription factor. Given the relatively high intracellular concentration of lipids and lipid metabolites (i.e. fatty acids, etc.) that can act as PPARβ/δ agonists [27, 28], antagonists or inverse agonists, the proportion of PPARβ/δ that is bound with these compounds is likely very high. This is consistent with the finding that apo-PPARβ/δ was not observed in the crystal structure as originally reported [29], due to the presence of fatty acids later found to occupy the ligand binding domain of PPARβ/δ [30]. Thus, it is not surprising that PPARβ/δ is found primarily in the nucleus in most tissues bound with its obligatory heterodimerization partner retinoic X receptor (RXR) [18], and is constitutively repressing and/or activating expression of a subset of target genes [25, 26]. It is important to note that the dynamic intracellular activity of PPARβ/δ can vary significantly based on numerous variables, including relative expression and localization of the receptor, relative expression of co-effector proteins, the relative concentrations of endogenous or exogenous agonists, antagonists or inverse agonists, and the relative proximity to binding sites on chromatin of regulatory regions of target genes [31, 32, 33]. Given the complex regulatory pathways that can influence PPARβ/δ, it is not surprising that there is a broad range of effects attributed to modulation of PPARβ/δ that may mediate effects that influence cancer.

Impact of PPARβ/δ activation on the hallmarks of cancer

There are six well accepted hallmarks of cancer (resisting programmed cell death, sustaining proliferative signaling, evading growth suppressors, enabling replicative immortality,

inducing angiogenesis, and activating invasion and metastasis), two emerging hallmarks of cancer (deregulating cellular energetics, and avoiding immune destruction), and two enabling characteristics of cancer (genomic instability and mutation, and tumor promoting inflammation) [1]. Ligand activation of PPARβ/δ or modulating the activity of PPARβ/δ using antagonists or inverse agonists may alter some of these hallmarks enabling full-scale carcinogenesis (Figure 2). However, results from these studies (Table 1) remain conflicting because they indicate that PPARβ/δ either inhibits or promotes tumorigenesis by modulating these hallmarks and enabling characteristics of cancer. In addition to Table 1, the reader is encouraged to examine other recent reviews that contrast in vivo and in vitro studies describing the effects of PPARβ/δ, and PPARβ/δ agonists and antagonists in many cancer models [4, 5, 6, 7]. By contrast, there is strong evidence that ligand activation of PPARβ/δ can induce terminal differentiation, which is known to reverse sustained cell proliferation and promote sensitivity to growth suppressors (reviewed in [4, 5, 6, 7, 8]). Further, there is a large body of evidence demonstrating that PPARβ/δ inhibits innate immune signaling, which may prevent tumor promoting inflammation (reviewed in [4, 5, 6, 7, 8]), an enabling characteristic of cancer [1].

One of the first studies describing a role for PPARβ/δ in promoting resistance to cell death, sustaining proliferative signaling and evasion of growth suppressors, used somatic cells that resembled primary mouse keratinocytes [34]. The proposed hypothesis postulated that PPARβ/δ directly up-regulated expression of **3-phosphoinositide dependent protein kinase-1** (PDPK1) and **integrin-linked kinase** (ILK) and down-regulated **phosphatase and tensin homolog** (PTEN), leading to increased phosphorylation of AKT1 and inhibition of apoptotic signaling [34]. Subsequent studies supported this hypothetical pathway in cancer models, while others did not (reviewed in [4, 5, 6, 7, 8]). In particular, studies using confirmed mouse primary keratinocytes, mouse skin and numerous cancer models showed no changes in the expression of these proteins and/or activity of this pathway (reviewed in [4, 5, 6, 7, 8]). More recent studies show that ligand activation does not increase many putative PPARβ/δ target genes including PDPK1 in primary keratinocytes, and no evidence of promoter occupancy of PPARβ/δ on or near these putative target genes was observed in the same model [26]. Indeed, other recent studies indicate that PPARβ/δ actually represses PDPK1, ILK and phosphorylation of AKT1 in oncogenic keratinocytes [35]. One possible explanation for these data suggesting that ligand activation of PPARβ/δ promotes anti-apoptotic activity is provided by a recent study showing that markers of early apoptosis are dose-dependently decreased in a human colon cancer cell line (DLD1) following co-exposure to hydrogen peroxide, but this change is actually associated with a decrease (not an increase) in viable cells, and a marked increase in late apoptotic/necrotic cells, as compared to controls [24]. This illustrates the need for future studies to include a thorough assessment of apoptosis to unravel the precise function of PPARβ/δ in this context. An ancillary hypothesis that all trans-retinoic acid (atRA), an agonist of RAR, can be differentially shuttled to activate PPARβ/δ rather than RAR, based on a relatively high ratio of intracellular **cellular retinoic acid binding protein II** (CRABPII) to **fatty acid binding protein** (FABP5) to promote PPARβ/δ-dependent anti-apoptotic activity has also been postulated but is not strongly supported by other studies, as previously discussed in detail (reviewed in [4, 5, 6, 7, 8]). It is noteworthy that this pathway is based on the hypothesis that

the relative expression of FABP5 and CRABPII are different [36], but this putative difference in relative intracellular expression has not been accurately quantified to date in any cell type. Moreover, the notion that breast cancer cells with higher FABP5 expression may be more sensitive to the chemopreventive activities of atRA [37], based on studies in a human keratinocyte cell line [36], is not supported by the finding that relative expression of FABP5 protein is not detected or is negligibly expressed in human breast cancer tissue, compared to non-transformed tissue [13].

Induction of angiogenesis is another hallmark of cancer and studies suggest that modulation of PPARβ/δ activity can influence this process. VEGF and ANGPTL4 are two proteins that can affect angiogenesis and there are opposing studies showing that PPARβ/δ affects VEGF, ANGPTL4 and angiogenesis in cancer models (Box 2). However, there is currently no consensus on how PPARβ/δ influences angiogenesis (reviewed in [4, 5, 6, 7, 8]). Two recent studies suggest that PPARβ/δ agonists can increase expression of *VEGF* mRNA modestly in cancer cells, but these studies did not measure angiogenic endpoints, and the effects were independent of PPARβ/δ, and therefore likely due to off-target effects of the agonists used (GW501516, L165041) [38, 39]. In contrast, secretion of VEGF was increased in a human colon cancer cell line by knockdown of PPARβ/δ, and ligand activation of PPARβ/δ inhibited secretion of VEGF, an effect that was mitigated by knockdown of PPARβ/δ [20]. Examination of **human umbilical vein endothelial cells** (HUVEC) cells revealed that L165041 also inhibits VEGF protein expression, tube formation and HUVEC proliferation and migration, and angiogenesis in vivo [40, 41]. However, the decrease is VEGF secretion and HUVEC migration were not mediated by PPARβ/δ, in one study [41]. While it is not clear that *VEGF* is a bona fide PPARβ/δ target gene, there is strong evidence that *ANGPTL4* is directly regulated by ligand activation of PPARβ/δ, and also by PPARα and PPARγ [42]. While some studies indicate that ANGPTL4 promotes tumorigenesis [43, 44, 45], other studies suggest that ANGPTL4 inhibits angiogenesis and tumorigenesis [46, 47, 48] (Box 2). A similarly disparate picture emerged for associations between *PPARD* and *ANGPTL4* mRNA expression and the clinical outcome of different cancers (Figure 2). Since all three PPARs can increase expression of ANGPTL4 [42] and PPARα and PPARγ agonists are currently being investigated as chemopreventive agents in humans (reviewed in [4, 5, 6, 7, 8]), it will be important to determine how ANGPTL4 influences cancer in response to changes in expression by PPARβ/δ agonists (Figure 1).

Box 2

Conflicting roles of ANGPTL4 in cancer

Secreted ANGPTL4 is cleaved by extracellular proteases into biologically active N-terminal (nANGPTL4) and C-terminal (cANGPTL4) fragments circulating through the blood stream [74]. Whereas a major function of nANGPTL4 is inhibition of lipoprotein lipase, cANGPTL4 has role in tumor progression and metastasis [75, 76, 77]. Thus, ANGPTL4 enhances cell migration [78, 79], cancer cell invasion [49] and angiogenesis [80]. ANGPTL4 also inhibits anoikis of circulating tumor cells [81] and increases the permeability of lung capillaries to promote their extravasation [78, 82] thereby promoting metastasis formation. This is consistent with the presence of *ANGPTL4* in gene

expression signatures indicative of metastasis and poor outcomes in humans [83] and the correlation of ANGPTL4 protein expression with venous invasion of gastric and colon carcinoma cells [44, 45]. It is therefor not surprising that multiple oncogenic signaling pathways regulate the *ANGPTL4* gene, including hypoxia [84], AP-1 [85] and TGFβ [79, 85].

By contrast, an inhibitory role for ANGPTL4 in angiogenesis has also been described, although in this system inhibition of migration is linked to diminished chemotaxis and decreased cell proliferation [86]. There is also a conflicting report suggesting an inhibitory role for ANGPTL4 in two mouse models that examined cell migration [46]. However, some of these data were not reproduced in other studies using different assays [49]. This discrepancy may be due to differences in the specific experimental approach used; Galaup and colleagues [46] used transfected cells overexpressing ANGPTL4 whereas Adhikary et al used soluble recombinant protein [49], as the relationship between the processing of ANGPTL4 and its different biological functions is poorly understood. It can also not be ruled out that different signaling mechanisms are involved, as suggested by studies using soluble [87] or matrix-bound ANGPTL4 [88]. Furthermore, the mouse *Angptl4* gene lacks functional SMAD binding sites and consequently is not inducible by TGFβ [89], which may cause species differences in the role of ANGPTL4 in angiogenesis.

An elegant study recently provided some novel insight that may explain this phenomenon. In MDA-MB-231 human breast cancer cells, *ANGPTL4* among all genes examined exhibited the largest increase in response to a PPARβ/δ agonist, and two inverse PPARβ/δ agonists markedly decreased expression of ANGPTL4 [49]. This change in *ANGPTL4* mRNA and ANGPTL4 protein expression by inverse PPARβ/δ agonism was associated with inhibition of MDA-MB-231 cell invasion, suggesting that inverse agonists may provide a suitable tool for interfering with cancer growth and progression. Surprisingly, MDA-MB-231 cell invasion was not enhanced by PPARβ/δ agonists, suggesting that the high expression of ANGPTL4 in these cells triggers invasion without the need for exogenous PPARβ/δ agonists [49]. However, it is important to note that there is considerable complexity associated with this type of effect and likely reflects different interactions between PPARβ/δ and endogenous agonists, antagonists and/or inverse agonists. That PPARβ/δ inverse agonism provides a new approach to prevent cell invasion, a hallmark of cancer associated with metastasis, is also supported by another recent study demonstrating that TAMs from human serous ovarian carcinoma ascites exhibit marked expression of *ANGPTL4* and other genes associated with cancer, presumably due to the high concentration of endogenous polyunsaturated fatty acids that act as PPARβ/δ agonists [28]. Interestingly, PPARβ/δ agonists have little influence on TAM gene expression, likely due to the occupancy of endogenous fatty acids, while inverse agonists caused a decrease in *ANGPTL4* and other genes associated with cancer [28]. Understanding the role of PPARβ/δ in tumorigenesis is further complicated by its potential role in host cells of the tumor microenvironment. Independent studies have shown a defect in tumor vascularization in *Ppard*-null mice [50, 51], and the publication discussed above reported the deregulation of potentially pro-tumorigenic PPARβ/δ target genes in TAMs of human ovarian cancer

patients [28]. In addition, recent findings suggest that PPARβ/δ agonists can influence VEGF expression through a mechanism that is not mediated by PPARβ/δ, and PPARβ/δ-dependent down-regulation of VEGF in cancer models can be observed. Collectively, there is increasing evidence that inverse agonists of PPARβ/δ may be suitable for targeting cancer cell invasion, which may inhibit this hallmark of cancer.

By contrast, PPARβ/δ has anti-inflammatory activities, suggesting that this receptor should be associated with anti-carcinogenic effects (reviewed in [4, 5, 6, 7, 8]). Intriguingly, a recent study contradicts a much larger body of evidence as it suggests that PPARβ/δ promotes pro-inflammatory signaling and tumor progression in a mouse model of colon cancer [52]. One possible explanation for this counterintuitive finding is that the activation of PPARβ/δ in macrophages is associated with strong anti-inflammatory gene expression and functional “signature”, but surprisingly, activation of PPARβ/δ in macrophages was also associated with a modest immune stimulatory component [53]. This striking observation suggests that there could be cell context-specific function involving immune cells and inflammation that could influence tumorigenesis in an undetermined way. Further studies are needed to address this hypothesis, as immune suppression and inhibition of inflammation are two hallmarks of cancer that could be modulated by PPARβ/δ.

Several recent studies have revealed a novel mechanism by which ligand activation of PPARβ/δ inhibits non-melanoma skin tumorigenesis by modulation of cell cycle progression and senescence. In mouse keratinocytes expressing an oncogenic form of **harvey sarcoma ras** (HRAS), ligand activation of PPARβ/δ can cause binding to the retinoblastoma protein family members p130 or p107 leading to G2/M arrest of the cell cycle [54]. In mouse keratinocytes expressing an oncogenic form of HRAS, ligand activation of PPARβ/δ can promote oncogene-induced senescence by repressing expression of PDPK1 and ILK causing increased phosphorylation of ERK and decreased phosphorylation of AKT1 [35]. This collectively increases p53/p27 causing enhanced cellular senescence and inhibition of HRAS-dependent tumorigenesis [35]. Interestingly, higher expression of PPARβ/δ also correlates with increased cellular senescence in human benign neurofibromas and colon adenomas [35]. Moreover, this increase in oncogene-induced senescence appears to be mediated in part by PPARβ/δ-dependent repression of endoplasmic reticulum-stress [55].

While there are many studies demonstrating that PPARβ/δ protects against oncogene-induced skin tumorigenesis (reviewed in [4, 5, 6, 7, 8]), there is a recent study suggesting that antagonizing PPARβ/δ inhibits ultraviolet-induced skin tumorigenesis, by activating the SRC pathway [56]. However, this study applied the PPARβ/δ antagonist prior to exposure to ultraviolet radiation. Since the PPARβ/δ antagonist used for this work (GSK0660) absorbs ultraviolet light with great efficacy, it cannot be ruled out that the observed anti-tumor activity was due to a sunscreen effect. Collectively the studies summarized above provide strong evidence that PPARβ/δ can modulate cell cycle progression and senescence in non-melanoma skin cancer, two mechanisms central to the hallmarks of cancer.

Concluding Remarks and Future Perspectives

It is now known that constitutive expression of PPARβ/δ is high in many tissues including the gut epithelium and keratinocytes, and that it is typically found in the nucleus where it functions to repress or activate target gene expression. Because of this relatively high expression and the fact that this transcription factor controls multiple genes, PPARβ/δ remains a viable molecular target for cancer chemoprevention (Outstanding Questions Box). Whether this will be accomplished by using natural or synthetic agonists, antagonists or inverse agonists remains to be determined. Since there is no known evidence that these chemicals are genotoxic, it is likely that effective targeting of PPARβ/δ will result in modulation of one or more molecular pathways involved in the hallmarks of cancer. Corroborating PPARβ/δ-dependent pathways suitable for new approaches for cancer chemoprevention and/or chemotherapy would likely be most effectively completed by co-operative collaborations between laboratories that have published opposing results. This type of approach may also be suitable for many other areas of controversy in cancer biology.

Outstanding questions

Is PPARβ/δ expression modulated by the APC/β-catenin/TCF4 pathway, or by other oncogene/tumor suppressor pathways?

Is the nuclear/cytosolic distribution of PPARβ/δ and/or expression increased, decreased or unchanged in cancer compared to normal somatic cells, or cells within the tumor microenvironment, during the different phases of cancer and is the same pattern observed for all cancers?

Given the heterogeneity in PPARβ/δ expression in cancer and normal cells, can expression patterns be useful for predicting clinical outcome of cancer patients, or the development of individualized therapies?

Is the activity of PPARβ/δ in cancers modulated by other transcription factors, signaling pathways, and/or the gut microbiome, which precludes a uniform model?

Is modulation of PPARβ/δ expression and/or activity a feasible approach for cancer chemoprevention?

Can consensus PPARβ/δ target genes and pathways related to cancer be fully determined and adequately validated by independent laboratories?

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Glossary

Adenomatous polyposis coli (APC)	a gene that when mutated, underlies the most common form of colon cancer.
Angiopoietin-like 4 protein (ANGPTL4)	a secreted multifunctional protein that is extra-cellularly processed to yield two products with different bioactivities. Induction of ANGPTL4 by gut lipids lowers lipid uptake into lymph node macrophages by inhibiting triglyceride hydrolysis, thereby preventing macrophage activation and foam cell formation and inhibiting saturated fat-induced inflammation. ANGPTL4 secretion from liver inhibits lipoprotein lipase activity and increase plasma triglycerides. Reported to have pro- and anti-angiogenic and tumor-promoting effects.
Catenin beta-1 (*CTNNB1*/β-CATENIN)	a subunit of the cadherin protein complex and signal transducer in the WNT signaling pathway. β-CATENIN levels are regulated by the APC protein.
Dextran sodium sulfate (DSS)	an inflammatory agent used to promote cell proliferation in the intestine in animal models of inflammatory bowel disease and colon cancer.
Familial adenomatous polyposis (FAP)	a phenotype used to describe patients with multiple colonic polyps and a predisposition to colon cancer due to a mutant *APC* gene.
Fatty acid binding protein 5 (FABP5)	a protein that binds non-esterified fatty acids and that has been implicated in shuttling agonists to PPARβ/δ.
Harvey sarcoma ras gene (HRAS)	a proto-oncogene that in a mutated form can contribute to the mutation “load” required to collectively drive a normal cell into a cancer cell.
Human umbilical vein endothelial cells (HUVEC)	endothelial cells derived from human umbilical veins that are often used to examine angiogenesis (the development of new blood vessels).
Integrin-linked kinase (ILK)	a kinase that interacts with integrins and PDPK1 to phosphorylate AKT1.
Peroxisome proliferator-activated receptor-β/δ (PPARβ/δ)	a ligand activated transcription factor that regulates target genes by repressing and activating expression. Also referred to as PPARδ, PPARβ, NUC1, PPARD, PPARd, PPARB and PPARb.
Peroxisome proliferator response element (PPRE)	a bipartite DNA motif with one direct repetition separated by a single nucleotide representing a binding site for PPARs to regulate transcription.

Phosphatase and tensin homolog (PTEN)	a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase that dephosphorylates phosphoinositide substrates and by doing so acts as a tumor suppressor by negatively regulating AKT1.
3-phosphoinositide dependent protein kinase-1 (PDPK1)	a kinase activated by growth factors that modulates cell proliferation and cell viability.
Retinoic acid receptor (RAR)	a nuclear receptor that is activated by natural and synthetic derivatives of vitamin A by forming homodimers and heterodimers with RXR.
Retinoic X receptor (RXR)	a nuclear receptor that is activated by natural and synthetic derivatives of vitamin A by forming homodimers and heterodimers with RAR.
T cell factor 4 (TCF4)	also known as TCF7L2 is a transcription factor encoded by the *TCF7L2* gene and central to canonical WNT/β-CATENIN signaling during cancer progression.
***Drosophila melanogaster* wingless gene, homolog of int-1 (WNT1)**	a human protein encoded by the *WNT1* gene. The canonical WNT pathway (or WNT/β-CATENIN pathway) causes accumulation of β-CATENIN and translocation into the nucleus to act as a transcriptional co-activator of transcription factors that belong to the TCF/LEF family. β-CATENIN is normally degraded via the ubiquitination/proteasome pathway by a complex of proteins: AXIN, APC, PP2A, GSK3 and CK1α. Disruption of this pathway (i.e. mutations in APC) allows nuclear accumulation of β-CATENIN and increased transcriptional activity of the TCF/LEF transcription factors.

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Trends Box

PPARβ/δ expression in cancers requires careful quantification and validation.

As PPARβ/δ is constitutively high expressed and regulates multiple pathways involved in carcinogenesis, and natural and synthetic agonists, antagonists and inverse agonists already exist, it has great potential as a target for cancer chemoprevention.

Dissecting the role of PPARβ/δ in tumor and host cells in the tumor microenvironment including in cancer patients where PPARβ/δ expression correlates with clinical outcomes is warranted.

Analyzing the role of PPARβ/δ in tumorigenesis in human models and different strains of *Ppard* transgenic mice will help exclude potential contributions by modifier genes.

Collaborations between laboratories describing opposing effects of PPARβ/δ in cancer could have a major positive impact in this field.

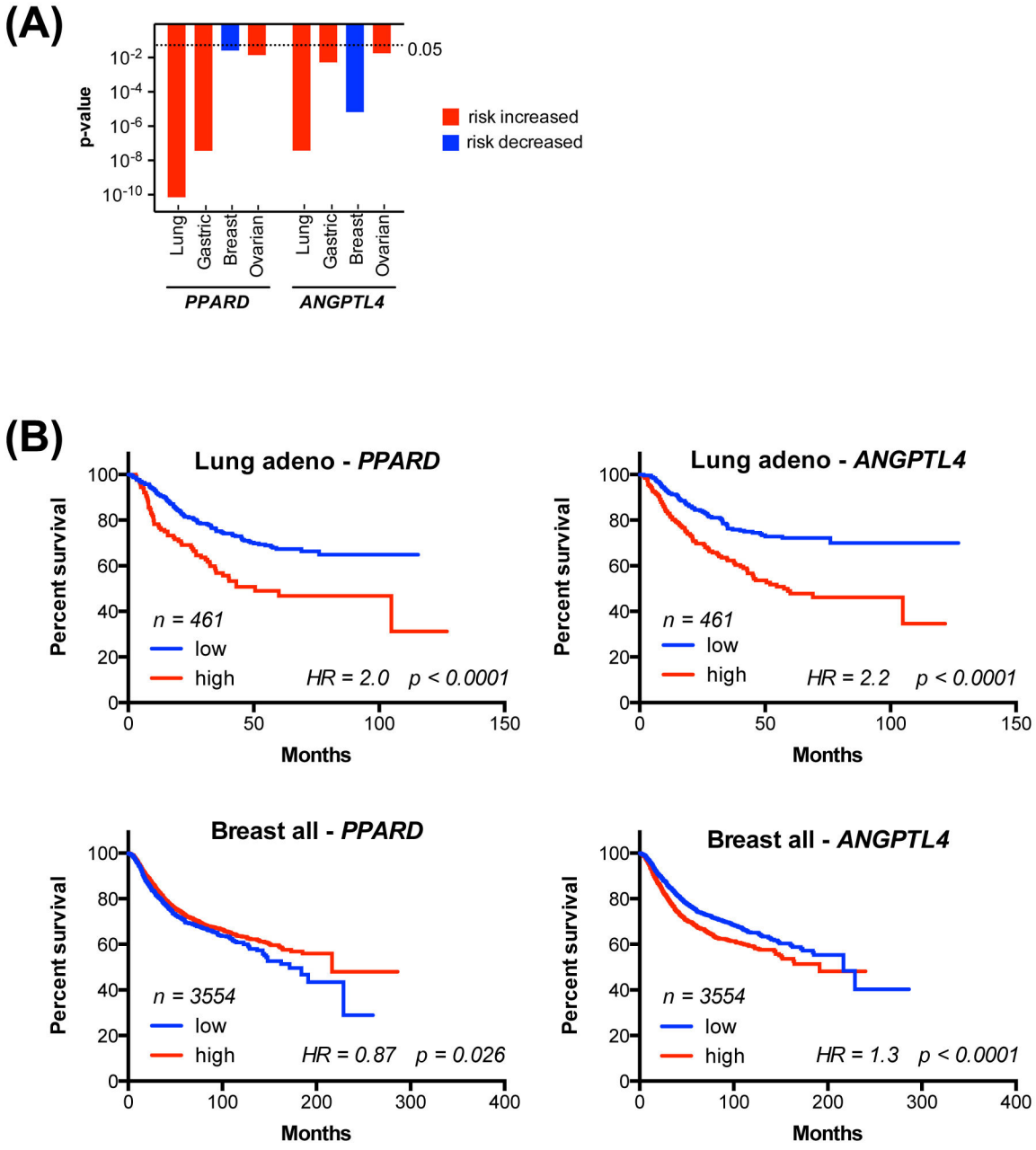

Figure 1.

Complex relationships between *PPARD* and *ANGPTL4* and the clinical outcome of cancer There is evidence based on correlative survival analysis using large microarray databases that PPARβ/δ has suppressive and/or pro-tumorigenic roles in human cancer. This is based on comparing relative *PPARD* mRNA in tumors and normal tissue from patients with different clinical outcomes. This is illustrated here using analyses of databases as described previously [57, 58, 59]. (**A**) Relapse-free survival (RFS) in different cancers is associated with relative expression of *PPARD* or *ANGPTL4* mRNAs. Note there is a significant association between the relative expression of *PPARD* and *ANGPTL4* mRNAs and RFS in lung (combined adenocarcinoma and squamous carcinoma), gastric, breast and serous

ovarian cancer but it can be increased or decreased. (**B**) Kaplan-Meier analysis illustrating an association between relative expression of *PPARD* or *ANGPTL4* mRNAs and lung adenocarcinoma patient RFS (upper panels). By contrast, Kaplan-Meier analysis indicates that while higher expression of *PPARD* mRNA in breast cancer patients is associated with longer RFS as compared to breast cancer patients with lower expression of *PPARD* mRNA, the inverse association is observed between breast cancer patients and relative expression of *ANGPTL4* mRNA (lower panels). However, associations between relative mRNA expression of *PPARD* mRNA does not necessarily account for the effects of endogenous or exogenous agonists, antagonists, or inverse agonists. Furthermore, this approach is only correlative because survival analyses using data from public databases (including TCGA) of mRNA analysis has significant limitations (discussed in text). This illustrates the need for more comprehensive analyses outlined in this review.

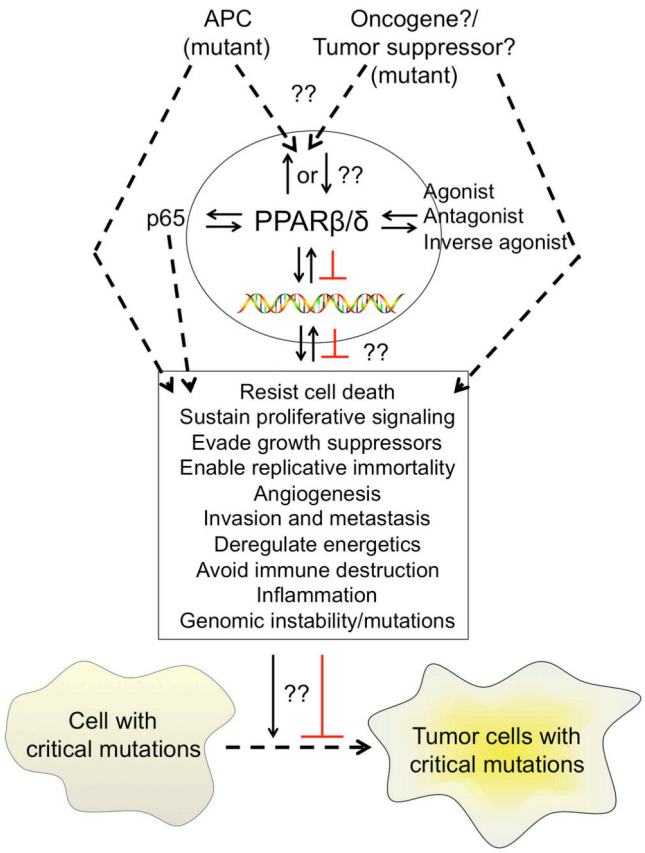

Figure 2.

Hypothetical mechanisms of PPARβ/δ-dependent regulation of carcinogenesis

Mutant APC may or may not cause increased expression of PPARβ/δ and other factors that drive proliferation of cells with critical mutations by overcoming a number of hallmark checkpoints that typically prevent mutant cells from growing into tumors. There is also evidence that APC does not cause up-regulation of PPARβ/δ and that targeting PPARβ/δ with agonists, antagonists and/or inverse agonists could be useful for modulating molecular pathways that promote cell replication of mutant cells transforming them into tumors. The effect of mutant oncogenes/tumor suppressors on expression of PPARβ/δ and/or functional roles of PPARβ/δ is unclear. Whether there are cell specific differences in: 1) expression of PPARβ/δ, 2) expression of co-effector proteins that interact with PPARβ/δ and chromatin, 3) the presence of endogenous agonists, antagonists and/or inverse agonists that could interact/interfere with exogenous agonists, antagonists and/or inverse agonists, or 4) molecular pathways that are regulated by PPARβ/δ is uncertain. PPARβ/δ remains a viable molecular target because it is expressed at high levels before cancerous tumors are observed, and the availability of natural and synthetic agonists, antagonists and/or inverse agonists is excellent. It is of particular interest to determine how inflammation modulates cancer via PPARβ/δ-dependent regulation because there is strong evidence that PPARβ/δ is mainly anti-inflammatory in nature, but that specific pro-inflammatory features may also interact in this system.

Table 1

Summary of select studies examining role of PPARβ/δ in cancer

Cancer type	Model	Outcome	Comments	Ref
Colon	Human tissue/ tumors, human cancer cell lines in vitro	PPARβ/δ is upregulated by APC pathway, pro-tumorigenic.	Limited to 4 human samples, not replicated by others.	[9]
Colon	Human/APC mutant mouse tissue/tumors	Expression of CYCLIN D1 is higher and PPARβ/δ is lower in mouse and human tumors compared to normal tissue.	*APC* genotype not examined in the human samples.	[24]
Colorectal	Human tissue/ tumors, human cancer cell lines in vitro	Survival of colorectal cancer patients is markedly greater in patients with relatively higher expression of PPARβ/δ as compared to patients with relatively lower expression of PPARβ/δ in their primary tumor.	Complemented by in vitro studies of human colon cancer cell lines; first retrospective study that focused on patient survival and PPARβ/δ expression.	[60]
Breast	Human cancer cell line in vitro	Inverse PPARβ/δ agonists inhibit invasion into a 3D collagen matrix by blocking *ANGPTL4* transcription.	Includes siRNA experiments to show PPARβ/δ dependence and link to *ANGPTL4*.	[49]
Lung	Human cancer cell lines in vitro	Ligand activation of PPARβ/δ increases expression of prostaglandin receptors, decreases expression of PTEN, increases PGC1α and increases PI3KA, AKT and NFκB activities to promote cell proliferation.	These papers were all retracted due to digital manipulations and image duplications without knowledge of all authors.	[61, 62, 63, 64, 65, 66]
Lung	Human cancer cell lines in vitro	Ligand activation of PPARβ/δ has no effect on expression of PTEN, AKT or cell proliferation.	Dose-dependent analysis.	[67]
Liposarcoma	Human tissue/ tumors, human cancer cell lines in vitro	Expression of PPARβ/δ is higher in tumors as compared to normal tissue, ligand activation of PPARβ/δ increased cell proliferation and migration by down-regulating LEPTIN in vitro.	Expression in human tumors examined by immunohistochemistry, which is not suitable for PPARβ/δ. Concentration of PPARβ/δ agonist required for the changes in cell proliferation and migration was 200 μM, much higher than that required to activate PPARβ/δ and high enough to cause non- specific effects.	[68]
Neuroblastoma	Human cancer cell line in vitro	Ligand activation of PPARβ/δ promotes differentiation of neuroblastoma cells and inhibits proliferation.	Consistent with many studies showing that PPARβ/δ promotes differentiation.	[69]
Melanoma	Human cancer cell lines in vitro	Ligand activation of PPARβ/δ increases expression of SNAIL promoting migration and invasion.	No dose-dependent analysis but siRNA control showing specificity.	[70]
Melanoma	Human cancer cell line in vitro	Ligand activation of PPARβ/δ inhibits cell proliferation.	Dose-dependent analysis of two agonists but no siRNA or antagonists used to demonstrate specificity.	[71]
Ovarian	Human ovarian cancer patients	Polyunsaturated fatty acids activate PPARβ/δ in TAMs in the tumor micro-environment and induce tumor promoting genes.	First retrospective study that focused on patient survival and PPARβ/δ function in TAMs in the tumor micro- environment.	[28]
Pancreatic	Human cancer cell lines in vitro	Ligand activation of PPARβ/δ inhibits cytokine- induced invasion and migration.	Included shRNA controls to demonstrate specificity.	[72]