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Sorting out the functional role(s) of peroxisome proliferator-activated receptor- β/δ (PPAR β/δ) in cell proliferation and cancer

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Abstract

Peroxisome proliferator-activated receptor- β/δ (PPAR β/δ) has many beneficial physiological functions ranging from enhancing fatty acid catabolism, improving insulin sensitivity, inhibiting inflammation and increasing oxidative myofibers allowing for improved athletic performance. Thus, given the potential for targeting PPAR β/δ for the prevention and/or treatment of diseases including diabetes, dyslipidemias, metabolic syndrome and cancer, it is critical to clarify the functional role of PPAR β/δ in cell proliferation and associated disorders such as cancer. However, there is considerable controversy whether PPAR β/δ stimulates or inhibits cell proliferation. This review summarizes the literature describing the influence of PPAR β/δ on cell proliferation, with an emphasis toward dissecting the data that give rise to opposing hypotheses. Suggestions are offered to standardize measurements associated with these studies so that interlaboratory comparisons can be accurately assessed.

Keywords

peroxisome proliferator-activated receptor- β/δ ; cell proliferation; apoptosis; differentiation; cancer

1. Introduction

Peroxisome proliferator-activated receptor- β/δ (also referred to as PPAR β and PPAR δ) is one of three PPARs, including PPAR α and PPAR γ that are part of the nuclear receptor superfamily of transcription factors. PPAR β/δ can be activated by endogenous compounds, which are thought to act as natural ligands (Fig. 1). For example, PPAR β/δ is activated by μ M concentrations of oleic acid and linoleic acid [1]. Additionally, PPAR β/δ may be activated by prostaglandin I₂ (PGI₂ or prostacyclin) [2] or stable analogues of prostacyclin such as carbaprostacyclin [1]. High affinity synthetic ligands for PPAR β/δ , produced by the pharmaceutical industry, include L165041, GW501516 and GW0742 (Fig. 1); all of which activate PPAR β/δ at concentrations in the low nM - μ M range [3,4]. Regulation of gene expression by PPAR β/δ is mediated by ligand activation of the receptor (reviewed in [5]). Briefly, ligand binding induces a conformational change in protein structure that causes

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dissociation of co-repressors, heterodimerization with RXR α , recruitment of co-activators and RNA polymerase, remodeling of histones near regions of DNA containing direct repeat 1 motifs (AGGTCANAGGTCA) and culminating with increased transcription of target genes (Fig. 2). In addition to this classic mechanism of gene regulation by transcription factors, PPAR β/δ can also interact directly with the p65 subunit of NF κ B leading to inhibition of NF κ B-dependent signaling and resultant anti-inflammatory activities (reviewed in [5]). Anti-inflammatory activities of PPAR β/δ may also be mediated through direct interaction with ERK5 or STAT3 [6,7]. Regulation mediated by direct interactions of PPAR β/δ with other transcription factors is one mechanism by which PPAR β/δ can cause down-regulation of target gene expression. There is also evidence that PPAR β/δ may repress transcription of some target genes through binding to DNA response elements in association with co-repressors [8]. Combined, there are a number of different mechanisms whereby PPAR β/δ can modulate cellular homeostasis including direct up-regulation of target genes and down-regulation of gene expression mediated by other transcription factors (Fig. 2).

2. Functional roles of PPAR β/δ

In addition to target gene specificity, tissue-specific expression patterns of the PPARs can distinguish their biological functions. In contrast to PPAR α and PPAR γ that are preferentially expressed in liver and kidney, and adipose tissues, respectively, PPAR β/δ is expressed in most tissues, with varying levels in certain cell types. For example, expression of PPAR β/δ is highest in small intestine, colon and liver where it is found predominantly in the nucleus and can be co-immunoprecipitated with its heterodimerization partner RXR α [9]. While expression of PPAR β/δ is not strikingly high in whole skin, it is found at very high level in keratinocytes, one of the main differentiated cell types found in skin [9]. Thus, there is the potential for PPAR β/δ to have functional role in tissues where it is expressed in high levels and even in tissues that may not exhibit high expression patterns by gross examination due to the low content of specific cell types.

Potential functional roles for PPAR β/δ have emerged in recent years. The first biological effect described for a PPAR β/δ agonist was an increase in serum high density lipoprotein cholesterol (HDLc) in diabetic mice following administration of L165041 [10]. An increase in serum HDLc was also found in obese non-human primates administered another potent PPAR β/δ ligand, GW501516 [11]. Further, ligand activation of PPAR β/δ also reduced serum low density lipoprotein cholesterol (LDLc), triglycerides and fasting insulin concentrations [11]. Studies in a mouse model of obesity determined that ligand activation of PPAR β/δ by GW501516 can increase expression of enzymes that catabolize fatty acids in skeletal muscle, and significantly decrease serum glucose concentrations [12]. Mice fed a high fat diet exhibit improvement in insulin sensitivity in response to ligand activation of PPAR β/δ , and this effect is not found in *Ppar β/δ* -null mice, thus demonstrating that PPAR β/δ is required to improve insulin sensitivity [13]. These observations demonstrate that PPAR β/δ ligands have great potential for the treatment of diseases associated with altered lipid and glucose homeostasis including diabetes. More recently, it was shown that combining ligand activation of PPAR β/δ with exercise increases the number of oxidative myofibers in muscle, resulting in enhanced exercise performance [14]. This observation suggests that PPAR β/δ ligands could be developed to improve athletic performance; this raises ethical questions regarding the potential for illicit use. PPAR β/δ and its ligand also have potent anti-inflammatory activities (reviewed in [5, 15]). The anti-inflammatory activities of PPAR β/δ and its PPAR β/δ ligands may be important for prevention of liver toxicity/cancer [6,16-19] or neurotoxicity [20].

In contrast to the mechanisms elucidated for PPAR β/δ in metabolism and inflammation, the role of PPAR β/δ in cell growth remains controversial due to conflicting reports in the literature. Some studies indicate that activating PPAR β/δ causes increased cell proliferation and anti-

apoptotic signaling, while others found that activating PPAR β/δ inhibits cell proliferation by inducing terminal differentiation and/or pro-apoptotic signaling. A critical analysis of these differences is the focus of this review. The remaining sections will focus on the role(s) reported for PPAR β/δ in specific cell types beginning with normal cell types and ending with cancer models.

3. Regulation of cell proliferation in skin and keratinocytes by PPAR β/δ

Regulation of cell proliferation in skin and keratinocytes has been extensively studied. There is evidence from three different null mouse models that PPAR β/δ inhibits cell proliferation in mouse keratinocytes [21-23]. A role for PPAR β/δ in cell proliferation in skin was revealed by the observations that phorbol ester-induced hyperplasia is exacerbated in PPAR β/δ -deficient mouse skin in vivo using two distinctly different *Ppar β/δ* -null mouse models [21,22]. Epidermal thickness and proliferating cell nuclear antigen (PCNA)-positive cells is also increased in mice where PPAR β/δ expression was conditionally disrupted in keratinocytes [23]. These observations suggest that PPAR β/δ attenuates cell proliferation in keratinocytes. In agreement with these studies, inhibition of cell proliferation was found in normal human epidermal keratinocytes, HaCaT keratinocytes, N/TERT-1 and NCTC 2544 human keratinocyte cell lines in response to ligand activation of PPAR β/δ [24-27]. Similarly, increased cell proliferation was found in *Ppar β/δ* -null mouse keratinocytes in vitro [28] and ligand activation of PPAR β/δ inhibits cell proliferation of cultured mouse keratinocytes through a mechanism that requires a functional receptor [29]. Exacerbated hyperplasia was also noted in *Ppar β/δ* -null mouse skin following treatment with phorbol ester [30,31] and ligand activation of PPAR β/δ inhibits phorbol ester-induced hyperplasia in mouse skin in vivo [30]. In one study, topical application of PPAR β/δ ligands had no effect on epidermal cell proliferation [32]. In contrast to these studies, one report suggested that ligand activation of PPAR β/δ increased cell proliferation of human keratinocytes. Treating cultured human keratinocytes from psoriasis patients with L165041 or GW501516 caused an increased in cell proliferation, and this increase was thought to be mediated by increased expression of heparin-binding EGF-like growth factor [33]. However, this study was limited to analysis of only one timepoint and a single concentration of either L165041 (1 μ M) or GW501516 (5 μ M); concentrations that are also known to cause activation of PPAR α and PPAR γ [3,34]. Thus, to date there are thirteen published studies that have specifically examined cell proliferation in skin and keratinocytes with eleven showing inhibition of cell proliferation of human and mouse keratinocytes by PPAR β/δ , one report showing no effect on cell proliferation and one report showing enhanced cell proliferation of human keratinocytes from psoriasis patients (Table 1). A number of mechanisms have been postulated to explain these effects.

3. 1. PPAR β/δ -dependent regulation of differentiation in keratinocytes

The first suggestion that PPAR β/δ regulates terminal differentiation in skin was revealed by increased expression of PPAR β/δ that correlated with elevated levels of skin terminal differentiation markers transglutaminase-1 and small proline-rich proteins [35]. Subsequent work by four independent laboratories supported this finding by showing that ligand activation of PPAR β/δ increased terminal differentiation of mouse and human keratinocytes using both in vivo and in vitro models [24-26,29,30,32,36]. Expression of mRNAs and proteins required for terminal differentiation of human and mouse basal keratinocytes to cornified keratinocytes was increased by PPAR β/δ ligands (L165041, GW0742, tetradecylthioacetic acid (TTA)) [25,26,29,30,32] and these changes in gene expression were also reflected by an increase in cornified envelopes [29], the functional end product of keratinocyte terminal differentiation. That these effects require PPAR β/δ was demonstrated by studies showing the absence of these effects in null mouse models [25,29,30,36]. These findings represent the most definitive response described to date for PPAR β/δ in cell proliferation and clearly establish that ligand

activation of PPAR β/δ promotes terminal differentiation in mouse and human keratinocytes. The mechanisms that mediate this effect remain unclear as the PPAR β/δ target genes that promote terminal differentiation have not been identified to date. PPAR β/δ -dependent induction of keratin 10 (K10) in keratinocytes [30,32,36] may contribute to the mechanisms underlying PPAR β/δ -mediated inhibition of cell proliferation in keratinocytes since increased expression of K10 inhibits cell proliferation of human HaCaT keratinocytes through a retinoblastoma-dependent mechanism involving the nonhelical terminal domain of K10 [37]. Since the induction of terminal differentiation is known to be associated with withdrawal from the cell cycle, it is likely that PPAR β/δ -mediated induction of terminal differentiation in keratinocytes is a key mechanism that causes inhibition of cell proliferation observed in most skin/keratinocyte models studied to date.

3.2. PPAR β/δ -dependent regulation of apoptosis in keratinocytes

There is a relative unique balance between differentiation and apoptosis in keratinocytes that could also influence cell proliferation of keratinocytes. Keratinocytes undergo terminal differentiation to form a cornified cell and this process is associated with increased activity of caspases including caspase 3 and caspase 14 [38,39]. Caspase 3 is known to be central in the process of apoptosis and increased caspase 3 activity occurs during keratinocyte terminal differentiation [39]. However, differentiating and fully differentiated keratinocytes (e.g. cornified cells) do not exhibit shrinkage, DNA fragmentation and are not phagocytosed [40, 41]. Additionally, caspase 14 does not participate in apoptotic signaling but instead modulates terminal differentiation [38]. These observations demonstrate that apoptotic signaling observed in differentiating keratinocytes is relatively unique as compared to most cells because differentiated (cornified) keratinocytes have a more sustained lifespan whereas apoptosis usually leads to cell death and phagocytosis. Consistent with these observations, a PPAR β/δ -dependent increase in caspase 3 activity and TUNEL-positive cells was found in phorbol ester-treated mouse skin [31]. Increased annexin V-positive human HaCaT keratinocytes, indicative of apoptosis, was also reported following treatment with GW0742 [24]. However, increased caspase 3 activity was not found in N/TERT-1 human keratinocytes following treatment with GW0742 or in three mouse skin tumor cell lines [25,30]. The effect of ligand activation of PPAR β/δ on the expression of caspase 14 in skin or keratinocytes has not been examined to date. Combined, there is compelling evidence for PPAR β/δ -dependent induction of apoptotic-like signaling in keratinocytes, that is likely related to induction of terminal differentiation and may also be involved in the inhibitory effects of PPAR β/δ on cell growth observed in mouse and human skin.

In contrast to strong evidence from multiple independent groups showing PPAR β/δ -mediated terminal differentiation of keratinocytes and the well-established relationship between terminal differentiation and apoptotic-like signaling, a paradoxical regulatory pathway has also been described suggesting that PPAR β/δ potentiates anti-apoptotic signaling in keratinocytes [42]. In response to ligand activation of PPAR β/δ by L165041 in cultured mouse keratinocytes or human HaCaT keratinocytes, the expression of phosphatase and tensin homolog deleted on chromosome Ten (PTEN) is decreased while expression of 3-phosphoinositide-dependent-protein kinase 1 (PDPK1) and integrin-linked kinase (ILK) is decreased [42]. The combined effect of this PPAR β/δ -dependent regulation led to increased phosphorylation of protein kinase B (AKT) causing inhibition of apoptosis and cell survival. Subsequent work by others using HaCaT human keratinocytes supports this putative anti-apoptotic signaling mediated by PPAR β/δ as shown by increased expression of PDPK1 and phosphorylation of AKT, but ILK or PTEN expression were not examined [43]. Inhibition of tumor necrosis factor- α (TNF α)-induced apoptosis has also been reported in response to L165041 in human HaCaT keratinocytes treated with and without epidermal growth factor (EGF) [44]. However, the high concentration of L165041 (10 μ M) used for these studies could potentially activate PPAR γ

[3] and thus it is not possible to conclude that this effect was due solely to activation of PPAR β/δ . Similar changes in the PTEN/PDPK1/ILK/AKT pathway are not consistently observed in response to ligand activation of PPAR β/δ in mouse and human keratinocytes. For example, increased expression of the known PPAR β/δ target gene adipocyte differentiation-related protein (*ADRP*) is increased in mouse keratinocytes or human N/TERT-1 keratinocytes in response to GW0742 but no changes in the expression of PTEN, PDPK1 or ILK, or phosphorylation of AKT were observed [25]. Similarly, expression of two known PPAR β/δ target genes, angiopoietin-like protein 4 (*ANGPTL4*) and *ADRP* were increased by GW0742 in human HaCaT keratinocytes but no change in the expression of PDPK1 or phosphorylation of AKT were noted [24]. There are other examples where no changes in this putative PPAR β/δ -dependent PTEN/PDPK1/ILK/AKT pathway were found (described elsewhere in this review). One likely explanation for the lack of concordance between laboratories is/are the difference(s) in the model used. The studies suggesting a putative PPAR β/δ -dependent PTEN/PDPK1/ILK/AKT pathway in mouse keratinocytes used cells that lacked the typical cell morphology of primary keratinocytes and importantly, did not exhibit basal expression of keratin 6 (K6) [36,42]. Since it is well documented that primary keratinocytes express K6 [45,46], this suggests that the putative PPAR β/δ -dependent PTEN/PDPK1/ILK/AKT pathway may only function in cells that have undergone some molecular changes not associated with normal keratinocytes. While there is some evidence that this pathway may function during wound healing, the evidence to date is more compelling that it is not functional in normal mouse or human keratinocytes in response to ligand activation of PPAR β/δ . Whether this pathways functions in other cells types is also uncertain as some reports suggest it does, while others indicate that it does not.

3.3. PPAR β/δ -dependent regulation of cell signaling in keratinocytes

In addition to the induction of terminal differentiation, there is also evidence suggesting that PPAR β/δ attenuates cell proliferation in keratinocytes by modulating kinase signaling. In phorbol ester-stimulated skin and keratinocytes, enhanced cell proliferation is observed in the absence of PPAR β/δ expression [21,22,28,30,31]. In *Ppar β/δ* -null mouse skin treated with phorbol ester, increased expression of *Ubiquitin C* does not occur leading to a significant decrease in free ubiquitin (UB) and reduced UB-dependent turnover of protein kinase C α (PKC α) [28,31]. Whether ligand activation of PPAR β/δ can increase UB-dependent turnover of PKC α or other kinases through this mechanism has not been examined. These findings suggest that this mechanism may contribute to PPAR β/δ -mediated inhibition of keratinocyte cell proliferation.

3.4. Effect of retinoic acid on cell proliferation in keratinocytes

Recent reports suggested that shuttling of retinoic acid between retinoic acid receptor (RAR) and PPAR β/δ occurs based on the relative expression of two RA-binding proteins, cellular retinoic acid binding protein II (CRABP-II) and fatty acid binding protein 5 (FABP5) [43, 47]. In this hypothetical model, retinoic acid is preferentially targeted to RAR in cells expressing more CRABP II leading to inhibition of cell growth, while retinoic acid is preferentially targeted to PPAR β/δ in cells expressing more FABP5 leading to enhanced cell growth [43]. This idea was supported by the reported increased *PDPK1* expression in human HaCaT keratinocytes treated with either retinoic acid or the PPAR β/δ ligand GW501516. In contrast to these findings, recent work shows that retinoic acid does not bind to or activate PPAR β/δ as shown in cells with a high FABP5:CRABP II ratio as well as with ligand-mediated binding assays [48]. Similarly, retinoic acid inhibits cell growth of keratinocytes independent of PPAR β/δ expression, and ligand activation of PPAR β/δ does not increase expression of *PDPK1* but rather inhibits proliferation of cells with a high FABP5:CRABP II ratio [24]. These observations are in line with inhibition of cell proliferation observed in HaCaT keratinocytes, other human keratinocyte cell lines, and various human cancers following treatment with

retinoic acid [49-53]. Retinoic acid also inhibits proliferation of mouse primary keratinocytes, which is consistent with previous studies [54] and with inhibition of skin cancer by retinoids observed in several mouse models [55-61]. Combined, these observations are highly inconsistent with the proposed pathway suggesting that activating PPAR β/δ by retinoic acid will enhance cell growth of human keratinocytes [43,47]. Given these inconsistencies, the aforementioned hypothesis that retinoic acid activates PPAR β/δ to promote cell survival should be rigorously re-evaluated.

4. Regulation of cell proliferation in other somatic cells by PPAR β/δ

Regulation of cell proliferation by PPAR β/δ in somatic cells other than skin and keratinocytes has also been studied, including endothelial cells, vascular smooth muscle cells, fibroblasts, hepatocytes and stellate cells. Blood vessels are composed of a number of cell types including endothelial cells (EC) that line the interior surface and vascular smooth muscle cells (VSMC) the major cell type in the cell walls. EC and VSMC can interact through the production of growth factors and signaling molecules that have paracrine and autocrine activities. Notably, EC produce prostacyclin, which may function as an endogenous PPAR β/δ ligand that could potentially act on both EC and VSMC. The effect of PPAR β/δ on cell proliferation in both of these cell types has been examined.

A stable prostacyclin analog (ciprostone) inhibits proliferation and associated ^3H -thymidine incorporation in bovine EC at a concentration of 50-100 μM [62]. In the absence of PPAR β/δ expression, mouse EC proliferation is significantly increased as compared to EC that express PPAR β/δ [63], but the effect of ligand activation was not examined in this model. The inhibitory effect of PPAR β/δ in mouse EC is mediated by PPAR β/δ -dependent up-regulation of *Cdkn1c*, which encodes the cell cycle inhibitor p57^{Kip2} [63]. Activation of PPAR β/δ by 100 μM carbaprostacyclin or 50 μM L165041 does not change annexin V-positive cells or caspase 3 activity in human EC [64]. In contrast to these reports, activation of PPAR β/δ with 0.1 μM GW501516 in human EC and an EC line (EAHy926) is reported to cause a marked increase in cell proliferation, with almost a doubling of cell number [65]. Interestingly, this effect is found only after 72 hours of treatment with no changes in cell proliferation being noted at earlier timepoints [65]. In another study, 0.01-0.1 μM GW501516 was reported to increase cell proliferation of human EC after up to 14 days of culture, but the kinetics of cell proliferation are uncertain because data from only one timepoint was reported [66]. The authors of this study suggested that the increased EC proliferation may be mediated by elevated expression of vascular endothelial growth factor (*VEGF*) and its receptor *FLT1* [66]. However, this hypothesis was not examined in detail and was based solely on mRNA data without further mechanistic evaluation. In endothelial progenitor cells (EPCs), an increase in cell proliferation and the percentage of cells undergoing the S phase of the cell cycle was found by 0.1-1.0 μM GW501516 treatment [67]. These changes found in cultured endothelial cells treated with GW501516 are reflected by angiogenic effects found in other models including increased endothelial cell tube formation, increased aortic ring outgrowths and increased tube formation in matrigel plug assays suggesting that activating PPAR β/δ with GW501516 stimulates angiogenesis by increasing expression of VEGF [65,67]. Thus, there is evidence that PPAR β/δ ligands have inhibitory and proliferative effects in EC (Table 2).

Anti-apoptotic activity resulting from activating PPAR β/δ was also reported in some EC models when apoptosis is induced. In human endothelial cells, 1-100 μM carbaprostacyclin and 10-50 μM L165041 decrease hydrogen peroxide-induced apoptosis as shown by decreased annexin V-positive cells, caspase 3 activity and PARP cleavage [64,68]. Increased expression of 14-3-3 ϵ mediated by activation of PPAR β/δ is thought to be the mechanisms underlying these effects [64,68], which causes inhibition of BAD translocation and downstream events thereby preventing apoptotic signaling that is typically induced in EC by treatment with

hydrogen peroxide. Ligand activation of PPAR β/δ was also reported to prevent hypoxia-induced apoptosis in human EPC by increasing phosphorylation of AKT, but this increase in phospho-AKT was not mediated by increased expression of ILK [67]. While expression of PDPK1 or PTEN was not assessed in this study, this is an example where the previously described PPAR β/δ -dependent modulation of PTEN/PDPK1/ILK/AKT does not appear to function as reported in a keratinocyte model [42].

There is contradictory evidence indicating that PPAR β/δ can either inhibit cell proliferation, potentiate cell proliferation or have anti-apoptotic activity under certain circumstances in EC. In contrast, reports examining the effect of PPAR β/δ in VSMC cell proliferation all indicate that activating this receptor will inhibit cell growth (Table 2). Prostacyclin is thought to be an endogenous PPAR β/δ ligand and synthetic prostacyclin analogs are routinely used for this purpose for the treatment of pulmonary hypertension. There is strong evidence from several laboratories that prostacyclin and prostacyclin analogs inhibit VSMC proliferation [62, 69-71]. In addition to membrane bound IP receptors that are known to mediate the effect of prostacyclin, recent evidence suggests that PPAR β/δ can mediate the inhibitory effect of prostacyclin analogs in VSMC [72,73]. For example, cell proliferation of rat VSMC is inhibited by 1 μ M beraprost and this effect is reduced by PPAR β/δ antagonism [73]. Additionally, platelet-derived growth factor (PDGF)-induced cell proliferation of rat VSMC was inhibited by 5-10 μ M L165041 and this inhibition was associated with reduced expression of CYCLIN D1, CDK4 and reduced phosphorylation of ERK1/2 [72]. No change in PDGF-induced phosphorylation of AKT was noted in response to L165041 [72].

There are a limited number of reports examining the effect of ligand activation in other somatic cell types (Table 2). TNF α causes an increase in bromodeoxyuridine (BrdU) labeling in primary mouse hepatocytes, and this effect is increased in *Ppar β/δ* -null hepatocytes [19]. Primary stellate cells from rat exhibit a modest increase in cell proliferation following treatment with 10 μ M L165041 [74]. In contrast, *Ppar β/δ* -null mouse primary stellate cells exhibit increased cell proliferation as compared to wild-type stellate cells [19]. The mechanisms underlying these effects have not been elucidated nor have the reason(s) for the differences observed between rat and mouse stellate cells. Cell proliferation of human lung fibroblasts is inhibited with the prostacyclin analog, Treprostinil, with concentrations greater than 0.1 μ M and with GW0742 at concentrations greater than 1.0 μ M [75]. PPAR β/δ is required for the inhibitory effect of Treprostinil in lung fibroblasts as this effect is mitigated in *Ppar β/δ* -null lung fibroblasts [75]. In cardiac fibroblasts, ligand activation of PPAR β/δ with 10 μ M GW501516 caused an inhibition in cell proliferation that was associated with PPAR β/δ -dependent inhibition of collagen synthesis [76]. Activation of PPAR β/δ by GW501516 in rat cardiac fibroblasts caused inhibition of BrdU incorporation and increased expression of the cell cycle inhibitor gene G0/G1 switch gene 2 (G0S2) [77]. While the former three studies indicate that ligand activation of PPAR β/δ in fibroblasts inhibits cell proliferation, there is one recent report showing increased cell proliferation in embryonic fibroblasts over-expressing PPAR β/δ [78]; however the effect of ligand activation in these cells was not reported. In the same study, a modest increase in cell proliferation of normal human thyroid cells was also observed and it was suggested to be due to a CYCLIN E-dependent mechanism [78]. Anti-apoptotic activity was also reported to occur in a human kidney cell line following co-treatment with hydrogen peroxide and 0.2-1.0 μ M L165041 [79] and in a rat cardiomyoblast cell line following co-treatment with hydrogen peroxide and 0.1-1.0 μ M GW501516 [80]. In contrast, overexpressing prostacyclin synthase, which increases intracellular prostacyclin, increases apoptosis in a human embryonic kidney epithelial cell line through a mechanism that requires PPAR β/δ [81]. In a mouse inner medullary collecting duct cell line, 10 μ M GW501516 inhibits cell proliferation whereas 0.01-1.0 μ M GW501516 has no effect on anisomycin-induced apoptosis [82].

5. Regulation of cell proliferation in cancer models and cancer cell lines by PPAR β/δ

The effect of activating PPAR β/δ in cancer models and cancer cell lines has been examined, and there are considerable differences in the responses reported in the literature (Table 3). Colon cancer models are among the more extensively studied and this subject has been reviewed in greater detail elsewhere [5]. There are two opposing mechanisms by which PPAR β/δ has been postulated to regulate colon cancer. One view is that PPAR β/δ is up-regulated by the adenomatous polyposis coli (APC)/ β -CATENIN/transcription factor 4 (TCF4) pathway, similar to what is observed with c-MYC and CYCLIN D1 [83]. It was hypothesized that PPAR β/δ could then be activated by cyclooxygenase-2 (COX2)-derived ligands such as prostacyclin [2,83], leading to expression of yet-to-be identified target genes that cause increased cell proliferation and tumor promotion. Consistent with this hypothesis, ligand activation of PPAR β/δ with GW501516 was reported to cause no change in colon tumorigenesis but an increase in small intestinal tumors in *APC*^{min} heterozygous mice [84], and cause a PPAR β/δ -dependent increase in intestinal and colon tumorigenesis in *APC*^{min} heterozygous mice [85]. Serum withdrawal-induced apoptosis was also reportedly inhibited by GW501516 (0.1-5.0 μ M) in LS174T and HCT116 colon cancer cell lines and mediated by PPAR β/δ -dependent up-regulation of VEGF and phosphorylation of AKT [84-86]. Interestingly, the changes observed in apoptotic signaling using serum withdrawal with in vitro models were in line with the observed reduction of TUNEL-positive cells in intestinal tumors from mice treated with GW501516 [84,85]. This is of interest because increased signaling for apoptosis is not typically found in tumors cells. However, other studies found no correlation between the APC/ β -CATENIN/TCF4 pathway and increased PPAR β/δ expression ([87], reviewed in [5]). Some studies revealed that ligand activation of PPAR β/δ had no effect on cell growth of HT29, SW480 and HCA7 cells [66]. Ligand activation of PPAR β/δ in LS174T, HCT116 or HT29 colon cancer cell had no effect on cell proliferation with 0.1 μ M GW501516 or GW0742 but inhibited cell proliferation at concentrations from 1.0 - 10.0 μ M of the same ligands [88]. Importantly, there was also no change in cell proliferation, expression of VEGF, PARP cleavage or phosphorylation of AKT in response to ligand activation of PPAR β/δ following serum-withdrawal [88]. Further, a PPAR β/δ -dependent increase in TUNEL-positive colonocytes was reported to occur in response to administration of GW0742 in mice [89]. Collectively, these findings and others (reviewed in [5]) are inconsistent with the idea that PPAR β/δ mediates anti-apoptotic signaling in colon cancer cell lines.

An alternative hypothesis has also been postulated to explain how modulation of PPAR β/δ activity influences colon cancer (reviewed in [5]). In this model, ligand activation of PPAR β/δ mediates terminal differentiation of colonocytes and inhibition of cell proliferation thereby preventing tumor promotion. In support of this view, silencing PPAR β/δ expression in HCT116 cells caused increased cell proliferation [90]. In vivo disruption of PPAR β/δ in mice caused an increase in colon tumorigenesis in both a genetic and chemically-induced model [91], and ligand activation of PPAR β/δ with GW0742 inhibited chemically-induced colon cancer [89, 92]. Bezafibrate administration was also reported to inhibit colon cancer in mouse models and human colon cancer patients [93,94]. This is of interest because while bezafibrate can activate PPAR α , PPAR β/δ and PPAR γ , the affinity of bezafibrate for human PPAR β/δ is greater as compared to human PPAR α and PPAR γ [34]. Well-characterized PPAR β/δ target genes associated with terminal differentiation of colonocytes are also increased by ligand activation of PPAR β/δ , including fatty acid binding protein (*Fabp*) and *Adrp* [89]. Since the induction of terminal differentiation is known to be associated with inhibition of cell proliferation, these findings are consistent with the observed inhibition of cell proliferation found in human colon cancer cell lines by 1.0-10.0 μ M GW501516 or GW0742 [88]. While induction of terminal differentiation that may be mediated in part by increased expression of the PPAR β/δ target

genes *FABP* and *ADRP* could contribute to the mechanisms leading to inhibition of tumor promotion in the colon, the involvement of other target genes remains uncertain.

Three studies have examined the effect of ligand activation of PPAR β/δ in breast cancer models. In estrogen receptor-positive human breast cancer cell lines MCF7 and T47D, ligand activation of PPAR β/δ by 0.01 μ M compound F or up to 0.05 μ M GW501516 caused an increase in cell proliferation after 12 days of culture, but this effect was not found in the estrogen receptor-negative human breast cancer cell lines BT20 and MDA-MB-231 [66]. The increase in cell proliferation was associated with an increase in cells undergoing S phase of the cell cycle, and increased expression of *CDK2*, *VEGF* and *FLT1* mRNAs in cells treated with 0.025 μ M GW501516 in medium with low (0.1%) serum [66]. In vivo, dietary administration of GW501516 at a concentration of 0.005% caused an increased in DMBA-induced mammary squamous cell carcinomas [95]. In tumors from these mice, PPAR β/δ co-immunoprecipitated with PDPK1 following GW501516 administration but this was only evaluated in two samples [95]. Critical examination of the functional significance of this putative interaction is uncertain and has not been examined in other cell types to date. In contrast to these reports, ligand activation of PPAR β/δ in MCF7 cells with either GW0742 or GW501516 does not increase cell proliferation, but rather, an inhibition of cell proliferation was observed with a concentration of 10 μ M for either ligand [96]. These effects were independent of either PPAR β/δ ligand and were not influenced by the presence or absence of culture medium serum. Since previous studies used low serum culture conditions [66], these recent findings [96] suggest that the differences observed between laboratories is not due to the differences in ligand used or the presence/absence of culture medium serum.

The first report examining the effect of PPAR β/δ ligands in a lung cancer model demonstrated inhibition of proliferation with an A549 human lung cancer cell line by 10-40 μ M L165041 that was associated with down-regulation of CYCLIN D1 and PCNA, and a block in the G1 phase of the cell cycle [97]. Combining ligand activation of PPAR β/δ with administration of indomethacin caused enhanced inhibition of cell proliferation in A549 cells that was associated with a block in the G1 phase of the cell cycle and increased apoptotic signaling [97]. These observations are consistent with results from in vivo models showing exacerbated Raf-dependent lung tumorigenesis in the absence of PPAR β/δ expression [98], and inhibition of lung tumorigenesis by increasing the production of the PPAR β/δ ligand prostacyclin [99, 100]. In contrast to these studies, others revealed that activating PPAR β/δ with GW501516 caused increased cell proliferation of human lung cancer cell lines. For example, ligand activation of PPAR β/δ with 0.001-10 μ M GW501516 was reported to cause increased proliferation of A549, H23, H157, H1838 and H2106 human lung cancer cell lines [101-103]. Several mechanisms were postulated to explain this effect including PI3 kinase/AKT-mediated up-regulation of the EP4 receptor for PGE₂, PPAR β/δ -dependent down-regulation of PTEN and increased phosphorylation of AKT [101-103]. However, analysis by others is inconsistent with these mechanisms as no changes in proliferation of A549 or H1838 cells are observed in response to 0.1-10.0 μ M GW0742 or GW501516, and no changes in expression of PTEN, PDPK1 or phosphorylation of AKT were found following ligand activation of PPAR β/δ in these cells [104].

Four studies have examined the effect of ligand activation in liver cancer models. Culturing the human hepatoma cell line HepG2 with 0.001-0.1 μ M GW501516 caused an increase in cell proliferation that was associated with elevated expression of COX2 [105]. A subsequent study reported similar findings with 0.005-0.05 μ M GW501516 causing an increase in proliferation of HepG2, Huh7 and Hep3B cells [106]. These authors provided evidence suggesting that ligand activation of PPAR β/δ caused an increase in COX2 expression thereby increasing PGE₂ and cytosolic phospholipase A2 (cPLA2) activity thus providing a potential feedback loop to drive tumor cell growth [106]. The same group proposed a similar mechanism for

cholangiocarcinomas [107]. However, this putative mechanism is based on the idea that ligand activation of PPAR β/δ increases expression of COX2 and when examined critically in the same liver cancer cell lines (HepG2 and Huh7), no change in COX2 expression was observed, despite confirming specific ligand activation of PPAR β/δ [88]. Further, only inhibition of cell proliferation by ligand activation of PPAR β/δ with 0.1-10.0 μ M GW0742 or GW501516 was observed in a separate study, while no increased cell proliferation was noted [88].

In addition to colon, breast, lung and liver cancer models, there are also limited reports describing the effect of ligand activation of PPAR β/δ in other cancer models. In a rat glioma cell line (BT4Cn), modestly increased cell proliferation is found in response to 1.0 μ M L165041 [108]. However, inhibition of BT4Cn cell proliferation was observed following culture in TTA [108], a compound that can activate PPAR β/δ . However, this study did not specifically determine whether this effect was due to PPAR β/δ but rather focused on PPAR γ and found that PPAR γ -independent mechanisms likely explained the growth inhibition by TTA. In a human melanoma cell line, UACC903, ligand activation of PPAR β/δ inhibits cell proliferation through an unknown mechanism [96]. Carbaprostacyclin inhibits cell proliferation of primary human myeloma cells and a human myeloma cell line and PPAR β/δ mediates inhibition of balcainein-induced inhibition of human myeloma cell proliferation, which may be due in part to inhibition of NF- κ B activity [109]. Culturing the human neuroblastoma cell line SH-SY5Y with 1.0 μ M GW0742 causes inhibition of cell proliferation that is associated with down-regulation of CYCLIN D1, PCNA, BCL2, increased expression of p16 and induction of terminal differentiation [110]. Inhibition of cell proliferation is reported in 786-O renal carcinoma cells following culture in 20-30 μ M GW501516 through unknown mechanisms [111]. Increased cell proliferation is found in some (PNT1A, LnCaP) but not other (DU145, PC3) human prostate cancer cell lines following culture in 0.01 μ M compound F [66].

6. Regulation of PPAR β/δ expression as a modulator of cell proliferation

Some reports examining the effect of PPAR β/δ have focused on the expression of the receptor rather than on ligand activation. Many of these studies have inherent limitations. For example, there are reports suggesting that increased expression of PPAR β/δ is negatively associated with cancer progression [2,112-116]. Some of these studies base the negative association between PPAR β/δ and cancer on mRNA expression or non-quantitative examination of PPAR β/δ protein. Importantly, none of these studies demonstrated that the relative presence of PPAR β/δ caused increased transcriptional activity of target genes. In the absence of these data, it is possible that the reported increase in PPAR β/δ mRNA is not reflected at the protein and/or functional level. Some studies have also suggested similar negative associations between PPAR β/δ expression and cancer using immunohistochemistry [114,115]. Unfortunately, even highly-specific PPAR β/δ antibodies exhibit significant non-specific immunoreactivity [9]. For this reason, immunohistochemical analysis of PPAR β/δ must be viewed with caution. Indeed, using samples obtained from the Ouyang study, recent collaborative findings demonstrate with quantitative western blotting, that expression of PPAR β/δ is not higher in *APC^{min}* heterozygous mouse colon [87], as previously suggested based solely on immunohistochemistry [115]. This also reinforces the notion that direct evidence of alterations in well characterized PPAR β/δ target genes should be included in analysis examining the effect of PPAR β/δ on cell growth. Lastly, some reports suggest that non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit cancer cell growth by down-regulating expression of PPAR β/δ [117,118]. However, these studies base their conclusions on extremely limited datasets and are inconsistent with more recent quantitative analysis showing either no change or increased expression of PPAR β/δ by NSAIDs in the same cancer cell lines [87]. Importantly, the latter studies also demonstrated consistent changes in expression of known PPAR β/δ target genes that correlated well with expression of PPAR β/δ [87], while the former studies provide no such comparison [117,118]. Combined, studies examining the role of PPAR β/δ in cell proliferation

that base their conclusions in part on expression of PPAR β/δ without direct evidence of distinct modulation of PPAR β/δ in their model system should be viewed with caution.

7. Gaps in Knowledge

It is clear from review of the literature that our understanding of the role of PPAR β/δ in cell proliferation and the mechanisms involved remain limited. There are a number of important questions that must be answered before this issue can be clarified. Why does ligand activation of PPAR β/δ cause inhibition of cell proliferation in normal mouse and human keratinocytes but increased cell proliferation in human keratinocytes from psoriasis patients? What are the direct target genes that mediate PPAR β/δ -dependent terminal differentiation in keratinocytes? How can PPAR β/δ inhibit cell proliferation in some models but increase cell proliferation in others? This is of particular interest for EC and VSMC since some studies indicate that ligand activation of PPAR β/δ will promote cell proliferation in EC but inhibit cell proliferation in VSMC. As PGI₂ is produced in EC and can function as a PPAR β/δ ligand, why are anti-apoptotic activity and/or increased cell growth observed in EC but inhibition of cell proliferation is found in VSMC that are juxtaposed to EC? Is there a molecular difference in PPAR β/δ between these cell types that explain this difference? Are there species differences in the response to ligand activation of PPAR β/δ ? This is a possibility that might explain why the literature suggests that ligand activation of PPAR β/δ in mouse and bovine EC may cause inhibition of cell proliferation while they increased proliferation in human EC. Severe pulmonary hypertension is associated with proliferation of ECs, and prostacyclin analogs are routinely used to treat this disease [119]. Since prostacyclin analogs can activate PPAR β/δ , why is EC proliferation not exacerbated in patients with severe pulmonary hypertension that are treated with prostacyclin analogs? Serum withdrawal-induced apoptosis in colon cancer cell lines can be inhibited by ligand activation of PPAR β/δ in some studies and this also reflected in vivo. Since the cellular milieu contains serum and growth factors, and apoptotic signaling in cancer cell lines is not typically affected by ligand activation of PPAR β/δ in the absence of apoptosis inducing agents, how can these differences be reconciled? Do anti-apoptotic effects observed in in vitro models when apoptosis is induced reflect physiological mechanisms? PPAR β/δ is expressed at high levels in liver, intestine and keratinocytes with evidence of constitutive gene activation activity, possibly due to the presence of endogenous ligands. There are a number of essential physiological roles for PPAR β/δ including regulating skeletal muscle fatty acid catabolism and glucose homeostasis. How can a nuclear receptor maintain essential physiological roles and also function to promote tumorigenesis? Is PPAR β/δ up-regulated by the APC/ β -CATENIN/TCF4 pathway? Given the redundancy in DNA response elements for all three PPARs, how could activation of PPAR γ inhibit tumorigenesis while activation of PPAR β/δ promotes tumorigenesis? Answering these questions with sound experimentation will provide significant insight for these disparities in the literature regarding the role of PPAR β/δ in cell proliferation.

8. Concluding remarks

Given the variation in responses reported for the same cell types and/or models, it is essential that critical examination of all endpoints be performed using highly stringent criteria, in order to clarify how activating PPAR β/δ influences cell proliferation. For example, specific demonstration of PPAR β/δ activity cannot rely solely on examination of mRNA expression of PPAR β/δ and should include examination of known PPAR β/δ target genes (e.g. *ADRP*, *FABP*, *ANGPTL4*, etc.). The use of knockout/knockdown approaches to confirm specificity is also highly suitable. The use of positive controls, in particular for examining PPAR β/δ expression since anti-PPAR β/δ antibodies exhibit significant non-specific immunoreactivity, should always be included.

There are a number of mechanisms described suggesting regulatory roles for PPAR β/δ in cell proliferation that have not been repeated and confirmed by independent laboratories. For example, the putative PPAR β/δ -dependent pathway described suggesting that PPAR β/δ causes down-regulation of PTEN expression, increased expression of ILK/PDPK1, increased phosphorylation of AKT and anti-apoptotic activity in keratinocytes [42] is based on data from cells that do not exhibit well characterized expression markers (e.g. K6). Careful re-examination of this pathway in mouse keratinocytes that express K6 demonstrated that this pathway does not function in normal mouse keratinocytes [25]. Changes in the PTEN/ILK/PDPK1/AKT pathway are also not found in human keratinocyte cell lines in response to ligand activation of PPAR β/δ [24,25]. However, there are many reports in the literature that incorrectly cite this report [42] as having conclusively demonstrated PPAR β/δ -dependent anti-apoptotic activity without critically examining the literature. Similar examples exist for other molecular pathways that have not been replicated by independent laboratories including the idea that PPAR β/δ is up-regulated by the APC/ β -CATENIN/TCF4 pathway ([83], reviewed in [5]), that PPAR β/δ up-regulates VEGF expression leading to increased phosphorylation of AKT and anti-apoptotic signaling [85,88], that NSAIDs inhibit PPAR β/δ expression leading to anti-apoptotic activity [87,117], that retinoic acid activates PPAR β/δ and potentiates cell proliferation [24,43,48], or other examples related to PPAR β/δ -dependent regulation of PDPK1 and AKT [102-104]. These reported differences emphasize the need to question all reported findings until they have been repeated and confirmed by independent laboratories. A collaborative effort between laboratories reporting disparate results is one approach that can clarify some of these misunderstandings. For example, the recent correction in the literature showing that PPAR β/δ expression is not up-regulated in *APC*^{min} mouse colon or down-regulated by an NSAID [87] as once suggested [115] is a significant advance. Similar collaborations would also move this field forward.

Given the large differences reported in the literature, there is a need to critically examine basic, functional roles for PPAR β/δ . Collaborative efforts focusing on the effect of ligand activation of PPAR β/δ in a large variety of cells and cell lines using complementary quantitative analysis including dose-dependent examination of cell proliferation over time by multiple methods (Coulter counting, BrdU labeling, ³H-thymidine uptake, and assorted cell based systems), examination of dose-dependent changes in cell cycle kinetics (flow cytometry) and comparative analysis of PPAR β/δ -dependent changes in known PPAR β/δ target gene expression is clearly needed. Until such analyses is undertaken and confirmed by multiple independent laboratories, it is likely that the literature will remain confusing to those who are not experts in the field.

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Fig. 1.
Chemical structure of ligands for PPAR β/δ .

Fig. 2. Modulation of gene expression by PPARβ/δ. (A) Transcriptional up-regulation of direct PPARβ/δ target genes. (B) Epigenetic regulation of gene expression by PPARβ/δ. PPARβ/δ can interfere with other transcription factors by physical interactions and inhibit expression of these gene products. This type of inhibition has been demonstrated for a number of inflammatory signaling cascades, including NF-κB, STAT3, BCL6 and AP1. (C) Transcriptional repression

Table 1Regulation of cell proliferation in skin and keratinocytes by PPAR β / δ .

Model	Effect	Reference
Homozygous <i>Ppar</i> β / δ -null mouse skin (disrupted by neomycin cassette in last exon of ligand binding domain) treated topically with phorbol ester	Enhanced hyperplasia in null mouse skin as compared to wild-type mouse skin	[22]
Heterozygous <i>Ppar</i> β / δ -null mouse skin (disrupted by neomycin cassette between exon 4 and 5 encoding the DNA binding domain) treated topically with phorbol ester	Enhanced hyperplasia in null mouse skin as compared to wild-type mouse skin	[21]
Homozygous <i>Ppar</i> β / δ -null mouse skin (disrupted by deleting exon 4 of the DNA binding domain)	Increased epidermal thickness and PCNA-positive keratinocytes in null mouse skin as compared to wild-type mouse skin	[23]
Mouse skin	Enhanced hyperplasia in null mouse skin as compared to wild-type mouse skin	[31]
Mouse skin	Enhanced hyperplasia in null mouse skin as compared to wild-type mouse skin, prevented by ligand activation of PPAR β / δ ; an effect not found in null mouse skin	[30]
Normal human keratinocytes	0.05 – 0.5 μ M L165041 and 0.2 – 5.0 μ M TTA inhibit cell proliferation	[26]
N/TERT-1 human keratinocyte cell line	1.0 μ M GW0742 inhibits cell proliferation	[25]
Human NCTC 2544 keratinocyte cell line	0.05 – 1.0 mM DEHP inhibits cell proliferation	[27]
Human HaCaT keratinocyte cell line	1.0 – 10 μ M GW0742 inhibits cell proliferation and increases apoptosis; no effect on serum withdrawal-induced apoptosis	[24]
Mouse primary keratinocytes	0.1 – 1.0 μ M GW0742 inhibits cell proliferation	[24]
Mouse primary keratinocytes	Enhanced cell proliferation in null keratinocytes as compared to wild-type	[28]
Mouse primary keratinocytes	0.01 – 0.1 μ M GW0742 inhibits cell proliferation	[29]
Mouse skin	Topical application of 4 mM GW1514 has no effect on epidermal cell proliferation	[32]
Human primary keratinocytes from psoriasis patients	1.0 μ M L165041 and 5.0 μ M GW501516 increases cell proliferation	[33]

Table 2Role of PPAR β/δ on cell proliferation and apoptosis in somatic cells

Cell type	Effect	Reference
Bovine endothelial cells	50 –100 μ M ciprostone (prostacyclin analog) inhibits cell proliferation	[62]
Mouse primary endothelial cells	Increased cell proliferation in <i>Pparβ/δ</i> -null endothelial cells as compared to wild-type	[63]
Human primary endothelial cells	100 μ M carbaprostacyclin or 50 μ M L165041 has no effect on annexin V-positive cells or caspase 3 activity	[64]
Human endothelial cells and a human endothelial cell line (EAHy926)	0.1 μ M GW501516 causes an increase in cell proliferation after 72 hours	[65]
Human primary endothelial cells	0.01 – 0.1 μ M GW501516 causes an increase in cell proliferation after 14 days	[66]
Human primary endothelial progenitor cells	0.1 – 1.0 μ M GW501516 causes an increase in cell proliferation after 8 days	[67]
Human primary endothelial cells	1 – 100 μ M carbaprostacyclin and 10-50 μ M L165041 decrease H ₂ O ₂ -induced apoptosis	[64,68]
Human primary endothelial progenitor cells	0.1 – 1 μ M GW501516 decrease hypoxia-induced apoptosis	[67]
Rat vascular smooth muscle cells	1.0 μ M iloprost (prostacyclin analog) inhibits cell proliferation	[71]
Rat vascular smooth muscle cells	Overexpression of prostacyclin synthase inhibits cell proliferation	[70]
Bovine vascular smooth muscle cells	3 –100 μ M ciprostone (prostacyclin analog) inhibits cell proliferation	[62]
Human vascular smooth muscle cells	0.1 – 10 μ M prostacyclin analogs (iloprost, cicaprost, UT-15) inhibits cell proliferation	[69]
Rat vascular smooth muscle cells	1.0 μ M beraprost (prostacyclin analog) inhibits cell proliferation; mediated by PPAR β/δ	[73]
Rat vascular smooth muscle cells	5 – 10 μ M L165041 inhibits PDGF-induced cell proliferation inhibits cell proliferation	[72]
Mouse primary hepatocytes	TNF α -induced incorporation of BrdU is enhanced in <i>Pparβ/δ</i> -null hepatocytes as compared to wild-type	[19]
Rat primary stellate cells	10 μ M L165041 increase cell proliferation	[74]
Mouse primary stellate cells	Increased cell proliferation in <i>Pparβ/δ</i> -null stellate cells as compared to wild-type	[19]
Human lung fibroblasts	0.1 – 10 μ M treprostinil (prostacyclin analog) and 0.1 – 100 μ M GW0742 inhibit cell proliferation	[75]
Mouse lung fibroblasts	100 μ M treprostinil (prostacyclin analog) inhibits cell proliferation; effect not found in <i>Pparβ/δ</i> -null fibroblasts	[75]
Rat cardiac fibroblasts	10 μ M GW501516 inhibits cell proliferation	[76]
Rat cardiac fibroblasts	10 μ M GW501516 inhibits cell proliferation	[77]
Mouse embryonic fibroblasts	Overexpression of PPAR β/δ increases cell proliferation	[78]
Human proximal tubular epithelial cell line (HK-2)	0.2 – 1.0 μ M L165041 decrease H ₂ O ₂ -induced apoptosis	[79]
Human embryonic kidney epithelial cell line (HEK-293)	Increased intracellular prostacyclin increases apoptosis; mediated by PPAR β/δ	[81]
Mouse inner medullary collecting duct cell line (IMCD-K2)	10 μ M GW501516 inhibits cell proliferation; 0.01 – 1.0 μ M GW501516 has no effect on anisomycin-induced apoptosis	[82]

Table 3Role of PPAR β/δ on cell proliferation and apoptosis in cancer cell lines

Cell type	Effect	Reference
HCT116 human colon cancer cell line	0.1 – 5.0 μ M GW501516 inhibits serum withdrawal-induced apoptosis	[84]
LS174T human colon cancer cell line	0.1 – 1.0 GW501516 inhibits serum withdrawal-induced apoptosis	[86]
LS174T human colon cancer cell line	1.0 GW501516 inhibits serum withdrawal-induced apoptosis	[85]
HT29, SW480 and HCA7 human colon cancer cell lines	0.01 μ M compound F has no effect on cell proliferation	[66]
LS174T, HCT116, HT29 human colon cancer cell lines	0.1 μ M GW501516 or GW0742 has no effect on cell proliferation. 1.0 – 10 μ M GW501516 or GW0742 inhibits cell proliferation. 0.1 – 10 μ M GW501516 or GW0742 has no effect on serum withdrawal-induced apoptosis.	[88]
HCT116 human colon cancer cell line	Silencing PPAR β/δ expression increases cell proliferation	[90]
MCF7, T47D human breast cancer cell lines (estrogen receptor-positive)	0.01 μ M compound F or up to 0.05 μ M GW501516 increase cell proliferation	[66]
BT20, MDA-MB-231 human breast cancer cell lines (estrogen receptor-negative)	0.01 μ M compound F has no effect on cell proliferation	[66]
MCF7 human cancer cell line	No effect of cell proliferation with 0.1 – 1.0 μ M GW501516 or GW0742, inhibition of cell proliferation with 10 μ M GW501516 or GW0742	[96]
A549 human lung cancer cell line	10 – 40 μ M L165041 inhibits cell proliferation	[97]
H157 human lung cancer cell line	1.0 – 10.0 μ M GW501516 increases cell proliferation	[101]
1838 human lung cancer cell line	1.0 μ M GW501516 increases cell proliferation	[102]
A549, H23, H157 human lung cancer cell lines	0.001 – 0.01 μ M GW501516 increase cell proliferation; 0.01 – 0.1 μ M GW501516 inhibits cisplatin-induced apoptosis	[103]
A549, H1838 human lung cancer cell lines	0.1 – 10.0 μ M GW501516 has no effect on cell proliferation	[104]
HepG2 human liver cancer cell line	0.001 – 0.1 μ M GW501516 increases cell proliferation	[105]
HepG2, Huh7, Hep3B human liver cancer cell lines	0.005 – 0.05 μ M GW501516 increases cell proliferation	[106]
CCLP1, HuCCT1, SG231 human cholangiocarcinoma cell lines	0.001 μ M – 0.05 μ M GW501516 increases cell proliferation	[107]
HepG2, Huh7 human liver cancer cell lines	No effect of cell proliferation with 0.1 – 1.0 μ M GW501516 or GW0742, inhibition of cell proliferation with 10 μ M GW501516 or GW0742	[88]
BT4Cn rat glioma cell line	1.0 μ M L165041 increases cell proliferation	[108]
BT4Cn rat glioma cell line	100 μ M TTA inhibits cell proliferation	[108]
UACC903 human melanoma cell line	1.0 – 10.0 μ M GW501516 or GW0742 inhibit cell proliferation	[96]
U266 human myeloma cell line and primary human myeloma cells	10 – 50 μ M carbaprostacyclin inhibits cell proliferation	[109]
SH-SY5Y human neuroblastoma cell line	1.0 μ M GW0742 inhibits cell proliferation	[110]
786-O human renal carcinoma cell line	20 – 30 μ M GW501516 inhibits cell proliferation	[111]
PNT1A, LnCaP human prostate cancer cell lines	0.01 μ M compound F increases cell proliferation	[66]
DU145, PC3 human prostate cancer cell lines	0.01 μ M compound F has no effect on cell proliferation	[66]