Critical roles of the nuclear receptor PPARβ (peroxisome-proliferator-activated receptor β) in skin wound healing

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Abstract
The PPARs (peroxisome-proliferator-activated receptors) α, β/δ and γ belong to the nuclear hormone receptor superfamily. While all three receptors are undetectable in adult mouse interfollicular epidermis, PPARβ expression and activity is strongly re-activated by inflammatory stimuli during epidermal injury. The pro-inflammatory cytokine TNFα (tumour necrosis factor α) stimulates transcription of the PPARβ gene via an activator protein-1 site in its promoter and it also triggers the production of PPARβ ligands in keratinocytes. This increase of PPARβ activity in these cells up-regulates the expression of integrin-linked kinase and 3-phosphoinositide-dependent kinase-1, which phosphorylates protein kinase B-α (Akt1). The resulting increase in Akt1 activity suppresses apoptosis and ensures the presence of a sufficient number of viable keratinocytes at the wound margin for re-epithelialization. Together, these observations reveal that PPARβ takes on multiple roles and contributes favourably to the process of wound closure.

Introduction
The PPAR (peroxisome-proliferator-activated receptor) subfamily includes three isotypes (α, β/δ and γ; NR1C1, NR1C2 and NR1C3, respectively) that play a major role in the sensing and metabolism of biologically active lipids. As sensor receptors, PPARs are activated by a wide range of fatty acids, oxidized fatty acids metabolites from the lipoxigenase and cyclo-oxygenase pathways, and selective synthetic compounds e.g. hypolipidaemic fibrates and insulin-sensitizing thiazolidinediones [1]. The biological roles of PPARα and γ have been well-documented and thus will not be reiterated herein [2,3].

The canonical pathway by which PPARs regulate gene transcription involves their initial activation by ligand binding and subsequent heterodimerization with the RXR (retinoid X receptor). The PPAR–RXR heterodimer binds to the DNA response element (called PPAR response element or PPRE) that resides in the promoter or intronic regions of target genes (Figure 1). Concomitantly, nuclear receptor co-activators interact with PPAR–RXR and mediate transactivation, in part by contributing to the recruitment and stabilization of active transcriptional complexes. Similar to other nuclear receptors, phosphorylation of PPARs can also modulate their transactivation potential [4]. Interestingly, PPARβ actions can also result in gene repression. Unliganded PPARβ was demonstrated to be associated with corepressors, such as SMRT (silencing mediator for retinoid and thyroid hormone receptors), SHARP (SMRT/HDAC (histone deacetylase) I-associated repressor protein), NCoR (nuclear receptor co-repressor) and class I HDACs [5,6]. In this non-activated state, PPARβ was shown to repress several PPARα and PPARγ target genes via competition for PPRE binding (Figure 1). Thus PPARβ can act as an intrinsic transcriptional repressor, a mechanism that is also shared by other nuclear receptors such as the thyroid hormone receptor (NR1A1, NR1A2), retinoic acid receptor (NR1B1, NR1B2, NR1B3), Rev-Erb (NR1D1, NR1D2) and COUP-TF (chicken ovalbumin upstream promoter-transcription factor; NRT2F3). Conversely, ligand-activated PPARβ results in the release of the co-repressors that might transrepress other non-PPRE-containing genes (Figure 1). This mechanism would widen the repertoire of genes and functions that are modulated by PPARβ. However, the physiological relevance of this mode of action has to be thoroughly investigated in vivo.

PPARβ is widely expressed with relatively high levels in the brain, gastrointestinal tract and colon [7,8]. Numerous studies have suggested that PPARβ might play a role in cholesterol efflux [9,10], fat metabolism [11,12], colon cancer [13], embryo implantation [14], placenta development [12], pre-adipocyte and hepatic stellate cell proliferation [15,16], oligodendrocyte maturation [17,18] and osteoclastic bone resorption [19]. Herein we focus on its multi-faceted roles during skin wound repair.

Wound re-epithelialization
The skin, which acts as a protective shield from the outside world, is subjected to constant aggression and insults that may result in the loss of its integrity. Following skin injury, the priority is to rebuild an effective epidermal barrier over...
Figure 1 | PPARβ regulates gene expression via multiple modes of action

Unliganded PPARβ can interact with co-repressors (CoRep) and heterodimerize with RXR [5,6]. (a) As a heterodimer associated with co-repressors, it can suppress the expression of several PPARα and PPARγ target genes by binding to their PPRE. (b) Upon ligand (L) activation, the CoRep dissociates from the heterodimer, that now recruits co-activators (CoAct) and increases transcription of target genes, such as ILK and PDK1 [24]. (c) The CoRep that is released from the heterodimer can also transrepress non-PPRE-harbouring genes [46,47]. De novo protein synthesis is not required for these modes of action. TF, transcription factor; RE, response element.

The multi-faceted role of PPARβ in wound repair

In situ hybridization on mouse skin revealed that all three PPARs are detectable in the epidermis of newborn pups, but their levels decline to become undetectable in the interfollicular keratinocytes 5–9 days after birth and in the adult mouse skin. In contrast, all three PPARs remain expressed in the hair follicle keratinocytes. Importantly, the expression of PPARβ is rapidly stimulated to high levels in the adult epidermis at the wound edges of a cutaneous injury, or after treatment with the tumour-promoting agent phorbol ester (PMA), or hair plucking [22].

The role of PPARβ in wound repair was revealed by a paradoxical observation in PPARβ heterozygous...
Figure 2 | Different stages of and various factors involved in wound re-epithelialization

After injury, the wounded area is filled with a blood clot invaded by neutrophils. At days 3–7 after injury, the majority of neutrophils have undergone apoptosis. Instead, macrophages are abundant in the wound tissue at this stage of repair. During this period (1–7 days), keratinocytes near the wound margin begin to proliferate while those at the wound edge begin to migrate down the injured dermis and above the provisional matrix. Pro-inflammatory cytokines such as TNFα and interleukin (IL)-1α/β are released from keratinocytes, neutrophils and macrophages. TNFα and IL-1α/β induce expression of PPARβ, and trigger production of PPARβ ligands in keratinocytes. TNFα and IL-1α/β also act on fibroblasts, and induce expression of keratinocyte growth factor (KGF) and transforming growth factor-β1 (TGF-β1) by these cells. At 1–2 weeks after injury the wound is completely filled with granulation tissue. Fibroblasts have transformed into myofibroblasts, leading to wound contraction and collagen deposition. The wound is completely covered with a neoeidermis.

(PPARβ+/−) mice. Despite the apparently normal skin architecture of these mice, wound closure was delayed by 2–3 days in female animals [22]. Ironically, further analysis revealed an enhanced keratinocyte proliferation response, which appears to be in apparent contradiction to the observed delay in wound closure. This delayed re-epithelialization might reflect defects in key processes including cell adhesion, migration, proliferation and apoptosis. In an attempt to resolve this paradox, we investigated the primary trigger that reactivates PPARβ expression and its subsequent actions in primary keratinocyte cultures. We showed that PPARβ is an important transcription factor relaying inflammatory signals at the cell surface into specific gene expression patterns, which define appropriate cellular responses in sudden stress situations. Pro-inflammatory signals, such as TNFα (tumour necrosis factor α), trigger the stress-associated signalling pathway, activate the transcription factor complex activator protein-1, and finally stimulate the PPARβ gene. Importantly, these signals also trigger the production of endogenous PPARβ ligands [23]. Two vital clues for understanding PPARβ functions came from the subsequent comparative analysis of PPARβ wild type (PPARβ+/+) and knockout (PPARβ−/−) primary keratinocytes in culture. Firstly, the PPARβ−/− keratinocytes displayed a defect in adhesion and migration [22]. In contrast with PPARβ+/+ cells that adhered to the culture dish within 24 h, PPARβ−/− keratinocytes adopted a rounded shape upon culturing and adhered only after about 4 days. In addition, the PPARβ−/− keratinocytes presented a defect in cell–cell contact as reflected by the ease of creating, by scraping, an in vitro wound in the monolayer of cultured PPARβ−/− keratinocytes. In a complementary manner, these in vitro scraping studies revealed impaired migration of these cells. Secondly, the PPARβ−/− keratinocytes were more susceptible to numerous apoptotic stimuli, such as anoikis, growth factor deprivation and TNFα treatment [23,24]. The increased apoptosis of the PPARβ−/− keratinocytes was also observed in vivo during wound healing in PPARβ+/− mice [23].
Figure 3 | Ligand-activated PPARβ potentiates Akt1 activity

In keratinocytes, the ILK and PDK1 genes are direct targets of PPARβ. PTEN is down-regulated by PPARβ via an indirect mechanism (dotted line). The co-ordinated increased expression of ILK and PDK1, with the reduction in PTEN expression, lead to an increase in Akt1 activity. The Akt1 signalling pathway plays a pivotal role in cell survival. The anti-apoptotic effect of Akt1 is mediated via the phosphorylation of several downstream target substrates, such as Bad and the forkhead family of transcription factors (FKHRs), which upon phosphorylation lose their pro-apoptotic activity.

Ultimately, while the PPARβ+/− mice showed increased keratinocyte proliferation, the mutant cells were unable to develop appropriate cell–matrix and cell–cell contacts that are important in vivo for cell migration at the wound edges. In addition, the balance between proliferation and apoptosis was tipped in favour of the latter, since despite a 2-fold increase in proliferating keratinocytes in the basal layer, a 10-fold increase in the number of apoptotic cells was concomitantly observed in the suprabasal layer [23]. These mutually non-exclusive defects in the PPARβ−/− keratinocytes could, in part, be responsible for the delayed wound closure in the PPARβ−/− animals.

Ligand-activated PPARβ modulates the Akt1 survival pathway

Adhesion, migration and apoptosis are processes that require tight regulation and synchronization for efficient wound repair. Several observations indicated that dysfunction of the Akt1 (protein kinase B-α) survival pathway could play a pivotal role in PPARβ−/− mutant cells. Firstly, the adhesion and migration defects of the PPARβ−/− keratinocytes are similar to those of integrin β1-deficient keratinocytes [25], suggesting that PPARβ may participate in integrin signalling. Integrin ligation triggers several downstream kinases, such as ILK (integrin-linked kinase), which participates in the regulation of Akt1 activity. Secondly, Akt1 exerts its anti-apoptotic effect by modulating both the mitochondrial and death receptor apoptotic pathways. It exerts anti-apoptotic effects either by inhibiting pro-apoptotic proteins, such as Bad and the forkhead family of transcription factors (FKHRs) via phosphorylation, or by inducing anti-apoptotic signals via the nuclear factor-κB [26].

Akt1 is a major downstream effector of phosphoinositide 3-kinase signalling. Maximal Akt1 activity requires phosphorylation of Thr-308 by PDK1 (3-phosphoinositide-dependent kinase-1), and of Ser-473 by ILK or other kinases. We showed that both ILK and PDK1 are target genes of PPARβ in keratinocytes, and that they contain a functional PPRE in their intronic and promoter region, respectively [24]. Thus PPARβ directly controls apoptosis in keratinocytes via a co-ordinated up-regulation of ILK.
and PDK1 expression and a down-regulation of PTEN (phosphatase and tensin homologue deleted on chromosome 10) expression, leading to increased Akt1 activation (Figure 3). The resulting higher Akt1 activity leads to increased keratinocyte survival following growth factor deprivation and anoikis. Finally, PPARβ also potentiates nuclear factor-κB activity, leading to increased matrix metalloproteinase-9 production, which can regulate keratinocyte migration.

The impact of PPARβ in modulating the Akt1 signalling pathway was underscored by the numerous overlapping phenotypes present in PPARβ−/− mice and mice deficient in various players of the Akt1 pathway. Both ILK- and PDK1-deficient mice are embryonic lethal [27,28]. ILK-null lethality is due to impaired epiblast polarization. Interestingly, ILK-null fibroblasts exhibit impaired cell spreading and diminished proliferation rates because of delayed formation of stress fibres and focal adhesion. PDK1 deficiency leads to multiple defects including lack of somites, midbrain and neural-crest-derived tissues [27]. Interestingly, these tissues express high level of PPARβ. Mice in which ILK has been deleted in bone as well as PDK1 hypomorphic mice have a reduced body size, similar to PPARβ−/− mice [27,29,30]. Mice deficient in Akt1, Akt2 or both also display phenotypes found in PPARβ−/− mice. The similarities between PPARβ−/− and Akt1−/− mice have been well described elsewhere [31]. Mice lacking Akt2 exhibit mild growth deficiency and lipatrophy [32]. Again compatible with a major role of PPARβ in the regulation of Akt signalling, Akt1 and Akt2 double-knockout mice exhibit dwarfism, impaired skin development, skeletal muscle atrophy, delayed bone development and impeded adipogenesis [33]. Importantly, PPARβ has been implicated in most of these biological processes (see Introduction).

Wound repair and carcinogenesis

In many aspects, wound healing closely resembles the phenotypic events that are observed during squamous cell carcinoma progression, where normal keratinocytes acquire a proliferative and migratory phenotype. Two crucial characteristics distinguish the two processes. Firstly, the phenotypic conversion of wound keratinocytes is triggered by factors external to the keratinocytes, such as epidermal injury or growth factors released after an injury, whereas that of carcinoma progression requires genomic mutations that are intrinsic to the keratinocytes. Secondly, whereas the activation of wound keratinocytes is transient and tightly regulated, and a phenotypic reversion takes place immediately after re-epithelialization is completed, the malignant transformation of keratinocytes during carcinoma progression is irreversible. It is thus tempting to speculate on possible roles of PPARβ during carcinogenesis.

Is PPARβ an oncogene or a tumour suppressor? Data supporting both will be presented below. Supporting the former, high dysregulated expression of PPARβ has been associated with numerous inflammatory hyperproliferative pathologies such as psoriasis [34] and cancers [35–38]. In addition, overexpression of activated oncogenic K-Ras in intestinal epithelial cells increases PPARβ expression and activity [39]. In accordance with the above, aberrant PPARβ expression may promote cell growth and accelerate cancer metastasis, which is consistent with the role of Akt1 signalling in mouse skin tumorigenesis [40,41]. Conversely, prolonged ligand-activation of PPARβ was shown to induce keratinocyte differentiation in vitro [23], a process that normally opposes cell proliferation. In addition, an important phenotype is the enhanced keratinocyte proliferation observed in PPARβ+/− mice upon skin wounding and exposure to PMA. Indeed, whereas topical exposure to PMA in PPARβ+/− mice results in a hyperplastic response, a similar treatment of PPARβ−/− and PPARβ+/− mice leads to an even more pronounced hyperplasia [18,22], suggesting a role for PPARβ in moderating the hyperplastic response. In the two-chemical induction of skin carcinogenesis in mice, PMA is used after initial priming with DMBA (9,10-dimethyl-1,2-benzanthracene). However, it is unclear if the PMA response to PMA is a cell-autonomous effect, since treatment with PMA of cultured primary keratinocytes or organotypic skin culture typically accelerates keratinocyte differentiation [42,43]. Thus, to date, there is evidence to support an involvement of PPARβ during carcinogenesis. However, the role it plays, either as an oncogene or as a tumour suppressor, is unclear. It might turn out that PPARβ assumes both roles, very much like transforming growth factor-β1 [44], depending on the various stages of transformation and the means of inducing cancer, e.g. ultraviolet light versus chemical induction.

Conclusions

PPAR dysfunction has been implicated in the manifestation of many diseases, ranging from obesity to cancer. We have conclusively shown that the up-regulation of PPARβ induced by inflammation is crucial for efficient skin wound healing. The anti-apoptotic role of PPARβ ensures a sufficient number of viable keratinocytes for wound re-epithelialization and participates in their migration. The multiple roles assumed by PPARβ during wound repair make it an important potential target for wound healing drugs [45]. Furthermore, while the roles of PPARβ in tissue repair are derived from the studies on the skin and keratinocytes, the pathway that has been deciphered may be more widespread and at work in several other epithelia. Thus, the implications of our findings are likely to be far-reaching. Despite our increased knowledge, however, more needs to be understood, particularly regarding the validity of the alternative pathways of gene regulation by PPARβ, as well as its roles in additional biological processes.

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