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The anti-apoptotic role of PPARβ contributes to efficient skin wound healing☆

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Abstract

PPARα and PPARβ are expressed in the mouse epidermis during fetal development, but their expression progressively disappears after birth. However, the expression of PPARβ is reactivated in adult mice upon proliferative stimuli, such as cutaneous injury. We show here that PPARβ protects keratinocytes from growth factor deprivation, anoikis and TNF-α-induced apoptosis, by modulating both early and late apoptotic events via the Akt1 signaling pathway and DNA fragmentation, respectively. The control mechanisms involve direct transcriptional upregulation of ILK, PDK1, and ICAD-L. In accordance with the anti-apoptotic role of PPARβ observed in vitro, the balance between proliferation and apoptosis is altered in the epidermis of wounded PPARβ mutant mice, with increased keratinocyte proliferation and apoptosis. In addition, primary keratinocytes deleted for PPARβ show defects in both cell–matrix and cell–cell contacts, and impaired cell migration. Together, these results suggest that the delayed wound closure observed in PPARβ mutant mice involves the alteration of several key processes. Finally, comparison of PPARβ and Akt1 knock-out mice reveals many similarities, and suggests that the ability of PPARβ to modulate the Akt1 pathway has significant impact during skin wound healing.
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Keywords: PPARs; Keratinocytes; Apoptosis; Skin wound healing

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of ligand-inducible transcription factors known as nuclear receptors. Three isotypes encoded by separate genes (PPARα or NR1C1, PPARβ/δ or NR1C2 and PPARγ or NR1C3) have been identified in amphibians, rodents and humans. PPARs heterodimerize with the retinoid X receptor (RXR), and regulate gene transcription upon ligand-dependent activation (fatty acids or fatty acid derivates). Each of the three PPARs exhibits unique tissue distribution and performs distinct roles in lipid metabolism, inflammation, diabetes and cancer [1]. Consistent with a potential role of PPAR ligands in epidermis development, PPARs are present both in rat and mouse fetal skin [2,3], and PPARα ligands can accelerate fetal epidermal differentiation [4]. In both species, the expression of PPARα and PPARβ decreases progressively after birth and it becomes undetectable in the interfollicular epidermis of adult rodents. These observations are consistent with the absence of apparent skin defects in adult PPARα null (PPARα−/−) and PPARβ−/− mice [3,5]. However, the expression of PPARβ is rapidly reactivated in the adult epidermis at the wound edges after a cutaneous injury. In accordance, wound repair is delayed by 2–3 days in the PPARβ heterozygous (PPARβ+/−) mice, probably due to impaired keratinocyte adhesion and migration [3]. Interestingly, increased apoptosis of the PPARβ mutant keratinocytes was also observed in vivo during wound healing, indicating a potential role of PPARβ in the control of keratinocyte survival [6].

Programmed cell death, or apoptosis, is a natural event that plays a crucial role in normal development and maintenance of tissue homeostasis in multicellular organisms. Indeed, apoptosis regulates the sequence of cellular events that are involved in the healing of all tissues [7]. Two major pathways control the apoptotic response: the mitochondrial pathway, which involves the Bcl-2 family of proteins, and the death receptor pathway, which includes receptors for the tumor necrosis factor (TNF) and the fas-ligand (Fas-L). Both pathways culminate in the activation of effector caspases and the fragmentation of nuclear DNA. Akt1 (also called protein kinase B), one of the major downstream effector of phosphatidylinositol-3-kinase (PI3K) signaling, plays a central role in the suppression of apoptosis induced by many cellular stresses. Akt1 exerts its anti-apoptotic effect by modulating both major apoptotic

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pathways. Its maximal activity requires its binding to 3-phosphatidylinositol-3,4,5-triphosphate (PIP3), the product of PI3K, and its phosphorylation by two different kinases, the 3-phosphoinositide-dependent kinase-1 (PDK1), and the integrin-linked kinase (ILK) [8].

We have recently demonstrated the important role of PPARβ in the protection of mouse primary keratinocytes against apoptosis [9]. This occurs via transcriptional upregulation of the ILK and PDK1 genes, leading to Akt1 activation. The present paper summarizes some of these findings and further extends the exploration of the anti-apoptotic role of PPARβ. We show that PPARβ protects mouse and human keratinocytes from stress-induced apoptosis, by modulating both early (Akt1 signaling pathway) and late (DNA fragmentation) apoptotic events. The PPARβ-mediated activation of the Akt1 pathway could explain many aspects of the phenotype of the PPARβ mutant mice, especially the delayed wound repair.

2. Materials and methods

2.1. Reagents and cell culture

The PI3K inhibitor LY294002 was from Cell Signaling. TNF-α was from CalBiochem. Cell culture media and supplies were obtained from Gibco, BRL.

HaCaT cells were maintained in DMEM containing 10% FCS and 5 μg/ml gentamycin. Mouse primary keratinocytes were isolated and cultured as described previously [6].

2.2. Plasmid constructs and transfection assays

All cDNAs were subcloned into the pcDNA3.1 expression vector (Invitrogen). Reporter constructs containing several copies of each PPRE were obtained by cloning oligonucleotides into the pGL2 luciferase promoter vector (Promega). Cells were transfected using Superfect reagent (Qiagen), and luciferase activity (Promega) was measured according to the manufacturers' instruction. β-Galactosidase activity was used for normalization.

2.3. RNase protection assay (RPA) and Western blot

Gene specific probes corresponding to PDK1, ILK, Akt1, ICAD-S, ICAD-L and L27 were subcloned into pGEM3Zf(+) (Promega). RPA was carried out as described [9]. All antisense riboprobes were synthesized using a ratio of 1:1 α^{32}-P-UTP to cold UTP, except for L27 (1:20). For Western blot, whole tissue extracts were prepared as described. Equal amounts of proteins (20 μg) were resolved by SDS-PAGE, transferred to PVDF membrane, and immunoblotted with the following antibodies: PDK1 (Transduction Laboratories; 1:1000), ILK (Santa Cruz Biotechnology; 1:2000), Akt1 (Cell Signaling; 1:1000) and β-tubulin (BD PharMingen; 1:2000).

2.4. Electrophoretic mobility shift assay (EMSA)

In vitro transcribed and translated (TNT, Promega) PPARβ and RXRα receptors were incubated with ^{32}P end-labeled oligonucleotides (20,000 cpm/μl) in 20 μl of binding buffer (20 mM HEPES, pH 7.5, 50 mM KCl, 0.1 mM EDTA, 5 mM $MgCl_2$, 5% glycerol, 1 mM DTT and 250 μg/ml poly dIdC). Bound DNA complexes were separated from free probes on a 5% Tris-glycine-EDTA gel containing 2.5% glycerol.

2.5. Proliferation and apoptotic assays

Anoikis was induced in cells using agarose-coated culture plates and serum-free medium. Apoptotic cells were stained using the ApoAlert Annexin V-EGFP apoptosis kit (Clontech), and monitored by fluorescence microscopy.

The detection of the Ki67 proliferative marker and the TUNEL assay were performed on frozen sections of skin wounds as described previously [6].

3. Results

3.1. PPARβ protects keratinocytes from stress-induced apoptosis

Increased keratinocyte death was observed in PPARβ-mutant mice subjected to wound healing experiments, indicating an anti-apoptotic role of PPARβ [6]. To assess this new function of PPARβ, primary keratinocytes derived from PPARβ+/+ and PPARβ−/− newborn mice were cultured and subjected to various apoptotic stimuli. When keratinocytes from both genotypes were cultured, a slight decrease (10–15%) in the survival of PPARβ mutant cells was observed. Interestingly, PPARβ−/− primary keratinocytes were significantly more sensitive than PPARβ+/+ cells to growth factors (GF) deprivation and anoikis (suspension-induced apoptosis), which invoke the mitochondrial pathway, as well as to TNF-α-induced apoptosis, which induces the death receptor pathway (Fig. 1A). Consistently, treatment of the human HaCaT keratinocyte cell line with the synthetic PPARβ agonist (L-165041) conferred a two- to four-fold increased resistance to apoptosis triggered by GF deprivation or anoikis (Fig. 1B). Together with our previous observations that PPARβ−/− keratinocytes are impaired in adhesion/migration, these results suggested a role of PI3K signaling as the possible underlying signaling mechanism responsible for this PPARβ-induced resistance to apoptosis. To address this hypothesis, we repeated the experiments with the HaCaT cells in the presence of a PI3K inhibitor (LY294002). As shown in Fig. 1B, the PI3K inhibitor abrogated the anti-apoptotic effect of PPARβ significantly, leading to an increase of the number of apoptotic cells, but did not completely reverse the protective effect of PPARβ. Similar results were

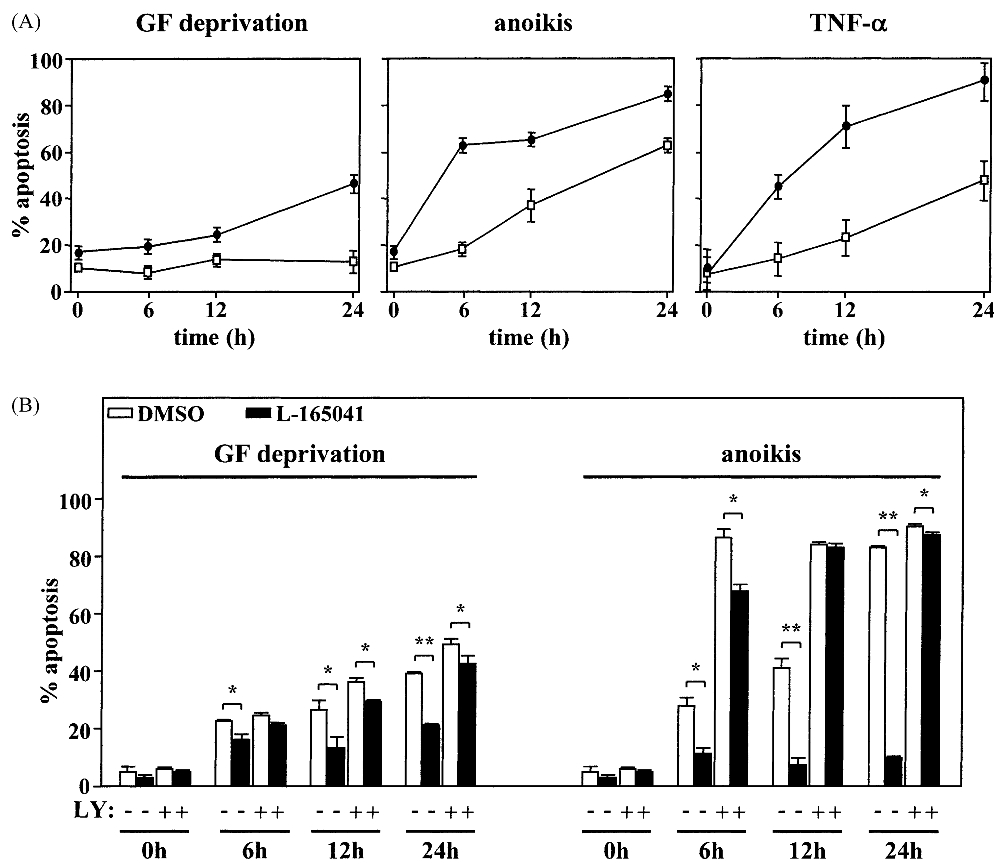

Fig. 1. PPARβ−/− keratinocytes are more susceptible to stress-induced apoptosis. (A) Apoptosis was induced in PPARβ+/+ (□) and PPARβ−/− (●) primary keratinocytes using growth factor- and serum-free medium (GF deprivation), plating on agarose-coated culture dishes (anoikis) or treatment with TNF-α (10 ng/ml). Apoptosis was measured at indicated time points by quantification of annexin V-EGFP positive cells (GF deprivation and anoikis), or by radioactive DNA fragmentation assay (TNF-α treatment). (B) Apoptosis was induced by GF deprivation or anoikis in HaCaT cells treated with vehicle (DMSO) or 1 μM of the PPARβ ligand L-165041 in the presence or absence of the PI3K inhibitor LY294002 (LY; 50 μM). Apoptosis was quantified at indicated time points using annexin V-EGFP (* $P < 0.05$; ** $P < 0.01$).

obtained using cells overexpressing the phosphatase and tensin homolog deleted on chromosome 10 (PTEN), the natural inhibitor of the PI3K [9].

Together with our previous observations [9], these results demonstrate that PPARβ protects mouse and human keratinocytes from mitochondrial- and death receptor-mediated apoptosis, mainly through a PI3K-dependent pathway. In addition, the PI3K inhibitor could not completely abolish the effect of PPARβ, suggesting the presence of a minor PI3K-independent anti-apoptotic pathway.

3.2. PPARβ regulates the expression of anti-apoptotic genes in keratinocytes

To further dissect the molecular events responsible for the anti-apoptotic role of PPARβ, we next identified PPARβ-regulated genes involved in apoptosis. Since the data presented above indicated that the PI3K signaling is required for protection against apoptosis, we first analysed the expression of genes involved in PI3K/Akt1 signaling, one of the most important anti-apoptotic pathway. The expression of such genes was determined both by RPA and Western blot, in PPARβ−/− versus wild-type primary keratinocytes. Expression of ILK and PDK1, involved in the phosphorylation hence activation of Akt1, was 1.8–2-fold lower at the mRNA level, and 2.5–4-fold lower at the protein level in the PPARβ−/− cells (Fig. 2A). In agreement with these observations, decreased phosphorylation and activity of Akt1 were observed in the mutant keratinocytes [9]. The analysis of other apoptotic genes also revealed a downregulation by two- to three-fold of the anti-apoptotic genes ICAD-S and ICAD-L (inhibitor of caspase-activated deoxyribonuclease short and long forms) in PPARβ−/− keratinocytes (Fig. 2B). These two genes are highly homologous to human DFF35 (DNA fragmentation factor 35) and DFF45, respectively, and play a critical role in the inhibition of DNA fragmentation during the final events of apoptosis [10]. These data indicate that PPARβ exerts its anti-apoptotic function by modulating both early apoptotic events via the ILK/PDK1/Akt1 pathway, as well as DNA fragmentation, which is a late apoptotic event.

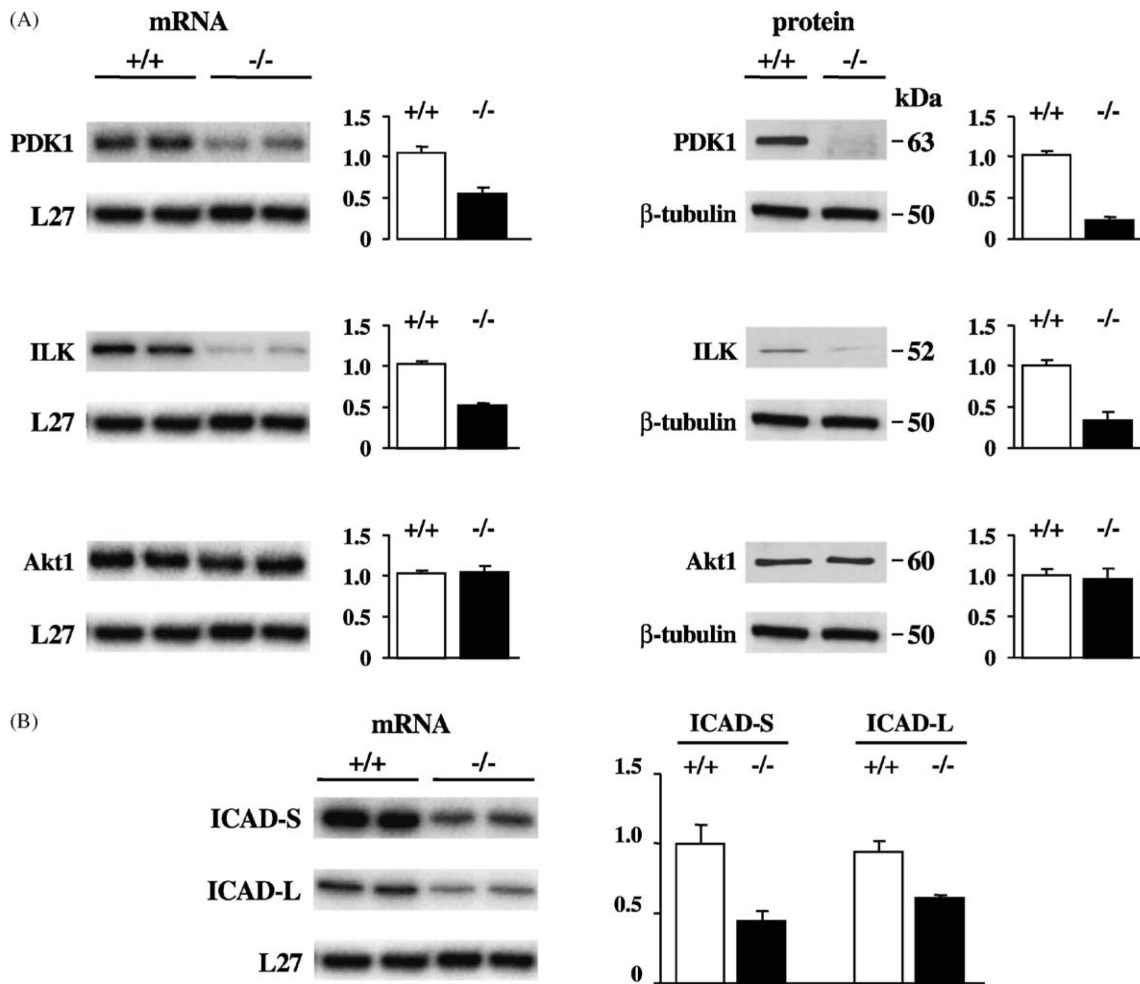

Fig. 2. PPARβ regulates anti-apoptotic genes in primary keratinocytes. (A) RPA (left panel) and Western blots (right panel) were performed using 5 μg of total RNA and 20 μg of proteins extracted from wild-type (+/+) and PPARβ null (−/−) primary keratinocytes, respectively. Quantification, indicated on the right, represent the mean of three experiments, after normalization to the wild type cells. L27 ribosomal protein (L27) mRNA and β-tubulin protein levels were used as internal controls. (B) RPA on ICAD-S and ICAD-L were carried out as in (A), using 2.5 μg of total RNA.

3.3. Human ILK, PDK1 and DFF45 are direct target genes of PPARβ

To explore the regulation of the above-mentioned apoptosis-associated genes by PPARβ, transient transfections were performed using reporter constructs harbouring putative PPREs residing in either the promoter or intronic sequences of these genes. We examined the 5′-region of the DFF45 human promoter (accession number NT_021937) for the presence of potential PPAR response elements (PPREs). We identified two putative PPREs in the far upstream region of the promoter, with a sequence similar to the consensus PPRE for known PPAR target genes, named PPRE1 (nucleotide −17,176 to −17,188) and PPRE2 (−31,912 to −31,924) (Fig. 3A). Similar analysis of human ILK gene also revealed two putative PPREs, ILK PPRE1 (nucleotides +1535 to +1547) and ILK PPRE2 (+4238 to +4250) in a long intron 2, whereas the analysis of the human PDK1 promoter sequence (accession number AC092117) revealed one putative PPRE (PDK1 PPRE1; nucleotides −5077 and −5065) [9].

To examine the functionality of these newly identified putative PPREs, transactivation studies were performed with luciferase reporter constructs containing two copies of each PPRE cloned upstream of the SV40 promoter. Consistent with our previous study [9], PPREs identified in the ILK (ILK PPRE1) and the PDK1 gene (PDK1 PPRE1) increased the luciferase reporter activity by four- to five-fold in response to PPARβ ligand (Fig. 3C). Similar analysis of the putative DFF45 PPREs revealed that only the DFF45 PPRE2 is functional (Fig. 3C). As positive control, three copies of the well known PPRE from the rat acyl-CoA oxidase (ACoA) promoter were used [11]. EMSA was also performed to demonstrate direct binding of PPARβ/RXRα heterodimers to these PPREs. As shown in Fig. 3B, neither PPARβ nor RXRα alone bound to any of the PPREs (lanes 3–4). However, PPARβ/RXRα heterodimers bound to the control ACoA PPRE, as well as to the ILK PPRE1, PDK1

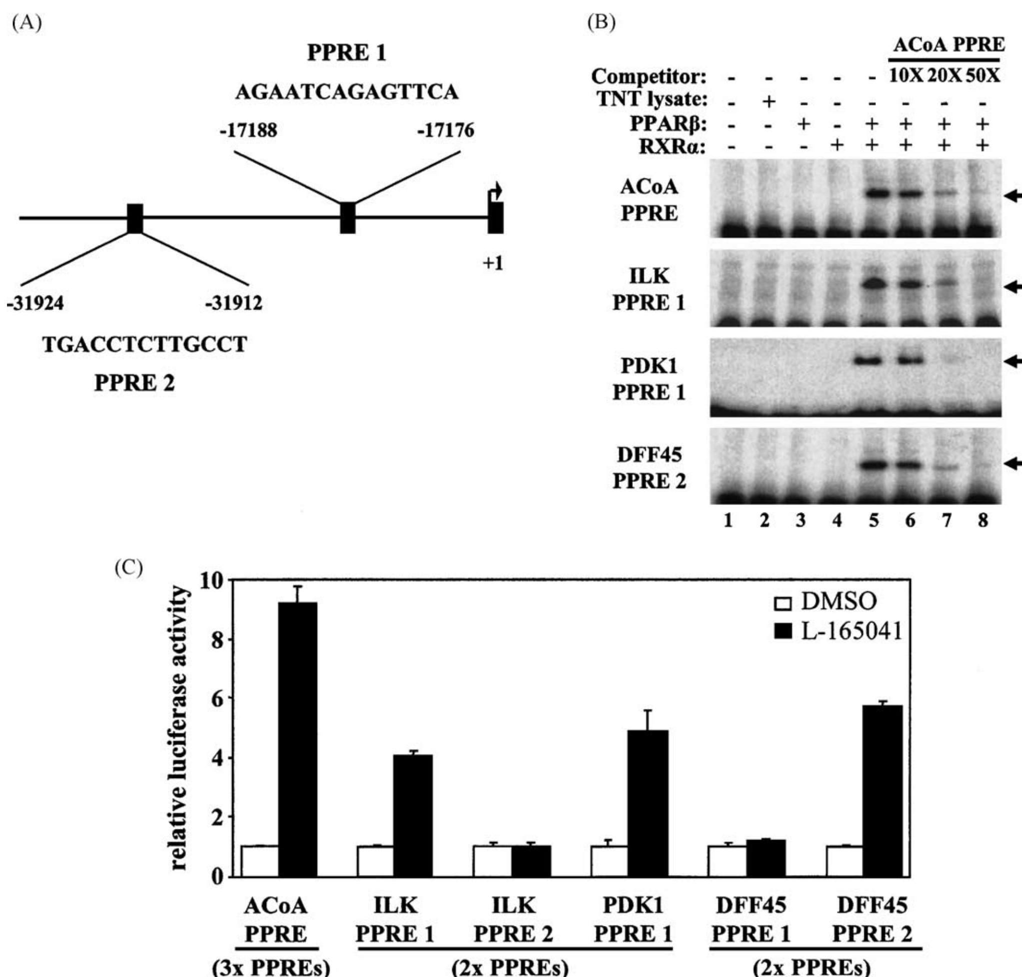

Fig. 3. ILK, PDK1 and DFF45 are direct target genes of PPARβ. (A) Relative positions with respect to the transcription start site (+1) of the two identified putative PPREs (PPRE 1 and PPRE 2) in the DFF45 promoter region are illustrated. (B) EMSA was performed using in vitro translated PPARβ and/or RXRα, and ^{32}P end-labeled PPRE oligonucleotides. The first and second lanes represent radiolabeled probe alone or with unprogrammed TNT reticulocyte lysate, respectively. Specific competition was performed with the indicated molar excess of unlabeled ACoA probe. Arrows indicate complexes comprising PPARβ/RXRα heterodimers. (C) HaCaT cells were transfected with luciferase reporter constructs containing two copies of each PPRE (2× PPREs) and treated or not (DMSO) for 24 h with 1 μM of the PPARβ ligand L-165041. A reporter containing three copies of the ACoA PPRE (3× PPREs) was used as positive control. Results represent means of at least three independent experiments, and are expressed as fold induction.

PPRE1 and DFF45 PPRE2 (lane 5). Specificity was established by competition with excess unlabeled ACoA probe (lanes 6–8). The functionality of the DFF45 PPRE2 in the chromatin context remains to be determined.

Together, these results demonstrate that PPARβ modulates cell death in keratinocytes by direct upregulation of anti-apoptotic genes, such as ILK, PDK1 and likely DFF45.

3.4. Activation of PPARβ contributes to efficient cutaneous wound repair

Skin wound healing can be defined by three distinct but partially overlapping phases: inflammation, re-epithelialization and tissue remodeling (Fig. 4). We have shown previously that, in response to cytokines released by injured skin during the inflammation phase, the expression of PPARβ and the production of its ligand are rapidly stimulated in interfollicular keratinocytes [6]. Activated keratinocytes become migratory and hyperproliferative in order to cover the wound with a neo-epithelium. Interestingly, the most significant differences in the rate of wound closure between PPARβ+/+ and PPARβ+/− mice are observed during this phase of re-epithelialization [3].

In accordance with the anti-apoptotic role of PPARβ observed in primary keratinocytes, the balance between proliferation and apoptosis is altered in the epidermis of wounded PPARβ+/− mice. Whereas there is only a two-fold increase in proliferating keratinocytes, an overwhelming 10-fold increase in the number of apoptotic wound edge keratinocytes was observed in the mutant mice (Fig. 4) [6]. These results suggest that the upregulation of PPARβ after skin injury is necessary for the survival of the keratinocytes involved in re-epithelialization in vivo.

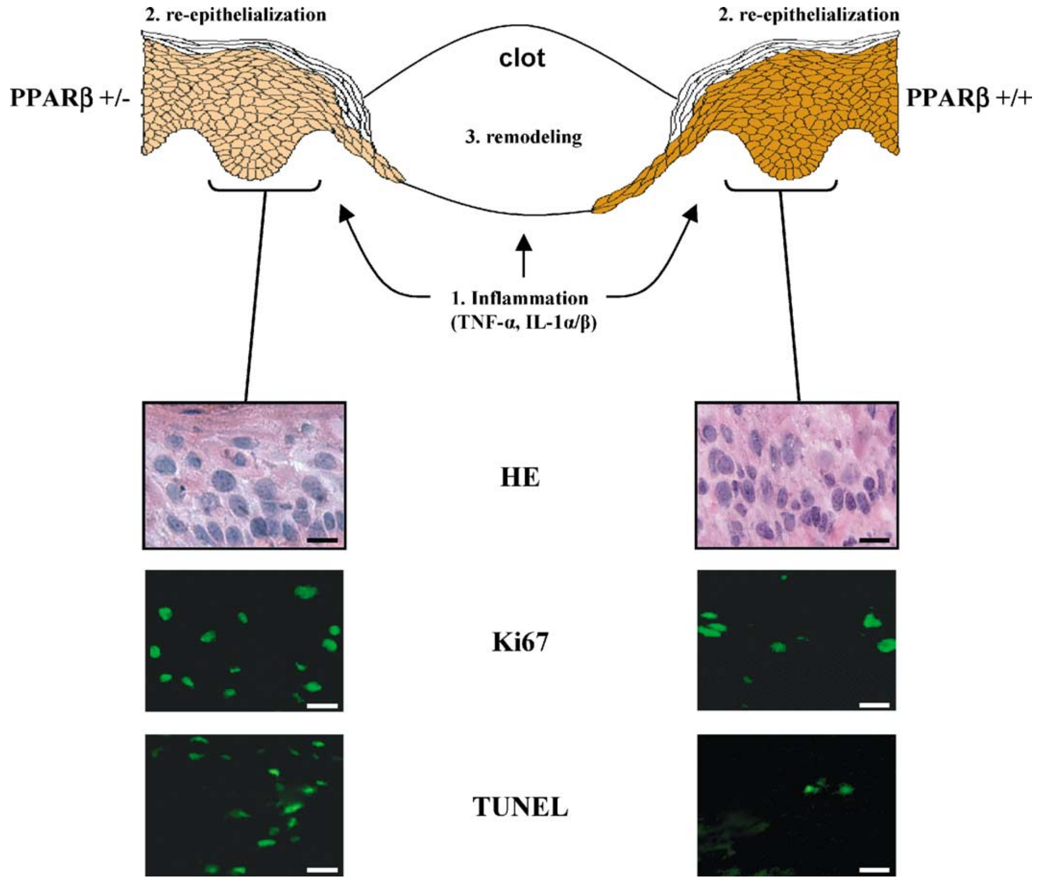

Fig. 4. PPARβ controls the balance between apoptosis and proliferation during wound repair. Skin wound healing occurs in three overlapping well-defined phases: inflammation, re-epithelialization and tissue remodeling. Difference in PPARβ expression between skin genotypes (PPARβ+/− and PPARβ+/+) is reflected by colour intensity (top panel). Representative fields of hematoxylin/eosin staining (HE), Ki67 and TUNEL fluorescent labelings obtained at the wound edges (7 days after injury) of PPARβ+/− and PPARβ+/+ skin are shown. Magnification bar, 25 μm.

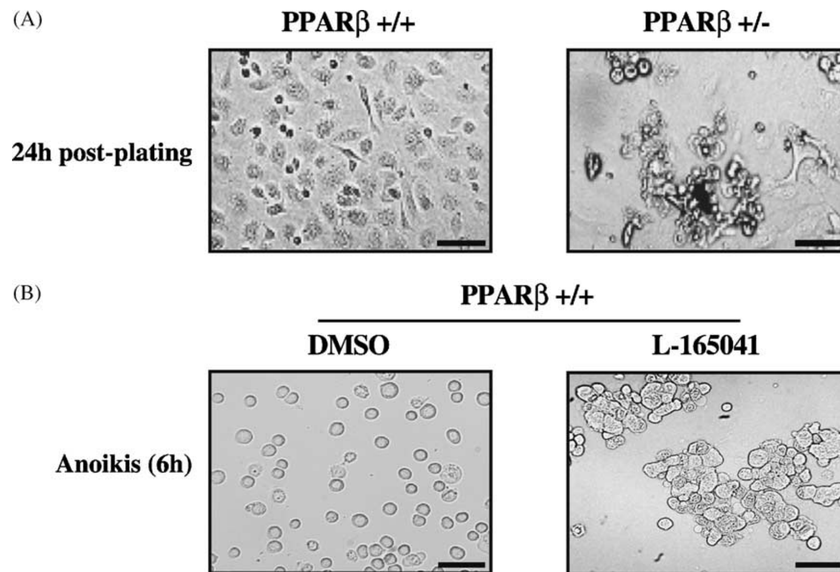

Fig. 5. PPARβ modulates cell adhesion in primary keratinocytes. (A) Representative fields of PPARβ+/+ and PPARβ+/− primary keratinocytes 24 h post-plating are shown. (B) Anoikis was induced in PPARβ+/+ keratinocytes treated either with vehicle (DMSO) or 1 μM of the PPARβ ligand (L-165041). PPARβ ligand-dependent aggregation of cells after 6 h of anoikis is shown. Magnification bar, 25 μm.

A comparative analysis of PPARβ+/+ and PPARβ mutant primary keratinocytes in culture revealed several phenotypes of the mutant cells that could also contribute to altered skin wound closure, in addition to impaired proliferation and apoptosis. Within 24 h after seeding, wild type keratinocytes spread and adhered to the culture dish. In contrast, mutant keratinocytes had impaired adhesion capacities, and adhered firmly only after 4 days of culture (Fig. 5A). In addition to these impaired adherence properties, in vivo scrapping experiments revealed migration defects in these mutated cells [3]. As reported for other keratinocyte cell lines, we also observed that treatment of PPARβ+/+ primary keratinocytes in suspension with the PPARβ agonist L-165041 led to a profound rearrangement of the cell monolayer culture, with the formation of multicellular aggregates (Fig. 5B) [9]. Our observations are consistent with aggregation-dependent cell survival previously reported [12], and show that PPARβ also modulates cell–cell contacts in keratinocytes.

In conclusion, delayed wound closure in the PPARβ mutated animals could be attributed to a rupture of the balance between proliferation and apoptosis, as well as to defects in cell adhesion and cell migration.

4. Discussion

Apoptosis is a natural event that contributes to the normal tissue homeostasis of mature organisms. It involves at least two major cascades, the death receptor and the mitochondrial pathways. Death receptors (TNF-α or Fas-L receptors) lead to the activation of the initiator caspase-8. The mitochondrial pathway involves a balanced ratio of pro- and anti-apoptotic cytoplasmic proteins of the Bcl-2 family of cytoplasmic proteins, which regulate the release of cytochrome *c* from mitochondria. Both pathways converge on the activation of effector caspases, such as caspase-3, responsible for the morphological and biochemical features of apoptosis. We have shown that PPARβ protects keratinocytes from both major apoptotic cascades, demonstrating the critical role of PPARβ for the survival of keratinocytes exposed to stress situations. The anti-apoptotic role of PPARβ could be necessary to protect the proliferative and migrating keratinocytes involved in re-epithelialization from pro-apoptotic cytokines released during wound healing, but could also influence later stages of wound repair such as tissue remodeling and/or scar formation [7]. The importance of cellular apoptosis for efficient wound repair is underscored by the observation that the inhibition of the pro-apoptotic molecule p53 leads to an accelerated wound closure [13]. Interestingly, overexpression of PTEN impairs injury healing by different mechanisms including increased apoptosis, arrest of cell proliferation and inhibition of cell migration [14].

In addition to regulate cell survival, the ability of PPARβ to modulate the PTEN/PI3K/Akt1 pathway has significant impact on various processes important during wound repair, such as keratinocyte proliferation, differentiation, adhesion and migration. Indeed, an increased proliferation and a delayed differentiation were also observed in PPARβ mutant skin [6]. We proposed that these alterations might be related to the Akt1-dependent dysregulation of the nuclear factor-κB (NF-κB) signaling [9], which plays an important role in epidermal differentiation. However, other molecules implicated in keratinocyte proliferation and/or differentiation may also be involved. Finally, impaired cell migration was observed in PPARβ−/− keratinocytes, due to changes in cell-to-matrix and cell-to-cell contacts. These changes may involve many factors including matrix metalloproteinases (MMPs) and integrins. In skin, MMP-9 is expressed in migrating keratinocytes at the wound edges, and its production is decreased in PPARβ mutant cells [9]. In addition, keratinocytes deficient in integrin β1 or PPARβ show similar migration defects [3,15]. Altogether, these results provide important insights into the functions of PPARβ during cutaneous wound repair, and suggest that the delayed wound closure observed in PPARβ mutant mice might involve the alteration of several key processes including cell apoptosis, migration/adhesion and proliferation/differentiation.

Although the ability of PPARβ to modulate the PTEN/PI3K/Akt1 signaling pathway was studied only in keratinocytes, it is interesting to note that PPARβ−/− and Akt1−/− mice [5,16–18] share many similar phenotypes (Table 1), suggesting a broader action of PPARβ via Akt1 signaling in several biological processes. Partial lethality of PPARβ−/− embryos due to placental defects from embryonic day 10.5 (E10.5) ([16], Nadra and Desvergne, unpublished observation) is consistent with the recent identification of Akt1 as a modulator of trophoblast cell differentiation [19]. In addition, embryonic lethality at the same period of gestation was observed in mice homozygous for a deletion in the catalytic subunit of the PI3K [20]. Another interesting phenotype is the growth retardation observed both in newborn and adult PPARβ knock-out, Akt1 knock-out (Table 1), and PDK1 hypomorphic mice [21]. Furthermore, both PPARβ−/− and Akt1−/− cells were more susceptible to apoptosis induced by TNF-α, GF deprivation and anoikis (Table 1). By contrast, cells deleted for the Akt1 inhibitor PTEN, which is downregulated by PPARβ [9], were more resistant to stress-induced apoptosis [22].

In human, the dysregulation of PPARβ has been associated with inflammatory hyperproliferative pathologies such as psoriasis [23]. Psoriatic epidermis is characterized by keratinocytes hyperproliferation accompanied by acanthosis and incomplete differentiation. In agreement with the anti-apoptotic role of PPARβ, aberrant expression of apoptosis-related molecules such as ICAD, whose expression is stimulated by PPARβ, was observed in psoriatic epidermis (herein and [24]). High PPARβ expression and Akt1 activation were also observed in many cancers [8,25], suggesting that PPARβ, by modulating the Akt1 pathway, may accelerate tumorigenesis and contribute to increased malignancy of tumors.

Table 1
Phenotype comparison between PPARβ−/− and Akt1−/− mice

	Strategies	
	PPARβ knock-out	Akt1 knock-out
	Peters et al. [5]: insertion into the last exon of the PPARβ gene (disruption of the ligand binding domain) Barak et al. [16]: disruption of the 5′ half of the DNA-binding domain (exon 4) Park et al. [26]: deletion encompassing the DNA binding domain (exons 4–6) Nadra and Desvergne[a]: disruption of the DNA-binding domain (exons 4–5)	Chen et al. [17]: deletion of the last six exons of the Akt1 gene (exons 8–13) Cho et al. [18]: replacement of the exons 4–8 with the neomycin resistance gene Stiles et al. [22]: deletion of the plekstrin homology domain (exons 2–3)
Phenotype	PPARβ−/−	Akt1−/−
Partial embryonic lethality	Sub-Mendelian ratio of PPARβ−/− pups [5,16][a]; ratio only restored (back-crossing) by Peters et al. [5] Placental defects in PPARβ−/− mice [16][a]	Partial lethality of Akt1−/− mice during midembryonic development [18]
Growth retardation	Smaller size of embryos from PPARβ−/− mice (10–20% reduced body weight) [5][a]	Smaller body weight (∼20% reduction) of newborn and adult mice [17,18]
Fertility	Both male and female mutants were fertile [5,16][a]	Abnormalities reported in the testes [17], but mice were fertile [17,18]
Metabolism	No consistent difference between genotypes in the serum [5] Reduced adiposity [16]	Normal glucose homeostasis and insulin levels [17,18]
Increased apoptosis	Increased apoptosis observed in PPARβ mutant mice during wound healing [6] PPARβ−/− keratinocytes were more susceptible to induced-apoptosis [9]	Increased spontaneous apoptosis observed in testes and the thymus [17] Akt1−/− MEFs were more susceptible to induced-apoptosis [17]
Tumorigenicity	Smaller size of intestinal polyps obtained by the breeding of PPARβ−/− and APCmin mice [16] Decreased ability of PPARβ−/− HCT116 cells to form tumors [26]	Akt1 deletion partially reversed the tumorigenesis of PTEN−/− ES cells (decrease of the size and of the vascularization of tumors) [22]

Reported strategies (top panel) and associated-phenotypes (bottom panel) for PPARβ and Akt1 knock-out mice are compared.
[a] Nadra and Desvergne, unpublished observation.

The expanding number of PPARβ functions in the skin highlights its central role in both normal epithelial processes and, possibly, in hyperproliferative pathologies. For this reason, we anticipate that much attention will be given to the identification of agonists and antagonists of PPARβ, to develop novel therapeutic interventions.

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