PPARδ Enhances Keratinocyte Proliferation in Psoriasis and Induces Heparin-Binding EGF-Like Growth Factor

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Psoriasis is a common skin disease involving keratinocyte proliferation and altered differentiation, as well as T-cell activation. Here, we show that altered gene transcription in psoriatic skin lesions is highly reproducible between independent data sets. Analysis of gene expression confirmed dysregulation in all expected functional categories, such as IFN signaling and keratinocyte differentiation, and allowed molecular fingerprinting of a previously characterized dendritic cell subset associated with psoriasis tumor necrosis factor alpha (TNF-α)- and inducible nitric oxide synthase (iNOS)-producing CD11b^{INT} DC (Tip-DC). Unexpectedly, a large group of dysregulated transcripts was related to fatty acid signaling and adipocyte differentiation, exhibiting a pattern consistent with the activation of peroxisome proliferator-activated receptorδ (PPARδ). PPARδ itself was strongly induced in psoriasis *in vivo*. In primary keratinocytes, PPARδ was induced by the transcription factor activator protein 1, in particular by junB, but not by canonical WNT signaling, in contrast to its regulation in colon carcinoma cells. Activation of PPARδ enhanced proliferation of keratinocytes, while this was inhibited by knockdown of PPARδ. Finally, heparin-binding EGF-like growth factor (HB-EGF), known to induce epidermal hyperplasia and itself overexpressed in psoriasis, was identified as a direct target gene of PPARδ. The present data suggest that activation of PPARδ is a major event in psoriasis, contributing to the hyperproliferative phenotype by induction of HB-EGF.

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INTRODUCTION

Psoriasis is a common inflammatory skin disease involving altered keratinocyte differentiation, for example (Gandarillas *et al.*, 1999; Hobbs *et al.*, 2004), accumulation of dendritic cells (DCs) (Lowes *et al.*, 2005), as well as T-cell activation (Boyman *et al.*, 2004; Kohlmann *et al.*, 2004). Genetically, psoriasis exhibits a polygenic as well as heterogeneous inheritance pattern (reviewed in Rahman and Elder, 2005). Clinically and histologically, however, the disease is quite uniform, despite case-to-case variability, suggesting that the interplay of susceptibility alleles and exogenous factors terminates in a common set of molecular changes.

Expression profiling has been used in several studies to characterize transcriptional changes in psoriasis (e.g., Oestreicher *et al.*, 2001; Nomura *et al.*, 2003; Zhou *et al.*, 2003; Kulski *et al.*, 2005; Quekenborn-Trinquet *et al.*, 2005). When profiling the transcriptome in whole skin samples, a sizable fraction, if not the majority, of dysregulated transcripts might be expected to constitute ''noise'' due to inhomogenous sampling. Therefore, microarray studies in psoriasis to date have analyzed lists of transcriptional changes by a candidate approach, assigning relevance to specific transcripts based on their involvement in well-established aspects of the disease, such as keratinocyte differentiation, IFN signaling, or influx of inflammatory cells. By this approach, however, a large portion of transcriptional changes may be dismissed as artefacts, precluding the identification of unrecognized relevant signaling pathways.

The transcription factor peroxisome proliferator-activated receptorδ (PPARδ) contributes to the regulation of adipogenesis, glucose metabolism, myogenesis, and macrophage function, and has previously been shown to be upregulated in psoriasis (Westergaard *et al.*, 2003). This transcription factor is expressed in basal and suprabasal epidermal keratinocytes (Westergaard *et al.*, 2001), is induced by IFN-γ, stress-activated kinase, or tumor necrosis factor-α (TNFα)

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Abbreviations: AP1, activator protein 1; DC, dendritic cell; HB-EGF, heparin-binding EGF-like growth factor; PPARδ, peroxisome proliferator-activated receptorδ; TNFα, tumor necrosis factor-α

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via activator protein 1 (AP1)-mediated transcription, and contributes to the so-called ''regenerative skin phenotype'' characterizing the late phase of wound healing (Tan *et al.*, 2001; Di-Poi *et al.*, 2003). Furthermore, PPARδ regulates keratinocyte differentiation, blocks apoptosis (reviewed in Icre *et al.*, 2006), and induces angiogenesis (Piqueras *et al.*, 2006), adding to an activity profile highly consistent with a role in psoriasis.

To be activated, all PPAR isoforms require binding of fatty acid ligands shuttled by fatty acid-binding proteins. One of these, psoriasis-associated fatty acid-binding protein (=FABP5), specifically interacts with PPARδ (Tan *et al.*, 2002), is upregulated in psoriasis (Madsen *et al.*, 1992), and is enriched in the highly proliferative transit amplifying keratinocyte subset (O'Shaughnessy *et al.*, 2000). Moreover, the retinoic acid shuttle cellular retoinic acid-binding protein II is overexpressed in psoriasis (Siegenthaler *et al.*, 1992), possibly activating PPARδ, since retinoic acid itself is a high-affinity PPARδ ligand (Shaw *et al.*, 2003). Furthermore, all PPAR isoforms require heteromerization with retinoid acid X-receptorα for full activity (Kojo *et al.*, 2003; Tan *et al.*, 2005).

The precise effects of PPARδ on PPARα/γ activity, and vice versa, have not been precisely defined *in vivo* to date because of superimposed ligand and co-regulator effects; but the outcome of PPARδ activation is clearly distinct from that of PPARα/γ activation. Specifically, PPARδ may act as a direct antagonist to PPARγ (Zuo *et al.*, 2006), or be effected in opposite fashion to PPARγ by the same ligand (Jarvis *et al.*, 2005). Furthermore, unliganded PPARδ itself can act as a dominant negative to the ligand-containing isoform (Lee *et al.*, 2006). In light of these data, it is not surprising that activation of PPARγ has an inhibitory effect on psoriasis, whereas this is not the case with PPARδ (Kuenzli and Saurat, 2003; Malhotra *et al.*, 2005).

Here, we show that the vast majority of all transcriptional changes in psoriatic skin lesions are highly reproducible across two independent studies. While all expected changes, such as DC- and IFN-related gene upregulation, were observed, a large group of altered genes was involved in fatty acid signaling, with a profile suggestive of PPARδ activation. Heparin-binding EGF-like growth factor (HB-EGF), an established inducer of epidermal hyperplasia (Kimura *et al.*, 2005), was identified as a direct target gene of PPARδ. Our data suggest that activation of PPARδ is a key element in psoriasis and identify deranged intermediary metabolism as a hitherto unrecognized feature of the disease.

RESULTS

Transcriptional dysregulation in psoriasis is highly reproducible

Expression profiling from lesional *versus* non-lesional psoriatic skin was performed in five patients with active disease, as detailed in Materials and Methods. Several types of analyses were carried out to rule out significant distortions and to confirm that non-lesional and lesional samples are clearly distinguishable in their expression profiles, that is that microarray analysis predicts disease status with certainty (Figure S1). Approximately, 3.6% of the 12,600 transcripts were up- and 2.1% downregulated by twofold or more when applying a threshold of $P<0.05$ in a Welsh-weighted *t*-test (see Supplementary Material ''Charité all transcripts''). To estimate the extent of artificial alterations due to inhomogenous sampling, we quantified the changes reported in a previous study employing a comparable experimental platform (Affimetrix U95 microarray; Zhou *et al.*, 2003). Those data include 326 transcripts upregulated by twofold or more in lesional skin. Applying the same threshold to the subset of validated translated genes, we were in fact able to confirm 80% of all tested transcripts (Table 1). When lowering the cutoff to ⩾1.5-fold upregulation, almost 90% of upregulated transcripts were observed in both data sets. Only four transcripts, not confirmed in the current data set, were significantly upregulated in the previous data set (⩾3-fold): IL-7 receptor, CLUAL protein, RASGRP2, RGS1. Three of these transcripts (IL-7 receptor, RASGRP2, RGS1) are T-cell associated, and two (RASGRP2 and RGS1) upregulated in $CD4^+$ T cells, suggesting that the discrepancy may be in fact be explained by uneven sampling of $CD4^+$ T cells known to localize preferentially subepidermally in psoriatic lesions. Likewise, approximately, 70% of transcripts reported to be downregulated by Zhou *et al.* (2003) were also downregulated in the current data set. Only 10 genes significantly downregulated (⩾2.5-fold) could not be confirmed. Thus, independent expression profiles comprising data from 17 patients exhibit an unexpected level of concordance. Other available microarray data sets (Oestreicher *et al.*, 2001; Itoh *et al.*, 2005; Quekenborn-Trinquet *et al.*, 2005) were not included in the present meta-analysis because of differences in experimental procedure, but collectively confirm the notion that transcriptional changes in psoriatic lesions are highly reproducible despite sampling complex cell-type mixtures. Thus, the majority of these transcripts do not represent random noise, but are probably related to disease pathogenesis, and must consequently be considered in data analysis.

Table 1. Concordance of transcriptional regulation in lesional psoriatic skin in two independent data sets[1]

	Upregulated		Downregulated	
	n	%	*n*	%
Translated mRNAs[2]	187		170	
Not tested[3]	50		74	
Confirmed of tested[4]	123	**89.7**	67	**69.8**
Significantly discrepant[5]	4	2.9	10	10.4

[1]As reported in http://hg.wustl.edu/bowcock/papers/hg2003/zhou/I_U_up_2.0.xls.
[2]ESTs, hypothetical ORFs, and multiply hybridized mRNAs were excluded from analysis.
[3]Transcript not present on microarray or signal below threshold.
[4]Cutoff⩾1.5-fold for upregulated and ⩾1.2-fold for downregulated transcripts (as used by Zhou *et al.*, 2003). When cutoff ⩾2.0-fold for upregulated and ⩾1.5-fold for downregulated transcripts were applied (as used by Zhou *et al.*, 2003), the concordance was 80.3%. Specific fold changes and *P*-values for all genes are detailed in the Supplementary Material (''Charité all transcripts'').
[5]Transcripts reported to be upregulated ⩾3.0-fold or downregulated ⩾2.5-fold by Zhou *et al.* (2003) but not confirmed in the current data set.

Psoriasis-specific gene regulation
In addition to identifying genes exhibiting consistent changes in independent data sets, we sought to uncover changes *specific* to psoriasis. To this end, we performed expression profiling of transcriptional changes in lesional *versus* non-lesional skin from atopic dermatitis and contact allergy, processing all samples identical to the psoriasis samples. We then filtered the entire data set to include genes regulated either exclusively in psoriasis or at least twofold less in the latter diseases. These genes (shown in Table 2) define a disease-specific pattern of gene dysregulation. Unexpectedly, the largest group of genes was related to fatty acid signaling. Thus, highly reproducible and disease-specific expression profiling suggests that aberrant fatty acid signaling is a central feature of psoriatic pathogenesis (see below).

DC-related genes
As expected, numerous type 1 IFN-induced genes were found upregulated in lesional psoriasis (Table 2, second group). While the best characterized source of IFN-α is the plasmacytoid DC subset (Gary-Gouy *et al.*, 2002), a myeloid CD11c$^+$ DC subset was recently characterized in psoriatic lesional skin (Lowes *et al.*, 2005). Therefore, we screened the present microarray data for transcripts allowing indirect fingerprinting of DC subsets. This analysis, summarized in Table 3, revealed upregulation of transcripts highly indicative of the myeloid CD14 CD1$^+$ DC subset as defined in Ahn *et al.* (2002) and consistent with the DC phenotype reported by Lowes *et al.* (2005), suggesting that this myeloid DC subset, and not plasmacytoid DCs, may in fact be the primary source of type 1 IFN secretion in psoriatic epidermis. Furthermore, the upregulation of kynuroninase and indoleamine dioxygenase points to a potential mechanism for the relative scarcity of CD4$^+$ T cells in psoriatic epidermis, as those genes are involved in DC-mediated inhibition of CD4$^+$ T cells (Marteau *et al.*, 2005). Finally, the strongly upregulated chemokine CCL20 has been identified as critical chemoattractant for CD11c+ DCs into the skin (Le Borgne *et al.*, 2006). Thus, the upregulated transcripts listed in Table 3, which are largely confirmed in independent studies, as a group defines a molecular profile of the expanded mature myeloid DC subset in psoriasis.

Dysregulation of genes related to fatty acid signaling in psoriasis
Transcripts congruently regulated in two independent data sets were grouped into functional categories. Although this procedure is somewhat arbitrary, it clearly showed that a large group of dysregulated genes were related to fatty acid signaling (Figure 1). Several of these were related to adipogenesis, fatty acid shuttling, the peroxisomal oxidative response, and sebocyte regulation, and are detailed in Table 4. All these processes are regulated by PPARδ, which was itself one of the most strongly upregulated transcripts (10.1-fold; $P=0.017$), confirming previous reports (Westergaard *et al.*, 2003). Additional transcripts dysregulated in both data sets were associated with other processes regulated by PPARδ, that is myogenesis and glucose turnover (listed in the Supplementary Material ''gene categories''). Taken together, a large fraction of dysregulated transcripts in lesional psoriasis strongly suggests activation of PPARδ, which, in addition, is also a key regulator of keratinocyte differentiation and apoptosis (Tan *et al.*, 2003, 2004; Schmuth *et al.*, 2004).

Expression of two PPARδ isoforms *in vitro*
Previously, a mixed nuclear and cytosolic expression of PPARδ has been shown in psoriatic epidermis on the protein level (Westergaard *et al.*, 2003). To study whether overexpression of PPARδ is maintained *in vitro*, and to study the subcellular distribution of PPARδ *in vitro*, we cultivated primary keratinocytes and prepared nuclear and cytosolic extracts. As shown in Figure 2a, PPARδ was expressed at widely varying levels in keratinocytes, which expanded from lesional or non-lesional psoriatic skin as well as from control skin. Thus, the established overexpression of PPARδ in lesional psoriasis does not represent a cell-autonomous phenotype of keratinocytes from psoriatic individuals. Interestingly, two isoforms were detected using an N-terminally directed antibody, migrating approximately 2–3 kDa apart at the expected size of 55 kDa. The shorter of these isoforms was clearly predominant in the nucleus. HaCaT keratinocytes only exhibited the smaller isoform both in cytosol and the nucleus (blot shown on right). The specificity of both bands was confirmed using peptide competition (Figure 2b). Next, we activated PPARδ by addition of the synthetic ligand L-165041 in primary keratinocytes from three donors. As shown in Figure 2c, while PPARδ was again found to range widely, addition of synthetic ligand did not result in consistent translocation to the nucleus. Whether the two isoforms represent differentially phosphorylated isoforms, analogous to PPARα/γ, is currently under investigation. Taken together, these data show that, as yet uncharacterized, PPARδ isoforms are enriched in different subcellular compartments and that the subcellular localization of PPARδ is independent of its activation status.

Localization of NF-κB is independent of PPARδ
Since p65/RelA and PPARδ have previously been reported to interact in a ligand-dependent manner (Westergaard *et al.*, 2003), we examined localization of p65 at steady-state conditions, as well as upon activation of PPARδ. As shown in Figure 2d, p65/RelA exhibited predominantly cytosolic localization in primary keratinocytes. Activation of PPARδ did not induce nuclear accumulation of p65/RelA. We conclude that in adult epidermal keratinocytes NF-κB translocation is not regulated by PPARδ ligation. Therefore, heterodimerization of p65/RelA and PPARδ is unlikely to contribute to PPARδ activity in the epidermis.

PPARδ is regulated through AP1 activation in keratinocytes
The transcriptional regulation of PPARδ is complex; several *trans*-acting factors have been described to date. In murine keratinocytes, a major transcriptional activation has been shown to initiated by IFN-γ, stress-activated kinase, and TNFα, all of these pathways being integrated by an AP1 site

Table 2. Genes-regulated disease – specifically in psoriasis[1]

	Symbol	Fold change			*P*-value[2]
		PS	AD	CA	
Adipogenesis, fatty acid signaling					
PPARδ	PPARB	10.1	3.9	1.7	0.017
Lipocalin 2	LCN2	7.7	1.9	1.3	0.010
Fatty acid-binding protein 5	FABP5	5.8	2.9	1.8	0.000
Cellular retinoic acid-binding protein II	CRABPII	5.0	1.6	1.6	0.003
12R-Lipoxygenase	ALOX12B	3.7	2.0	1.5	0.002
Thyroxine deiodinase II, skeletal muscle	DIO2	3.0	1.1	1.3	0.012
Retinoic acid-receptor responder 1	RARRES1	−2.3	−1.3	1.4	0.001
Carnitine acetyltransferase	CRAT	−5.4	−2.0	−2.7	0.014
Protein phosphatase 1, regulatory subunit 3C	PPP1R3C	−2.9	1.4	2.0	0.007
Protein kinase C-like 1 (PRK1)	PRKCL1	−2.1	−1.1	−1.1	0.006
Interferon induced					
Interferon-stimulated protein, 15 kDa	ISG15	7.2	1.6	2.6	0.000
2′,5′-Oligoadenylate synthetase 1	OAS1	6.6	2.3	2.6	0.000
MX 1 (IFN-inducible p78)	MX1	5.9	1.6	2.5	0.001
IFNα-inducible protein (IFI-6-16)	G1P3	4.1	2.0	1.1	0.003
DC related					
CD24 antigen	CD24	5.0	1.4	2.6	0.000
Cystatin A (stefin A)	CSTA	4.3	1.4	2.1	0.001
ADAM-like (decysin 1)	ADAMDEC1	4.0	1.1	1.4	0.000
LAMP3 (DCLAMP)	LAMP3	3.8	1.5	2.1	0.000
Chemokine (C–C motif) ligand 20	CCL20	3.3	1.1	1.2	0.004
Mitosis/cell cycle					
Ki67	MKI67	4.4	1.5	2.0	0.001
M-phase phosphoprotein 6	MPHOSPH6	4.1	1.8	1.7	0.004
KIAA0101 gene product	KIAA0101	3.8	1.6	2.0	0.000
Mitogen-activated protein kinase 6	MAPK6	3.8	1.4	1.7	0.006
DKFZP434C212 protein	GAPVD1	3.4	1.1	1.8	0.010
MAD2 mitotic arrest deficient-like 1	MAD2L1	2.7	1.3	1.3	0.006
HRAS-like suppressor 3	HRASLS3	−4.2	1.0	−1.4	0.009
Keratinocyte differentiation/desquamation					
Kallikrein 6 (neurosin)	KLK6	10.5	1.6	3.4	0.002
Kallikrein 13	KLK13	6.2	1.0	1.2	0.000
kallikrein 10	KLK10	4.6	2.0	1.6	0.006
Small proline-rich protein 2C	SPRR2C	24.7	1.8	2.2	0.000
Transglutaminase 1	TGM1	4.4	1.9	1.8	0.000
Paired-like homeodomain transcription factor 1	PITX1	5.1	2.8	1.2	0.007
Adhesion					
S100 calcium-binding protein A2	S100A2	4.3	1.9	1.6	0.000
Olfactomedin 1	OLFM1	3.8	1.7	1.6	0.000
Collagen XIVα 1 (undulin)	COL14A1	2.6	1.2	1.0	0.003

Table 2 is continued on the following page

Table 2. continued

	Symbol	Fold change PS	Fold change AD	Fold change CA	*P*-value[2]
Apoptosis					
H2A histone family, X	H2AFX	4.5	1.4	1.4	0.009
Flap structure-specific endonuclease 1	FEN1	3.5	1.4	1.9	0.005
Wound response					
Secretory leukocyte protease inhibitor	SLPI	3.1	1.1	1.3	0.012
TGFβ receptor III=betaglycan	TGFBR3	−3.5	−1.8	−1.7	0.012
Miscellaneous (putative role indicated in parentheses)					
IL-1β	IL1B	5.9	1.6	1.8	0.013
Uridine phosphorylase (TNF induced)	UP	7.1	1.8	1.8	0.001
WNT 5a (differentiation)	WNT5A	4.5	2.2	2.0	0.000
Inhibitor of DNA binding 1 (angiogenesis)	ID1	3.0	1.6	1.2	0.014
LIV-1 (estrogen regulated)	LIV-1	2.7	1.3	1.3	0.011
Transcobalamin I (neutrophils)	TCN1	16.9	2.1	1.4	0.002
LIM domain protein (cytoskeleton)	RIL	−3.0	−1.3	−1.5	0.001
Chemokine (C–C motif) ligand 27 (Th 2 recruitment)	CCL27	−7.1	−2.1	−1.3	0.000
Solute carrier family 1 member 6 (peripheral nerves)	SLC1A6	−2.6	−1.4	−1.1	0.010
MGC24447 (unknown function)	C14orf147	3.1	1.1	1.3	0.001

IFN, interferon; PPARδ, peroxisome proliferator-activated receptorδ; TGFβ, transforming growth factor; TNF, tumor necrosis factor.
[1]Genes specifically up- or downregulated in psoriasis skin lesions (n=5), but not in lesional atopic dermatitis (AD, n=4) or experimentally induced contact allergy (CA, n=4). The genes listed in the table satisfied the following criteria: (1) fold change >2.5 and P-value <0.02 for psoriasis; (2) relative change at least two-fold higher in psoriasis; (3) P-values >0.01 in AD and CA. Sample processing, data acquisition, and analysis was performed as specified in Materials and Methods.
[2]Only P-values pertaining to psoriasis are shown since transcripts with P-values <0.01 in AD or CA were considered non-specific for psoriasis and omitted by the filter settings.

in the PPARδ promoter (Tan *et al.*, 2004). To assess the contribution of this *cis*-acting element, we blocked expression of two AP1 subunits active in adult epidermis, c-jun and junB, by RNA interference in HaCaT keratinocytes. As shown in Figure 3a, knockdown of junB greatly reduced steady-state PPARδ expression, whereas c-jun knockdown had only a minor effect. By contrast, knockdown of β-catenin, which has been reported to strongly regulate PPARδ expression in colon carcinoma cells (He *et al.*, 1999), had a negligible effect on PPARδ expression (Figure 3b), both in normal and in calcium-free medium. This was not due to incomplete cell infection, as shown in Figure 3c. Thus, PPARδ expression in human keratinocytes is primarily regulated via AP1 activation, specifically by junB, whereas canonical Wnt signaling has no effect.

PPARδ enhances proliferation in keratinocytes

Keratinocyte proliferation is greatly increased in psoriasis. Since PPARδ can either block or enhance cellular proliferation, depending on cellular context, we examined the effect of PPARδ on proliferation in human keratinocytes. To this end, HaCaT keratinocytes, which are fast cycling under steady-state conditions, were infected with a lentivirus containing an RNAi sequence recognizing PPARδ or control lentivirus. As shown in Figure 4a, the infection ratio for both vectors, quantified by FACS analysis of the green fluorescent protein reporter was approximately 94% for both vectors. However, 13 days after infection, the percentage of green fluorescent protein-positive cells had decreased significantly only after infection with PPARδ^{RNAi}, but not after control virus, indicating a proliferative disadvantage in the absence of PPARδ. In a complementary approach, adult primary epidermal keratinocytes, which are proliferating much slower than HaCaT cells, were exposed to the PPARδ-specific ligands L-165041 or GW-501516. As shown in Figure 4b and c, stimulation with either ligand caused a highly significant increase in cell number in keratinocytes expanded from three independent donors. These data indicate that PPARδ augments, rather than inhibits, keratinocytes proliferation, suggesting that this effect may contribute to keratinocyte hyperproliferation in psoriasis.

Heparin-binding EGF-like growth factor is a target of PPARδ

We next sought to identify target genes activated by PPARδ activation involved in enhanced proliferation. To this end, primary keratinocytes from four independent donors were

Table 3. Upregulation of dendritic cell-related transcripts in psoriatic lesional skin

Gene name	Symbol	*P*-value	Fold change
Genes specific for CD1⁺CD14⁻ subset[1]			
Cystatin A	CSTA	0.001	4.3
Lysosomal-associated membrane protein 3	LAMP3	0.000	3.8
Act. RNA pol II transcription cofactor 4	PC4	0.014	3.7
B-cell linker	BLNK[2]	0.000	2.9
Matrix metalloproteinase 12	MMP12	0.114	2.6
Annexin A2	ANXA2[2]	0.320	2.1
Isocitrate dehydrogenase 3	IDH3A[2]	0.000	2.0
DC associated[3]			
Indoleamine dioxygenase	INDO[2]	0.011	2.1
Kynureninase	KYNU	0.059	3.4
Inducible NO synthase	NOS2a	0.021	3.3
CD47 antigen	CD47	0.004	3.3
C-type lectin 12 =Dectin 1	CLECSF12	0.175	2.5
Regulator of G-protein signaling 20	RGS12	0.000	4.3
Fc-γ receptor IIIb	FCGR3B	0.067	2.4
CD24 antigen	CD24	0.000	5.0
RNA helicase	RIG-I	0.024	2.2
decysin 1	ADAMDEC1	0.000	4.0
CCL20=MIP3α[4]	CCL20	0.003	3.3

DC, dendritic cells.
[1]As defined by Ahn *et al.* (2002).
[2]Not reported to be upregulated in psoriasis to date: PC4, BLNK, ANXA2, IDH3A, and INDO.
[3]Independently confirmed in Bowcock *et al.* (2001) and Zhou *et al.* (2003).
[4]Confirmed in Nomura *et al.* (2003).

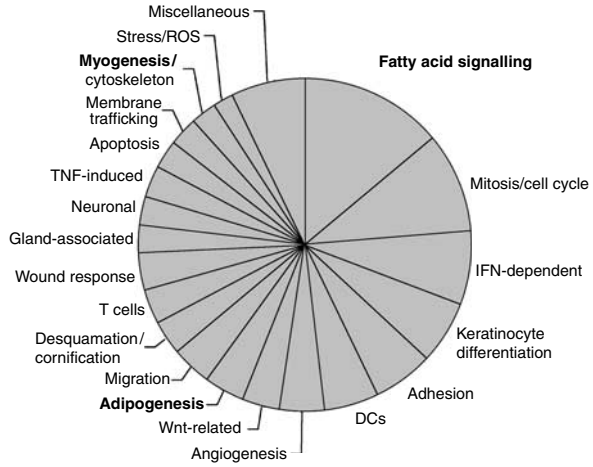

Figure 1. Functional distribution of transcripts with altered expression in lesional psoriatic skin in two independent data sets. (Shown are current data and Zhou *et al.*, 2003; total number of genes classified: $n = 175$). Genes with known roles in multiple categories were simultaneously assigned to all relevant categories. For an annotated list of all changes, see Supplementary Material ''Charité-gene categories''. ''Miscellaneous'' contains the following categories: IL-1 related; stem cell differentiation associated; glucose metabolism; neutrophil related.

grown in the presence of L-165041, or vehicle, for 48 hours. Total gene expression was then analyzed using the PIQUOR SkinPatho array, which contains approximately 1,100 transcripts relevant to epidermal biology. The magnitude of gene induction by PPARδ has been found to lie in the range of 1.5–3-fold in previous microarray studies (Tan *et al.*, 2001; Tanaka *et al.*, 2003; Tachibana *et al.*, 2005). Since this moderate induction is close to the background level, we applied a threshold for a minimum fold change of greater than three standard deviations of the mean fold change observed across all genes (illustrated in Figure 5a). Although this conservative approach reduced sensitivity, thus preventing detection of further potential target genes, it largely eliminates identification of false positives. Table 5 summarizes the observed changes. Several of the listed genes pertaining to fatty acid metabolism have previously been identified as targets in other cell types (Tanaka *et al.*, 2003; Tachibana *et al.*, 2005), thus confirming the present data.

One gene, HB-EGF was of particular interest because it is also upregulated 3.6-fold *in vivo* in lesional psoriasis (Table 5) and because it is known to enhance keratinocyte proliferation. We, therefore, confirmed the observed upregulation of HB-EGF by quantitative real-time PCR. As a positive control, we used CIDE-A, an proapoptotic gene previously shown to be indirectly downregulated by PPARδ (Tan *et al.*, 2001). Expression of PPARδ itself was used as negative control. As shown in Figure 5b, both HB-EGF and CIDE-A were moderately, but statistically significant induced by PPARδ activation in four independent patients, whereas expression of PPARδ itself did not change, in confirmation of the microarray data. On the protein level, HB-EGF expression was increased on activation of PPARδ (Figure 5c, left) and decreased after lentivirus-mediated knockdown of PPARδ in primary keratinocytes (Figure 5c, right). Finally, we addressed whether HB-EGF is directly or indirectly induced by PPARδ. To this end, primary keratinocytes were incubated in the absence, or presence, of L-165041, with or without cycloheximide. Subsequently, HB-EGF expression was determined by reverse transcription-PCR (RT-PCR). ACADVL, an established PPARδ target gene was amplified as positive control. CIDE-A, previously shown to be indirectly induced by PPARδ (Tan *et al.*, 2001), was used as a negative control. As shown in Figure 5d, induction of CIDE-A by PPARδ activation could be suppressed, whereas this was not the case for ACADVL and HB-EGF. Thus, HB-EGF is a direct target gene of PPARδ.

DISCUSSION

Several independent lines of evidence implicate PPARδ in psoriasis. PPARδ is highly, and specifically, upregulated in psoriasis. The activity profile of this nuclear hormone receptor includes antiapoptotic and proangiogeneic effects,

Table 4. Genes dysregulated in psoriatic lesions related to fatty acid signaling[1]

Gene name	FC (Zhou *et al.*, 2003)	FC (this report)	Putative functional relationship to PPARδ	Adipogenesis	PPAR ligand related	PPARα/γ modulation	Oxidative response	Peroxisome	Sebaceous glands
Upregulated									
Lipocalin 2	9.9	7.7	PKB/Akt target in adipocyte differentiation (Baudry *et al.*, 2006)	x					
FABP5	5.3	5.8	Ligand shuttle, psoriasis specific (see Introduction)		x				
ALDH1A3	3.4	4.8	Retinoic acid synthesis		x				
t-PA (PLAT)	2.3	3.9	Adipocyte differentiation (Seki *et al.*, 2001) and angiogenesis (Merchan *et al.*, 2003)	x					
HIF1A	2.4	3.8	Induces VEGF and *ABCA*, oxidative stress, inhibits PPARα, PPARγ2			x	x		
ALOX12B	2.7	3.7	Psoriasis-specific and ligand synthesis (see Discussion)		x				
SOD2	3.2	3.1	Adipocyte differentiation inhibitor (Lechpammer *et al.*, 2005), PPARδ target	x			x		
MAP17 (DD96)	3.1	2.9	Increases HDL, binds *PDZK1* (Silver, 2003)						
SLC6A14	7.4	2.2	Associated with obesity (Suviolahti, 2003)						
SLC16A1	2.1	2.1	Short-chain FA transport (Gill *et al.*, 2005), muscle mitochondra (Butz *et al.*, 2004)						
HIP2 (E2-25 K)	2.6	1.7	LDL-induced, antiapoptotic, ubiquitin conjugase						
APOL1*	2.5	1.5	Endothelial-cell specific, HDL associated (Monajemi *et al.*, 2001)						
Downregulated									
FMO5	−2.0	−1.6	Detoxification, progesteron induced						
HELO1*	−2.5	−1.7	Lipogenesis, polyunsaturated C16-C20 synthesis (Leonard, 2000)	x	x				
ALOX15B*	−5.0	−1.9	Activator of PPARα γ			x			
PXF (PEX19)	−2.0	−1.9	Peroxisome membrane trafficking (Hoepfner *et al.*, 2005)					x	
GATA3	−2.5	−1.9	Adipocyte differentiation suppressor (Tong, 2005)	x					
HIBADH	−2.5	−2.1	Fibroblast differentiation toward lipocytes (Kedishvili *et al.*, 1994)	x					
ALDH3A2	−2.0	−2.2	FA aldehyde oxidation, 12R-LOX related, lipoxin synthesis		x		x		
ABCA8	−2.0	−2.2	Cholesterol and phospholipid shuttle to apolipoproteins		x		x		
PDZK1*	−10.0	−2.3	Binds *MAP17*						
FASN*	−3.3	−2.4	Stratum granulosum (Uchiyama *et al.*, 2000), associated with glands						
ID4	−3.3	−2.5	Adipocyte differentiation associated (Chen *et al.*, 1999)	x					
CYP1B1	−2.0	−2.6	Adipocyte differentiation, induced by PPARα,γ; oxidative stress (Cho, 2005)	x		x	x		
transferrin*	−2.5	−3.0	Adipocyte differentiation enhancement (Hemmrich *et al.*, 2005)	x					

Table 4 is continued on the following page

Table 4. continued

Gene name	FC (Zhou *et al.*, 2003)	FC (this report)	Putative functional relationship to PPARδ	Adipo-genesis	PPAR ligand related	PPARα/γ modu-lation	Oxidative response	Per-oxisome	Sebaceous glands
EPHX1	−2.0	−3.2	Activates PPARα, peroxisomal and microsomal location			x			
APOE	−3.3	−3.6	Sebaceous glands						x
APOD	−2.5	−3.9	Sebaceous glands						x
RBP4	−3.3	−5.2	Shuttles retinoic acid; mediates insulin resistance (Yang *et al.*, 2005)		x				
CRAT	−2.5	−5.4	Peroxisomal and mitochondrial β-oxidation					x	
FABP7	−5.0	−6.6	Induced by PPARδ in stellate cells (Hellemans *et al.*, 2003)						
FADS1	−10.0	−7.7	Induced by SREB1 and PPARα (Matsuzaka *et al.*, 2002)			x			
APOC1	−2.0	−8.1	Apocrine and sebaceous glands						x
FADS2*	−5.0	−13.8	Sebaceous gland-specific (Ge *et al.*, 2003)						x

HDL, high-density lipoprotein; LDL, low-density lipoprotein; PPARδ, peroxisome proliferator-activated receptorδ; VEGF, vascular endothelial growth factor.
[1]Genes congruently regulated in Zhou *et al.* (2003) and the current data set with a documented function in fatty acid signaling. (*) marks genes with a *P*-value of 0.05–0.2 (Welsh-weighted *t*-test) in the Charité data set (see Supplementary Material ''Charité all transcripts''). All other genes have $P<0.05$ in both data sets.

as well as regulation on keratinocyte differentiation. PPARδ is activated by TNFα, a central mediator of the inflammatory response in psoriasis. Its interaction partners FABP5 and cellular retinoic acid-binding protein II are upregulated in psoriasis (Table 2). To this list, we here add (1) that a large group of dysregulated genes in psoriasis lesions *in vivo* are related to PPARδ function; (2) that PPARδ enhances keratinocyte proliferation; (3) that it directly induces HB-EGF. The role of PPARδ on proliferation is, in fact, highly controversial and other recent papers report a growth-inhibitory effect (Burdick *et al.*, 2007). However, using primary adult epidermal human keratinocytes, we observe a growth-stimulatory role using two different PPARδ ligands. Since the stimulatory time period used was 7 days, the observed increase in cell number is highly unlikely to result exclusively from inhibited apoptosis. Therefore, it appears that PPARδ activation in human keratinocytes indeed enhances proliferation. HB-EGF represents a highly relevant target, since it drives keratinocyte proliferation, regulates wound healing, and induces epidermal hyperplasia, thus replicating important elements of the psoriatic phenotype *in vivo* (Zheng *et al.*, 2003; Kimura *et al.*, 2005; Shirakata *et al.*, 2005). Moreover, we define two regulatory aspects of PPARδ activity in keratinocytes: its expression is independent of canonical Wnt signaling and its localization and activity is independent of p65/RelA.

A discussion of all disease-specific changes in psoriasis (Table 3) is beyond the scope of the current report. However, the genes involved in fatty acid signaling as a group (Table 3, top section) constitute a disease-specific determinant of PPARδ activity in psoriasis. Thus, FABP5 and cellular retoinic acid-binding protein II regulate PPARδ activity by fatty acid ligands. Moreover, FABP5 enhances keratinocyte motility in wounds (Kusakari *et al.*, 2006). 12R-Lipoxygenase catalyzes the formation of 12R-HETE, which specifically accumulates in psoriasis and has been shown to activate PPARδ (Westergaard *et al.*, 2003). Lipocalin 2 is junB induced, modulates adipocyte differentiation, and wound healing (Florin *et al.*, 2006). Dio2 regulates brown fatty tissue homeostasis (Christoffolete *et al.*, 2004). RARRES1, downregulated in psoriasis (Table 3), blocks keratinocyte proliferation (DiSepio *et al.*, 1998). Carnitine acyltransferase regulates fatty acid β-oxidation. PPP1R3C (PTG) regulates glycogen metabolism (Printen *et al.*, 1997). The serine/threonine kinase PRK1, found to be downregulated, inhibits phosphorylation of PKB/AKT by lipids (Wick *et al.*, 2000; Lim *et al.*, 2005), which is activated by PPARδ. Taken together, these genes underscore the complex regulation of adipogenesis, lipid, and glycogen metabolism exerted by PPARδ, and reinforce the concept of psoriasis as a dysregulated wound response program.

The unexpectedly large group of dysregulated genes involved in fatty acid metabolism positions intermediary metabolism as a central aspect of the disease, connected to, but distinct from its inflammatory nature. Thus, increased body mass index is an independent risk factor for psoriasis (Naldi *et al.*, 2005) and may have an effect on disease severity (Marino *et al.*, 2004), as well as TNFα-related inflammatory activity (Hamminga *et al.*, 2006). Since the prevalence of metabolic syndrome is increased in psoriasis (Sommer *et al.*, 2006), a combination of insulin resistance, obesity, or chronic inflammation may trigger the expression of PPARδ, which in turn contributes to a non-terminated regenerative skin phenotype. This disease mechanism would be expected

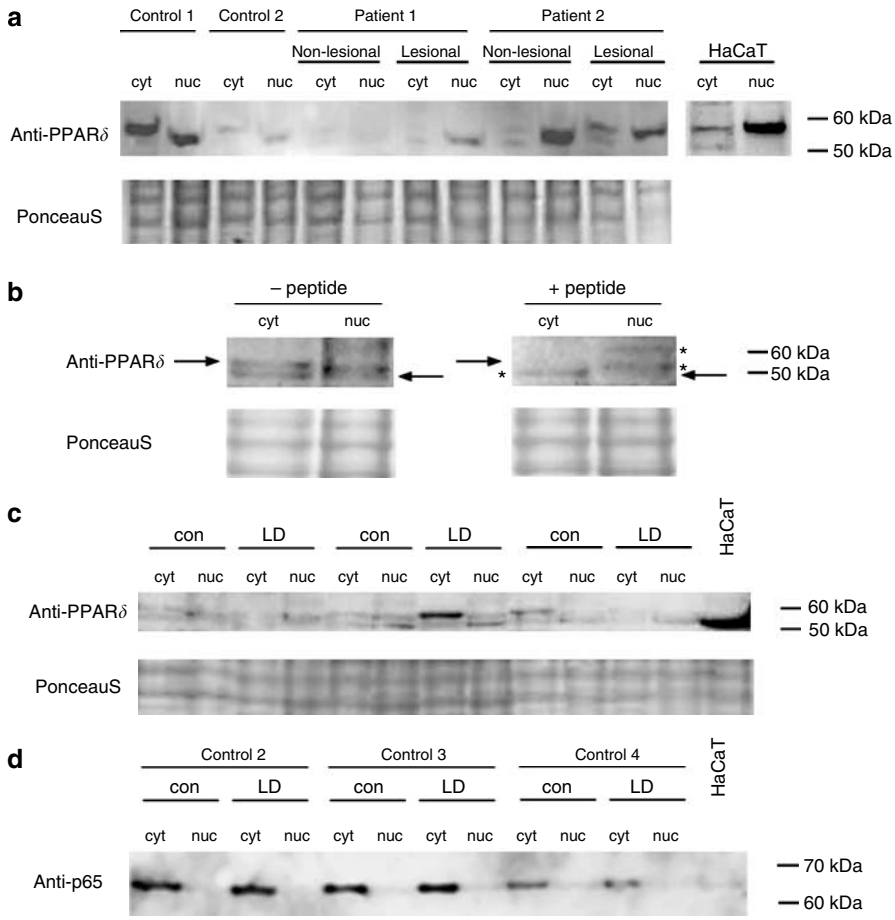

Figure 2. Expression of PPARδ and p65/RelA in primary adult keratinocytes. (**a**) Western blot of nuclear and cytosolic fractions from keratinocytes expanded from non-lesional, or lesional skin, as well as from healthy control skin, as indicated. Twenty micrograms of nuclear and cytosolic fractions were loaded per lane. PonceauS staining is shown on the bottom as control for even loading. (**b**) Cytosolic and nuclear fractions of primary keratinocytes from healthy control skin were prepared and processed exactly as in panel (**a**), except that anti-PPARδ was pre-incubated with 10 μg/ml of antigenic peptide (+peptide) for 30 minutes before incubation of the blot with antibody. Non-specific bands not competed by peptide are marked by (*). (**c**) Western blot of nuclear and cytosolic fractions from keratinocytes from three control individuals stimulated with the PPARδ-specific ligand L-165041 (LD) or vehicle (con) for 48 hours. Nuclear extract from HaCaT keratinocytes was included as positive control (rightmost lane). (**d**) The blot shown in **b** was re-probed using p65/RelA antibody.

to be aggravated by acute inflammation, or stress via the induction of PPARδ by TNFα and stress-activated kinase (Tan *et al.*, 2001). Clinically, these factors are precisely the most potent triggers of disease flares. Thus, activation of PPARδ in psoriasis offers a pathogenetic concept unifying various clinical aspects as well as transcriptional changes in psoriasis. Therapeutically, since caloric restriction can decrease the expression level of PPAR isoforms (Masternak *et al.*, 2005), trials to this end appear warranted to explore the efficacy of caloric restriction as a treatment option in appropriately selected psoriasis patients. Conversely, currently on-going trials employing PPARδ agonists for the treatment of metabolic syndrome and obesity should be carefully scrutinized for increased incidence of psoriasis.

Mechanistically, PPARδ may act as an antagonist to other nuclear hormone receptor heterodimers. Thus, the RXR/RAR heterodimer activates, rather than inhibits apoptosis (Chiba *et al.*, 1997; Monczak *et al.*, 1997). Another competitive antagonism may be exerted toward PPARγ, since activation of this isoform directly inhibits STAT3 (Wang *et al.*, 2004) which, when overexpressed, causes a psoriasis-like phenotype *in vivo* (Sano *et al.*, 2005). Furthermore, the antiapoptotic activity of PPARδ may be most pronounced in the context of type 1 IFN activity, since PPARδ is expressed in DCs (Jakobsen *et al.*, 2006). In fact, we have observed that PPARδ can be induced by IFN-α (N.a.Y and J.F., paper in preparation), adding yet further support for a role of PPARδ in psoriasis.

The upregulation and activity of PPARδ in lesional psoriasis suggests how the psoriasis-like phenotype in K14-vascular endothelial growth factor transgenic mice relates to the human disease, since vascular endothelial growth factor is a target gene of PPARδ (Xia *et al.*, 2003; Sano *et al.*, 2005). It is also intriguing that, in contrast to human skin, PPARδ is not expressed in adult interfollicular mouse epidermis, perhaps explaining why psoriasis form dermatitis does not occur spontaneously in mice. Finally, it should be pointed out that the PPARδ gene is located 4.2 Mb telomeric of the

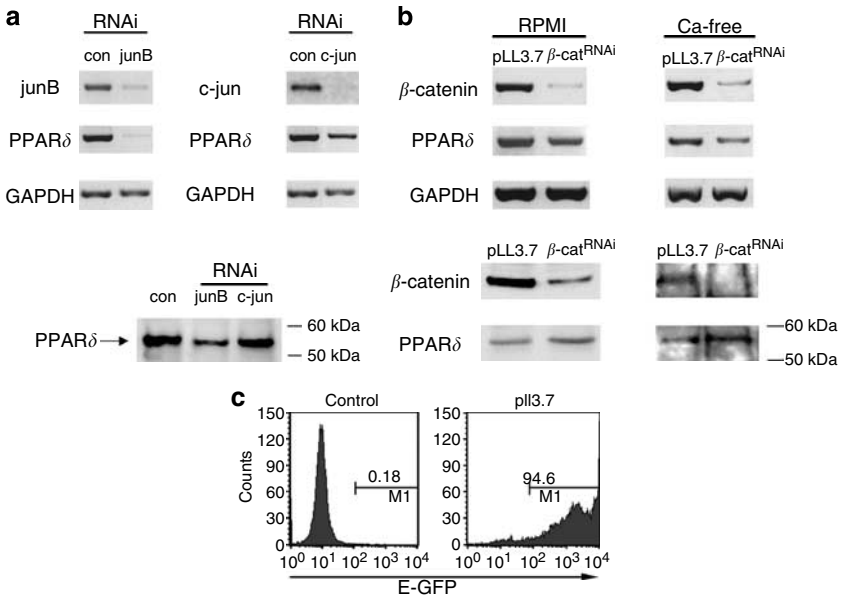

Figure 3. Transcriptional regulation of PPARδ expression in human keratinocytes. (**a**) Human HaCaT keratinocytes were transfected in the absence (con) or presence of siRNA specific for junB or c-Jun, as indicated. Forty-eight hours after transfection, RNA was analyzed by RT-PCR (top panel), and protein by Western blot (bottom) for the expression of PPARδ. (**b**) HaCaT cells grown under standard conditions or in calcium-free medium were infected with empty control lentivirus (pLL3.7) or lentivirus harboring a β-catenin-specific siRNA sequence, as indicated in the figure. Forty-eight hours after infection, RNA was analyzed by RT-PCR (top) and protein by Western blot (bottom) for the expression of β-catenin and PPARδ. (**c**) FACS analysis of the E-green fluorescent protein reporter for the control virus.

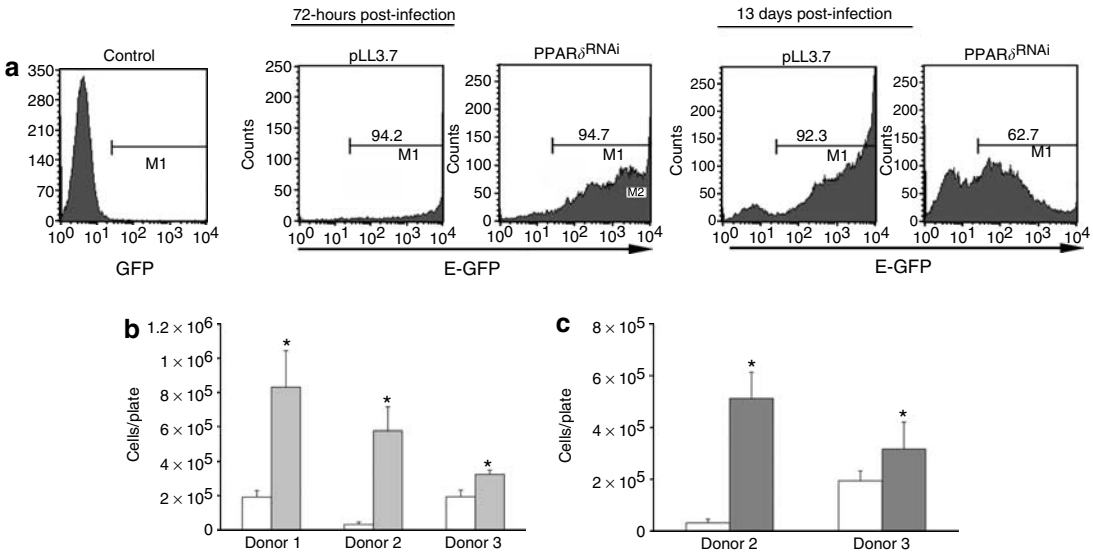

Figure 4. Effect of PPARδ on keratinocyte proliferation. (**a**) HaCaT keratinocytes were infected with control virus (pLL3.7) or lentivirus harboring a PPARδ-specific siRNA sequence (PPARδ^{RNAi}). The frequency of infected cells was determined by FACS analysis of the E-green fluorescent protein reporter gene. (**b**) Proliferation of adult primary keratinocytes *in vitro* grown for 7 days in the presence of 1 μM of the PPARδ-specific ligand L-165041 (shaded columns) or vehicle (white). Data represent mean ± SD of experiments performed in duplicate or triplicate with cells from three independent donors. *$P<0.01$. (**c**) As in panel (**b**) except that the PPARδ-specific ligand GW-501516 was used at 5 μM concentration.

putative PSORS1 locus at HLA-C on chromosome 6p21.2. Since earlier genome-wide scans were performed using microsatellites located actually closer to the PPARδ locus than to HLA-C and yielding a positive signal (Nair *et al.*, 1997; Trembath *et al.*, 1997; Enlund *et al.*, 1999), PPARδ formally constitutes a candidate susceptibility gene for the disease.

MATERIALS AND METHODS

Patients

All work with patient materials presented in the current report was carried out in accordance with the Declaration of Helsinki Principles. All patients undergoing biopsy gave prior written consent. Psoriasis and atopic dermatitis patients sampled for expression profiling were undergoing in-patient treatment for active disease at

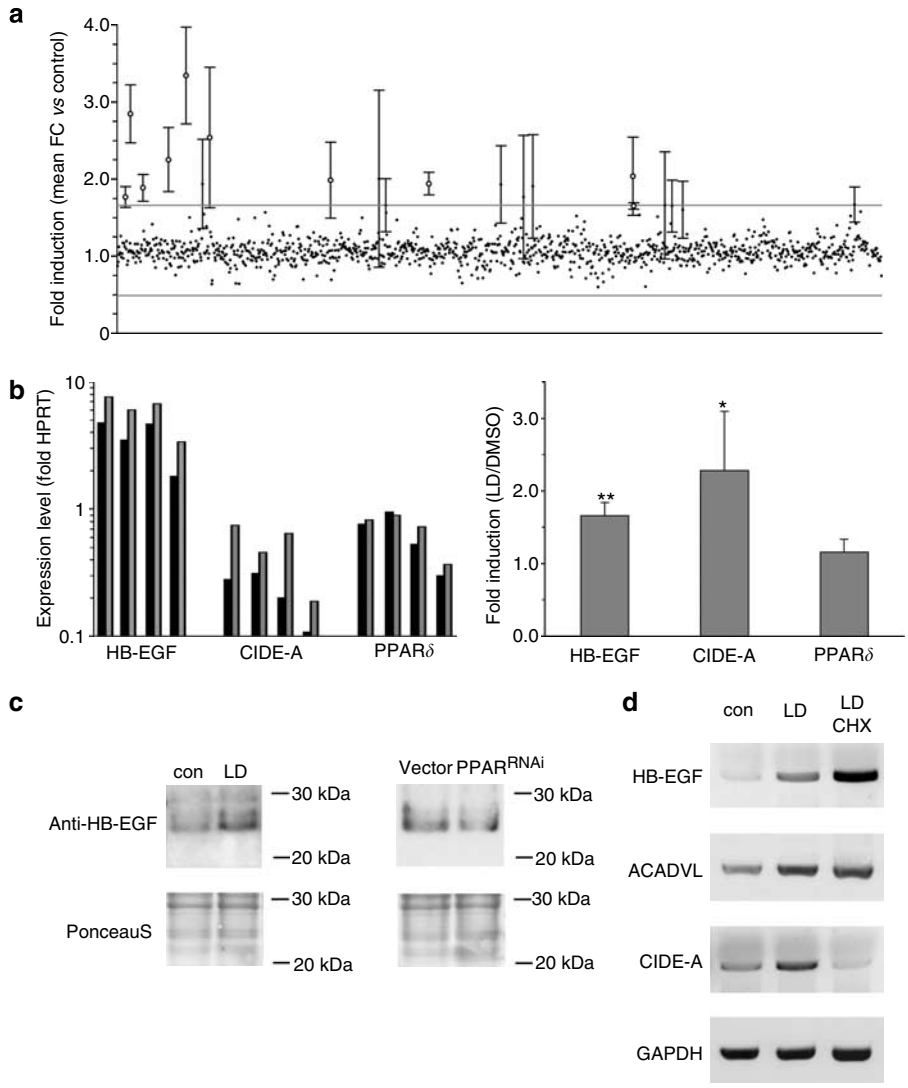

Figure 5. HB-EGF is a PPARδ target gene. (**a**) Scatter plot of mean fold changes induced by stimulation of primary adult epidermal keratinocytes with the PPARδ-specific ligand L-165041. Shaded horizontal lines indicate the threshold applied for significant changes (three SD of the mean change across all genes). Error bars indicate SD for all genes exceeding the threshold. Open symbols denote genes listed in Table 5. (**b**) Left, quantitative PCR showing gene expression relative to hypoxanthine ribosyltransferase in primary keratinocytes from four donors stimulated with L-165041 for 48 hours. Right, mean ± SD of the changes for all individuals. *$P<0.01$; **$P<0.001$. (**c**) Western blot analysis: left, primary keratinocytes were stimulated as in panel (**b**), whole-cell lysates separated by SDS-PAGE and blots probed with anti-HB-EGF. PonceauS staining is shown as a loading control. Right, primary keratinocytes were infected either with empty control virus (vector) or virus containing a PPARδ RNAi-sequence ($PPAR^{RNAi}$) for 48 hours before cell harvesting. (**d**) RT-PCR analysis of primary keratinocytes were stimulated with L-165041, or vehicle (0.5% DMSO), or 5 μM cycloheximide (CHX) for 24 hour.

the time of biopsy. Contact allergy was elicited by standard patch testing for 72 hours on the back.

Expression profiling

Full-thickness lesional and non-lesional 5-mm punch biopsies were snap-frozen in liquid nitrogen. RNA was purified using the RNeasy kit (Qiagen, Hilden, Germany). Reverse transcription, second-strand synthesis, cRNA labeling, and hybridization to the Affimetrix U95A array was performed according to Mishra *et al.* (2002). Microarray processing is detailed in the supplement (legend to Figure S1).

Cell culture

Expansion of adult epidermal keratinocytes on feeder layer was performed modified according to Rheinwald and Green (1975). The detailed protocol is specified in the Supplementary Material. Briefly, full-thickness punch biopsies were incubated with dispase solution overnight at 4°C. Epidermal sheets were carefully removed and incubated in trypsin solution for 15 minutes at 37°C. Trypsin was inactivated by addition of 5 ml keratinocyte medium (DMEM low glucose; HAM's F12 adenine PenStrep plus HICE cocktail (hydrocortisone, insulin, cholera toxin, and EGF)). Cells were re-suspended and seeded on plates with prepared feeder layer (consisting of mitomycin C-treated adult fibroblasts). Medium was first replaced after 2 days. At confluence (7–9 days), cells were split 1:2 or 1:3, depending on confluence, and re-seeded on fresh feeder layer. After a second passage 7 days later (splitting ratio 1:2), cells were subjected to experiments. HaCaT keratinocytes were cultured in RPMI 1640-containing GLUTAMAX™-I (substituted on a molar

Table 5. Genes induced by PPARδ activation in keratinocytes[1]

Gene ID	Name	FC	*P*-value[2]	FC *in vivo*[3]
ABCC3	Multispecific organic anion transporter 2	1.8±0.1	<0.001	**2.4**
ACADVL	Acyl-CoA dehydrogenase	2.8±0.4	<0.001	NS
AKR1B1	Aldose reductase	1.9±0.2	<0.001	NS
ATP12A	K-transporting ATPase (non-gastric)	2.3±0.4	0.001	NT
BG1	Very long-chain acyl-CoA synthase	4.6±0.6	0.001	NT
CAT	Catalase	2.5±0.9	0.008	NS
ECHB	3-ketoacyl CoA thiolase	2.0±0.5	0.012	NT
HB-EGF	Heparin-binding EGF-like growth factor	1.9±0.1	0.009	**3.6**[4]
KRT75	Cytokeratin type II (K6HF)	1.9±0.5	<0.001	NS
OACT5	*O*-acyltransferase domain containing 5	2.0±0.1	0.002	NS

PPARδ, peroxisome proliferator-activated receptorδ.
[1]Epidermal keratinocytes were stimulated with the PPARδ-specific ligand L-165041 or vehicle (DMSO) for 48 hours and gene expression profiling performed using PIQUOR skin-patho microarrays as detailed in Materials and Methods. Data shown represent mean±SD of fold changes (L-165041 *vs* 0.1 % DMSO) for cells from four independent donors.
[2]As calculated by a two-sided paired student's *t*-test.
[3]Upregulation in lesional *versus* non-lesional psoriatic skin, as determined by microarray-based expression profiling (see Materials and Methods, complete data set in Supplementary Methods "Charité all transcripts"). NS, non-significant ($P>0.05$), NT, transcript not present or not detected on U95A microrray.
[4]$P=0.0026$ for ABCC3; HB-EGF is not included in Table 3 because of the formally non-significant *P*-value (0.08, cf Supplementary Methods "all transcripts").

equivalent basis for L-glutamine), 100 U/ml Pen/Strep (Invitrogen-Gibco, Karlsruhe, Germany), 10% fetal calf serum, and 0.4 mM calcium.

Synthetic ligand stimulation

Cells were seeded 5×10^5 cells/58 cm^2 dish and incubated overnight at 37°C in a humidified atmosphere of 5% CO_2. The selective PPARδ agonists L-165041 and GW-501516 (Calbiochem, Darmstadt, Germany) were diluted and aliquoted in DMSO under N_2 atmosphere. Cells were stimulated with 1 μM (L-165041) or 5 μM (GW-501516) for the time span specified in Results. Medium with fresh ligand was added every 24 hours. Control cells received identical volume of vehicle (0.05% v/v final DMSO).

Western blotting

Nuclear and cytoplasmic protein fractions were prepared using NE-PER reagents (Pierce, Rockford, IL, no. 78833). For HB-EGF blots, whole cell extracts were prepared with buffer containing CHAPS exactly as described in Klose (1999). Protein extracts were aliquoted, shock-frozen in liquid N_2 and stored at −80°C. Protein concentrations were determined using BCA Protein Assay kit (Pierce, no. 23225). Twenty micrograms of protein extract was loaded per lane, separated on 7.5% acrylamide-SDS gels, and blotted onto cellulose-nitrate membranes (Whatman Schleicher & Schell, Dassel, Germany, no. 10 402 594). Blots were blocked for 30 minutes with 4% non-fat milk in Tris-buffered saline with 0.05% Tween 20, and subsequently incubated with primary and secondary antibodies in 4% non-fat milk/Tris-buffered saline with 0.05% Tween 20 as follows. PPARδ: rabbit anti-human PPARδ polyclonal purified IgG (Cayman Chemical Europe, Tallinn, Estonia, no. 101720; antigenic peptide corresponds to aa 39-54 (SSSYTDLSRSSSPPSL)), diluted at 1:300 overnight at 4°C; p65/RelA: (Santa Cruz Biotechnology, Santa Cruz Biotech, CA, no. sc-109G) 1:1,000, overnight at 4°C. HB-EGF: (R&D Systems, Minneapolis, MN, AF-259-NA) 1:500, overnight at 4°C. Secondary antibodies were anti-rabbit-HRP (Amersham Biosciences, Buckinghamshire, England, no. NA934) at 1:2,000, anti-goat-HRP (Santa Cruz Biotechnology, no. SC-2922) 1:6,000. Incubation with appropriate secondary antibody was carried for 1 hour at room temperature. Blots were developed with ECL Plus Western Blotting Detection system (Amersham Biosciences, no. RPN2 132).

RT-PCR

RNA extraction was performed using the NucleoSpin II extraction kit (Machery-Nagel, Düren, Germany, no. 740 955). All reagents used for cDNA synthesis (SuperScript II Reverse Transcriptase, dNTP, RnaseOut, $Oligo(dT)_{12–18}$, dithiothreitol) were purchased from Invitrogen-Gibco (Karlsruhe, Germany). GoTaq DNA Polymerase (Promega, Mannheim, Germany, no. M3001) was used at 1 U/25 μl for amplification. The annealing temperature for all PCR reactions was 55°C except for PPARδ (54°C). Primer sets, number of cycles, and amplicon lengths are detailed in the Supplementary Methods. For quantitative real-time PCR, cDNA synthesis was performed using Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT). Amplification was performed in a TaqMan SDS7700, using Assay-on-Demand kits (ABI) for hypoxanthine ribosyltransferase (Hs99999909_m1); CIDE-A (Hs00154455_m1), HB-EGF (Hs00181813_m1), and PPARδ (Hs00602622_m1) according to the manufacturer's instructions. Reactions were performed in triplicate and expression levels normalized to hypoxanthine ribosyltransferase.

PPARδ target gene identification

Primary keratinocyte culture, stimulation with L-165041, and RNA preparation was performed as detailed above. Samples were labeled either with Cy3 or Cy5, hybridized to Piquor SkinPatho microarrays (Memorec, Cologne, Germany), and scanned by the Charité in-house microarray service facility. Dye swab experiments were performed to rule out uneven green/red intensities. Data with at least twice background intensity and %CV of less than 30% between

the four replicas on each array were further analyzed as detailed in Table 5.

Lentiviral transfer vectors

pLL3.7 [14] was a kind gift of Luk van Parijs. Short hairpin RNAi sequence for PPARδ : (5′-tGTCGTACGATCCGCATGAAttcaagagaTTCATG-CGGATCGTACGACttttttc) or β-catenin: (5′-tGCTGAAACATGCAGTTGTAttcaagaga-TACAACTGCATGTTTCAGCttttttc; target sequence underlined) were synthesized in both strands, annealed, and cloned immediately downstream of the U6 promoter using HpaI/XhoI restriction sites. Parent plasmid served as control. An alternative shRNAi sequence for PPARδ (CCACTACGGTGTTCATGC) was synthesized analogously with MluI and ClaI restriction sites and cloned between under the control of H1 promoter of pLVTH (Szulc *et al.*, 2006), which was kindly provided by Didier Trono, as well as pPAX2 and pMD2.G for second generation packaging. Production of VSV-G pseudotyped replication-deficient lentivirus was carried out according to Mitta *et al.* (2005). Lentivirus infection was performed at a multiplicity of infection (MOI) of three for 12 hours at 37°C.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Figure S1. Validation of microarray data.

Supplementary Methods.

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