Linking Induction and Transrepression of PPARβ/δ with Cellular Function

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Authors' contributions

This work was carried out in collaboration between both authors. Author NPD performed the literature searches and produced the initial draft, which was developed by author LM. Both authors read and approved the final manuscript

ABSTRACT

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. PPARβ/δ is ubiquitously expressed and has a central role in homeostasis, and has been suggested as a therapeutic target for a number of metabolic and cardiovascular disorders. This important nuclear receptor controls transcription under different modes of molecular activity which directly control the cellular function and fate of tissues. This complex activity of induction and transrepression of gene expression (with and without exogenous ligands) is poorly understood and yet understanding this molecular control through novel drug development would led to control over a key molecular switch in all cells. This review outlines the main molecular mechanisms of PPARβ/δ, and links the modes of activity to the signalling pathways in inflammation, proliferation and senescence, with the goal to understand how this will translate into novel drug design to control the PPARβ/δ molecular switch.

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ABBREVIATIONS

apoE: Apolipoprotein E; Bcl6:B cell lymphoma-6; ECs: Endothelial cells; GSK: Glaxo Smith Kline; HDL cholesterol: High Density Lipoprotein; IL-6: Interleukin 6; LDL cholesterol: Low Density Lipoprotein; MAPK: Mitogen-activated protein kinase; NF-κB: Nuclear factor κB; PDGF: Platelet derived growth factor; PPARs: Peroxisome proliferator-activated receptors; PPRE: peroxisome proliferator-response elements; ROS: Reactive oxygen species; RXR: Retinoid X receptor; TNF-α: Tumour necrosis factor alpha; VSMCs: Vascular smooth muscle cells; VCAM-1: Vascular cell adhesion protein 1.

1. INTRODUCTION

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. PPARs were originally identified as members of the nuclear receptor superfamily that are activated by peroxisome proliferators, whereby they received their name [1]. The PPAR family consists of three isoforms, PPARα, PPARβ/δ and PPARγ with different tissue expression profiles: PPARα is expressed in tissues of high fatty acid catabolism, most importantly the liver, kidneys, heart and brown adipose tissue; PPARγ is found as three isoforms, PPARγ$_1$ is expressed in the gut, brain, vascular cells and immune cells, PPARγ$_2$ in adipose tissue, and PPARγ$_3$ in adipose and large intestine [2]; PPARβ/δ is ubiquitously expressed, although more highly active in skeletal muscle, arteries and endothelium [3,4]. PPARβ/δ is present in all animal cells, from *C. elegans* and drosophila to all mammals tested [5], however the biology of how this ubiquitous protein controls gene expression is complex and poorly understood and is yet to be fully explored. Signalling via PPARβ/δ has a number of effects on cell function, including lipid metabolism [6], glucose metabolism [7], insulin sensitivity [7], inflammation [8] and cell proliferation [9], suggesting that the control over such an important molecular switch is a desirable goal for research.

There are a large number of PPARβ/δ agonists, both endogenously produced (prostacyclin, fatty acids including the 'omega 3' fatty acids, arachidonic acid and linoleic acid [10]) although very little is known about their biological activity within the cell via PPARβ/δ. The PPARβ/δ agonist GW501516 completed proof-of-concept clinical trials successfully for dyslipidaemia [11] and hypercholesteremia [6], indicating that the control of PPARβ/δ activity has a significant effect on lipid profiles in metabolic syndrome-like patients. However, further clinical trials with GW501516 were halted due to a suspected link with tumour development [6,12] and Glaxo Smith Kline (GSK) did not continue investigating with GW501516. Other studies have suggested PPARβ/δ agonists (GW501516 and GW0742) as treatments for pulmonary hypertension [13], atherosclerosis [14,15] and kidney disease [16]. Taken together, it is clear that our understanding of PPARβ/δ in health and disease is not sufficient to rule out PPARβ/δ as a therapeutic target.

2. PPARβ/δ MULTIPLE MECHANISMS OF ACTION

2.1 Induction Mode

PPARβ/δ forms a complex with retinoid X receptor (RXR) and together as a heterodimer, regulate the target genes by binding to the promoter PPRE (peroxisome proliferator-response elements). The promoter is composed of the repetition of the consensus sequence AGGTCA separated by one nucleotide, whereas PPARs are orientated to the 5'-end and RXR to the 3'-end [17]. In the absence of ligand, co-repressor proteins are bound to the heterodimer and prevent binding to the PPRE sites. The presence of ligand induces a conformational change in PPARs that releases the co-repressor proteins and allows the binding to the promoter [3,4].

2.2 Transrepression Mode

PPARβ/δ regulates gene expression in a PPRE-independent manner through the suppression of other transcription factors such as nuclear factor κB (NF-κB) [18], AP-1 [19] and B cell lymphoma-6 (Bcl6) [14,20]. Once PPARβ/δ is non-ligand bound it binds to the nuclear factor and suppresses the production of numerous cytokines, chemokines, and pro-inflammatory genes such as VCAM-1 and E-selectin [14].

Control over other nuclear factors can occur different ways:

1) Direct competition between PPARs and other transcription factors for limiting amounts of shared co-activators [19].
2) Direct interaction of the PPAR-RXR heterodimer with other transcription factors preventing binding with their promoters and thus inhibiting gene transcription [21].
3) PPAR-RXR heterodimer inhibition of mitogen-activated protein kinase (MAPK) phosphorylation and activation, resulting in the inhibition of transcription factors [22].

2.3 Direct, Non-genomic Actions of PPARβ/δ

Surprisingly, PPARs also have non-genomic and off-target effects (Table 1). For example, the activated PPARβ/δ receptor binds directly to PKCα in platelets to inhibit aggregation [23], inducing vasodilatation in arteries, possibly mediated by direct interaction with RhoA [13,24], activation of PI3-Akt-eNOS pathways [25] or activation of K^+ channels [26].

2.4 Transcriptomics

A key insight into the complexity of transcription and transrepression has been provided through microarray analysis of mouse keratinocytes, where GW0742 was used as the principle agonist in PPARβ/δ$^{+/+}$ and PPARβ/δ$^{-/-}$ mice. Khozoie et al. [28] organised the genes into four main groups plus a further 4 minor groups (constituting 5% of responses collectively) (Fig. 1).

This model compares well with a study in human myofibroblasts, where Adhikary et al. [29]. used gene silencing to parallel the use of knockouts by Khozoie et al. [28]. By exposing cells to GW501516 and comparing the results from siPPARβ/δ to normal untreated cells, they developed a three mode of action model for PPARβ/δ: Group I; Transrepression no exogenous ligand, Group II; induction or repression with exogenous ligand, and Group III; induction no exogenous ligand [29]. Strikingly, both studies [28,29] indicate that PPARβ/δ induces gene transcription when there is no exogenous ligand (Fig. 1), although this is explained by Khozoie et al. [28] as likely to be due to gene induction by endogenous ligands found within the cell; however, few studies have shown the extent to which these ligands induce cellular effects by binding to PPARβ/δ. The models so far proposed to explain the different modes of PPARβ/δ action may be further complicated by endogenous ligands, which may account for induction with no exogenous ligand, suggesting that signalling by different ligands would have a large impact on the functional outcome for the cell [28,30]. While this is a novel concept to explain the diverse range of effects of PPARβ/δ in the control of cellular function, a parallel argument has been made on the cause of the side effects of glucocorticoids. It has been argued that subtle changes to transcriptional activity of glucocorticoid receptors would alter the related side effects of these drugs as opposed to the traditional view that the benefits of glucocorticoids are due to the inhibition of transcription and the side effects due to activation of transcription by the glucocorticoid receptor [31]. Implications for drug design for PPARβ/δ therefore needs to take this difference into account. It is tempting to think that direct control of the PPARβ/δ molecular switch would be possible by tipping the balance between endogenous-like ligands and exogenous ligands.

Table 1. Non-genomic effects of PPARβ/δ activation

| Species	Tissue	Effect	References
Cell line	HEK 293 cells	Coronary vasoconstriction.	[24]
Rat	Endothelium from aorta rings	Relaxation of aorta.	[25]
Rat	Heart	Increment of cardiac contractility but not heart rate.	[27]
Rat and mice	Pulmonary arteries, aorta and mesenteric arteries	Relaxation of vessels.	[13]
Human and rat	Platelets	Inhibits platelet activation and aggregation.	[23]
Human	Umbilical vein endothelial cells (HUVECs)	NO production and phosphorylation of eNOS.	[25]
Human	PASMCs and pulmonary arteries	Vascular relaxation.	[26]
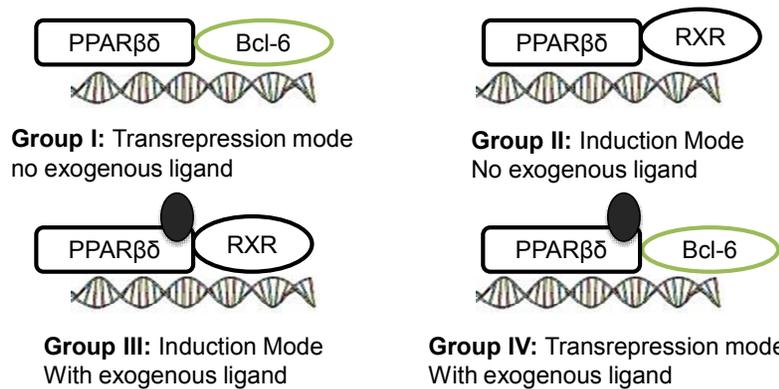
Fig. 1. Transrepression and induction modes of PPARβ/δ; the type of molecular control exerted by the receptor relates to the functional outcome of the cell; based on published microarray data [28]

Group I: Repression with no exogenous ligand (185 genes; involved in cell adhesion and communication, developmental processes and fatty acid and lipid transport, eg. Slc43a2).
Group II: Induction with no exogenous ligand (297 genes; involved in calcium signalling, cell communication, proliferation and differentiation, oncogenesis and a wide variety of other functions egSlc26a9).
Group III: Induction with exogenous ligand (71 genes; fatty acid beta oxidation and metabolism, lipid fatty acid steroid metabolism egCes5 and Hdhb).
Group IV: Repression of other nuclear receptors (such as BCl6) with exogenous ligand (28 genes; including Pmp22 and Morn4)

Understanding how PPARβ/δ switches between modes of action, and how this determines cellular function is therefore of great interest and could hold the key to the development of many non-communicable diseases. Whether differences in endogenous and exogenous ligands induce slightly different genes in different cells is a great possibility, and one that has not been so far explored. The question that needs to be addressed is not whether activation leads to proliferation and cancer, but whether the type of agonist activation and the subsequent molecular control can be adjusted to place the cell into a non-proliferative and non-inflammatory state, i.e. with a Group III profile [28] of gene expression. A parallel argument has been made on the cause of the side effects of glucocorticoids; the type of ligand determines whether the glucocorticoid receptor forms a homodimer, and the subsequent type of transrepression and transcriptional control exerted leads to change in cellular function [31].

3. CONTROL OF METABOLIC DISEASES

The benefits of PPARβ/δ agonist GW501516 are highlighted by their control of dyslipidaemia [11] and hypercholesteremia [6] in patients, with significant changes to the lipid profiles including increases in HDL cholesterol and reductions in LDL cholesterol, triglycerides, apoB and free fatty acids [6]. Other studies have shown the key importance PPARβ/δ has in controlling metabolism; agonists for this receptor alleviate hyperglycaemia and improve insulin sensitivity in diabetic *db/db* mice [7], and expression of PPARβ/δ is decreased significantly in hearts from streptozotocin (STZ) treated rats (a Type I model of diabetes). GW501516 significantly reduced the size of established atherosclerotic lesions and increased HDL in apoE^{-/-} mice, although they had no effect on total cholesterol levels [32]. This phenomenon was not mirrored in hypercholesterolemia mice with advanced lesions, where PPARα and PPARγ agonists but not PPARβ/δ agonist inhibited the development of atherosclerosis and foam cells formation [33].

3.1 Role of PPARβ/δ in the Inflammatory Responses

Several cellular pathways that have been recently identified describe powerful PPARβ/δ anti-inflammatory effects, and transrepression of Bcl6 is likely to be a key mechanism. In the absence of ligand, PPARβ/δ acts in the transrepression mode by sequestering Bcl6, which leads to a pro-inflammation response. Ligand activation of PPARβ/δ releases Bcl6, which is free to repress inflammatory gene expression (Fig. 2). Therefore care needs to be given when using experiments involving PPARβ/δ[-/-], where Bcl6 would be permanently in the activated free state, thus falsely resembling

the anti-inflammatory effects of PPARβ/δ activation [34].

In addition, the Akt/GSK-3β/NF-kB inflammatory pathway has been shown to be involved in PPARβ/δ activation [34]. Akt is a member of the phosphoinositide 3-kinases signal transduction enzyme family that reduces apoptosis and inflammation when phosphorylated [35]. Ligand activated PPARβ/δ contributes to the phosphorylation of Akt, and hence, contributes to the anti-inflammatory effects. On the other hand, GSK-3β is a serine-threonine kinase which is inactivated by phosphorylation, and it is regulated by multiple signalling pathways including the Akt pathway [35]. NF-kB is a transcriptional factor that regulates the transcription of genes involved in local and systemic inflammation, such as cytokines, chemokines, cell adhesion molecules, apoptotic factors, and other mediators [36] and several studies have reported an association between GSK-3β and NF-KB [37]. Taking this together, it is likely that activation of PPARβ/δ phosphorylates and activates the Akt pathway [34], which goes on to phosphorylate and hence inhibit GSK-3β, resulting in the inhibition of NF-κB and its subsequent pro-inflammatory effects.

The tumour necrosis factor alpha (TNF-α) induces the expression of the pro-inflammatory molecules VCAM-1 and E-selectin in endothelial cells (ECs), which is suppressed by PPARβ/δ activation [38]. Interestingly, the 5'-flanking regulatory regions of VCAM-1 and E-selectin genes lack PPRE, which means that these proteins are regulated via transrepression rather than induction mechanism of PPARβ/δ, and transcription factors such as NF-kB might play an important role [38].

There are a few studies involving interleukin 6 (IL-6) that suggest a similar anti-inflammatory mechanism. IL-6 is a molecule related to the development of rheumatoid arthritis and other inflammatory disorders, atherosclerosis, osteoporosis and septic shock [39]. The activation of PPARβ/δ inhibits IL-6-mediated inflammatory responses and subsequent acute phase reaction in the liver by increasing the phosphorylation of STAT-3 [34,40]. Activation of PPARβ/δ in adipocytes also inhibits NF-kB and consequently IL-6 expression [41].

Changes in PPARβ/δ activity leads to changes in the induction of gene expression of three important anti-oxidative stress enzymes, SOD1, catalase and thioredoxin, which are key in the elimination of reactive oxygen species (ROS) from the cell [41]. A new mechanism (induction and transrepression) for the anti-inflammatory effects of PPARβ/δ in ECs has been proposed, namely that the activation of PPARβ/δ leads to the activation of the target genes, including the antioxidative enzymes SOD1, catalase and thioredoxin (induction), and releases Bcl6, which represses the transcription of pro-inflammatory genes such as VCAM-1 and E-selectin (transrepression). Such a synergistic action leads to a potent inhibition of endothelial activation and therefore to the vascular protection. On the contrary, the activation of PPARα and PPARγ, but not PPARβ/δ leads to the inhibition of allergen-induced airway inflammation in a murine model of asthma, and what is more, this inhibition does not involve the NF-KB pathway [42]. Additionally, in other cell types such as epithelial cells, eosinophils, neutrophils, and lymphocytes, the PPARβ/δ activation was ineffective in inhibiting inflammatory processes [42].

3.2 Role of PPARβ/δ in Cell Proliferation, Migration and Angiogenesis

A number of growth factors and cytokines participate in the processes of abnormal vascular remodelling, inflammation, and cell proliferation involved in vascular diseases. PDGF is a potent mitogen involved in cell proliferation and migration that induces the expression of CDK2, CDK4, Cyclin D1 and Cyclin D3, enzymes that stimulate quiescent vascular smooth muscle cells (VSMCs) to enter the cell cycle [43].It was reported that PDGF increases the expression of PPARβ/δ in VSMCs from rats and humans; moreover, the over expression of PPARβ/δ promotes the proliferation of *in vitro* VSMCs from rat [44,45]. However, ligand-activation of PPARβ/δ inhibits PDGF, and hence, inhibits cell proliferation and migration of *in vitro* human and rat arterial VSMCs as well as *in vivo* rat VSMCs [45,46]. This suggests that PPARβ/δ regulates cell proliferation through the modulation of cell cycle regulatory genes. Interestingly, it has described a non-transcriptional inhibitory effect of L-165041 after 1 h treatment [46]. The authors of this study proposed the cooperation between the non-genomic effect and the conventional genomic effect of L-165041 to produce anti-proliferative effect of L-165041 through a Src and ERK1/2 dependent pathway.
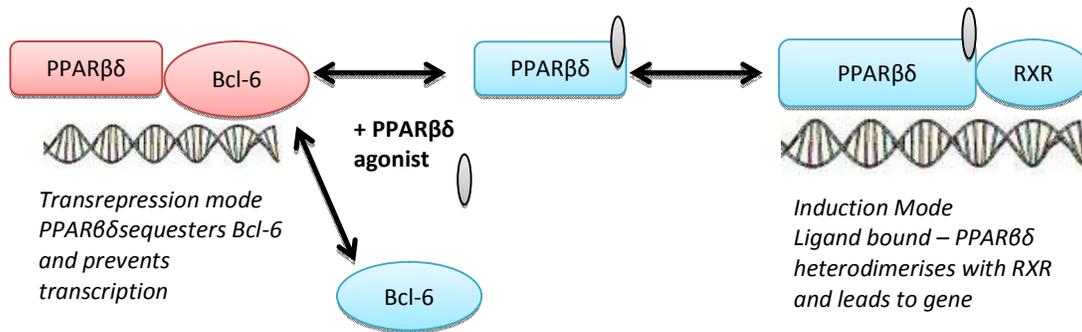
Fig. 2. Switch between transrepression and induction mode of PPARβ/δ

PPARβ/δ has a profound effect on skin wound healing, having a direct effect on two phosphatidylinositol 3-kinase-dependent pathways, namely, the Akt and the Rho-GT Pase pathway by amplifying keratinocyte responses to chemotactic signalling and inducing cell migration [47] and protect cells against apoptosis [48].

IL-1β also induces proliferation and migration of VSMCs through a TGF-β- and IL-1Ra mediated process, which is attenuated by GW501516. Furthermore, it was found that ERK is also involved in this pathway, since inhibition of ERK significantly reduced the effects of GW501516 [49]. On the contrary, the ligand-activation of PPARβ/δ induces proliferation and angiogenesis of human endothelial cells through a VEGF-dependent mechanism [50]. In addition, proliferation of cells after PPARβ/δ-activation has also been reported in human keratinocytes [51] and colorectal tumourcells of mice [52].

3.3 Role of PPARβ/δ in Senescence

It has been demonstrated that angiotensin II (Ang II) promotes senescence of vascular cells, leading to vascular remodelling and atherosclerosis [53]. Ligand-activated PPARβ/δ prevents the Ang II-induced cellular senescence through two different mechanisms, induction and transrepression. The induction mode was described both *in vitro* (human aortic VSMCs) and *in vivo* (mice) [53]. It was shown that ligand-activated PPARβ/δ reduces the intracellular ROS by the binding of PPARβ/δ to the PPRE of the PTEN gene, which up-regulates anti-oxygen genes and protects against senescence; at the same time, the transrepression of Bcl6 by PPARβ/δ also plays a role in senescence. A recent study in rat cardiac cells showed that the PPARβ/δ ligand L-165041 increased Bcl6

expression via p38, JNK and Akt activation, and also induced the release of Bcl6 from PPARβ/δ, thereby enabling Bcl6 to bind to its anti-senescent genes [54]. This study went on to show that the protective effects of PPARβ/δ exogenous agonists involve mitogen-activated protein kinases (MAPKs) and Akt activation.GW501516 ligand-activation of PPARβ/δ also produces anti-senescence effect in cultured human coronary artery endothelial cells by up-regulation of SIRT-1 [53]. Although the transcriptional regulation of SIRT1 is poorly understood, it is known to be PPRE-independent; hence, it could be another example of trans-repression mode. In this context, PPARβ/δ may serve as an anti-senescent mediator in age-related vascular changes such as atherosclerosis.

3.4 Dual Effect of PPARβ/δ

There are great discrepancies in the literature, which may be due to differences in experimental variables. PPARβ/δ receptor appears to be a sensitive molecular switch, that has both endogenous and exogenous ligands, and which controls cellular function through changes in very small concentration range [55]. Added to this, in any cell or tissue, the activity of PPARβ/δ may also depend on its promoter activity and relative expression, as well as presence and activity of co-repressor and co-activator proteins [3,28]. It is clear though that PPARβ/δ has a dual effect in the cell and indeed acts as a molecular switch having both pro- and anti- effects in inflammation, proliferation and differentiation. It has been shown that GW0742 is capable of behave as a PPARβ/δ agonist and antagonist, activating transcription at lower concentrations (in the order of nM) and inhibiting this effect at higher concentrations(in the order of μM) [56]. The mechanism by which GW0742 inhibits

activity is not absolutely clear, but it has been suggested that GW0742 inhibits PPARβ/δ by competing with the co-activators [56]. Alternatively the large ligand binding pockets of the receptor that can accommodate more than one ligand and high GW0742 concentrations could result in unusual PPAR: ligand-stoichiometries that could trigger inactive receptor conformations [56], an issue that requires further investigation.

4. CONCLUSION

The models of molecular control exerted by PPARβ/δ have given us a valuable insight into the complex actions of this receptor on control of key regulatory gene expression. However, these models are based on the effects of addition of exogenous ligands and do not provide any insight as to the extent of endogenous ligand binding and resultant gene expression. Exploring the pathways associated with PPARβ/δ signalling in inflammation and senescence indicates that there is a great deal to yet understand in terms of mode of action and cellular function, and moves the study of this interesting nuclear receptor away from the metabolic diseases and towards the cardiovascular. How we control the molecular switch with different types of ligands is an area that needs a great deal of consideration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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