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Regulatory mechanisms mediated by peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in skin cancer

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Abstract

Considerable progress has been made during the past twenty years towards elucidating the role of peroxisome proliferator-activated receptor-β/δ (PPARβ/δ) in skin cancer. In 1999, the original notion that PPARβ/δ was involved with epithelial cell function was postulated based on a correlation between PPARβ/δ expression and the induction of mRNAs encoding proteins that mediate terminal differentiation in keratinocytes. Subsequent studies definitively revealed that PPARβ/δ could induce terminal differentiation and inhibit proliferation of keratinocytes. Molecular mechanisms have since been discovered to explain how this nuclear receptor can be targeted for preventing and treating skin cancer. This includes the regulation of terminal differentiation, mitotic signaling, endoplasmic reticulum stress, and cellular senescence. Interestingly, the effects of activating PPARβ/δ can preferentially target keratinocytes with genetic mutations associated with skin cancer. This review provides the history and current understanding of how PPARβ/δ can be targeted for both non-melanoma skin cancer and melanoma, and postulates how future approaches that modulate PPARβ/δ signaling may be developed for the prevention and treatment of these diseases.

Keywords

peroxisome proliferator-activated receptor-β/δ; skin cancer; cell cycle; differentiation

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1. INTRODUCTION

Peroxisome proliferator-activated receptor-α (PPARα) was the first PPAR identified in 1990[1] and later confirmed to be the key ligand-activated transcription factor that mediates the pleiotropic effects induced by PPARα including serum lipid lowering and rodent-specific hepatocarcinogenesis.[2–7] Two more PPAR isoforms were discovered shortly thereafter and termed PPARβ/δ (also known as PPARδ or PPARβ), and PPARγ.[8,9] Initially, the regulation of ligand-activated transcription factors including nuclear receptors such as PPARβ/δ was described as a static mechanism (Fig. 1). This mechanism was thought to be initiated by ligand binding to PPARβ/δ complexed with proteins that caused a conformational change in PPARβ/δ. (reviewed in[10]) This conformational change allowed for dissociation of co-repressor proteins that have histone deacetylase activity and recruitment of co-activator proteins that have histone acetyltransferase activity, scaffolding proteins, and RNA polymerase. The ligand bound, activated PPARβ/δ complex could then regulate expression of target genes that was dependent on PPARβ/δ-specific response elements in the DNA regulatory region of genes, usually upstream of transcription start sites. However, it is now known that this static mechanism of nuclear receptor-mediated regulation of transcription, such as that by PPARβ/δ, is actually dynamic in nature (Fig. 1) and constitutively occurring in cells due to the presence of endogenous and exogenous ligands that bind to and modulate PPARβ/δ activity (reviewed in[10]). This has been shown in many cell types including keratinocytes by studies showing that PPARβ/δ is localized in the nucleus,and can be co-immunoprecipitated with PPARβ/δ and its heterodimerization partner retinoic acid X receptor.[11] In other words, the presence of endogenous ligands allows for PPARβ/δ to bind to and modulate activity/expression of target genes in chromatin that becomes available by the activities of other regulatory proteins in the nucleus.[10] This is supported by studies showing in the absence of PPARβ/δ in keratinocytes, expression of PPARβ/δ target genes is markedly increased and decreased without treating the cells with exogenous ligands.[10] Thus, PPARβ/δ is able to regulate homeostasis based on the daily changes in the intracellular flux of endogenous ligands, levels of expression of the receptor, and activities of chromatin remodeling proteins that occurs in response to fasting and feeding, as well as exposure to different physiological conditions or environmental factors.

Since their discovery, the role of PPARs in cellular physiology has emerged extensively due in large part to technological advances such as the generation of highly specific and unique agonists and antagonists, transgenic animal and cell-based models, genome editing facilitated by CRISPR/Cas9 models, and other molecular biology and biochemical models developed in the past 20–30 years. It is well accepted that PPARα is a central regulator of fatty acid catabolism, but also has other roles in normal homeostasis and in rodent-specific hepatocarcinogenesis. (reviewed in[12–15]) By contrast, PPARγ is one of a number of transcription factors that modulates adipogenesis, and has also been shown to have good potential of targeting for cancer chemoprevention and chemotherapy[15,16]. Whereas the role of PPARα, PPARγ, and PPARβ/δ in normal physiology is also clear, how PPARβ/δ modulates cancer remains less certain in most instances due to contradictory studies. (reviewed in[15,17–23]) However, the functional roles of PPARβ/δ in skin function and cancer have been elucidated and the findings are less contentious (Table 1).

2. PPARβ/δ-DEPENDENT REGULATION OF TERMINAL DIFFERENTIATION AND PROLIFERATION OF KERATINOCYTES

The first observation made suggesting that PPARβ/δ was involved in skin homeostasis was the correlation between increased expression of the mRNAs encoding this nuclear receptor and several proteins involved in squamous differentiation (e.g. transglutaminase I (TGI), small proline rich proteins (SPR; also known as CORNIFIN) in normal human keratinocytes in response to phorbol ester.[24] Increased expression of *Pparb/d* mRNA was also noted in CD-1 mouse skin following treatment with phorbol ester as compared to control.[24] Considerable interest was generated by these results. Subsequent studies with a *Pparb/d*-null mouse model more definitively determined whether PPARβ/δ was required to mediate these effects. Surprisingly, while topical administration of phorbol ester markedly increased expression of PPARβ/δ that was associated with increased expression of TG-1 and SPRs, these changes were also observed in similarly treated *Pparb/d*-null mouse skin.[25] This suggested that PPARβ/δ was not required for phorbol ester-induced terminal differentiation in skin. However, subsequent studies reported that treatment of both mouse and human keratinocytes with the PPARβ/δ ligands GW501516 or L165041[26] caused an increase in the expression of proteins that mediate terminal differentiation and promote improved barrier function.[27,28] That ligand activation of PPARβ/δ promotes terminal differentiation was definitely proven by demonstrating that the increase in expression of genes required for inducing terminal differentiation by another PPARβ/δ ligand GW0742 and the associated increased in cornified cells were only observed in wild-type mice but not in similarly treated *Pparb/d*-null mouse skin and keratinocytes.[29] Thus, one mechanism by which PPARβ/δ can regulate skin homeostasis is through promoting terminal differentiation (Fig. 2A). This is of interest because ligand activation of PPARβ/δ also induces terminal differentiation in many other cell types. (reviewed in[21,23,30])

Given that ligand activation of PPARβ/δ promotes terminal differentiation in skin, and the induction of terminal differentiation is associated with a concomitant inhibition of cell proliferation,[31,32] it is not surprising that early studies also revealed that PPARβ/δ attenuates keratinocyte proliferation. This was first demonstrated with in vivo models where topical application of phorbol ester caused exacerbated epidermal proliferation in *Pparb/d*-deficient mouse skin as compared to controls.[25,33] Moreover, expression of proliferating cellular nuclear antigen (PCNA) was also higher in *Pparb/d*-null mouse skin as compared to wild-type mouse skin.[34] These observations were extended by studies showing that ligand activation of PPARβ/δ with GW0742 or L165041 indeed inhibits keratinocyte proliferation in both in vitro and in vivo models. It is important to note that these effects were found in three different *Pparb/d*-deficient mouse models, mouse primary keratinocytes, and human and mouse cell lines,[29,35–39] thus providing strong independent evidence of reproducibility.

The physiological role of PPARβ/δ in promoting terminal differentiation and inhibiting keratinocyte proliferation made it a promising target for skin cancer chemoprevention and/or chemotherapy.

3. PPARβ/δ AND KERATINOCYTE PROGRAMMED CELL DEATH

In contrast to the well-established roles for PPARβ/δ in regulating keratinocyte and differentiation and proliferation, the role of PPARβ/δ in keratinocyte programmed cell death is not as clear. The continual induction of terminal differentiation of basal keratinocytes into cornified keratinocytes (envelopes) is known to be associated with a form of programmed cell death, leading to the formation of the anucleated keratinocytes of the stratum corneum. While the differentiation process shares some similarities with apoptosis (another form of programmed cell death), it is important to note that the type of programmed cell death signaling in differentiating keratinocytes is highly specialized to the cornification process and differs substantially from apoptosis.[40–42] For example, differentiating keratinocytes is mediated in part by caspase 14, rather than classical caspase 3, 6 or 7.[41] It is thus surprising when others suggested that ligand activation of PPARβ/δ with L165041 caused down-regulated expression of phosphatase and tensin homologue deleted on chromosome ten (PTEN) and upregulated expression of 3-phosphoinositide-dependent kinase-1 (PDPK1) and integrin-linked kinase-1 (ILK1), that collectively caused an increase in the phosphorylation of protein kinase B (AKT1) and markedly inhibited apoptosis in keratinocytes.[43] Since these effects were observed in primary keratinocytes from wild-type mice but not in primary keratinocytes from *Pparb/d*-null mice, this implied that these effects were mediated by PPARβ/δ; but they are contradictory to the known unique form of programmed cell death in keratinocytes.[40–42] Additionally, the hypothetical pathway that ligand activation of PPARβ/δ promotes anti-apoptotic signaling in keratinocytes through the proposed PTEN/PDPK1/ILK1/AKT1 pathway is not supported by data showing that ligand activation of PPARβ/δ with GW0742 in human N/TERT-1 keratinocytes increased expression of the known PPARβ/δ target gene adipose differentiation-related protein (*ADRP*) but caused no change in the expression of PTEN, PDPK1, ILK1 or phosphorylation of AKT1 as compared to controls.[38] Importantly, these analyses included temporal examination over a twenty-four hour period.[38] In the same studies, ligand activation of PPARβ/δ with GW0742 in mouse primary keratinocytes also caused a marked increase in the expression of *Adrp* mRNA but no changes in the expression of PTEN, ILK1, PDPK1, or phosphorylation of AKT1 as compared to controls were noted.[38] Additionally, changes in the expression of PTEN, ILK1 or PDPK1 are not observed following treatment with GW0742, whereas numerous PPARβ/δ target genes are induced by GW0742 in wild-type but not *Pparb/d*-null keratinocytes.[10] One likely explanation for the differences between these two studies is that the primary keratinocytes used by Di-Poi and colleagues did not exhibit the morphology of primary keratinocytes[44] and the constitutive expression of keratin 6 (K6), a standard marker of hyperproliferation typically observed in primary keratinocytes was lacking,[45] whereas expression of this marker was present in the cells used in the latter studies.[29] Thus, the cells used by Di-Poi and colleagues that resembled keratinocytes do not appear to be keratinocytes and this may explain the observed disparity between studies. Other studies also do not support the hypothesis that ligand activation of PPARβ/δ down-regulates expression of PTEN and upregulates expression of PDPK1 and ILK1, that collectively caused an increase in the phosphorylation of AKT1 and inhibition of keratinocyte apoptosis as suggested by others.[43] For example, ligand activation of PPARβ/δ in human HaCaT cells caused no change in AKT1 phosphorylation, and while no change in apoptosis was observed

in HaCaT cells following ligand activation of PPARβ/δ at relatively low concentration of the PPARβ/δ ligand GW0742, a relatively higher concentration of this PPARβ/δ ligand caused an increase in early apoptosis in human HaCaT keratinocytes compared to controls.[37] Over-expression and/or ligand activation of PPARβ/δ with GW0742 did not have any influence on staurosporine- or ultraviolet (UV) light-induced apoptosis in human HaCaT cells as compared to controls.[46] Further, ligand activation of PPARβ/δ with GW501516 was reported to increase expression of PTEN and inhibit phosphorylation of AKT1 in human keratinocytes.[47] Moreover, bioinformatic analyses of microarray and chromatin immunoprecipitation sequencing (ChIP-seq) data revealed that ligand activation of PPARβ/δ with GW0742 did not cause changes in PTEN/ILK1/PDPK1 expression or promoter occupancy of PPARβ/δ on any of these genes in primary keratinocytes following ligand activation of PPARβ/δ[10] as suggested by a previous study.[43] These studies are also consistent with others experiments showing that ligand activation of PPARβ/δ with GW0742 or GW501516 in other cell types may actually increases PTEN expression and inhibit phosphorylation of AKT1.[48–50] Combined, the evidence that ligand activation of PPARβ/δ inhibits apoptosis in keratinocytes via modulation of PTEN/ILK1/PDPK1/AKT1 is inconsistent with many other studies, including those that have shown there is a unique form of programmed cell death associated with keratinocyte differentiation that differs substantially from apoptosis.[40–42]

4. PPARβ/δ-DEPENDENT REGULATION OF NON-MELANOMA SKIN CANCER: MULTIPLE REGULATORY MECHANISMS

The first evidence that PPARβ/δ could be targeted for non-melanoma skin cancer was the observation that *Pparb/d*-null mice exhibited exacerbated chemically-induced skin carcinogenesis using the classical two-stage chemical carcinogenesis bioassay[51] (a single topical application of dimethylbenzo[*a*]pyrene (DMBA) followed by multiple, weekly topical application of a tumor promoter such as phorbol ester). This model was used for many years to study non-melanoma skin cancer as it allows for dissecting the role of initiation of DNA damage versus tumor promotion. The mechanisms underlying the cancer chemopreventive effect modulated by PPARβ/δ have since been examined extensively in the two-stage chemical carcinogenesis and related models.

To determine if PPARβ/δ influenced DNA damage induced by DMBA, the effect of a topical application on the expression of xenobiotic metabolizing enzymes required for bioactivation of polycyclic aromatic hydrocarbons (PAH) to a mutagenic metabolite was examined in wild-type and *Pparb/d*-null mice. Expression of cytochrome P450s (CYPs) required for bioactivation of PAH was markedly reduced in *Pparb/d*-null mouse skin following topical application of different PAHs as compared to similarly treated wild-type mice.[52] This study suggested that PPARβ/δ influenced the function of the aryl hydrocarbon receptor (AHR). This difference was not due to altered expression of AHR, aryl hydrocarbon receptor nuclear transporter, or heat shock protein 90.[52] The relative ability of PAH to bind to the AHR and the temporal translocation of the AHR into the nucleus also did not explain the observed differences.[52] However, occupancy of AHR on the *Cyp1a1* enhancer region was increased only in wild-type mouse skin but not in *Pparb/d*-null mouse skin by topical

exposure to a PAH.[52] The difference in the ability of the AHR to bind to the enhancing element of the xenobiotic metabolizing enzyme CYP1A1 in *Pparb/d*-null mouse skin was likely due to differences in the methylation pattern in this region that was mediated by a mechanism that required PPARβ/δ.[52] These results suggest that the absence of PPARβ/δ expression modulates the epigenome. This was supported by functional analysis showing that the number of DNA adducts following topical application of PAH was lower in *Pparb/d*-null mouse skin as compared to similarly treated wild-type mice.[52] Since *Pparb/d*-null mice exhibit enhanced non-melanoma skin cancer as compared to wild-type mice in the two-stage chemical carcinogenesis bioassay, this suggests that PPARβ/δ likely regulates mechanisms that modulate tumor promotion rather than initiation of DNA damage (Table 1).

Since the absence of PPARβ/δ expression leads to enhanced non-melanoma skin cancer as compared to wild-type mice in a two-stage chemical carcinogenesis bioassay,[51] this suggested that ligand activation of PPARβ/δ may inhibit chemically-induced skin cancer. Indeed, multiple bioassays were performed revealing that activation of PPARβ/δ by topical application of GW0742 can markedly inhibit the onset of skin tumor formation, and decrease tumor multiplicity, and this effect is not found in similarly treated *Pparb/d*-null mice.[35,36,51,53] While it was of interest to determine whether ligand activation of PPARβ/δ could inhibit malignant conversion of benign papillomas to squamous cell carcinomas (SCC), the number of SCC observed in two-stage chemical carcinogenesis bioassays is low when the genetic background of the mice is C57BL/6. An alternative strategy was used to examine this hypothesis by determining the effect of ligand activation of PPARβ/δ using mouse cell lines that exhibited phenotypes ranging from benign papillomas to SCC (308 cells, SP1 cells, and Pam212 cells). Ligand activation of PPARβ/δ with GW0742 inhibited proliferation of all three cell lines as compared to controls,[35,36] so these models did not distinguish between effects that might be induced during early tumorigenesis versus those that could be induced when a cell is more transformed in nature. These observations also suggested that ligand activation of PPARβ/δ was likely effective for both cancer chemoprevention and chemotherapy; consistent with past studies examining both of these model systems.[35,36,51,53]

The role of PPARβ/δ has also been examined using A431 cells, a human SCC cell line with mutant *EGFR* and *TP53* (the latter a common mutation observed in UV light-induced non-melanoma skin cancer). The growth of ectopic xenografts derived from stable A431 cells over-expressing PPARβ/δ was markedly inhibited and essentially negligible as compared to controls A431 cells.[54] Interestingly, ligand activation of PPARβ/δ with GW0742 did not further influence this striking inhibition.[54] These observations collectively provide support that PPARβ/δ also inhibits human non-melanoma skin cancer associated with mutant *TP53* and suggest that there are endogenous ligands for PPARβ/δ that may promote this phenotype in cells that exhibit relatively high expression of PPARβ/δ such as keratinocytes.[10,11]

One of the first mechanisms examined to determine how ligand activation of PPARβ/δ inhibits non-melanoma skin cancer was the induction of terminal differentiation (Fig. 2B, Table 1). This is due to the fact that inducing terminal differentiation is known to cause withdrawal from the cell cycle,[31] a feature that has been targeted for impeding the growth of cancer cells.[55] Increased expression of gene products required for the induction of terminal

differentiation has been observed in non-melanoma skin cancer models, both in vivo and in vitro, in response to ligand activation of PPARβ/δ with GW0742 and the in vivo effects were not found in similarly treated *Pparb/d*-null mice.[35,36,53] These studies indicate that PPARβ/δ may be used to prevent and treat non-melanoma skin cancer because activating this receptor causes terminal differentiation of the solid tumors. Examination of target genes directly regulated by PPARβ/δ using bioinformatic analyses of microarray and ChIP-seq data revealed that none of the differentiation associated genes whose expression is increased by PPARβ/δ activation are directly regulated by PPARβ/δ in mouse primary keratinocytes.[10] Thus, further studies are still required to determine how ligand activation of PPARβ/δ mediates the induction of terminal differentiation. This is most likely due to secondary effects mediated by direct target genes regulated by PPARβ/δ based on the former data.[10]

To begin to determine the mechanisms by which ligand activation of PPARβ/δ inhibits early tumorigenesis, a series of studies were undertaken using models that utilized a Harvey sarcoma ras (HRAS) retrovirus to introduce the key genetic mutation required for keratinocytes to undergo malignant conversion in chemical carcinogenesis models.[56] Ligand activation of PPARβ/δ with GW0742 inhibited proliferation of HRAS-expressing primary keratinocytes, but not in HRAS-expressing keratinocytes from *Pparb/d*-null mice (Table 1).[39] Proliferation of HRAS-expressing keratinocytes from *Pparb/d*-null mice was also markedly increased as compared to HRAS-expressing keratinocytes from wild-type mice. These observations are consistent with in vivo results showing that ligand activation of PPARβ/δ inhibits cell proliferation in wild-type mice but not in similarly treated *Pparb/d*-null mice in two-stage chemical carcinogenesis bioassays.[35,36,53] The PPARβ/δ-dependent inhibition of HRAS-expressing keratinocyte proliferation was due to a block in the G2/M phase of the cell cycle.[39] Gene expression associated with promoting mitosis was markedly repressed by ligand activation of PPARβ/δ with GW0742 in HRAS-expressing keratinocytes from wild-type mice and this effect was not found in HRAS-expressing *Pparb/d*-null keratinocytes.[39] Results from these studies also showed that ligand activation of PPARβ/δ targeted HRAS-expressing keratinocytes as compared to controls, and selectively caused HRAS-expressing keratinocytes to undergo a block in the G2/M phase of the cell cycle. This is interesting because the human SCC cell line A431 also exhibits a block in the G2/M phase of the cell cycle in response to ligand activation of PPARβ/δ with GW0742,[54] since these cells are mutant for *TP53* a common etiological factor in UV-induced non-melanoma skin cancer. More detailed analyses of HRAS-expressing keratinocytes demonstrated that this was mediated by E2F crosstalk with PPARβ/δ. Ligand activation of PPARβ/δ with GW0742 led to direct binding of p130/p107 with PPARβ/δ causing enhanced nuclear translocation and increased promoter occupancy of p130/p107 on E2F target genes following resulting in repression of gene products that promoted mitosis causing G2/M phase arrest in HRAS-expressing keratinocytes.[39] This is consistent with the known roles of p130/p107 in repressing E2F target genes.[57] These findings provide support for a combinatorial approach to prevent non-melanoma skin cancer using mitosis inhibitors such as Paclitaxel with specific PPARβ/δ ligands; an idea supported by synergistic effects observed in the former studies.[39]

PPARβ/δ can also inhibit non-melanoma skin cancer by modulation of oncogene-induced senescence (Fig. 2B, Table 1). HRAS-expressing primary keratinocytes typically exhibit

calcium-induced differentiation and cell cycle arrest in vitro. Interestingly, HRAS-expressing *Pparb/d*-null keratinocytes developed calcium-resistant foci that contained a high percentage of cells that were undergoing replicative DNA synthesis, whereas HRAS-expressing wild-type keratinocytes did not.[58] Since HRAS can induce senescence to prevent malignant transformation in vitro,[59,60] it is of interest to note that HRAS-expressing wild-type keratinocytes cultured in high calcium medium exhibited a phenotype that resembled senescent cells that was not found in HRAS-expressing *Pparb/d*-null keratinocytes.[58] This was consistent with a higher percentage of cells undergoing replicative DNA synthesis and a lower percentage that were senescent in HRAS-expressing *Pparb/d*-null keratinocytes as compared to wild-type keratinocytes. Ligand activation of PPARβ/δ with GW0742 decreased both the percentage of senescent cells and keratinocytes undergoing replicative DNA synthesis in HRAS-expressing wild-type but not in *Pparb/d*-null keratinocytes demonstrating that these effects required PPARβ/δ.[58] Loss-of-function and gain-of-function studies confirmed that PPARβ/δ promotes senescence in HRAS-expressing wild-type keratinocytes by increasing phosphorylated mitogen-activated protein kinase kinase (pMEK), pERK and HRAS GTP, as well as proteins involved in promoting senescence (p53, p21, p27) as compared to HRAS-expressing *Pparb/d*-null keratinocytes.[58] By contrast, higher levels of pAKT1 were observed in HRAS-expressing *Pparb/d*-null keratinocytes compared to HRAS-expressing wild-type keratinocytes. The mechanism underlying the PPARβ/δ-dependent increase in expression of p53, p21 and p27 was mediated by repression of pAKT caused by PPARβ/δ that led to enhanced forkhead box O (FOXO) activity and decreased mouse double minute 2 homolog (MDM2).[58] PPARβ/δ modulated expression of both negative and positive protein regulators of HRAS (RASGAP120 and RASGRP1), and follow-up studies showed that these changes contributed to the PPARβ/δ-dependent increase in promotion of cellular senescence.[58] Moreover, PPARβ/δ repressed expression of ILK1, and increased pERK and decreased pAKT1 and mediated the increase in cellular senescence by PPARβ/δ. In vivo tumors also exhibited higher expression of ILK1 and pAKT1 and the percentage of dividing cells in tumors from *Pparb/d*-null mice as compared to controls was consistent with the notion that pAKT1 inhibits FOXO and p27 expression. Through this mechanism, PPARβ/δ promotes senescence and is anti-tumorigenic in a non-melanoma skin cancer model by repressing ILK1 and pAKT1 signaling (Table 1).[58]

Senescence can also be induced in non-melanoma skin cancer by PPARβ/δ-dependent repression of HRAS-induced endoplasmic reticulum (ER) stress (Table 1), thus suppressing carcinogenesis.[61] HRAS expression caused enhanced ER-stress in *Pparb/d*-null keratinocytes as compared to wild-type keratinocytes and this effect was mediated by ER-stress activation of the unfolding response (UPR), in particular two of the three primary UPR pathways: 1) ATF6, and 2) IRE1 but not the PERK pathway.[61] These observations were confirmed in both mouse and human models using loss-of-function, gain-of-function models, and pharmacological inhibition. HRAS-dependent promotion of ER stress through these pathways was mediated by increased pAKT1 activity in HRAS-expressing *Pparb/d*-null keratinocytes as compared to HRAS-expressing wild-type keratinocytes.[61] Additional studies showed that the differential phosphorylation of AKT1 was mediated by mammalian target of rapamycin (mTOR), and that ER stress-induced UPR maintained higher pAKT1 in part through a cell surface BiP-dependent mechanism.[61] Interestingly, a transient increase in

ER stress was sufficient to cause evasion of cellular senescence and malignant conversion in an in vitro model of carcinogenesis. More importantly, ER stress also attenuated senescence and promoted non-melanoma skin carcinogenesis an in vivo allograft model, and ER stress was negatively correlated with cellular senescence in human benign colon lesions.[61] Collectively, cellular senescence in non-melanoma skin cancer can be modulated by PPARβ/δ-dependent regulatory mechanisms that involve regulation of pAKT, pERK, and ER stress, and may be particularly important for cancer involving oncogenic RAS signaling.[58,61]

Lastly, the original observation that *Pparb/d*-null mouse skin exhibits enhanced inflammation following topical application of phorbol ester,[25,51] suggests that PPARβ/δ may also function to prevent non-melanoma skin cancer by inhibiting inflammation (Fig. 2B, Table 1)). This is supported by other studies using different *Pparb/d*-deficient mice that also exhibited exacerbated epidermal inflammation following topical treatment with phorbol ester.[33,34] While it is well established that PPARβ/δ has many anti-inflammatory activities,[62] the precise mechanism by which PPARβ/δ inhibits inflammation in non-melanoma skin cancer models requires further studies.

The two-stage chemical carcinogenesis model of non-melanoma skin cancer provides a useful model for dissecting the mechanisms of this disease and investigating approaches to prevent and treat this disease. The UV-induced model of non-melanoma skin cancer more closely reflects the etiology of non-melanoma skin cancer in humans. However, few studies have been published to date that have examined the role of PPARβ/δ in modulating UV-induced non-melanoma skin cancer. In one study, UV exposure to skin caused an increase in expression of *Pparb/d* mRNA.[63] This is consistent with what is found in mouse skin/keratinocytes exposed to phorbol ester and is likely to due to in part to increased activity of AP1 since there is an AP1 response element in the mouse promoter for this gene.[44] Surprisingly, the onset of tumor formation, the percentage of mice with tumors, and tumor multiplicity were all inhibited in *Pparb/d*-null mice crossed with SKH1 hairless mice as compared to wild-type controls.[63] This is surprising because presumably the parent line of *Pparb/d*-deficient mice used for these studies exhibited enhanced hyperplasia in response to phorbol ester as compared to controls,[33] consistent with what was observed with two other lines of *Pparb/d*-null mice.[25,34] This difference in tumorigenicity was attributed to PPARβ/δ-dependent regulation of oncogenic SRC.[63] However, there are several weaknesses to this study that preclude understanding whether PPARβ/δ-dependent regulation of SRC has a critical role in UV-induced skin cancer.[63] First, the effect of a topical application of a PPARβ/δ ligand was not examined, so it is unclear from this study if ligand activation of PPARβ/δ has any influence on UV-induced tumorigenicity as observed with the two-stage chemical carcinogenesis model. SRC is also known to be a proto-oncogene and functions more effectively in malignant conversion when there is an oncogenic mutation in the Y527 residue or in other genes that act on this phosphorylation site.[64] Since the investigators did not distinguish between the SRC proto-oncogene versus the oncogenic form of SRC, and only measured SRC and SRC activities, it remains possible that the oncogenic properties of SRC are unrelated to the observed phenotype. Additionally, while topical application of the PPARβ/δ antagonist GSK0660 prevented the changes in SRC expression, and GSK0660 was applied post-irradiation, this approach does not account for the sunscreen effect since it is known that this compound strongly absorbs UV light and thus could prevent induction of

any UV-induced effects. Lastly, the genetic background of the *Pparb/d*-null mice used for these studies was mixed (Sv/129/C57BL) and it is well known that the genetic background can markedly influence the outcome of skin cancer bioassays.[65,66] In contrast to this study, preliminary studies have shown that ligand activation of PPARβ/δ with GW0742 or sodium oleate inhibits the onset of skin tumor formation, the incidence of tumor formation, and tumor multiplicity in SKH1 hairless mice exposed to UVB, and these effects are not observed in similarly treated SKH1 mice on a *Pparb/d*-null background.[67] Since the latter study applied topical PPARβ/δ ligands post-irradiation, the results from this study controlled for the sunscreen effect and provide a better degree of clarity than the former study[63] that has multiple weaknesses. Additionally, inhibition of ectopic xenograft growth from A431 SCC cell line over-expressing PPARβ/δ provide further evidence that PPARβ/δ can inhibit tumorigenesis of a human SCC cell line-derived xenografts that contain mutant *TP53*;[54] a signature mutation causally linked to UV-induced skin carcinogenesis. Thus, there remains a need for more studies to determine the role of PPARβ/δ in UV-induced non-melanoma skin cancer because results obtained from studies examining the effect of chemopreventive and chemotherapeutic agents in two-chemical skin carcinogenesis models and xenograft studies are typically similar to those observed in UV-induced skin cancer models.

5. PPARβ/δ-DEPENDENT REGULATION OF MELANOMA

There are limited peer-reviewed publications focusing on the role of PPARβ/δ in melanoma. The first study to examine the effect of ligand activation of PPARβ/δ with GW0742 in a melanoma model showed concentration-dependent inhibition of cell proliferation by a PPARβ/δ-specific ligand in a human melanoma cancer cell line as compared to controls.[68] This inhibition of cell proliferation by ligand activation of PPARβ/δ with either GW0742 or GW501516 was also found in both mouse and human melanoma cell lines and mediated by repression of Wilm's tumor suppressor 1 (WT1) that may in turn regulate expression of NESTIN and ZYXIN.[69] These findings are of interest to note because ligand activation of PPARβ/δ with GW0742 and/or over-expression of PPARβ/δ inhibited growth of ectopic xenografts derived from a human melanoma cell causing a block in cell proliferation at the G2/M phase of the cell cycle and induction of apoptosis as compared to controls.[70] Moreover, since the melanoma cell line used for two of these studies[68,70] was UACC903, which expresses mutant PTEN and an active AKT3, these results demonstrate that ligand activation and/or over-expression of PPARβ/δ are capable of preventing the negative effects of mutant PTEN and active AKT3, and possibly other mutations known to exist in this melanoma cell line.[71–73] Consistent with these studies, genetic ablation and pharmacological inhibition of PPARβ/δ demonstrated that PPARβ/δ inhibited epithelial-mesenchymal transition (EMT), migration, adhesion, and invasion of a mouse melanoma cell line, and that PPARβ/δ prevented metastasis in a syngeneic mouse model of melanoma.[74] By contrast, one published study suggests that ligand activation of PPARβ/δ with GW501516 promotes migration and invasion of a human melanoma cancer cell line.[75] Combined, there is a stronger weight of evidence that targeting PPARβ/δ for melanoma chemoprevention and progression is a promising molecular target, although further studies are needed in more melanoma models to increase the preclinical evidence to support this hypothesis.

6. CONCLUSIONS

The role of PPARβ/δ in some cancer remains controversial due to conflicting reports with some suggesting that PPARβ/δ promotes carcinogenesis and others suggesting that PPARβ/δ inhibits carcinogenesis. (reviewed in[15,20–22,30,76]) By contrast, the role of PPARβ/δ in skin cancer is clearer. There is strong evidence from multiple laboratories using a variety of models showing that PPARβ/δ promotes terminal differentiation of both mouse and human cells and this is associated with a concomitant inhibition of proliferation by inducing a block at the G2/M phase of the cell cycle in response to ligand activation of PPARβ/δ. There is also strong evidence that PPARβ/δ inhibits inflammatory responses in skin and related models. Results from chemical carcinogen models and a xenograft model demonstrate that PPARβ/δ inhibits non-melanoma skin cancer by inducing differentiation, inhibiting proliferation and preventing inflammation (Fig. 2B, Table 1). At the molecular level, there are several mechanisms that have been delineated that include crosstalk with E2F signaling causing increased shuttling of PPARβ/δ with p130/p107 into the nucleus causing repression of E2F target genes that collectively inhibit mitosis and cause a block at the G2/M phase of the cell cycle. There are also at least two mechanisms by which PPARβ/δ promotes oncogene-induced senescence. The first involves regulation of pAKT and pERK and the other converges on the ER stress response that is also influenced by pAKT signaling. Combined, these mechanisms explain at least in part how targeting PPARβ/δ can be used for the prevention and treatment of non-melanoma skin cancer (Fig. 2B, Table 1).

While the two-stage chemical carcinogenesis skin cancer bioassay provides an invaluable tool to dissect out effects mediated by initiation and promotion of tumorigenesis, the primary causal factor of non-melanoma skin cancer in humans is exposure to UV light. However, whereas the two-stage chemical carcinogenesis model causes non-melanoma carcinogenesis through mechanisms that are different than those elicited by exposure to UV light (e.g. *HRAS* mutations versus *TP53* mutations as initiating events), the effects of most, if not all, chemopreventive agents are typically effective in both models as they likely act on molecular pathways downstream from the initial DNA damage. Thus, while there remains a need for more studies to study the role of PPARβ/δ in UV-induced non-melanoma skin cancer models, it is more likely that activation of PPARβ/δ in UV-induced non-melanoma skin cancer will also prove to be effective as a chemopreventive or chemotherapeutic approach for this disease. This is supported by a study showing growth inhibition of ectopic xenografts from A431 SCC cells with a mutant TP53 gene by over-expression of PPARβ/δ.

The role of PPARβ/δ in melanoma is only beginning to emerge. The consensus from the majority of studies performed to date indicate that ligand activation of PPARβ/δ may be suitable for targeting for melanoma chemoprevention or chemotherapy, but further studies are needed in models that better reflect the human disease (genetically modified animal models).

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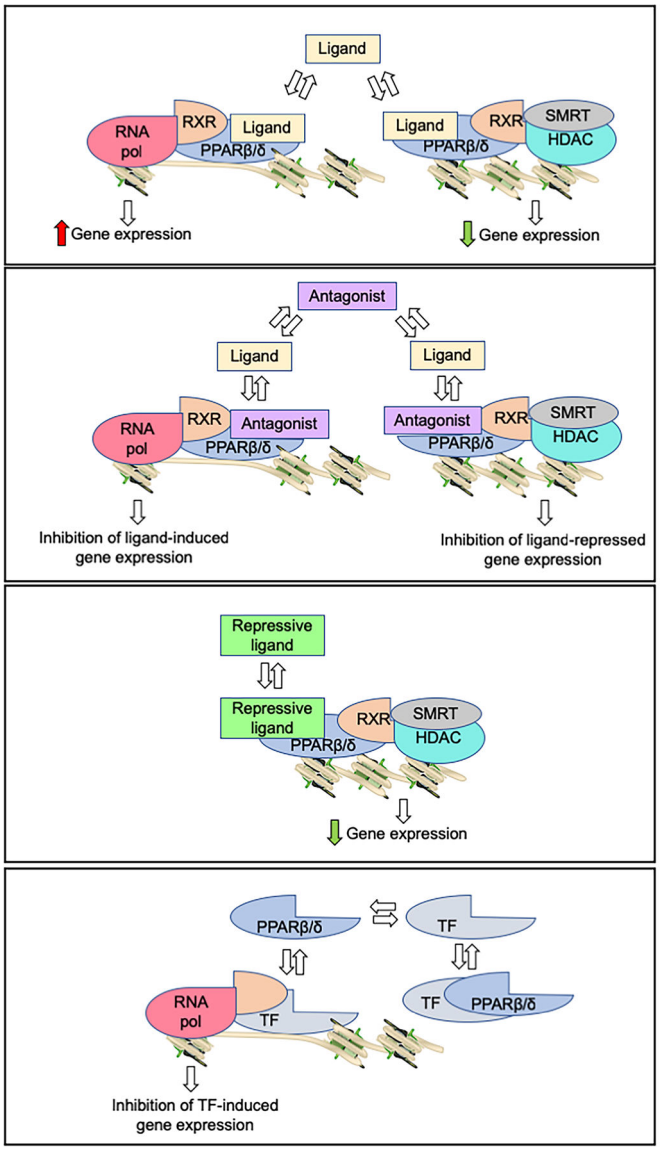

Figure 1.

Modulation of cellular signaling by PPARβ/δ. PPARβ/δ dynamically binds with endogenous and exogenous ligands, antagonists, and repressive ligands forming complexes with retinoic acid X receptor (RXR) causing recruitment of co-activators with histone acetyl transferase activities, histone de-acetylase activities, scaffolding proteins, and/or RNA polymerase leading to increased or decreased expression of target genes. Target gene expression in turn modulates cellular homeostasis. This dynamic regulation occurs constitutively due to the presence of endogenous compounds that can bind to PPARβ/δ and modulate its transcriptional activity and is particularly high in epithelial cells such as keratinocytes or colonic epithelium.[10,11] Moreover, the binding of PPARβ/δ to response elements in chromatin is often accompanied by co-recruitment of other transcription factors such as ATF4 that in turn modulate PPARβ/δ target gene expression.[10] (A; upper panel) Typical endogenous and exogenous PPARβ/δ ligands dynamically cause increased and decreased

expression of target genes containing response elements usually near the transcription start site through the mechanisms described above. (B; second panel below top) PPARβ/δ antagonists dynamically compete with endogenous and exogenous ligands and can prevent increased or decreased expression of target genes by outcompeting ligand binding and preventing the ligand activated complex from modulating transcription.[77] (C; third panel below top) Selective, repressive ligands (also referred to as ‘inverse agonists”) selectively recruit co-repressors and cause down-regulation of PPARβ/δ target genes by preventing ligands from increasing expression due to the formation of this repressive complex. This type of compound has not been examined in a skin cancer model to date (D; bottom panel) PPARβ/δ can also interact with other proteins such as the p65 subunit of the NF-kB complex and in turn down-regulate inflammatory signaling by decreasing NF-kB-dependent gene expression.

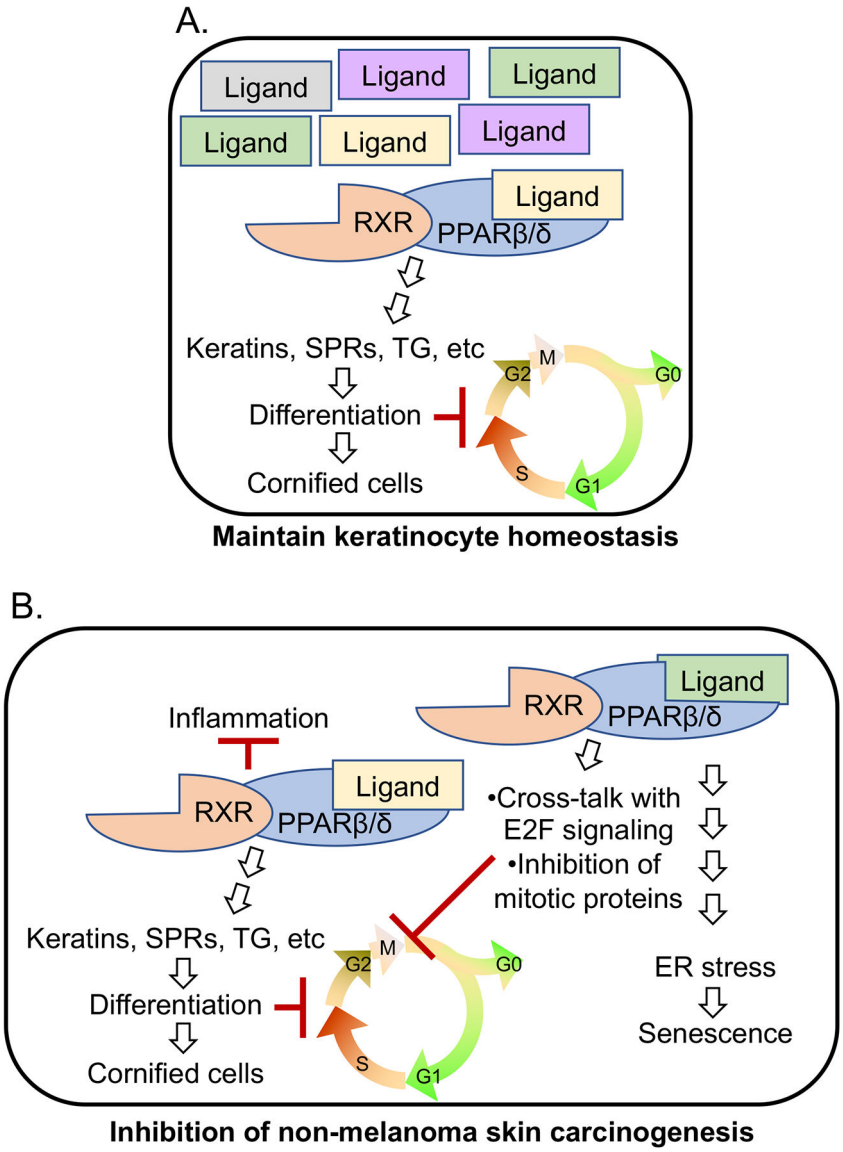

Figure 2.

PPARβ/δ-dependent regulation of non-melanoma skin cancer. (A) Expression of PPARβ/δ is high in keratinocytes,[11] and is likely chronically activated by the presence of different endogenous ligands.[10] Ligand activation of PPARβ/δ promotes terminal differentiation by increasing expression of keratins, small proline-rich proteins (SPRs), transglutaminase-I (TG), etc. and inhibits keratinocyte proliferation by doing so. This signaling likely helps to maintain skin homeostasis and a mechanism to help protect cells from exogenous agents such as chemical carcinogens and UV light. (B) Ligand activation of PPARβ/δ inhibits non-melanoma skin cancer by inducing terminal differentiation, inhibiting cell proliferation, inhibiting inflammation, and can also promote senescence through two mechanisms: 1) crosstalk with E2F signaling whereby PPARβ/δ shuttles p130/p107 across the nuclear membrane causing repression of E2F target genes and a block at the G2/M phase of the cell

cycle, and 2) PPARβ/δ-dependent repression of oncogene-induced ER stress that promotes senescence.

TABLE 1

Summary of effects mediated by PPARβ/δ in skin and skin cancer models

Effect	Reference(s)
Activation of PPARβ/δ causes increased terminal differentiation	24,27–29
Activation of PPARβ/δ causes inhibition of proliferation	25,29,33–39
PPARβ/δ modulates bioactivation of chemical carcinogens	52
Ligand activation of PPARβ/δ inhibits non-melanoma skin cancer	35,36,51,53,54
Inhibition of non-melanoma skin cancer by PPARβ/δ mediated by enhanced terminal differentiation	35,36,53
Inhibition of non-melanoma skin cancer by PPARβ/δ mediated by a block in the G2/M phase of cell cycle	39,54
Inhibition of non-melanoma skin cancer by PPARβ/δ mediated by enhanced senescence	58
Inhibition of non-melanoma skin cancer by PPARβ/δ mediated by inhibition of ER stress	61
Inhibition of non-melanoma skin cancer by PPARβ/δ influenced by inhibition of pro-inflammatory signaling	25,33,34,51
Ligand activation of PPARβ/δ inhibits melanoma	70,71,74,75