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Peroxisome Proliferator-Activated Receptors (PPARs): from metabolic control to epidermal wound healing

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Summary

Peroxisome proliferator-activated receptors control many cellular and metabolic processes. They are transcription factors belonging to the family of ligand-inducible nuclear receptors. Three isotypes called PPARα, PPARβ and PPARγ have been identified in lower vertebrates and mammals. They display differential tissue distribution and each of the three isotypes fulfills specific functions. PPARα and PPARγ control energy homoeostasis and inflammatory responses. Their activity can be modulated by drugs such as the hypolipidaemic fibrates and the insulin sensitising thiazolidinediones (pioglitazone and rosiglitazone). Thus, these receptors are involved in the control of chronic diseases such as diabetes, obesity, and atherosclerosis. Little is known about the main function of PPARβ, but it has been implicated in embryo implantation, tumorigenesis in the colon, reverse cholesterol transport, and recently in skin wound healing. Here, we present recent developments in the PPAR field with particular emphasis on both the function of PPARs in lipid metabolism and energy homoeostasis (PPARα and PPARγ), and their role in epidermal maturation and skin wound repair (PPARα and PPARβ).

Key words: lipid metabolism; metabolic diseases; inflammation; skin; keratinocytes

Introduction

In developed societies, diseases of metabolic origin such as hyperlipidemia, diabetes, and obesity have become increasingly prevalent. These disorders have a complex etiology involving genetic and nutritional factors. Intense research over the past decade has yielded evidence that a group of nuclear hormone receptors, called peroxisome proliferator-activated receptors (PPARs), are attractive target for pharmaceutical intervention of these diseases [1]. More recently, these receptors have also been shown to be involved in epidermal wound repair during the different phases of the healing process. Furthermore, they stimulate keratinocyte migration and differentiation and protect them from cytokine-induced apoptosis, suggesting that they promote keratinocyte survival after an injury [2]. It is mainly these two aspects of PPAR biology, control of lipid metabolism and energy homoeostasis and function in epidermal differentiation and repair, which will be discussed below.

Expression and molecular mode of action of PPARs

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The PPARs were first cloned as nuclear receptors that mediate the effects of synthetic compounds called peroxisome proliferators because these compounds stimulate peroxisome proliferation in the liver of rodents. PPARs are transcription factors that directly modulate gene activity (review in [3]). Their molecular mode of action is the same as that of many nuclear hormone receptors. They can be activated by specific ligands and then modulate DNA transcription by binding to defined nucleotide sequences in the promoter region of target genes. Thus, in a simplified view, the effector function of the nuclear receptors in a cell is to adapt the gene expression program in response to signals received in form of lipophilic ligands. Nuclear receptors share a common modular

structural organisation. A poorly structured N terminal domain that may comprise a ligand-independent transactivation domain is followed by the DNA binding domain (DBD) folded in two zinc fingers, which is the hallmark of members of the nuclear receptor family. A hinge region then links the DNA binding domain to the ligand binding domain (LBD) that comprises a ligand binding pocket and a ligand-dependent transactivation domain. Nuclear receptors bind to DNA in form of dimers, either homodimers or more often heterodimers with the receptor for 9-cis retinoic acid known as retinoid X receptor (RXR), which is indeed the obligate partner of PPARs. The DNA response element of nuclear receptors comprises two short hexameric sequence motifs corresponding to or closely related to AGGTCA. The organization of these motifs in direct repeats or palindromes and the length of the spacing between the two hexamers determine the binding specificity of the response elements towards each kind of family member dimers. The peroxisome proliferator response element, which binds the PPAR:RXR heterodimer, is a direct repeat of two of these motifs with a spacing of one nucleotide. Transcriptional gene regulation via nuclear receptors is believed to occur in at least two steps. In absence of ligand, the nuclear receptor dimer is associated with a co-repressor(s), a protein that inhibits its transactivation properties. In presence of a ligand, or after activation by an alternative pathway, such as phosphorylation, the co-repressor is released and co-activators are recruited, allowing further contacts to be made with the transcription machinery, which lead to an enhancement of transcription of the target genes (reviewed in [3] and [4]). Whereas the association of the unliganded PPAR:RXR heterodimer with a co-repressor in a physiological context remains to be assessed, several co-activators binding to this heterodimer have been identified [4].

Three PPAR isotypes are known to date, α, β (also called δ), and γ (NR1C1, NR1C2, NR1C3, respectively) [5]. The patterns of expression of these three PPARs contribute to the specificity of their functions. The levels of PPARα are highest in brown adipose tissue and in the liver, then come the heart, the kidney, and enterocytes. PPARβ expression is more general in terms of tissue distribution, with varying levels in different organs. Alternate promoter usage and splicing generate two main PPARγ isoforms, PPARγ1 and PPARγ2. PPARγ1 is mainly expressed in the white and brown adipose tissues, but is also detected in the colon, the spleen, the retina, and hematopoeitic cells. PPARγ2 has been found mainly in the white and brown adipose tissues [6].

The PPARα isotype is the cellular target for fibrates such as gemfibrozil, bezafibrate, and fenofibrate, which are hypolipidaemic drugs widely used for the treatment of cardiovascular diseases. The PPARγ isotype is the target for a group of drugs called thiazolidinediones (TZDs), such as the marketed rosiglitazone (Avandia) and pioglitazone (Actos), which are used in the treatment of type 2 diabetes. TZDs are effective glucose-lowering drugs that produce modest effects on lipids in these patients. Lastly, a PPARβ selective ligand, GW501516, causes a dramatic dose-dependent rise in serum high density lipoprotein cholesterol while lowering the levels of small-dense low density lipoprotein, fasting triglycerides, and fasting insulin in insulin-resistant middle-aged obese thesus monkeys [7].

A search for natural ligands revealed that PPARα is activated by a variety of long-chain fatty acids, in particular by polyunsaturated fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, linoleic acid, linolenic acid, and arachidonic acid. Competition binding and other assays revealed that the latter three are ligands for PPARα PPARβ and PPARγ albeit with various affinities. Fluorescence spectroscopy, an equilibirum method that does not require separation of free from bound ligand, established that K_ds for these fatty acids is in the nanomolar range. Only PPARα binds to a wide range of saturated fatty acids [8]. Screening of arachidonate derivatives revealed that some eicosanoids are PPAR ligands. First, the prostaglandin (PG) D2 metabolite 15-deoxy-Δ12, 14 PGJ2 was identified as a specific PPARγ ligand, and 8(S)-hydroxy-eicosatetraenoic acid (HETE) and leukotriene (LT) B4 were identified as PPARα ligands. Oxidised metabolites of linoleic acid present in oxidised low-density lipoproteins, i.e. 9-hydroxy-octadecadienoic acid (9-HODE) and 13-HODE, have been identified as PPARγ ligands. A specific potent PPARβ ligand among eicosanoids has not yet been found, although endogenously synthesised prostacyclin (PGI2) might serve as a ligand for PPARβ [9].

The ability of PPAR to bind such a diverse variety of synthetic and natural compounds has been puzzling. Partial explanation for this ability to bind multiple ligands came with the description of the crystal structure of the ligand binding domain of PPARγ [10] and PPARβ [8], and recently of PPARα [11]. A common structural three-dimensional fold, which consists of an antiparallel α-helical sandwich of 12 helices (Helix 1 to Helix 12) organised in three layers with a central ligand binding hydrophobic pocket, had been previously characterised in classical hormone receptors, such as the thyroid hormone receptor (TR), the retinoic acid receptor (RAR), and RXR [12–14]. Upon ligand binding, the ligand binding pocket closes according to a so-called "mouse trap model". The ligand binding pocket of PPARs is much larger than that of other nuclear receptors with a volume of ~1300 $Å^3$, of which the ligand occupies only about 30 to 40%. This cavity is larger than in other known LBDs (compare with the cavity in TR ~600 $Å^3$, most of this volume, ~530 $Å^3$, being occupied by the triiodothyronine molecule [14]). Overall, PPARs appear to have evolved as nuclear receptors adapted for binding to multiple natural ligands with relatively low affinity. However, the

comparison of the crystal structures of the ligand binding domain of the three PPARs revealed molecular determinants of isotype specificity which should aid in the design of drugs for the treatment of metabolic and cardiovascular diseases [11] (see below).

Functions of PPARs in lipid metabolism and energy homoeostasis

The opposite and complementary roles of PPARα and PPARγ

As can be expected from "sensor" molecules for a variety of fatty acids and their derivatives, PPARs regulate most of the pathways associated with lipid metabolism. A most fascinating recent finding is that the two isotypes α and γ have balanced regulatory actions in fatty acid oxidation in the liver via PPARα, and in fatty acid storage in the adipose tissue via PPARγ (fig. 1). The role of PPARβ remains more elusive and will be discussed later.

In addition to the identification of ligands, there are two main and complementary approaches to study the functions of nuclear receptors: characterization of their target genes to unveil the regulated pathways, and generation of null allele mutant mice that are then subjected to adequate physiological challenges. These approaches have contributed to most of the present knowledge of PPAR functions as will be presented below.

PPARα has been mainly studied in the liver where it is highly expressed and because peroxisome proliferation in rodent mainly occurs in this organ after prolonged treatment with peroxisome proliferators. In the liver, PPARα target genes form a comprehensive ensemble of genes which participates in many if not all aspects of lipid catabolism (fig. 1). It includes transport of fatty acids in the circulation, their uptake by the hepatocytes, intracellular binding by fatty acid binding proteins, activation by the acyl-CoA synthase, as well as catabolism by β-oxidation in the peroxisomes and mitochondria, and ω-oxidation in the microsomes (review in [3]). PPARα null mice are viable and do not exhibit an obvious phenotype when kept under normal laboratory confinement and diet [15]. However, these mice experience serious difficulties during fasting, a situation that normally results in an enhanced fatty acid mobilisation and increased β-oxidation in the liver as fatty acids represent the major energy source. Confronted to such a metabolic challenge, PPARα null mice are not capable of enhanced fatty oxidation and rapidly suffer from hypoketonemia, hypothermia, and hypoglycemia [16, 17]. This latter observation underscores the link between fatty acid and glucose metabolism, which is essential in general energy homoeostasis that is dependent on precise metabolic adjustments.

In contrast, PPARγ is clearly involved in the adipocyte differentiation program and lipid storage [18] (fig. 1). It is a late marker of adipocyte differentiation and its forced ectopic expression suffices to push fibroblasts into the adipogenic program. Direct target genes of PPARγ include those coding for the adipocyte fatty acid binding protein (aP2), lipoprotein lipase, acyl-CoA synthase, and fatty acid transport protein. PPARγ null mice are not viable, due to placental defects ([19], our own unpublished results). However, a thorough analysis of PPARγ heterozygous mice revealed that they are less prone to develop insulin resistance when chronically under high fat diet [20]. Again the link

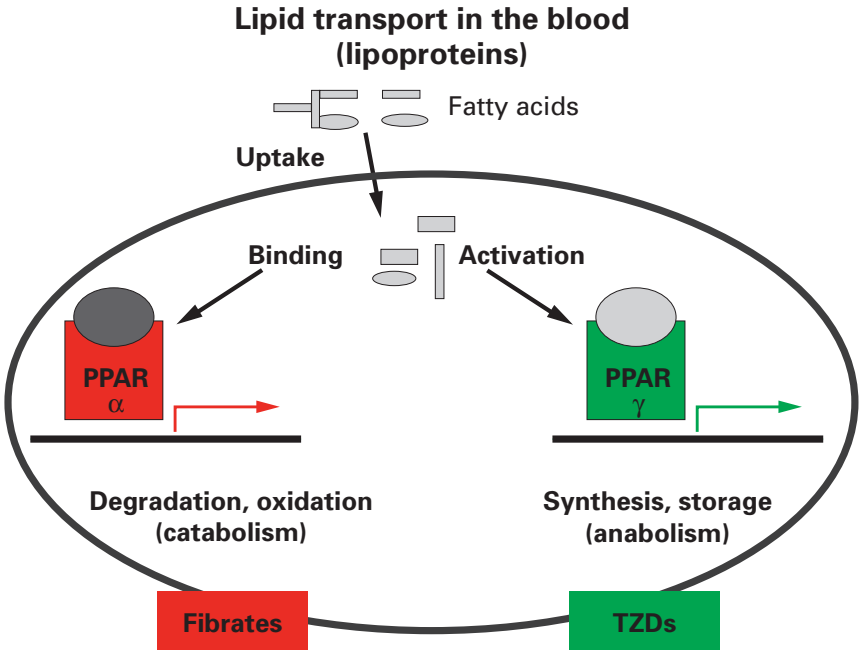

Figure 1

Examples of PPARα and PPARγ functions in energy homoeostasis. PPARα and PPARγ are regulators of opposite but complementary functions. They are involved in the control of genes regulating the transport of fatty acids in the circulation, their uptake by cells (hepatocytes, adipocytes), intracellular binding by fatty acid binding proteins, activation by the acyl-CoA synthase, as well as catabolism by β oxidation in the peroxisomes and mitochondria and ω-oxidation in the microsomes (PPARα, hepatocytes) or storage as triglycerides (PPARγ, adipocytes). PPARs are receptors for natural compounds (fatty acids and their derivative) or synthetic coumpounds (hypolipidaemic fibrates for PPARα and insulin sensitising TZDs for PPARγ).

between glucose and lipid metabolism is obvious, but further investigation is necessary to unveil the underlying molecular mechanisms.

Not surprisingly, PPARγ agonists such as TZDs, which are insulin sensitisers (see above), also induce both adipocyte differentiation in cell culture models and weight gain in rodents and humans. We recently identified a new synthetic PPARγ antagonist, dimethyl α-(dimethoxyphosphinyl)-p-chlorobenzyl phosphate (SR-202), which inhibits both TZD-stimulated recruitment of a transcriptional coactivator and TZD-induced transcriptional activity of PPARγ. Functional studies using cultured cells showed that SR-202 can indeed antagonise TZD or hormone-induced adipocyte differentiation. *In vivo*, a decrease of PPARγ activity obtained either by treatment with SR-202 or by invalidation of one allele of the PPARγ gene, leads to a reduction of both high-fat diet-induced adipocyte hypertrophy and insulin resistance. The smaller size of adipocytes in mice with lower PPARγ activity was associated with a decrease of both TNFα and leptin secretion and lower plasma free fatty acid levels, which can contribute to enhanced insulin sensitivity. Thus, SR-202 is a new selective PPARγ antagonist, which is effective both *in vitro* and *in vivo*. Because it yields both anti-obesity and anti-diabetic effects, SR-202 may be a lead for new compounds to be used in the treatment of obesity and type 2 diabetes [21].

The peroxisome proliferator activated receptor α regulates amino acid metabolism

Our recent work suggests that PPARα does not function exclusively as a regulator of lipid metabolism. It also influences the expression of numerous genes implicated in major pathways of amino acid metabolism, indicating that it is a key controller of intermediary metabolism [22]. This control includes the transcriptional regulation of genes involved in transamination, deamination, urea cycle (all five enzymes), oxidation of alpha keto acids, amino acid inter-conversions, and synthesis of amino acid derived products. With the exception of hydroxypyruvate/glyoxylate reductase and arginase, PPARα suppresses the expression of all these genes, leading to an overall decrease in amino acid degradation.

Contrary to common understanding, oxidation of amino acids contributes to a large extent to energy production in several organs, including liver and gut [23]. In addition, amino acid oxidation is dramatically elevated during conditions such as sepsis and cachexia, and after severe trauma and burns. These catabolic diseases are characterized by massive net body protein breakdown, leading to a negative nitrogen balance. Despite the clinical importance of amino acid metabolism, and unlike lipid and glucose metabolism, little information is available about its regulation at the genetic level. It has been demonstrated that glucocorticoids and glucagon increase expression of urea cycle enzymes [24–26]. Furthermore, recent work has established the important role of the transcription factor C/EBPα in stimulating the expression of urea cycle enzymes [27]. Our data point towards another global transcriptional regulator, PPARα, which inhibits amino acid degradation and has an effect directly opposite to that of C/EBPα. In agreement with these data, plasma urea levels are increased in fasted PPARα null mice compared to wild-type mice.

As fatty acids are ligands for PPARα the suppressive effect of PPARα on urea cycle enzymes may provide a potential explanation for the inhibitory effect of fatty acids on ureagenesis [28] and ammonia detoxification [29]. Fatty acids have also been shown to suppress arginino succinate synthase and carbamoyl phosphate synthase expression in cell culture [30]. This mechanism may account for the abnormal expression of urea cycle enzymes observed in carnitine-deficient juvenile visceral steatosis, a disease characterized by defective fatty acid uptake into mitochondria and associated accumulation of fatty acids in the cytosol [31].

Why would the same transcription factor that stimulates hepatic fatty acid oxidation suppress amino acid degradation and ureagenesis? During prolonged fasting fatty acid oxidation becomes the major source of energy for the liver, an effect mediated by PPARα [16, 17]. At the same time, the relative contribution of amino acid metabolism to hepatic ATP production, which is dominant in the fed state, declines [23]. In mice, this is associated with a decreased expression of several amino acid metabolising and urea synthesising enzymes during fasting. The reciprocal relationship between fatty acid oxidation and nitrogen metabolism is illustrated by comparing the plasma ketone body concentration, which reflects the rate of fatty acid oxidation, and the plasma urea concentration, which in the absence of changes in renal clearance is indicative of the rate of amino acid metabolism and subsequent urea synthesis. It is conceivable that the simultaneous increase in ketone body concentration and decrease in urea concentration during fasting in mice are actually due to the action of a single factor, PPARα which balances the activities of the two pathways by altering the expression of genes involved.

Finally, in addition to amino and fatty acid metabolism, recent evidence also implicates PPARα in the regulation of carbohydrate metabolism. It was shown that PPARα up-regulates the expression of several genes involved in gluconeogenesis. Overall, this suggests that PPARα acts as a global regulator of energy metabolism in the liver, which coordinates the rates of utilization of the various energy substrates in relation to food availability.

This short overview of PPARα and PPARγ functions emphasizes the regulatory network that these two receptors govern in the organism between the liver and the adipose tissue, and between fatty acid and glucose metabolism, allowing for a proper adaptive response to the everyday alter-

nance of fasting and feeding periods [1]. The main characteristic of PPARs resides in their ability to function as sensors of the metabolic needs of the organism. Any perturbation in the pathways they control is likely to provoke metabolic alterations such as those seen in diabetes type 2, obesity, and dyslipidaemia; thus, the strong interest of the pharmaceutical industry in developing specific PPAR ligands that could be used as drugs aimed at these disorders. In addition, other pathways, noticeably those underlying inflammatory reactions, are also major research targets with respect to PPAR functions due to the fact that the lipid mediators involved are PPAR ligands.

Functions of PPARs in epidermal maturation and repair

As the outermost layer of the skin, the epidermis affords protection against mechanical and chemical aggressions. It is a multistratified epithelium. Progenitor undifferentiated keratinocytes which migrate from the basal to the uppermost layer undergo a vectorial differentiation. This program includes a biochemical differentiation, the sequential expression of various structural proteins (e.g. keratins, involucrin and loricrin), and the processing and reorganization of lipids (e.g. sterols, free fatty acids, and sphingolipids), which will provide a hydrophobic barrier to the body. We hypothesized that PPARs have specific roles in these complex processes.

The epidermal maturation process

The epidermis matures during the latest stages of vertebrate foetal development and becomes fully developed before term. Skin development is regulated by several nuclear hormone receptors and their respective ligands. For instance, oestrogen, thyroid hormones, and glucocorticoids accelerate the skin barrier maturation, whereas testosterone delays the process [32–34]. Furthermore, retinoids are also known to influence keratinocyte differentiation. Consistent with this observation, the specific elimination of RXRα expression in the murine epidermis by conditional knock out of the gene has severe consequences on the hair follicle cycle and the epidermal maturation [35, 36].

PPAR gene expression during epidermal differentiation

Like the nuclear receptors mentioned above, PPARα, β and γ are also present in rodent keratinocytes [2, 6, 37–39]. Each of the three isotypes has a specific pattern of expression, suggesting non redundant functions during development and in the various layers of the epidermis. PPARα, β and γ transcripts are already present in the mouse epidermis at foetal day 13.5 [39]. Their expression in the interfollicular epidermis during mouse foetal development parallels all the major events of the maturation of the epidermal barrier such as, for example, the expression of differentiation markers (involucrin, loricrin, filaggrin) and changes in lipid metabolism (apparition of the lipid granules). Whether some of these events are regulated through PPARs remains to be demonstrated. PPAR expression decreases after birth to become undetectable in the interfollicular epidermis of the adult mice. In contrast, all three isotypes remain expressed in the hair follicles. Interestingly, expression of PPARα and PPARβ can be reactivated in the adult epidermis by stimuli inducing keratinocyte proliferation (topical application of TPA, hair plucking) [39]. The three PPAR isotypes have been observed in human keratinocytes as well [40–42], and PPARβ seems to be the prevalent isotype. Its expression remains high during the differentiation of human keratinocytes. PPARα and γ are expressed at lower levels, which seem to increase during differentiation.

PPAR ligands and keratinocyte differentiation in cell culture models

The expression of the three PPAR isotypes in the epidermis during rodent foetal development has prompted studies on the effects of PPAR ligands on keratinocyte differentiation. In the rat, PPARα ligands accelerate rat epidermal maturation in *in vitro* cultured keratinocytes [43–45] and *in utero* [38], whereas PPARβ and γ activators had no effects. In addition, PPARα ligands induce epidermal differentiation and restore epidermal homoeostasis in hyperproliferative mouse epidermis. In human keratinocyte cell lines however, PPARα activators seem to have no effect on cell differentiation [42], but are able to influence lipid metabolism in an *in vitro* human skin model [40]. In contrast, a selective PPARβ ligand was reported to induce the expression of differentiation markers in a human keratinocyte cell line, whereas the PPARγ ligand rosiglitazone had a negligible effect. Interestingly, PPARβ and γ ligands, when added simultaneously, have a synergistic effect on human keratinocyte cell line differentiation. Finally, we recently demonstrated that in mouse keratinocyte primary cultures a PPARβ ligand induces an acceleration of the differentiation of the cultured cells (see below). Overall, the effects of the PPAR ligands on keratinocyte differentiation appear to be quite different across species for reasons which remain to be elucidated. However, the PPAR isotypes exhibit important species specificity in ligand binding, which in addition to differences in the experimental models used could account, at least in part, for these apparent discrepancies.

PPAR functions in epidermal differentiation in vivo

Important information on the role of PPARs in epidermis homoeostasis has been obtained from PPAR mutant mouse models. To address the hypothesis of the involvement of PPAR in the differentiation of the epidermis during mouse foetal development, skin maturation was studied in PPAR mutant embryos during late foetal development, at the time of the formation of a competent epidermal barrier. PPARα null or PPARβ heterozygous mutant embryonic skin showed a normal architecture upon histological staining at all the embryonic stages examined. All the characteristic layers, with no major defect in their thickness and organization, were present in the epidermis of these PPARα and β mutant embryos. Similarly, the expression of epidermal differentiation markers in the PPARα and β deficient epidermis remained unchanged. Together, these results suggest that the mouse foetal epidermis is able to undergo normal maturation in the absence of PPARα, and in conditions where PPARβ expression is decreased by half. Similarly, the epidermis of PPARγ heterozygous animals, or of the PPARγ null mice born after placental rescue, did not exhibit any obvious maturation defect either [19, 39]. Moreover, PPARγ null mutant cells were able to participate in the development of the epidermis in mouse chimeras comprising PPARγ null and wild type cells, suggesting no or little contribution of PPARγ in epidermal tissue differentiation [46].

In accordance with earlier characterization of the PPARα null mice [15, 47], we did not detect any major defect in skin sections of PPARα null adult animals. In contrast, comparison of PPARβ heterozygous mice with wild type control animals revealed a significant increase in the keratinocyte proliferation rate in the epidermis of PPARβ adult mutant mice [39]. This difference was even more striking after topical application of TPA on the epidermis of these animals. The well characterized hyperplastic response observed after TPA treatment of the epidermis was indeed much more pronounced in the PPARβ mutant animals, strongly suggesting a defect in the control of keratinocyte proliferation in these heterozygous animals. Similarly, an impaired control of keratinocyte proliferation was also observed in a PPARβ null mouse model [48]. These *in vivo* data demonstrate that the PPARβ isotype has a role in the control of keratinocyte proliferation in the whole animal.

These observations prompted us to test whether PPARs are involved in adult skin wound repair. In a skin injury the mature epithelium is disrupted, and the covering of the wound by a new epithelium starts within hours after the event. A fully differentiated epithelium, and thus a competent protective epidermis, will eventually be reconstituted at the wounded place. This re-epithelialisation involves initially the migration of keratinocytes, their proliferation, stratification, and differentiation/maturation [49]. Using *in situ* hybridisation, we demonstrated that PPARα and β, but not PPARγ expression is upregulated in the keratinocytes at the wound edges of the damaged skin. PPARα is re-expressed transiently in this area during the early inflammatory phase of the healing, whereas PPARβ remains expressed until completion of the process [39]. Consistent with this pattern of PPAR expression during wound healing, and using PPARα, β and γ mutant mice models, PPARα and β, but not PPARγ, are necessary for the normal healing of an excisional skin wound.

In PPARα null mice there is a transient retardation of the healing process during the inflammatory phase. In the PPARβ mutant mice, completion of healing is postponed for 2 to 3 days compared to the wild type animals. Interestingly, in both models, the delay observed in skin repair is consistent with the pattern of expression of the respective PPAR isotype as analysed during skin injury repair [39]. Thus, there are important but non redundant roles for PPARα and β during the regeneration of the skin in the adult mouse. Importantly, our finding revealed that a PPARα or β mutation has no obvious effect during normal foetal development of the epidermis, but affects epidermal regeneration after an injury at the adult stage.

Mechanisms of PPAR action during wound repair

Wound repair requires the integration of interdependent processes and signals that involve, among others, soluble mediators, inflammatory cytokines produced by a variety of cell types, cell proliferation and migration, cell differentiation, and production of extracellular matrix components. As mentioned above, it comprises three successive main phases, i.e. inflammation, re-epithelisation, and tissue remodelling. During the initial phase, keratinocytes are exposed to many pro-inflammatory cytokines and bioactive lipids. The hypothesis that PPARα participates in the control of the inflammatory response is reinforced by the following observations: a) in agreement with the *in vivo* study, there is a transient increase in PPARα expression in cultured primary keratinocytes following exposure to inflammatory signals, b) inflammatory eicosanoids, e.g. LTB_4 and 8S-HETE, which are produced after an injury are PPARα ligands [50], and c) there is an alteration in the recruitment of inflammatory cells to the wound bed in PPARα-/-mice [39].

Interestingly, while keratinocyte differentiation occurs during both normal epidermis renewal and wound repair, PPARβ up-regulation is observed only in the latter. We have demonstrated that the up-regulation of the PPARβ gene is closely associated with necrosis and the inflammatory response it triggers. Pro-inflammatory cytokines, e.g. TNF-α, can both increase PPARβ expression via the stress kinases signaling pathway and trigger the production of ligands for this receptor (fig. 2). Consistent with an important role of PPARβ in mediating inflammation-induced keratinocyte dif-

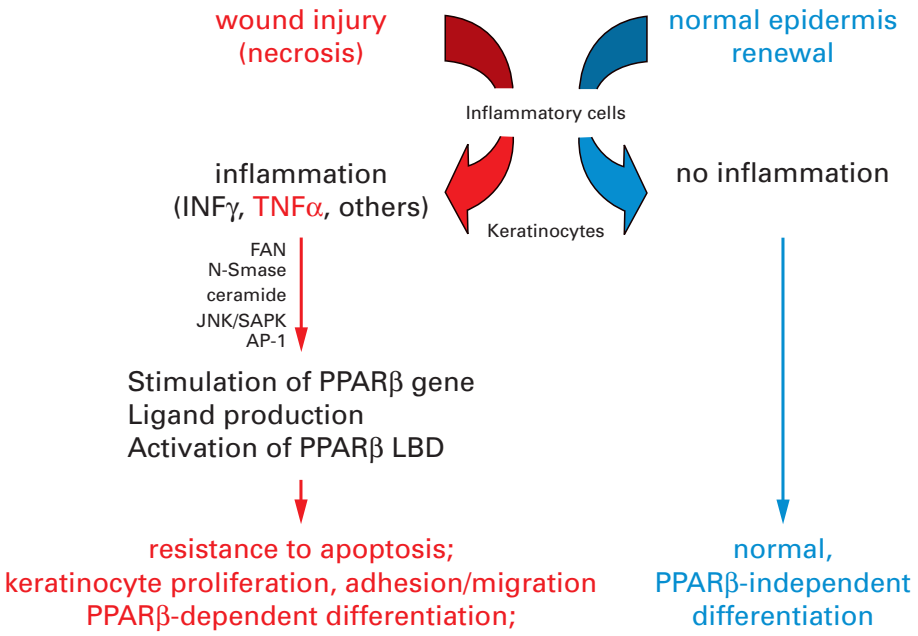

Figure 2

Role of PPARβ during wound healing. Upon injury cell necrosis triggers the production of inflammatory cytokines by immune cells. PPARβ is an important transcription factor relaying these signals (TNFα, IFNγ) at the cell surface into specific gene expression patterns that participate into the appropriate cellular responses to sudden stress situations. TNF-α or IFN-γ activate the stress-associated signaling pathway leading to the stimulation of PPARβ gene expression via an AP-1 site located in its promoter. These signals also trigger the production of PPARβ ligands that activate the receptor. The resulting increase in PPARβ transcriptional activity accelerates the differentiation of keratinocytes and increases their resistance to apoptotic signals. Increased proliferation and death of keratinocytes at the edges of epidermal wounds in PPARβ mutant mice most likely participate in the healing delay observed in these animals [39, 52].

ferentiation, keratinocytes derived from PPARβ-/- mice are both severely delayed in inflammatory cytokine-stimulated differentiation. Importantly, the injury-triggered release of pro-inflammatory cytokines is not only important for haemostasis, recruitment of macrophages and removal of infectious agents, but these cytokines are also apoptotic signals [51]. Interestingly, recent results indicate that keratinocytes derived from PPARβ-/-mice are more sensitive to TNF-α-induced apoptosis [52]. Therefore, one of the roles of PPARβ expression after injury induced by cytokines and bioactive lipids, would be to confer resistance against apoptotic signals, thereby providing a critical window for the action of other factors such as KGF, which modulate keratinocytes behavior [53]. A deficiency in PPARβ during this phase would result in an increase in the apoptosis of keratinocytes, hence reducing the number of proliferating and migrating cells that are vital for wound closure. Observations made at the wound edges of PPARβ+/-mice are indeed in agreement with this hypothesis. Although there is higher keratinocyte proliferation rates at the wound edges in these mutant mice, the number of apoptotic cells were increased dramatically [52]. Accordingly, the most significant differences in the rate of wound closure between wild type and PPARβ+/-mice are observed during the first week of wound repair [39]. As wound repair enters into the re-epithelisation phase, migrating keratinocytes have an important role in rapid wound closure. The accelerated keratinocyte differentiation sustained by elevated and prolonged activation of PPARβ as seen in keratinocyte cultures, is likely to be important during the re-epithelialisation phase, during which PPARβ might have a specific spatio-temporal role. For example, we have observed that PPARβ+/- keratinocytes in culture are defective in substrate adhesion [39]. At present, it is unclear which are the PPARβ target genes that contribute to this phenotype. It is worth noting, with respect to the role of PPARβ, that the expression of PPARα in response to inflammatory signals is very similar in wild-type and PPARβ-/-keratinocytes, indicating that PPARα does not compensate for the lack of PPARβ It also indicates that PPARα expression is not controlled by PPARβ

In conclusion, PPARα and PPARβ expression and activation might participate in the sequential regulatory events taking place during wound healing or inflammatory challenges [54]. As deviation from this pattern may result in skin disorders, e.g. psoriasis [55], the exploration of the role of PPARα and PPARβ in skin disorders might open important therapeutic perspectives and lead to the discovery of additional so far unknown functions of these PPAR isotypes.

Conclusions

The study of the PPAR expression profiles, the identification of target genes and ligands, and the utilization of PPAR mutant mouse models have unveiled distinct and often complementary physiological functions of the PPARs (fig. 3). Since the first description of the mouse PPARα as the mediator of peroxisome proliferation in the liver and as illustrated herein, PPARs have shown their importance in several vertebrate physiological pathways, such as the maintenance of energy homoeostasis and the control of the inflammatory response. The evidence that PPARs are also impli-

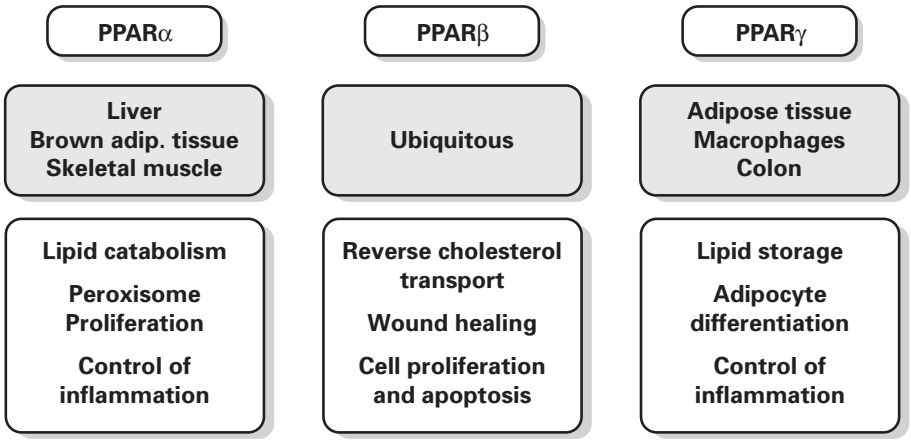

Figure 3

Expression and functions of the three PPAR isotypes. The main sites of PPAR expression and their principal functions are listed. Detailed information can be found at ref. [1–3, 56–59].

cated in cell fate is growing rapidly, and it is becoming clear that PPARs participate in the control of cell proliferation and differentiation. However, the molecular mechanisms by which PPARs coordinate the regulation of these processes remain largely unknown, and unveiling this aspect of PPAR biology is of high interest. In addition, like for many other nuclear receptors, the involvement of PPARs in tissue differentiation *in vivo* might still be underestimated, because of functional redundancies or lethality in the null mouse lines. In these cases the analysis of mouse lines in which specific tissues are deficient for a given PPAR isotype will most likely reveal additional unexpected functions for these nuclear receptors.

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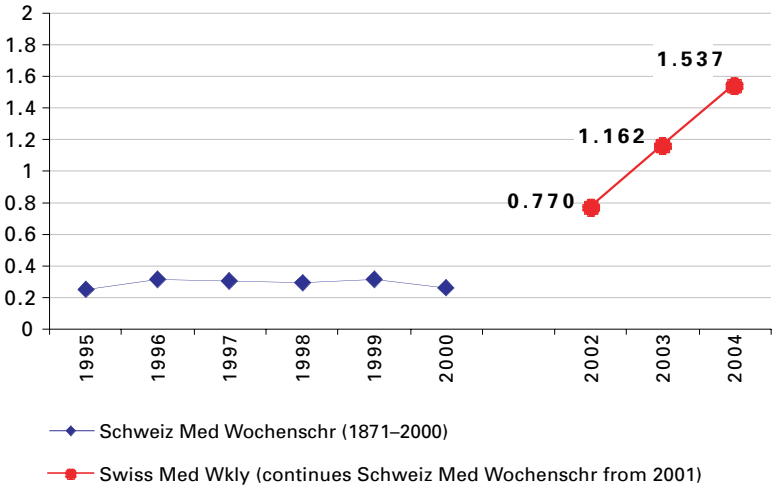

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