

Activation of PPAR β/δ Causes a Psoriasis-Like Skin Disease In Vivo

Malgorzata Romanowska^{1,9}, Louise Reilly^{1,9}, Colin N. A. Palmer², Mattias C. U. Gustafsson³, John Foerster^{1*}

1 Division of Experimental Medicine, University of Dundee, Dundee, United Kingdom, **2** Biomedical Research Institute, University of Dundee, Dundee, United Kingdom, **3** Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, Lund, Sweden

Abstract

Background: Psoriasis is one of the most frequent skin diseases world-wide. The disease impacts enormously on affected patients and poses a huge financial burden on health care providers. Several lines of evidence suggest that the nuclear hormone receptor peroxisome proliferator activator (PPAR) β/δ , known to regulate epithelial differentiation and wound healing, contributes to psoriasis pathogenesis. It is unclear, however, whether activation of PPAR β/δ is sufficient to trigger psoriasis-like changes in vivo.

Methodology/Principal Findings: Using immunohistochemistry, we define the distribution of PPAR β/δ in the skin lesions of psoriasis. By expression profiling, we confirm that PPAR β/δ is overexpressed in the vast majority of psoriasis patients. We further establish a transgenic model allowing inducible activation of PPAR β/δ in murine epidermis mimicking its distribution in psoriasis lesions. Upon activation of PPAR β/δ , transgenic mice sustain an inflammatory skin disease strikingly similar to psoriasis, featuring hyperproliferation of keratinocytes, dendritic cell accumulation, and endothelial activation. Development of this phenotype requires the activation of the Th17 subset of T cells, shown previously to be central to psoriasis. Moreover, gene dysregulation in the transgenic mice is highly similar to that in psoriasis. Key transcriptional programs activated in psoriasis, including IL1-related signalling and cholesterol biosynthesis, are replicated in the mouse model, suggesting that PPAR β/δ regulates these transcriptional changes in psoriasis. Finally, we identify phosphorylation of STAT3 as a novel pathway activated by PPAR β/δ and show that inhibition of STAT3 phosphorylation blocks disease development.

Conclusions: Activation of PPAR β/δ in the epidermis is sufficient to trigger inflammatory changes, immune activation, and signalling, and gene dysregulation characteristic of psoriasis.

Citation: Romanowska M, Reilly L, Palmer CNA, Gustafsson MCU, Foerster J (2010) Activation of PPAR β/δ Causes a Psoriasis-Like Skin Disease In Vivo. PLoS ONE 5(3): e9701. doi:10.1371/journal.pone.0009701

Editor: Jacques Zimmer, Centre de Recherche Public de la Santé (CRP-Santé), Luxembourg

Received: December 31, 2009; **Accepted:** February 23, 2010; **Published:** March 16, 2010

Copyright: © 2010 Romanowska et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The work presented here was in part funded by Cancer Research United Kingdom (role: fellowship for JF), the Anonymous Trust (role: provision of funds for consumables), and Tenovus Scotland (role: provision of funds for consumables). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: j.foerster@dundee.ac.uk

9 These authors contributed equally to this work.

Introduction

Psoriasis is one of the most frequent skin diseases world-wide, affecting approx. 2% in Caucasian, and 1% in African populations [1]. The disease represents a life-long affliction of affected patients. About 60% of psoriasis patients suffer from moderate to severe disease, i.e. more than 10% of the body surface area is covered by psoriatic plaques [2]. These patients are largely excluded from participation in activities involving public skin exposure due to stigmatization. Moreover, they exhibit increased rates of depression and alcohol consumption causing secondarily increased mortality [3,4]. Besides high direct treatment-related costs, absence from work-related indirect cost is enormous [5] and lack of employment is attributed to the disease in one-third of psoriasis patients [6]. Thus, psoriasis does not kill, but it impacts enormously on those affected and poses a huge financial burden on health care providers worldwide.

Among psoriasis patients, the prevalence of metabolic syndrome is increased [7] and an increased body mass index is a strong risk factor for psoriasis [8]. Although the molecular mechanisms underlying this association are unknown, it likely involves the existence of overlapping signalling pathways in psoriasis and other disorders of metabolism and chronic inflammation. The nuclear hormone receptor peroxisome proliferator activator (PPAR) β/δ has well established roles both in metabolism and in the skin. On the one hand, PPAR β/δ is a key regulator of adipogenesis and glucose metabolism [9]. On the other hand, it regulates keratinocyte differentiation [10]. The PPAR subfamily of nuclear hormone receptors also includes PPAR α (target of fibrate class lipid lowering drugs) and PPAR γ (target of the rosiglitazone-family of anti-diabetes drugs), all of which form heterodimers with the RXR α subunit of retinoid receptors and require binding of ligands in order to bind cognate promoters and transactivate distinct set of target genes. All three isoforms have been extensively reviewed

elsewhere (e.g. [11]). Table S9 lists selected information on ligands. Several lines of evidence support a role for PPAR β/δ in psoriasis. It is upregulated in psoriatic skin [12,13], induced by TNF α [14,15], stimulates proliferation and blocks apoptosis in keratinocytes [16], and induces angiogenesis [17], all of which is consistent with a disease-promoting role in psoriasis. Thus, induction of PPAR β/δ in the context of metabolic dysregulation might underlie the observed clinical association of psoriasis with metabolic disease.

PPAR β/δ represents an isoform of the peroxisome – proliferator activator receptor subfamily of nuclear hormone receptors.

The inflammatory patches of psoriasis exhibit a number of characteristic properties which are important clues to the underlying pathogenesis. Macroscopically, they are inducible by wounding or other mechanical skin trauma, indicating that challenges to the skin barrier trigger specific response pathways. Histologically, they are marked by increased keratinocyte proliferation, as well as a block in terminal differentiation. Accordingly, markers of late differentiation, including filaggrin, are decreased [18]. Besides keratinocyte biology, psoriasis is marked by complex pattern of immune system activation, including expansion of CD11c⁺ dendritic cells [19], upregulation of interferon signalling, and influx of T cells. Specifically, the Th17 subset of T cells has recently emerged as central for the disease [20]. In addition, endothelial cells are activated, bactericidal proteins accumulate, and a variety of soluble mediators are overexpressed (reviewed in [21]). Finally, the IL12/23p40 gene, the IL23 receptor, the β -defensin locus, as well as the HLA-C region harbour genetically predisposing variants [22], suggesting that quantitative differences in immune response pathways affect disease penetrance.

The combination of proliferative changes and a distinctive immune response pattern in psoriasis has long been recognized for its similarity to the wound response. Thus, like wound response pathways, the development of inflammatory psoriasis plaques are triggered by mechanical skin trauma, as well as infection. Therefore, in many respects psoriasis represents a proliferative wound response failing to terminate, suggesting the existence of molecular feed-forward circuits fuelling a vicious circle. In this regard, too, the upregulation of PPAR β/δ is notable since it is an important regulator of the wound response [10].

We report here that PPAR β/δ activation is sufficient to trigger a skin disease replicating many elements of psoriasis. Our findings identify PPAR β/δ as a molecular link between metabolism, keratinocyte differentiation, and the epidermal immune response.

Results

Overexpression of PPAR β/δ in psoriasis

We and others have previously shown that PPAR β/δ is overexpressed in psoriasis. In order to independently confirm those results, we re-analyzed two publicly available large gene expression datasets, totalling 58 paired lesional and non-lesional skin samples, for the expression of all PPAR isoforms. As shown in figure 1a, both data sets confirm highly significant upregulation of PPAR β/δ in psoriatic skin whereas both PPAR α and PPAR γ are downregulated, consistent with the notion that PPAR β/δ acts antagonistically to PPAR γ in psoriasis, as previously proposed [12]. Furthermore, we localized the site of maximal PPAR β/δ accumulation in the skin by immunohistochemistry. As shown in figure 1b, PPAR β/δ is found in the cytosol of the lower epidermis both in normal skin and psoriasis. By contrast, strong nuclear

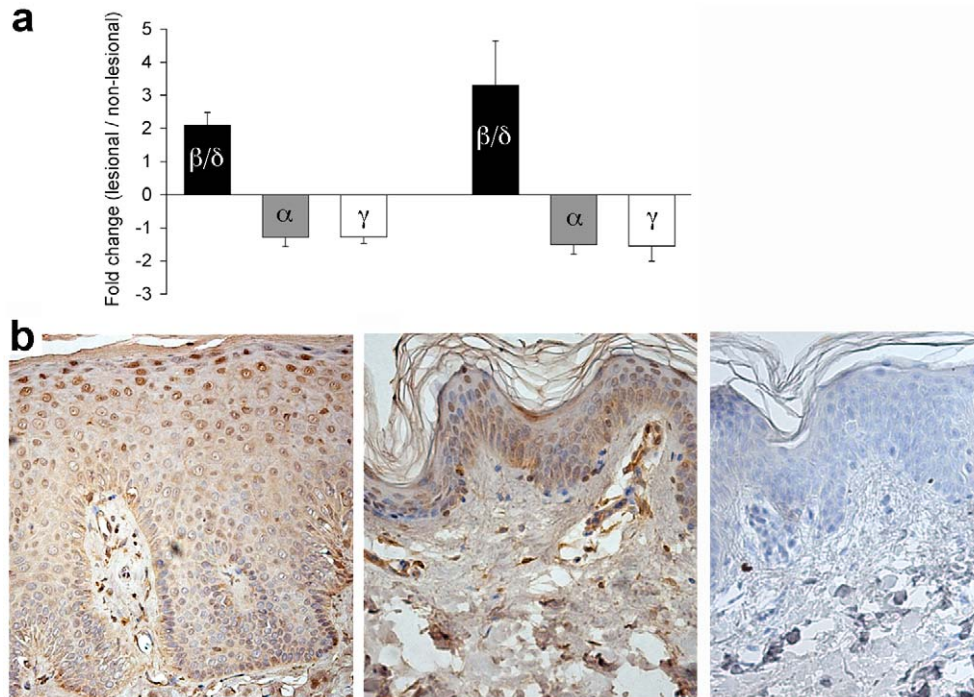


Figure 1. Overexpression of PPAR β/δ in psoriasis. A. Fold-change of mRNA expression of PPAR β/δ (black columns), PPAR α (shaded), and PPAR γ (white) between lesional and non-lesional skin. Data shown represent mean \pm s.d. from the GAIN dataset (left, n=30) and the GSE14905 dataset (right, n=28). $p < 10^{-3}$ for all data points shown. For each PPAR isoform, the probe yielding the highest hybridization signal was used to calculate the data shown (probesets 37152_at, 226978_at, 208510_at, respectively). **B.** Nuclear accumulation of PPAR β/δ in suprabasal epidermis in psoriasis skin lesions. Representative immunohistochemistry of paraffin-embedded lesional (left) and control (middle) skin samples stained with anti-PPAR β/δ , as well as staining control (right). Magnification 200 \times . doi:10.1371/journal.pone.0009701.g001

expression in the upper spinous layer was only seen in psoriasis. This pattern was highly reproducible (found in all eight lesional skin samples examined, figure S1a). These data confirm that upregulation of PPAR β/δ is a consistent feature of psoriasis and define the suprabasal epidermis as major site of its activation.

Targeting PPAR β/δ expression to suprabasal skin in mice in vivo

In mice, PPAR β/δ is not expressed in inter-follicular epidermis beyond the postnatal period [23]. To model the suprabasal expression of PPAR β/δ observed in psoriasis in humans, we initially intended to target transgenic PPAR β/δ expression using a “conventional” promoter active in suprabasal epidermis, e.g. the involucrin promoter. However, a transgenic line expressing PPAR β/δ under the control of the rat CYP1A1 promoter was already available, and turned out to afford skin-specific PPAR β/δ activation, as follows. The CYP1A1 promoter allows expression induced by the aryl hydrocarbon receptor (AhR) [24]. This promoter activity is mediated by a well-documented so-called “DXE/XRE” sequence cluster conferring responsiveness to AhR [25]. In order to bind to the DXE/XRE cluster, the AhR must first be ligand-activated, which can be achieved by employing specific synthetic chemicals such as indole-3-carbinol (I3C). However, even in the absence of AhR activation and binding to the CYP1A1 promoter, a EGFP reporter gene placed under the control of the CYP1A1 promoter was found to be constitutively and strongly active in skin-associated sebaceous glands [26]. We were able to identify a G/C rich enhancer element most likely responsible for this sebaceous-specific expression, since this element had previously been shown to direct strong sebaceous-gland specific expression in the keratin 5 promoter [27]. Indeed, a screen of the GEO database showed this G/C element to be highly conserved in the promoters of multiple genes belonging to the top 10% of all genes expressed in sebaceous glands (fig. 2a, bottom). Not surprisingly then, the CYP1A1 promoter also conferred high constitutive sebaceous-specific expression of PPAR β/δ in the absence of AhR activation (figure 2b). Human, rather than murine PPAR β/δ , was chosen as transgene to facilitate subsequent drug screening applications. We next observed that, unexpectedly, administration of the highly selective PPAR β/δ -agonist GW501516 to the chow of PPAR β/δ -transgenic mice induced subsequent additional expression of the PPAR β/δ -transgene in the epidermis (fig. 2c). Thus, functional activation of PPAR β/δ expressed at high level in the sebaceous glands causes secondary transgene expression in the epidermis. (RT-PCR analysis of PPAR β/δ -transgene expression revealed a borderline-detectable expression in whole-skin samples, consistent with the sebaceous glands forming a small minority of all skin associated cells, Figure S8). It is known that ligand-mediated activation of PPAR β/δ in the sebaceous gland triggers sebocyte differentiation [28,29] and delivery of sebum to the skin [30], containing lipoxygenase-derived bioactive lipids that can bind and activate the AhR, such as LXA4 or 5,6-DiHETE [31,32]. Once ligand-bound, the AhR is then able to transactivate the expression of the CYP1A1-controlled PPAR β/δ transgene in the epidermis via the AhR-responsive DXE/XRE element (shown in fig. 2d). In confirmation of this proposed mechanism, the transcriptional induction of the CYP1A1-controlled PPAR β/δ transgene in the epidermis could be replicated by direct topical cream application of the AhR ligand indole-3-carbinol (I3C) to the skin (I3C, fig. 2e). Furthermore, expression of transgenic PPAR β/δ was epidermis-specific and was not detectable in dermal fibroblasts, endothelia, skin – associated T cells, or any other organ screened, including intestine, muscle, liver, spleen (Fig. S1b), confirming that activation of AhR only occurred in the skin. Taken together, use of the rat

Cyp1A1-driven expression of PPAR β/δ and ligand-mediated activation by the specific PPAR β/δ agonist GW501516 promoter affords a tightly controlled epidermis-specific inducible expression of PPAR β/δ . Although we have not identified the endogenous AhR ligand(s) mediating secondary induction of the transgene in the epidermis, the net effect is a distribution and expression level of PPAR β/δ rather similar to that observed in human psoriasis (fig. 2f).

Psoriasis-like skin disease in PPAR β/δ transgenic mice

As early as seven days after initiation of PPAR β/δ - activation by GW501516 (GW), scaling, inflammation, and skin thickening was notable in all mice (figure 3a–c). Skin roughening (“hyperkeratosis”) and concomitant hair loss was maximal in regions subjected to mechanical friction, such as abdomen (fig. 3b, S2), the paws (fig. 3a), or the chin (fig. S2). While psoriasis-like plaques also developed on the back in some mice (fig. 3c), changes on the dorsal skin were mostly limited to scaling (fig. S2). Thus, the overall distribution of skin changes suggest that mechanical friction contributes a trigger effect similar to that characteristic of psoriasis. Histology showed epidermal thickening (fig. 3e), dilation of dermal vessels (black arrowhead), and abundant lymphocytes (white arrowheads). Moreover, Ki67 staining demonstrated massive hyperproliferation in the basal layer of the epidermis (fig. 3g). All of these changes are highly similar to those found in psoriasis. In contrast to psoriasis, the granular layer was prominent (fig. 3f, white arrowhead), consistent with the known effect of PPAR β/δ on epidermal differentiation [33]. In order to exclude that AhR activation as such contributed to the development of skin disease, we also administered the AhR ligand I3C in the chow at a very high concentration (0.5% w/w) in the absence of GW501516 administration, which did not induce a skin phenotype. Likewise, skin disease could be effectively replicated by topical cream-based, instead of systemic, application of GW501516 + I3C to the skin, but not by I3C alone (fig. 3h), consistent with the observation that I3C induces transcription of the CYP1A1-driven PPAR β/δ transgene (fig. 2e), but does not activate it. Finally, C57Bl/6j wild type mice fed GW501516 did not exhibit skin changes. Thus, the psoriasis-like skin disease in PPAR β/δ transgenic mice is triggered solely by activation of PPAR β/δ overexpressed in the skin, but not by endogenous murine PPAR β/δ .

Immune system activation and involvement of Th17 cells in PPAR β/δ dependent skin disease

In order to further explore overlaps of the skin phenotype in PPAR β/δ transgenic mice with psoriasis, we next characterized immunological changes after disease induction. As shown in figure 4a, there was a massive influx of CD4⁺ T cells into the dermis and, to a lesser extent, of CD8⁺ T cells into the epidermis. CD11c⁺ dermal dendritic cells were abundant, while CD11c⁺-epidermal Langerhans cells were not found. Activation of endothelial cells was also evident by staining with CD31. All of these changes are highly consistent with those typical of psoriasis. Co-immunofluorescence studies revealed that the PPAR β/δ transgene was not found in either endothelial cells or dermal dendritic cells (fig. S3), further confirming that the skin disease in PPAR β/δ transgenic mice is driven by expression of the transgene in suprabasal epidermal keratinocytes, but not other cell types.

Th17 cells are required, but not sufficient, for phenotype development

Since the Th17-subset of T cells is of central importance in the immune activation of psoriasis, we next quantified these cells using intracellular FACS analysis. Indeed, Th17-cells, marked by

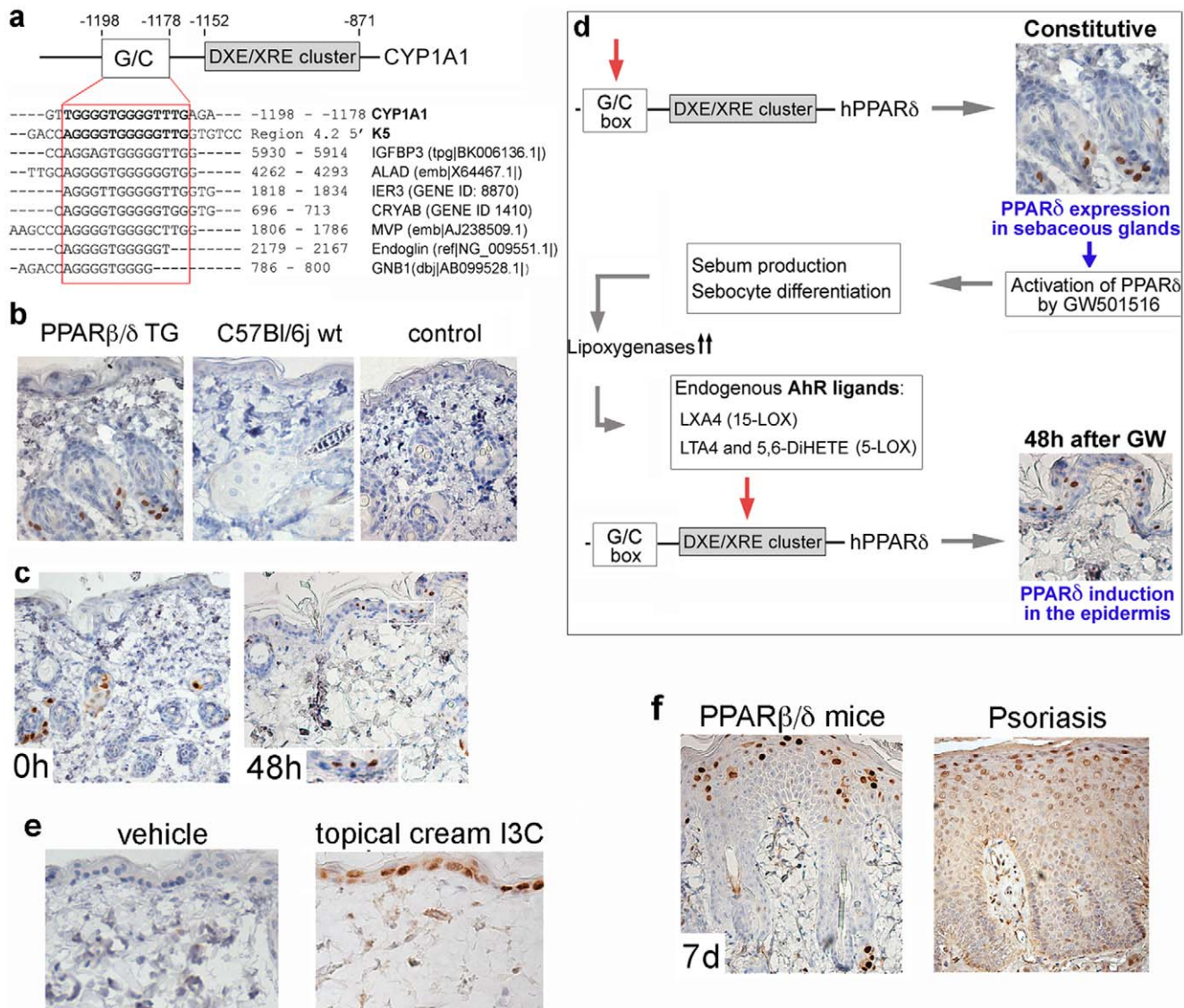


Figure 2. Inducible expression of PPAR β/δ in mouse epidermis. A. Cis-regulatory elements in the rat CYP1A1 promoter used to drive inducible expression of human PPAR β/δ . Upper panel: Map of the sebaceous – specific G/C-box element and the AHR – responsive DXE/XRE cluster in the *cyp1A1* promoter as well as the human human K5 promoter. Bottom panel: ClustalW alignment of promoters identified by a BLAST search using the 20 bp G/C element of the *Cyp1A1* promoter. All of the genes shown were found in the top 10% percentile of all transcripts expressed in human sebaceous glands (GDS3215 at the NCBI GEO website www.ncbi.nlm.nih.gov/geo/). **B.** Immunohistochemistry using anti-PPAR β/δ of mice transgenic for human PPAR β/δ driven by the rat CYP1A promoter (PPAR β/δ TG), as well as wild type mice. Magnification 200 \times . **C.** Immunohistochemistry of skin samples from PPAR β/δ transgenic mice taken before, or 48 h after initiation of PPAR β/δ activation by oral administration of the synthetic ligand GW501516 in the chow; (inset in “48 h”: 400 \times); **d.** schematic illustrating the mechanism regulating constitutive expression of transgenic PPAR β/δ driven by the rat CYP1A1 promoter in sebaceous glands, as well as delayed PPAR β/δ expression in the epidermis after ligand-mediated activation of PPAR β/δ . **e.** PPAR β/δ immunohistochemistry 48 h after topical cream application of indole-3-carbinole (I3C) to the skin of PPAR β/δ transgenic mice at 400 \times (right) magnification. **f.** Immunohistochemistry of PPAR β/δ in the skin of PPAR β/δ - transgenic mice after seven days of feeding of the PPAR β/δ ligand GW501516 in the chow (left) and in human psoriasis skin (right). doi:10.1371/journal.pone.0009701.g002

expression of IL17, were significantly expanded in the psoriasis-like plaques of PPAR β/δ mice, whereas the Th1 subset, marked by IFN γ^+ expression, was not (fig. 4b,c). A small, but statistically significant expansion of IL17 $^+$ cells was also noted in peripheral lymphoid organs upon GW501516 stimulation (fig. S4). To assess the requirement of Th17 cells for PPAR β/δ - mediated skin disease, we depleted them *in vivo* by intraperitoneal injection of anti-IL12/23p40, analogous to the monoclonal antibody (ustekinumab) used to treat psoriasis. We extended this experiment to include the effect of injection using anti-TNF α . Blockade of TNF α

is an established treatment for psoriasis. Since TNF α itself induces PPAR β/δ expression [14], blocking of TNF α should not be able to completely abrogate skin phenotype development in PPAR β/δ -transgenic mice since PPAR β/δ expression is enforced downstream of TNF α in this model. As shown in fig. 4d, treatment with anti-IL12/23p40, but not with anti-TNF α , effectively suppressed expansion of Th17 cells in GW501516-treated PPAR β/δ transgenic mice, verifying that the treatment had the expected effect. Strikingly, Th17-depletion caused a significant reduction of disease severity, as shown in fig. 4e and S5. By contrast, the effect

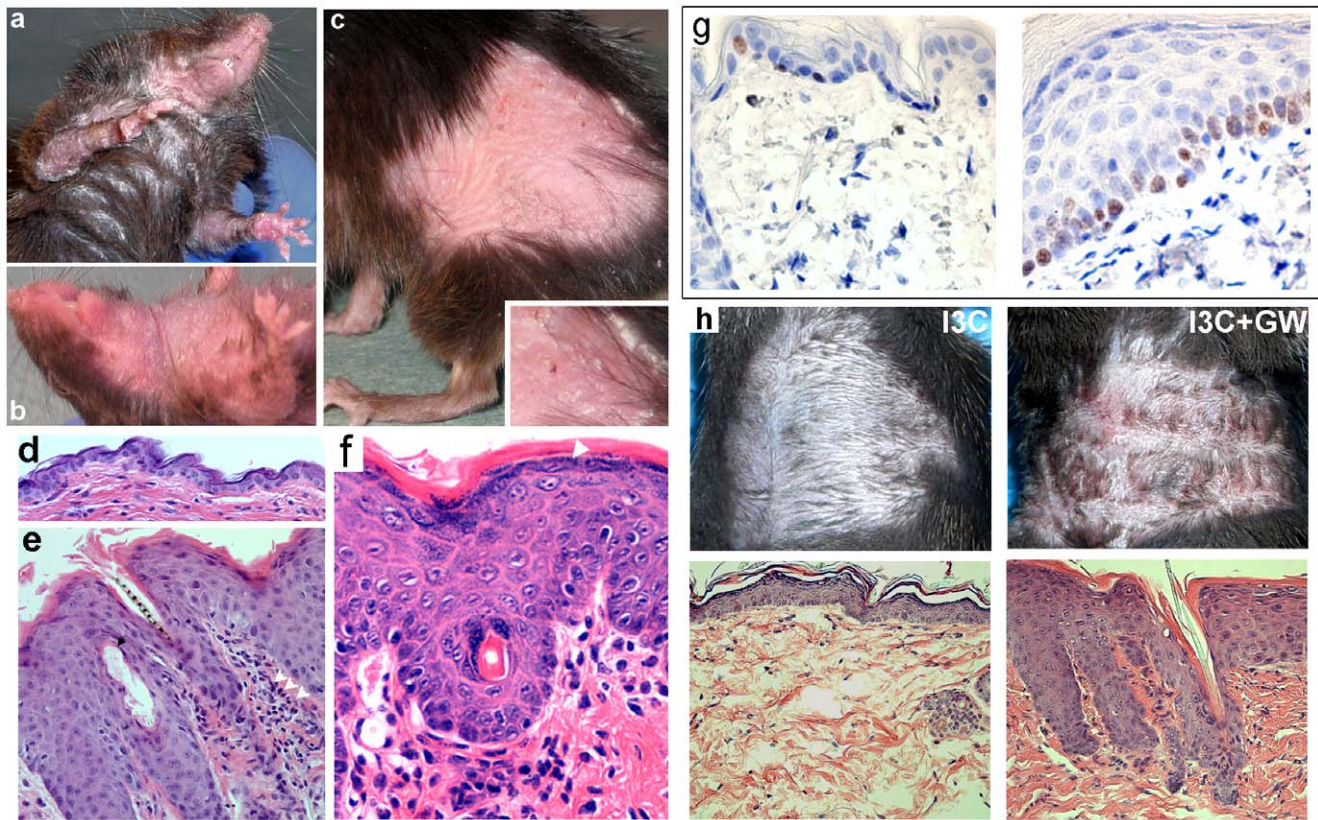


Figure 3. Skin phenotype in PPAR β/δ TG mice twenty days after GW501516 (GW) administration for twenty days. (a–c) Gross morphology, (d–e) H&E histology of control mice not treated with GW (d), or fourteen days after induction (e–g). Magnification 200 \times (d,e) or 400 \times (f). The white arrowhead in (f) denotes the granular layer. (g) Immunostaining for Ki67 of skin from PPAR β/δ TG mice maintained in the absence (left) or presence (right) of GW. Magnification 200 \times . (h) Induction of skin disease by topical application of either 0.3% of indole-3-carbinole (I3C, left) or I3C plus 0.3% GW501516 once daily to shaved abdominal skin. Gross macroscopic phenotype (top) and H&E histology of treated skin (bottom) was documented 10 days after beginning of treatment.
doi:10.1371/journal.pone.0009701.g003

of anti-TNF α was much less pronounced, as expected. Thus, Th17 cells are required for full disease expression in PPAR β/δ transgenic mice. In order to clarify whether they are sufficient to trigger disease development, we performed adoptive transfer of splenocytes from PPAR β/δ transgenic mice with active disease to wild-type mice, which had previously been depleted of endogenous CD4 $^+$ cells. This treatment failed to induce any skin phenotype even after GW501516 administration to the recipient mice. Moreover, when GW501516 was administered to naïve wild type C57Bl/6j mice, they did exhibit a modest increase of Th17 cells in peripheral organs (fig. S4), indicating that endogenous murine PPAR β/δ also stimulates Th17 cell expansion. Wild type mice did not, however, develop skin disease. Thus, Th17 cells are required, but not sufficient for development of psoriasis-like disease in PPAR β/δ transgenic mice, although we cannot rule out that their presence in the skin in high numbers might allow disease development.

Psoriasis-like gene dysregulation in PPAR β/δ transgenic mice

Although psoriasis lesions are complex, involving various cell types and a multitude of dysregulated genes, the observed changes in gene expression are remarkably reproducible between different patients. This is demonstrated by the tight correlation between two large independent expression profiling datasets (Fig. S6), thus yielding a consistent psoriasis-specific pattern of global gene

dysregulation. We studied to what extent this pattern is reflected in PPAR β/δ mice. As shown in figure 5a, most of the top 50 genes upregulated in lesional skin of PPAR β/δ mice were found congruently upregulated in human psoriasis. Quantitative real-time-PCR of selected genes confirmed that the changes observed by microarray-based expression profiling were reproducible (figure S7). Indeed, 56% of all upregulated and 33% of all downregulated genes in PPAR β/δ mice were found congruently regulated in psoriasis, respectively (figure 5b, clusters I and VI). Conversely, appr. 30% of all genes dysregulated in human psoriasis were found to be regulated congruently in PPAR β/δ mice (table S3). Geneset Enrichment analysis (GSEA) independently confirmed a highly significant enrichment of those genes upregulated in psoriasis (defined as gene-set) in lesional skin of PPAR β/δ mice (figure 5c). Only two small subsets of genes (8.3% of all, clusters III and IV) displayed inverse regulation between psoriasis and PPAR β/δ mice. When analysing the functional profile of these, we observed that cluster III, containing genes upregulated in PPAR β/δ mice but downregulated in psoriasis, was enriched for markers of late epidermal differentiation (e.g. FLG, PCDH21), indicative of cells in the so-called granular layer, which is prominent in PPAR β/δ mice (fig. 3f) but absent in psoriasis. Cluster IV, containing genes upregulated in psoriasis but downregulated in PPAR β/δ mice, was highly enriched for interferon-signalling (fig. 5b, table S3), where we were able to identify the mechanism underlying this discrepancy (see below). Taken together, expression profiling

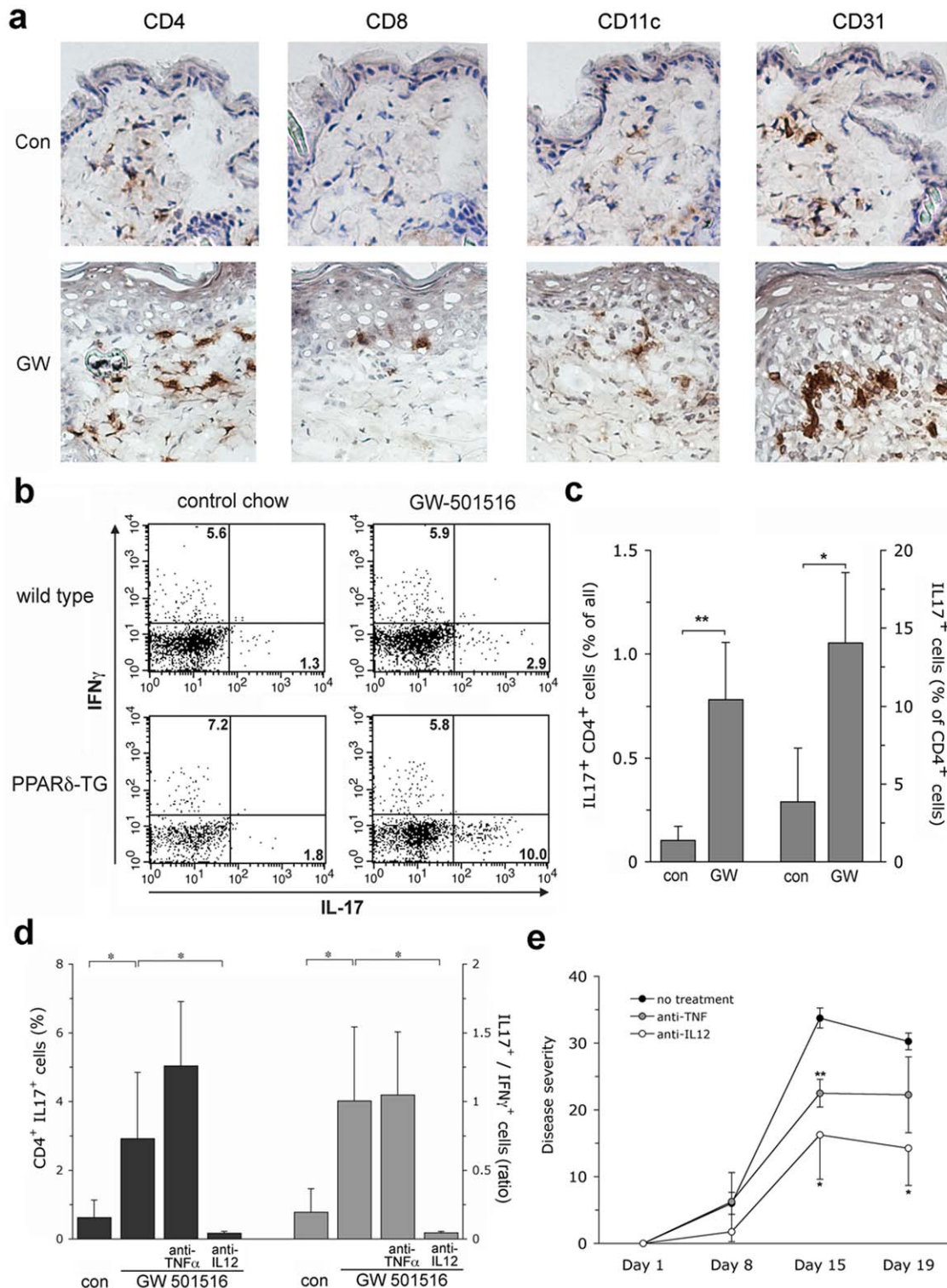


Figure 4. Immune activation in PPAR β/δ -mediated skin disease. (a) Immunohistochemistry for CD4, CD8, CD11c, and CD31 (Pecam 31) of skin from PPAR β/δ transgenic mice maintained in the absence (top) or presence of GW501516. Magnification 200 \times , (b) flow cytometry analysis showing intracellular FACS-staining for IFN γ and IL17 of skin cells (gated for CD4) from wild type and PPAR β/δ transgenic mice maintained in the presence or absence of GW501516, respectively. Numbers in quadrants indicate frequency of positive cells, (c) frequency of CD4 $^+$ IL17 $^+$ of IL17 $^+$ cells (expressed as percent of all CD4 $^+$ gated cells) in PPAR β/δ transgenic and C57Bl/6 wild type mice maintained in the presence or absence of GW501516 (n=4 per group), as determined by flow cytometry. * p<0.01; ** p<0.001, (d) frequency of CD4 $^+$ IL17 $^+$ Th17 cells (left y-axis, black columns) and ratio of IL17 $^+$ and IFN γ $^+$ cell frequencies (right y-axis, grey columns) in the skin of PPAR β/δ mice maintained in the absence or presence of GW501516 with or without i.p. injection of anti-TNF α , or α IL12/23p40 (n=4, see Methods), (e) disease severity, expressed as mean \pm s.d., assessed by the degree of erythema, thickening, scaling, and hair loss (see Methods, representative photographs of mice on day 19 post induction are shown in figure S6) in PPAR β/δ transgenic mice GW501516 – containing chow with or without additional intraperitoneal injection of anti-TNF α or α IL12/23p40 (anti-IL12). * p<0.01, ** p<0.001 (treatment vs. control). doi:10.1371/journal.pone.0009701.g004

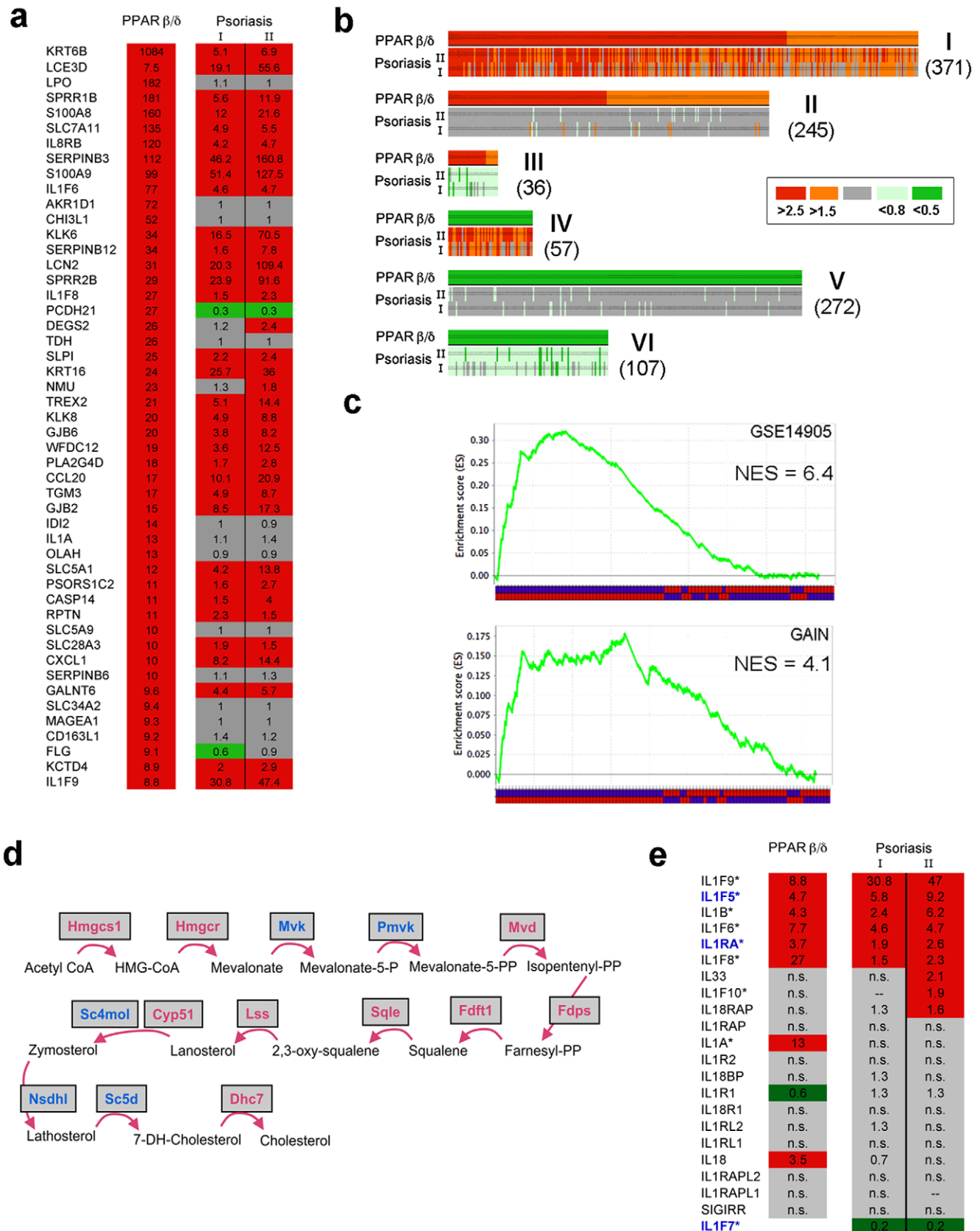


Figure 5. Congruent gene dysregulation in PPAR β/δ mice and psoriasis. (a) Fold-change between the lesional skin of PPAR β/δ mice after administration of GW501516 and control mice ($n = 3$ per group), and between lesional and non-lesional skin samples from psoriasis obtained through the GAIN (I) and the GSE14905 (II) datasets, respectively, as detailed in Methods. Red: FC >1.5 , Green: FC <0.8 . Shown are the top 50 upregulated genes. The complete dataset is given in table S1. (b) Heat map showing all genes dysregulated in GW501516-fed PPAR β/δ mice ($n = 1077$), clustered

by congruence with psoriasis. Color codes for $-$ fold change are indicated. The genes in all clusters are detailed in table S2. **(c)** gene-set enrichment analysis (GSEA), performed using the top 500 genes upregulated in psoriasis lesions from the GSE14905 dataset (top), or the GAIN dataset (bottom), as genesets, respectively, and the complete mouse array collapsed to single genes as expression dataset. Analysis was run with 100 permutations and a classic statistic, NES = normalized enrichment score. The blue-red lines on the bottom represent heat-map of human genes found to be upregulated (blue on top) or downregulated in the mouse set. **(d)** Induction of cholesterol biosynthesis, conjugation, and channeling by PPAR β/δ . Red: upregulated in psoriasis and PPAR β/δ transgenic mice, blue: upregulated only in PPAR β/δ transgenic mice. Shaded boxes: repressed by Foxo1. **(e)** Induction of IL-1 signalling by PPAR β/δ . Datasets and color codes are as in (a). “n.s.”: $p > 0.01$; “-”: fold change between 0.8–1.2., blue print: anti-inflammatory. * gene located within the IL1 cluster on chr. 2q between 113.2–113.7 Mb. (IL1F7 has only been identified in *homo sapiens* and *bos taurus*, the closest homologue in mice is IL1F5). doi:10.1371/journal.pone.0009701.g005

confirms a large overlap in gene regulation between psoriasis and the skin disease in PPAR β/δ mice, strongly suggesting that the phenotype similarities described above are not a phenocopy but involve overlapping signalling pathways.

PPAR β/δ -dependent regulation of specific pathways

When extending analysis of gene expression to functional processes, we found that processes concordantly regulated in psoriasis and PPAR β/δ transgenic mice included lipid-metabolism, differentiation, and proliferation (table S4), which confirmed the expected, given the known activity profile of PPAR β/δ . Likewise, the complete set of genes involved in cholesterol biosynthesis was strongly co-upregulated (fig. 5d, table S6), as were a number of proliferation-associated kinases (table S8). Unexpectedly, however, both the human and the murine datasets exhibited a highly consistent upregulated IL1-signalling module, which, remarkably, not only includes pro-inflammatory transcripts but also the anti-inflammatory components IL1F5, and the IL1-receptor antagonist (fig. 5e, table S7). Importantly, wild type C57Bl/6 mice administered GW501516 did not exhibit these changes (table S1), but did show the expected upregulation of genes involved in lipid metabolism (table S5), thereby confirming that the observed induction of IL1-signalling was triggered by the transgene rather than endogenous murine PPAR β/δ . These results strongly suggest that a number of transcriptional programs known to be dysregulated in psoriasis are regulated by PPAR β/δ .

Critical role of STAT3 in PPAR β/δ dependent skin disease

STAT3 is phosphorylated in psoriasis [34], as well as in a wound-response type model of psoriasis induced by serum response factor-deficiency [35]. Accordingly, we analyzed STAT3 activation in PPAR β/δ mice. Tyr-705 phosphorylation of STAT3 was markedly increased in lesional skin of PPAR β/δ transgenic mice (figure 6a) and localized to the nuclei of suprabasal cells in the epidermis (figure 6b). Moreover, inhibition of STAT3 phosphorylation by the JAK2 inhibitor WP1066 led to a marked attenuation of skin disease, demonstrating the relevance of this pathway for the development of clinical disease, further demonstrating the overlap in pathogenesis between psoriasis and the current model (figure 6c).

STAT3 activation mediates suppression of interferon-target genes

As described above, the single group of genes upregulated in psoriasis but downregulated in PPAR β/δ mice were the interferon response genes (fig.5b, cluster IV). Strikingly, precisely this set of genes was previously shown to be repressed by STAT3 in vivo (fig. 6d, dark shaded columns). We therefore hypothesized that the notable repression of interferon-response genes in the skin of PPAR β/δ transgenic mice with skin disease was mediated by activation of STAT3. Indeed, repression of STAT3 activation by use of the JAK2 inhibitor significantly blocked the down-regulation of one of the most repressed transcripts, IFI27 (fig. 6e).

This STAT3-dependent effect was specific to interferon-response genes, since the dysregulation of another inflammatory pathway, exemplified by IL1 β , remained unaltered by STAT3 inhibition (fig. 6e). These data show the inhibition of IFN signalling in PPAR β/δ transgenic mice is mediated by STAT3 as part of what has previously been termed the “anti-inflammatory response” [36].

Discussion

We here show that PPAR β/δ is activated in the upper epidermis of human psoriatic skin and that recapitulation of this event in mice is sufficient to elicit major elements of psoriasis. PPAR β/δ transgenic mice exhibit not only down-stream immunological changes but also psoriasis – specific gene dysregulation, thereby defining subsets of genes regulated by PPAR β/δ in psoriasis. Although the current transgenic model exhibits important differences to psoriasis (see below) and cannot recapitulate all features of a polygenetic disease, it does thus indicate that activation of PPAR β/δ in the upper spinous layer of the epidermis initiates a number of inflammatory and immunological changes seen in psoriasis.

One major implication of the present results is that they suggest a molecular explanation for the clinical overlap between psoriasis and metabolic, as well as cardiovascular disease [37]. Thus, PPAR β/δ expression is increased in chronic inflammation and regulated by caloric intake [38,39]. Specifically, factors such as TNF α which are known to directly induce PPAR β/δ expression are increased in the chronic inflammation accompanying metabolic syndrome [40]. Therefore, obesity, chronic inflammation, and dyslipidemia may increase the penetrance of psoriasis by inducing PPAR β/δ expression. Conversely, it is tempting to speculate that weight reduction or correction of other PPAR β/δ - inducing factors leads to suppression of PPAR β/δ expression in the skin, thus dampening disease severity. This may well be a contributory factor in the clinical observation that the response to low-dose cyclosporin, an established psoriasis treatment, is improved in psoriasis patients undergoing weight loss [41].

The role of PPAR β/δ in inflammation has been extensively and controversially studied, several papers suggesting anti-inflammatory properties (e.g. [42]), while others find that it stimulates pro-inflammatory cytokine synthesis including IL-8 and IL1 β in macrophages [43]. Here, we show that, in the skin, PPAR β/δ induces a specific IL-1 signalling “module” both in human psoriasis and in PPAR β/δ transgenic mice. This module includes pro-inflammatory mediators such as IL1 β , which is known to stimulate Th17 differentiation [44], and IL1F8, which stimulates pro-inflammatory mediators in fibroblasts [45], but also anti-inflammatory cytokines such as IL-1F5, which actually inhibits inflammatory skin disease [46], as well as the IL1 receptor antagonist (IL1RA). The latter has recently been shown to be a direct target of PPAR β/δ [47] and to be upregulated in psoriasis [48], thereby corroborating our findings. Thus, the PPAR β/δ - mediated induction of IL1-family cytokines in psoriasis defies a

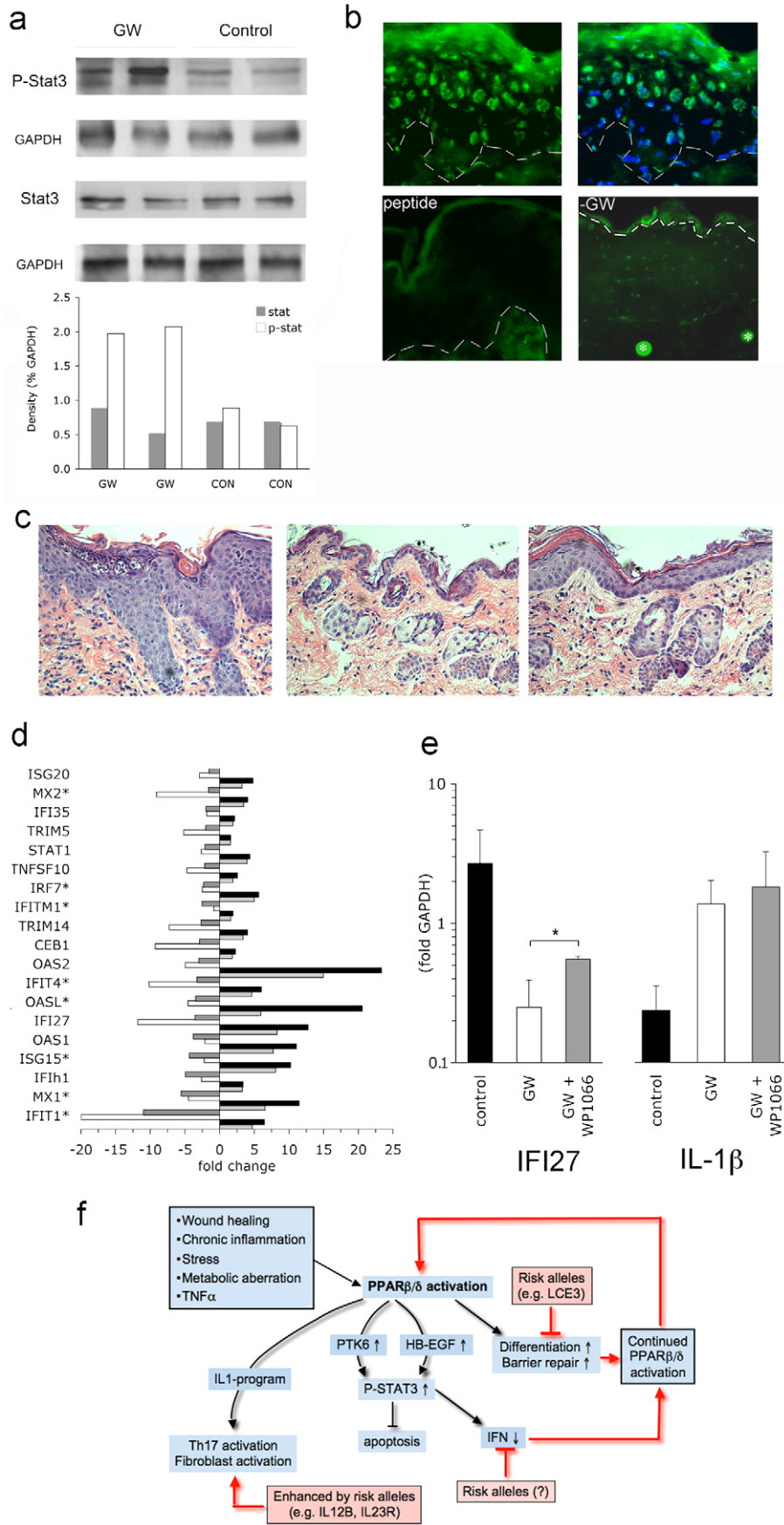


Figure 6. Activation of STAT3 by PPAR β/δ . (a) Western blot of whole skin samples from two GW501516-treated (GW) and two control PPAR β/δ transgenic mice, respectively, probed with anti phospho-STAT3 (top) and anti-STAT3 (bottom) along with anti-GAPDH loading controls (top-band of the STAT3 doublet represents STAT3 α , bottom-band STAT3 β , respectively), semi-quantitative densitometry performed using ImageJ is included on the bottom, (b) immunofluorescence with anti phospho-STAT3 of GW-treated skin (upper left), upper right: same with DAPI counterstain to verify nuclear localisation, bottom left: control stain performed in the presence of blocking peptide, lower right: PPAR β/δ transgenic mouse not treated with GW. White dashed lines mark the dermo-epidermal boundary; all samples at 400 \times magnification. * = hair shaft (c) H&E histology of skin from GW-treated (left panel), untreated (middle), GW-treated mice concurrently receiving intraperitoneal injections of the STAT3 inhibitor WP1066 (right) at 200 \times magnification, (d) fold change of genes previously shown to be repressed by activated STAT3 (dark grey columns, data taken from [56]) and their regulation in GW501516-fed vs. control PPAR β/δ transgenic mice (white), lesional vs. non-lesional skin from psoriasis patients in the GSE14905 (black), as well as the GAIN (light grey) datasets, respectively. * denotes genes that are not contained in cluster IV (table S2) since they did not meet the $p < 0.01$ cut-off. (e) Taqman-based qPCR of IFI27 and IL1 β from whole skin of untreated (black columns), GW-fed (white), and GW-fed + WP1066-injected PPAR β/δ mice (grey), respectively (n=3 mice per group), * $p < 0.05$; (f) schematic illustrating the PPAR β/δ /STAT3 /IL-1 pathway identified here, the role of PPAR β/δ in maintaining chronically active psoriasis, as well as disease-enhancing role of predisposing genomic risk alleles. The box on the upper left lists factors known to trigger both PPAR β/δ induction as well as clinical psoriasis flares. doi:10.1371/journal.pone.0009701.g006

simplified concept of purely “pro-“ or “anti-” inflammatory. Clearly, these results would signal some caution regarding the proposed use of PPAR β/δ agonists to treat a variety of conditions [49].

We here identify activation of STAT3 as a novel pathway targeted by PPAR β/δ . PPAR β/δ activation evidently causes psoriasis-like disease not solely through STAT3 activation since (i) the phenotype is not completely reversed by inhibition of STAT3 and (ii) overexpression of STAT3 alone causes a less widespread psoriasis-like phenotype with a much longer latency [34]. Regarding the mechanism of STAT3 activation, STAT3 can be phosphorylated by a number of kinases. Of these, at least two appear to be involved. First, the two EGF-family ligands TGF α and HB-EGF, previously identified as a direct transcriptional target of PPAR β/δ [12], are highly upregulated in PPAR β/δ as well as in psoriasis, suggesting that EGF-receptor activation contributes to STAT3 phosphorylation. Second, PTK6 kinase, which also phosphorylates STAT3 [50], is the most highly upregulated kinase in psoriasis and PPAR β/δ mice (table S8). Thus, at least two kinase pathways converge on STAT3 phosphorylation both in psoriasis and PPAR β/δ mice.

An obvious difference between the skin disease induced by activation of PPAR β/δ and psoriasis in humans is the regulation of IFN signalling. While IFN response genes are strongly induced in psoriasis they are repressed in PPAR β/δ transgenic mice. On the other hand, subsequent downstream events, including CD4+ and CD8+ T-cell influx, endothelial activation, dendritic cell accumulation, as well as Th17 activation are all recapitulated preserved in this model. Therefore, the present data suggest that upregulation of interferon response genes is not, as commonly assumed, required for sustained disease. Furthermore, while the upregulation of IFN response genes could be taken for granted in the milieu of a wound-response, our data show that they should actually be repressed by the so-called anti-inflammatory response mediated by STAT3 [36,51]. Their continuous upregulation despite STAT3 activation suggests the existence of as yet to be identified factors actively inhibiting STAT3 repression of IFN target genes (schematically shown in fig. 6f).

Psoriasis is a genetically determined disease and genomic variants at the PPAR β/δ genomic locus have not so far been associated with psoriasis. Although this might be regarded as evidence against a role for PPAR β/δ in psoriasis, our data clearly show that overexpression of PPAR β/δ in psoriasis skin lesions is a common phenomenon occurring in the vast majority of psoriasis patients (fig. 1). Thus, upregulation of PPAR β/δ appears to occur downstream of individually variable genomic risk, offering a potential target for treatment relevant for most patients.

Apart from interferon response genes, the other major difference in gene expression between psoriasis and the phenotype

in PPAR β/δ mice is terminal epidermal differentiation, which is blocked in psoriasis, but increased in the mouse model, which confirms the established pro-differentiation activity of PPAR β/δ [52] and the fact that it triggers differentiation in wound healing [28,53]. In psoriasis, on the other hand, late epidermal differentiation is disturbed and the skin barrier disrupted. Based on the data presented here, one may speculate that the suppression of terminal differentiation and the block in skin barrier repair, aggravated by genomic risk alleles such as the recently described LCE3 variant [54], act as stimuli to maintain sustained upregulation of PPAR β/δ in the upper epidermal layers. The net effect would be the establishment of a vicious cycle, schematically shown in fig.6f, which is able to account for the chronic persistent course typical of psoriasis.

In conclusion, we here identify a central role for PPAR β/δ in the pathogenesis of psoriasis and identify IL1 and STAT3 signalling as novel pathways regulated by PPAR β/δ . Our data suggest novel approaches to psoriasis treatment. Finally, our results underscore that PPAR β/δ activation as a treatment strategy for metabolic diseases might harbour the risk of pro-inflammatory effects or autoimmune activation.

Methods

Ethics Statement

All work involving animals was approved by the Tayside Ethics Committee. Storage and use of all tissues included in the work presented here was approved by the Tayside Committee on Medical Research Ethics B (REC ref. Nr. 07/S1402/90).

PPAR β/δ immunohistochemistry

Paraffin-embedded samples were obtained from the Tayside Tissue bank. Prior to biopsy, patients gave written consent to storage and analysis of biopsy samples. Sections from paraffin embedded tissue (nominally 4 microns thick) were cut onto superfrost plus slides (VWR International Ltd) and dried for 1 hr at 60 °C before being de-paraffinised in Histoclear (National Diagnostics) and then rehydrated through a graded alcohol series. 10 mM Citric acid buffer, pH 6.0 was used as standard microwave-based antigen retrieval methods. Sections were microwaved in a pressure cooker for 15 min before being immunostained on a DAKO autostainer using Vectastain[®] ABC kits (Vector Labs) according to the manufacturer’s protocol. Briefly, sections were blocked in either normal goat, rabbit or horse serum containing 10%(v/v) from stock avidin solution (Vector Labs) for 20 min followed by 1 hr incubation with anti-PPAR β/δ (R&D, PP-K9436) at 1:100 overnight at 4°C for human samples (incubation for 1 h at room temperature yielded comparable results.), and 1:1000 for mu-

rine samples. Sections were washed 3 \times for 15 min in TBS, pH 7.6, followed by anti-mouse-biotin, antibody for 30 min followed by Vectastain Elite ABC reagent for another 30 min. Liquid Diaminobenzidine (DAB) (DAKO) was applied for 5 min and sections were counterstained with Mayer's haematoxylin.

H&E histology

Paraffin-embedded samples were heated for 15 minutes. Samples were treated with 3 washes of Xylene, followed by a series of graded alcohol washes. Samples were washed with water followed with staining with Harris' Haematoxylin. Wash 2 with water. Samples incubated in 0.1% acid alcohol for 1 minute followed by a 3rd wash with water. Samples were then incubated in STWS for 1 minute followed by a 4th wash with water. Samples stained with Shandon Eosin for 30 seconds followed by a 5th wash. Samples were re-hydrated with a series of graded alcohol washes followed by 3 washes with Xylene. Sections were mounted with DPX.

Generation of PPAR β/δ transgenic mice

PPAR β/δ transgenic mice were generated by cloning full-length human PPAR δ downstream of the human CYP1A1 promoter. Plasmids encoding human PPAR δ were prepared as follows. The PPAR δ coding sequence was amplified using primers PRMG15 (5'-CTAGTCTAGA**ATGGAGCAGCCACAGGAGGAAGC**-3') and PRMG3 (5'-CTAGTCTAGATTAGTACATGTCCTTG-TAGATCTCCTG-3'), respectively (*Xba*I-sites underlined, ATG start codon in bold). PCR products were cleaved with *Xba*I and cloned in plasmid pUHD10-3 (M. Gossen, unpublished, Genbank accession number U89931) creating pMGD7 (PPAR δ). The integrity of the inserts was confirmed by sequencing and cleaved out using *Bam*HI and ligated into plasmid pAHIR1- β -gal (Campbell, 1996) cleaved with *Bgl*II, resulting in the plasmid pMGD72 (PPAR δ). Proper insert orientation was confirmed by restriction endonuclease analysis and sequencing. Transgenic mice were generated by microinjection of the expression unit (*Not*I fragment) of the plasmid pMGD72 into pro-nuclei of C57BL/J6 x CBA F1 fertilized eggs. Mice were maintained under standard animal house conditions.

Disease induction

PPAR β/δ mediated skin disease was induced either by administration of powdered standard RMI-chow containing 0.003% GW501516 (w/w, custom – synthesized by AF-Pharmaceuticals, UK, to \geq 98% purity), or topical application of 0.3% (w/w) GW501516 in 10% (w/w) DMSO in Hydromol ointment (Alliance, UK); for topical induction, control mice received 10% DMSO in Hydromol.

Flow cytometry and intracellular measurement of IL17

Skin samples were shaved, trimmed of associated fat, cut to appr. 10–15 mm³ size using a scissor, incubated in 2 mg/ml collagenase IV (Roche, cat-nr. 110880855001), 1.1 U/ml dispase I (Roche, cat-nr. 04942086001) in HBSS for 30' at 37°C. Subsequently, samples were incubated in RPMI incl. Pen/Strep and 10% FCS, 0.5 μ g/ml PMA, 0.5 μ g/ml ionomycin for 3 h. For the last hour, Brefeldin A was added at 2 μ g/ml. Surface and intracellular staining for CD4-FITC (Pharmingen, clone RM4-4), CD8-PerCP/Cy5.5b (Pharmingen, 53–6.7), IFN γ -APC (Pharmingen, cat-nr. 554413), and IL17-PE (Pharmingen, cat-nr. 559502) and analysis on a FACS-Calibur was done according to standard procedures.

TNF α and IL-12 antibody treatment

70 μ g of anti-TNF α (Millipore, cat-nr 05-168), anti-IL12/23p40 (BioLegend, Clone C17.8, cat-nr. 505304), or PBS, respectively, were injected on three times per week, beginning on day 1 of GW501516 administration. Mice were sacrificed on day 22 for tissue analysis. For disease severity, erythema, scaling, palpable hyperkeratosis, and hair loss were scored as absent (0), weak (1), moderate (2), or severe (3), respectively, and the sum calculated for index regions (chin, forepaws, abdomen) chosen in order to allow hand-held analysis of mice during on-going treatment.

Expression profiling was performed as detailed in the supporting information (Method S1).

Western blot STAT3, P-STAT3

Nuclear extracts were made using the NE-PER kit (Pierce). Protein concentration was determined by Bradford assay. 40 μ g of protein loaded per well, subjected to SDS-PAGE gel and transferred to nitrocellulose membrane. Primary antibodies: 1:1000 dilution of Phospho-Stat3 (Tyr705) Antibody (New England BioLabs UK, 9131S) and 1:1000 dilution of anti-Stat3 (New England BioLabs UK, 9132) followed by HRP-conjugated anti-rabbit Ig, ECL Plus (GE Healthcare, Amersham), and detection using a CCD camera.

Immunofluorescence P-STAT3

5 μ m thick sections of snap-frozen skin were fixed in methanol, followed by incubation with anti-Phospho-Stat3 (Tyr705) (D3A7) (New England Biolabs UK, 9145S) with or without blocking peptide (New England Biolabs UK, 1195) for 1 hour at RT. Secondary antibody was Alexa Fluoro[®] 488 donkey anti-rabbit IgG (H+L) (Invitrogen). Coverslips were mounted using ProLong[®] Gold antifade reagent with DAPI (Invitrogen, Cat.no.: P-36931).

Treatment with WP1066

WP1066 (Calbiochem, order-nr 573097) was dissolved in DMSO/PEG 600 (20/80) according to [55] at 1.25 μ g/ μ l. Mice were injected with WP1066 or vehicle at 75 μ l intraperitoneally three times a week.

Supporting Information

Table S1 Synopsis of dysregulated genes in PPAR β/δ transgenic mice and psoriasis. Sheet "Changed in PPAR δ mice": all genes found dysregulated in PPAR δ mice, as detailed in the file Method S1. Sheet "PPAR δ mice vs psoriasis": all genes dysregulated in PPAR β/δ mice (orange shaded cells) that are also present on the two gene expression sets representing psoriasis (green shaded cells). FC = fold change lesional vs. non-lesional (psoriasis), or induced vs. non-induced (PPAR β/δ transgenic mice).

Found at: doi:10.1371/journal.pone.0009701.s001 (0.65 MB XLS)

Table S2 Clustering of genes dysregulated in PPAR β/δ transgenic mice. The table contains all 1077 genes listed in the synopsis between dysregulated genes in PPAR β/δ transgenic mice and psoriasis, color-coded as up- (red/orange) or down-regulated (light-green/dark green), as shown in Figure 5b.

Found at: doi:10.1371/journal.pone.0009701.s002 (0.17 MB XLS)

Table S3 Concordance of gene dysregulation between psoriasis and PPAR β/δ transgenic mice.

Found at: doi:10.1371/journal.pone.0009701.s003 (0.03 MB DOC)

Table S4 Genes concordantly regulated between PPAR β/δ transgenic mice and psoriasis, listed for the functional categories lipid-metabolism, differentiation, and cell-cycle.

Found at: doi:10.1371/journal.pone.0009701.s004 (0.23 MB DOC)

Table S5 Genes induced by the PPAR β/δ agonist GW501516 in the skin of C57Bl/6 wild type mice.

Found at: doi:10.1371/journal.pone.0009701.s005 (0.06 MB DOC)

Table S6 Expression of genes involved in cholesterol biosynthesis in PPAR β/δ transgenic mice and psoriasis.

Found at: doi:10.1371/journal.pone.0009701.s006 (0.07 MB DOC)

Table S7 Interleukin-1 related genes in PPAR β/δ transgenic mice and psoriasis.

Found at: doi:10.1371/journal.pone.0009701.s007 (0.09 MB DOC)

Table S8 Expression of kinase genes in PPAR β/δ transgenic mice and psoriasis.

Found at: doi:10.1371/journal.pone.0009701.s008 (0.04 MB PDF)

Table S9 PPAR isoforms and ligands.

Found at: doi:10.1371/journal.pone.0009701.s009 (0.06 MB DOC)

Method S1 Expression profiling.

Found at: doi:10.1371/journal.pone.0009701.s010 (0.80 MB DOC)

Figure S1 (a) Immunohistochemistry of PPAR β/δ in a panel of eight paraffin-embedded samples from psoriasis skin lesions, counterstained with hematoxylin. The inset in the right upper panel in addition demonstrates expression in dermal fibroblasts and endothelial cells. Magnification 200 \times in each case. (b) Immunohistochemistry of PPAR β/δ in PPAR β/δ -transgenic mice treated with GW501516 for seven days, counterstained with hematoxylin. Panels in lower row were taken from slides stained with secondary antibody only. Magnification 200 \times for all panels. Found at: doi:10.1371/journal.pone.0009701.s011 (3.17 MB TIF)

Figure S2 Macroscopic changes in PPAR β/δ transgenic mice upon ligand-mediated activation of PPAR β/δ by administration of the ligand GW501516 in the chow. Pictures shown were taken 14 (left) or 20 days (middle, right) after disease induction. Note the sharp demarcation of hyperkeratosis on the abdomen (middle). Panel on upper right represents illustrates the scalp, exhibiting heavy scaling, but no marked erythema.

Found at: doi:10.1371/journal.pone.0009701.s012 (1.79 MB TIF)

Figure S3 Confinement of PPAR β/δ transgene expression to suprabasal epidermal keratinocytes. Co-immunofluorescence with anti-PPAR β/δ visualized with Alexa288 and either CD11c, visualized with TexasRed, was performed as described in Methods. The white dashed line indicates the dermo-epidermal boundary. Magnification 400 \times .

Found at: doi:10.1371/journal.pone.0009701.s013 (1.93 MB TIF)

Figure S4 Expansion of Th17 cells upon activation of PPAR β/δ . PPAR β/δ transgenic mice were maintained in the presence (black columns) or absence (dark shaded) of GW501516, as were C57Bl6/j wild type (light shaded: GW; white: control) and Th17 cell frequencies determined by intracellular FACS, as described in

Methods. Data show mean \pm s.d. of Th17 cells (top), as well as the ratio between IL17⁺ and IFN γ ⁺ cells (bottom) in the lymphocyte gate for n = 3 mice per group. * p < 0.05.

Found at: doi:10.1371/journal.pone.0009701.s014 (0.21 MB TIF)

Figure S5 Inhibition of PPAR β/δ -mediated skin disease by depletion of Th17 cells. PPAR β/δ transgenic mice were maintained in the absence (control) or presence (all other groups) of GW501516 and additionally treated by injection of either anti-IL12/23p40, or anti-TNF α , as described in Methods. Pictures shown were taken nineteen days after disease induction. Mice were manually restrained to allow for comparable positioning during photography, thereby causing artificial tightening of abdominal skin.

Found at: doi:10.1371/journal.pone.0009701.s015 (5.20 MB TIF)

Figure S6 Reproducibility of gene dysregulation in psoriasis. Fold-change of gene expression between lesional and non-lesional skin in two independent datasets. The left panel shows all genes, the right panel all genes significantly upregulated (p < 0.001) in both datasets. R² = 0.93 for both panels. The dashed line indicates theoretically equal up-regulation in the two datasets. Both datasets were obtained using the same platform (using the Affymetrix HU133 Plus 2.0 array). The dataset from the GAIN cohort was obtained from the dbGaP website (www.ncbi.nlm.nih.gov/sites/entrez?db=gap). The CEL files are also available at the GEO website of NCBI (GEO dataset GSE13355). In the initial release, whole-skin expression profiles from paired lesional/non-lesional samples of 31 psoriasis patients were available which was used for the present analysis. The CEL files containing the dataset GSE14905 (n = 28 patients) were also downloaded from the GEO website. The data show the extend of reproducibility of gene dysregulation across patients and also indicate that -fold changes obtained with the GSE14905 dataset are consistently slightly higher than those observed in the GAIN data.

Found at: doi:10.1371/journal.pone.0009701.s016 (0.15 MB DOC)

Figure S7 Upregulation of psoriasis-associated genes in lesional skin of PPAR β/δ transgenic mice. The expression level for the representative genes shown, previously found to be upregulated in the skin of PPAR β/δ mice treated with GW501516 by microarray-based expression profiling (see main text), was quantified using TaqMan-based real-time PCR using Assays-on-Demand kits obtained from ABI according to the manufacturer's instruction (LCE3f: Mm02605425, Il1 β : Mm01336189, Hb-EGF: Mm004-39305, CRABP1: Mm00801693, ALOX12b: Mm00507782, m1: MM00436999, ATP12a: Mm00446786). The data shown represent mean \pm s.d. of GAPDH-calibrated expression levels obtained from n = 3 mice for each group (GW-fed, red columns, vs. control, blue columns). For all genes, p < 0.001 in a two-sided independent t-test. Found at: doi:10.1371/journal.pone.0009701.s017 (0.09 MB TIF)

Figure S8 Expression of transgenically overexpressed PPAR β/δ in murine skin. Whole skin samples from C57Bl/6j wild type (WT) or PPAR β/δ transgenic mice fed control chow (Tg) or GW501516-containing chow (TG+GW) were taken, genomic DNA digested, and total RNA isolated, followed by cDNA synthesis. RT-PCR was performed for the indicated number of cycles using primers specific for the transgene. Two mice for each condition were used.

Found at: doi:10.1371/journal.pone.0009701.s018 (0.25 MB TIF)

Author Contributions

Conceived and designed the experiments: MR JF. Performed the experiments: MR LR JF. Analyzed the data: MR LR JF. Contributed reagents/materials/analysis tools: CNAP MCUG. Wrote the paper: JF.

References

- Gelfand JM, Stern RS, Nijsten T, Feldman SR, Thomas J, et al. (2005) The prevalence of psoriasis in African Americans: results from a population-based study. *J Am Acad Dermatol* 52: 23–26.
- Dubertret L, Mrowietz U, Ranki A, van de Kerkhof PC, Chimenti S, et al. (2006) European patient perspectives on the impact of psoriasis: the EUORPSO patient membership survey. *Br J Dermatol* 155: 729–736.
- Wu Y, Mills D, Bala M (2008) Psoriasis: cardiovascular risk factors and other disease comorbidities. *J Drugs Dermatol* 7: 373–377.
- Poikolainen K, Karvonen J, Pukkala E (1999) Excess mortality related to alcohol and smoking among hospital-treated patients with psoriasis. *Arch Dermatol* 135: 1490–1493.
- Finlay AY, Coles EC (1995) The effect of severe psoriasis on the quality of life of 369 patients. *Br J Dermatol* 132: 236–244.
- Schmitt JM, Ford DE (2006) Work limitations and productivity loss are associated with health-related quality of life but not with clinical severity in patients with psoriasis. *Dermatology* 213: 102–110.
- Sommer DM, Jenisch S, Suchan M, Christophers E, Weichenthal M (2006) Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. *Arch Dermatol Res* 298: 321–328.
- Naldi L, Chatenoud L, Linder D, Belloni Fortina A, Pescricio A, et al. (2005) Cigarette smoking, body mass index, and stressful life events as risk factors for psoriasis: results from an Italian case-control study. *J Invest Dermatol* 125: 61–67.
- Barish GD, Narkar VA, Evans RM (2006) PPAR delta: a dagger in the heart of the metabolic syndrome. *J Clin Invest* 116: 590–597.
- Short B (2009) Time (and PPARbeta/delta) heals all wounds. *J Cell Biol* 184: 767.
- Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, et al. (2006) International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol Rev* 58: 726–741.
- Romanowska M, al Yacoub N, Seidel H, Donandt S, Gerken H, et al. (2008) PPARdelta enhances keratinocyte proliferation in psoriasis and induces heparin-binding EGF-like growth factor. *J Invest Dermatol* 128: 110–124.
- Westergaard M, Henningsen J, Johansen C, Rasmussen S, Svendsen ML, et al. (2003) Expression and localization of peroxisome proliferator-activated receptors and nuclear factor kappaB in normal and lesional psoriatic skin. *J Invest Dermatol* 121: 1104–1117.
- Tan NS, Michalik L, Noy N, Yasmin R, Pacot C, et al. (2001) Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes Dev* 15: 3263–3277.
- Di-Poi N, Michalik L, Tan NS, Desvergne B, Wahli W (2003) The anti-apoptotic role of PPARbeta contributes to efficient skin wound healing. *J Steroid Biochem Mol Biol* 85: 257–265.
- Icre G, Wahli W, Michalik L (2006) Functions of the Peroxisome Proliferator-Activated Receptor (PPAR) alpha and beta in Skin Homeostasis, Epithelial Repair, and Morphogenesis. *J Invest Dermatol* 126 Suppl: 30–35.
- Piqueras L, Reynolds AR, Hodivala-Dilke KM, Alfranca A, Redondo JM, et al. (2006) Activation of PPAR{beta}/delta Induces Endothelial Cell Proliferation and Angiogenesis. *Arterioscler Thromb Vasc Biol*.
- van der Vleuten CJ, de Jong EM, van de Kerkhof PC (1996) Epidermal differentiation characteristics of the psoriatic plaque during treatment with calcipotriol. *Arch Dermatol Res* 288: 366–372.
- Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, et al. (2005) Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* 102: 19057–19062.
- Di Cesare A, Di Meglio P, Nestle FO (2009) The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 129: 1339–1350.
- Sabat R, Philipp S, Hoflich C, Kreutzer S, Wallace E, et al. (2007) Immunopathogenesis of psoriasis. *Exp Dermatol* 16: 779–798.
- Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, et al. (2009) Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* 41: 199–204.
- Di-Poi N, Ng CY, Tan NS, Yang Z, Hemmings BA, et al. (2005) Epithelium-mesenchyme interactions control the activity of peroxisome proliferator-activated receptor beta/delta during hair follicle development. *Mol Cell Biol* 25: 1696–1712.
- Campbell SJ, Carlotti F, Hall PA, Clark AJ, Wolf CR (1996) Regulation of the CYP1A1 promoter in transgenic mice: an exquisitely sensitive on-off system for cell specific gene regulation. *J Cell Sci* 109 (Pt 11): 2619–2625.
- Robertson RW, Zhang L, Pasco DS, Fagan JB (1994) Aryl hydrocarbon-induced interactions at multiple DNA elements of diverse sequence—a multicomponent mechanism for activation of cytochrome P4501A1 (CYP1A1) gene transcription. *Nucleic Acids Res* 22: 1741–1749.
- Rowe JM, Welsh C, Pena RN, Wolf CR, Brown K, et al. (2008) Illuminating role of CYP1A1 in skin function. *J Invest Dermatol* 128: 1866–1868.
- Kaufman CK, Sinha S, Bolotin D, Fan J, Fuchs E (2002) Dissection of a complex enhancer element: maintenance of keratinocyte specificity but loss of differentiation specificity. *Mol Cell Biol* 22: 4293–4308.
- Michalik L, Wahli W (2007) Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease. *Biochim Biophys Acta* 1771: 991–998.
- Rosenfield RL, Kentsis A, Deplewski D, Ciletti N (1999) Rat preputial sebocyte differentiation involves peroxisome proliferator-activated receptors. *J Invest Dermatol* 112: 226–232.
- Trivedi NR, Cong Z, Nelson AM, Albert AJ, Rosamilia LL, et al. (2006) Peroxisome proliferator-activated receptors increase human sebum production. *J Invest Dermatol* 126: 2002–2009.
- Machado FS, Johndrow JE, Esper L, Dias A, Bafica A, et al. (2006) Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent. *Nat Med* 12: 330–334.
- Chiaro CR, Morales JL, Prabhu KS, Perdew GH (2008) Leukotriene A4 metabolites are endogenous ligands for the Ah receptor. *Biochemistry* 47: 8445–8455.
- Schmuth M, Jiang YJ, Dubrac S, Elias PM, Feingold KR (2008) Thematic review series: skin lipids. Peroxisome proliferator-activated receptors and liver X receptors in epidermal biology. *J Lipid Res* 49: 499–509.
- Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, et al. (2005) Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 11: 43–49.
- Koegel H, von Tobel L, Schafer M, Alberti S, Kremmer E, et al. (2009) Loss of serum response factor in keratinocytes results in hyperproliferative skin disease in mice. *J Clin Invest* 119: 899–910.
- Murray PJ (2006) STAT3-mediated anti-inflammatory signalling. *Biochem Soc Trans* 34: 1028–1031.
- Azfar RS, Gelfand JM (2008) Psoriasis and metabolic disease: epidemiology and pathophysiology. *Curr Opin Rheumatol* 20: 416–422.
- Sun L, Ke Y, Zhu CY, Tang N, Tian DK, et al. (2008) Inflammatory reaction versus endogenous peroxisome proliferator-activated receptors expression, re-exploring secondary organ complications of spontaneously hypertensive rats. *Chin Med J (Engl)* 121: 2305–2311.
- Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, Bonkowski MS, Panic JA, et al. (2005) Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. *J Gerontol A Biol Sci Med Sci* 60: 1238–1245.
- Sutherland JP, McKinley B, Eckel RH (2004) The metabolic syndrome and inflammation. *Metab Syndr Relat Disord* 2: 82–104.
- Gisondi P, Del Giglio M, Di Francesco V, Zamboni M, Girolomoni G (2008) Weight loss improves the response of obese patients with moderate-to-severe chronic plaque psoriasis to low-dose cyclosporine therapy: a randomized, controlled, investigator-blinded clinical trial. *Am J Clin Nutr* 88: 1242–1247.
- Barish GD, Atkins AR, Downes M, Olson P, Chong LW, et al. (2008) PPARdelta regulates multiple proinflammatory pathways to suppress atherosclerosis. *Proc Natl Acad Sci U S A* 105: 4271–4276.
- Hall JM, McDonnell DP (2007) The molecular mechanisms underlying the proinflammatory actions of thiazolidinediones in human macrophages. *Mol Endocrinol* 21: 1756–1768.
- Kryczek I, Wei S, Vatan L, Escara-Wilke J, Szeliga W, et al. (2007) Cutting edge: opposite effects of IL-1 and IL-2 on the regulation of IL-17+ T cell pool IL-1 subverts IL-2-mediated suppression. *J Immunol* 179: 1423–1426.
- Magne D, Palmer G, Barton JL, Mezin F, Talabot-Ayer D, et al. (2006) The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes. *Arthritis Res Ther* 8: R80.
- Blumberg H, Dinh H, Trueblood ES, Pretorius J, Kugler D, et al. (2007) Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation. *J Exp Med* 204: 2603–2614.
- Chong HC, Tan MJ, Philippe V, Tan SH, Tan CK, et al. (2009) Regulation of epithelial-mesenchymal IL-1 signaling by PPARbeta/delta is essential for skin homeostasis and wound healing. *J Cell Biol* 184: 817–831.
- Debets R, Hegmans JP, Croughs P, Troost RJ, Prins JB, et al. (1997) The IL-1 system in psoriatic skin: IL-1 antagonist sphere of influence in lesional psoriatic epidermis. *J Immunol* 158: 2955–2963.
- Kang K, Hatano B, Lee CH (2007) PPAR delta agonists and metabolic diseases. *Curr Atheroscler Rep* 9: 72–77.
- Liu L, Gao Y, Qiu H, Miller WT, Poli V, et al. (2006) Identification of STAT3 as a specific substrate of breast tumor kinase. *Oncogene* 25: 4904–4912.
- El Kasmi KC, Holst J, Coffre M, Mielke L, de Pauw A, et al. (2006) General nature of the STAT3-activated anti-inflammatory response. *J Immunol* 177: 7880–7888.
- Schmuth M, Haqq CM, Cairns WJ, Holder JC, Dorsam S, et al. (2004) Peroxisome proliferator-activated receptor (PPAR)-beta/delta stimulates differentiation and lipid accumulation in keratinocytes. *J Invest Dermatol* 122: 971–983.
- Michalik L, Desvergne B, Tan NS, Basu-Modak S, Escher P, et al. (2001) Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J Cell Biol* 154: 799–814.
- de Cid R, Riveira-Munoz E, Zecunen PL, Rorborge J, Liao W, et al. (2009) Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis. *Nat Genet* 41: 211–215.
- Kong LY, Abou-Ghazal MK, Wei J, Chakraborty A, Sun W, et al. (2008) A novel inhibitor of signal transducers and activators of transcription 3 activation is

efficacious against established central nervous system melanoma and inhibits regulatory T cells. *Clin Cancer Res* 14: 5759–5768.

56. Dauer DJ, Ferraro B, Song L, Yu B, Mora L, et al. (2005) Stat3 regulates genes common to both wound healing and cancer. *Oncogene* 24: 3397–3408.