Introduction

1. Clinical and biochemical picture of psoriasis vulgaris.

Psoriasis vulgaris (OMIM 177900) is a common, inflammatory skin disease which affects about 25 million people in North America and Europe. The initial onset of the disease is first observed in late adolescence or early adulthood and is then followed by chronic relapses, but more or less severely manifested psoriasis usually persists for life (Lowes *et al.*, 2007). Psoriasis is clinically characterized by red, scaly plaques (psoriatic lesions) affecting mostly the scalp, elbows or knees. Elongated/hyperplastic blood vessels in the papillary dermal region (between epidermal rete) with marked dilation accounts for the visible redness of psoriatic skin lesions. The histopathological picture of the disease is defined by a hyperproliferation of epidermal keratinocytes and hyperkeratosis, altered keratinocytes differentiation, tissue inflammation with neutrophils in the stratum corneum as well as infiltration of immunocytes along with angiogenesis and endothelial cell activation. (Reviewed in (Lowes *et al.*, 2005; Schon & Boehncke, 2005))

To the complexity of the psoriatic phenotype contribute various cell types physiologically present in dermis and epidermis such as T lymphocytes (Boyman *et al.*, 2004; Kohlmann *et al.*, 2004) and dendritic cells (DC) (Lowes *et al.*, 2005), endothelial cells (Bhushan *et al.*, 1999), fibroblasts (Debets *et al.*, 1996) and keratinocytes (Hobbs *et al.*, 2004). Each of those cell types plays a role in maintaining skin homeostasis and each can combine to produce a pathological condition. Inflammation processes ongoing within psoriatic lesions are mediated by plasmacytoid DCs, which produce high levels of interferon-α (IFN-α) upon activation as well as TIP-DCs (TNF- and iNOS-producing DCs). The latter express an immunomodulatory protein tumor necrosis factor (TNF) (Lowes *et al.*, 2007). TNF-α in turn is a downstream target of a transcription factor nuclear factor-κB (NF-κB). Constitutive activation of NF-κB due to deficiency of inhibitor of κB (IκB) leads to a disorder that closely resembles psoriasis as demonstrated with the mouse model (Chen *et al.*, 2000).

Apart from inflammatory events psoriasis is characterized by keratinocytes hyperproliferation with a marked thickening of the epidermis. At the same time the differentiation of keratinocytes is extensively altered. The granular layer of the epidermis, in which terminal differentiation begins, is greatly reduced or absent. Excess of highly proliferative keratinocytes which fail to complete their differentiation produces a histological picture of psoriatic lesion which closely resembles the one observed during the wound healing process (Lowes *et al.*, 2007).

In the suprabasal layers of uninvolved epidermis from psoriasis patients and in the marginal part of psoriatic lesions heparin-binding epidermal-growth-factor-like growth factor (HB-EGF) overexpression was reported (Zheng *et al.*, 2003). HB-EGF is a potent mitogen for many cell types, including human epithelial cells and human keratinocytes (Nolan *et al.*, 2004). HB-EGF is also known to be involved in the wound healing process in the skin. Soluble HB-EGF induces migration and proliferation of keratinocytes, fibroblasts, and smooth muscle cells to fill the injured area and thus promotes reepithelialization and granulation tissue formation in the wound (Kanda & Watanabe, 2005; Shirakata *et al.*, 2005).

2. Insight into psoriasis pathogenesis.

In spite of intensive study, the pathogenesis of psoriasis currently remains controversial as to whether the disorder results from a primary dysfunction of the immune system (Wrone-Smith & Nickoloff, 1996; Schon & Boehncke, 2005) or from epidermal keratinocytes deregulation (Sano *et al.*, 2005). In order to investigate the matter multiple transgenic, knockout, and xenograft models of psoriasis have been developed over the past two decades. These models have clearly shown that events related to the immune system and keratinocytes dysregulation are closely interrelated (recently reviewed in (Gudjonsson *et al.*, 2007)). The psoriasis-like phenotype was observed to develop spontaneously in older transgenic mice generated with VEGF-A targeted to the basal layer of the epidermis using human keratin 14 promoter (K14) (Xia *et al.*, 2003). Histologically, the inflammatory lesions in these mice demonstrated hyperplastic and inflamed dermal blood vessels, epidermal thickening with impaired keratinocyte differentiation, and accumulation of dermal $CD4^+$ T-lymphocytes and epidermal $CD8^+$ lymphocytes as well as lymphatic vessel proliferation and enlargement.

K14-VEGF-A transgenic mice are unable to downregulate experimentally induced skin inflammation. This leads to chronic psoriasis-like inflammation (Kunstfeld *et al.*, 2004). Since it has been demonstrated that increased expression of VEGF is induced via activation of the latent transcription factor STAT3 (Platt *et al.*, 2005), a phenotype resembling human psoriasis in STAT3 overexpressing mice is to be expected. Indeed, a transgenic mouse model was recently developed, in which a constitutively active STAT3 mutation was expressed in basal keratinocytes under control of a bovine keratin 5 promoter (KRT5). As expected, these mice developed skin lesions resembling psoriasis either spontaneously or in response to wounding by tape-stripping. The development of skin lesions in this model is proven to be dependent not only on STAT3 activation in keratinocytes, but also on the presence of activated T-cells in skin (Sano *et al.*, 2005).

Although the etiology of psoriasis has not been elucidated, the development of psoriatic skin lesions has been shown to be associated with ongoing inflammation process as well as group A streptococci infection or physical trauma, that may trigger psoriatic lesions at sites of injury (Koebner's phenomenon) (Schon & Boehncke, 2005). A broad spectrum of environmental events has been observed to induce clinical disease flares. In particular, chronic exposure to psychological stress is regarded as risk factors for psoriasis (Buske-Kirschbaum *et al.*, 2007). Moreover, the risk of psoriasis is directly related to Body Mass Index. Thus, 16% of all the psoriasis cases was found to be attributable to elevated BMI in an Italian study. Additionally, the risk of the disease was nearly 2 times higher for obese individuals (BMI $\geq$ 30) (Marino *et al.*, 2004; Naldi *et al.*, 2005). In addition to environmental factors, there is a strong genetic component to the disease (reviewed in (Bowcock & Krueger, 2005)). Briefly, one locus consistently identified in studies of psoriasis is the class I region of the major histocompatibility locus antigen cluster (MHC) named psoriasis susceptibility 1 (PSORS1) which is associated with human leukocyte antigen (HLA) Cw6 on chromosome 6p21 (Trembath *et al.*, 1997). The additional psoriasis susceptibility loci identified and confirmed to date include: PSORS2 on 17q, PSORS3 on 4q, PSORS4 on 1cenq21, PSORS5 on 3q21, PSORS6 on 19p, PSORS7 on 1p, and PSORS9 on 4q31 (Rahman & Elder, 2005). In general, psoriasis is thought to result from a combination of multiple susceptibility loci interaction and various environmental factors (Sterry, 2003; Lowes *et al.*, 2005).

Recently published expression profiling data from lesional versus non-lesional psoriatic skin showed that the large group of transcripts dysregulated in psoriasis is related to fatty acid signaling. Several of affected gene groups are related to

adipogenesis, fatty-acid shuttling, the peroxisomal oxidative response, and sebocyte regulation suggesting involvement of some regulatory protein as an orchestrating factor (Romanowska *et al.*, 2007). Since all these processes are regulated by peroxisome proliferator activated receptor beta/delta (PPARδ), this transcription factor may contribute to psoriatic pathogenesis. This all the more, as since PPARδ itself is known to be strongly induced in psoriasis in vivo (Westergaard *et al.*, 2003).

3. Characterization of PPARδ.

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors and members of the nuclear hormone receptor superfamily. PPARs belong to the largest subfamily 1 (Class II receptors), which in turn comprises 11 groups of receptors including retinoic acid receptors (RARs) and PPARs (Nuclear Receptors Nomenclature Committee1999). Three closely related PPAR isotypes have been identified: PPARα (NR1C1), PPARβ/δ, (NR1C2; called PPARδ here), and PPARγ (NR1C3). Each isoform is encoded by a separate gene and PPARγ is expressed as two isoforms, PPARγ1 and PPARγ2, differing at their N terminus. PPARδ has also been identified as two isoforms produced by alternative splicing: 441 amino acid-long isoform 1 and isoform 2, which lacks 88 aa from the C-terminus (The M. G. C. Project Team2004). A schematic representation of protein sequence aligment for all PPARs is shown in figure 1A. During the evolution of PPARs, the first gene duplication gave rise to PPARγ. The second gene, which was subsequently duplicated yielded PPARα and PPARδ (Michalik *et al.*, 2006).

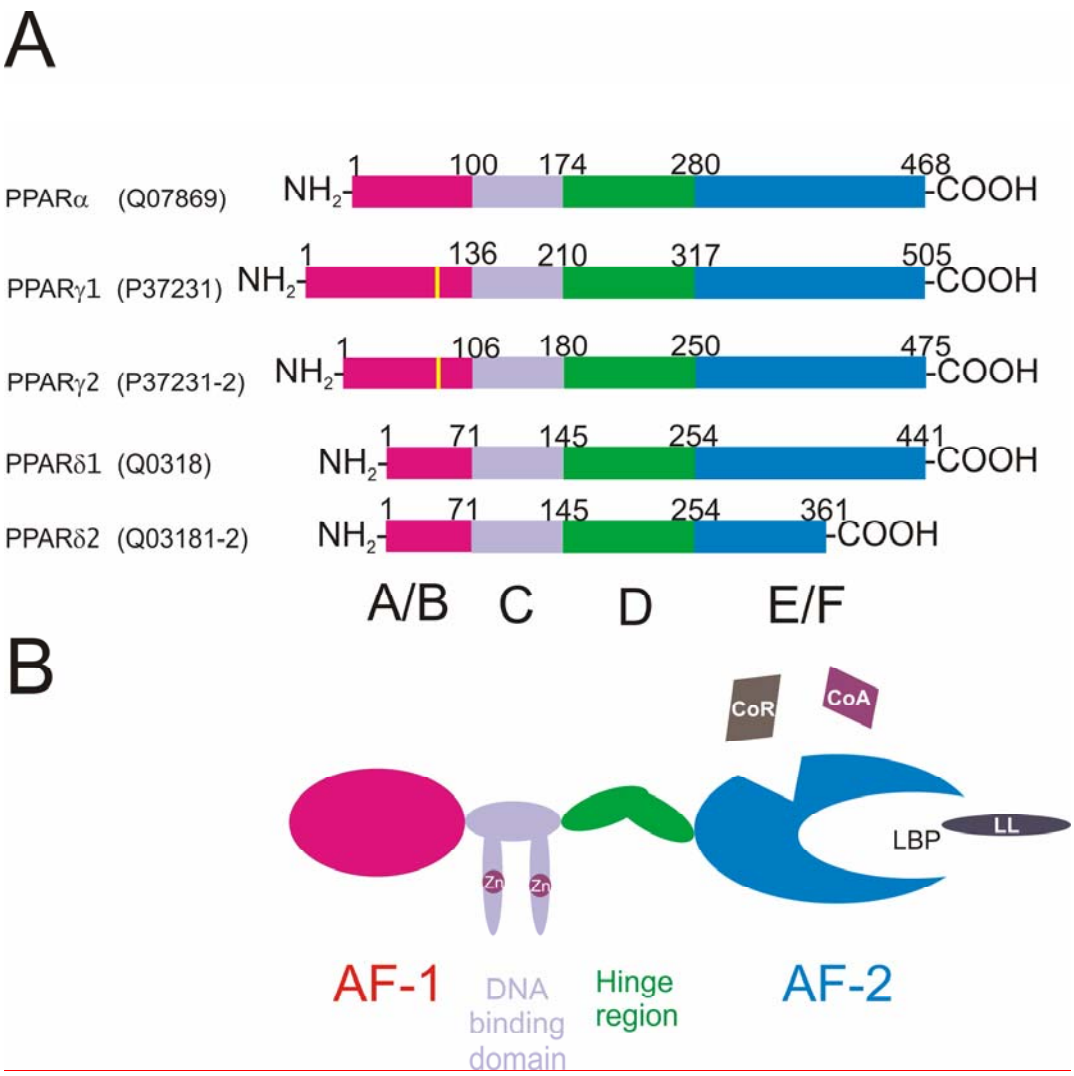

Fig 1. *Structural organization of PPARs.* **A**. Schematic representation of protein sequence aligment for PPARs. The sequence aligment was done with ClustalW (Chenna *et al.*, 2003) and displayed up to scale. Phosphoserine 112/82 (PPARγ1/PPARγ2) is marked in yellow. Swiss-Prot database (Bairoch *et al.*, 2004) accession numbers for each protein are noted in brackets next to respective protein name. **B.** Schematic representation of the functional domains of PPARs. A/B – transactivation and phosphorylation domain; C - DNA-binding domain with two zinc fingers which binds to PPAR response element in promoter regions of responsive genes; D – hinge region; E/F - ligand-binding and hetero-dimerization domain. AF-1 - ligand-independent activator function; AF-2 - ligand-dependent activator function, LBP - ligand-binding pocket, CoA - co-activator, CoR - co-repressor, LL – lipid ligand.

All PPAR proteins, and other nuclear hormone receptors share a common structure consisting of four domains named A/B, C, D and E/F (Fig. 1B). The amino-terminal region (domain A/B) contains a ligand-independent activation function 1 (AF-1). Within this domain a consensus MAPK site was identified in PPARγ. It has been confirmed that phosphorylation by ERK2 and JNK significantly inhibits both ligand-independent and ligand-dependent transcriptional activation by PPARγ (Adams *et al.*, 1997). Similar phosphorylation sites in mouse PPARα, but not PPARδ, isoforms have been reported (Lazennec *et al.*, 2000). For a recent review on PPAR phosphorylation, please refer to (Burns & Vanden Heuvel, 2007). The DNA binding domain (C) is structurally conserved across the nuclear receptor superfamily and is folded into two zinc fingers conferring DNA binding specificity (Fig. 2A). Short hinge region (D) connects DNA binding domain to the C-terminal trans-activating domain (E/F) known also as ligand binding domain (LBD). Apart from ligand binding, this domain is also involved in heterodimerization with 9-cis retinoic acid receptors (RXRs) and interaction with transcriptional co-factors. In response to ligand binding, the LBD undergoes a permissive conformational change required for transcriptional activation - activation function-2 (AF-2). This function is physically connected with helix H12 closing on the ligand-binding site (Friedmann *et al.*, 2005; Kota *et al.*, 2005).

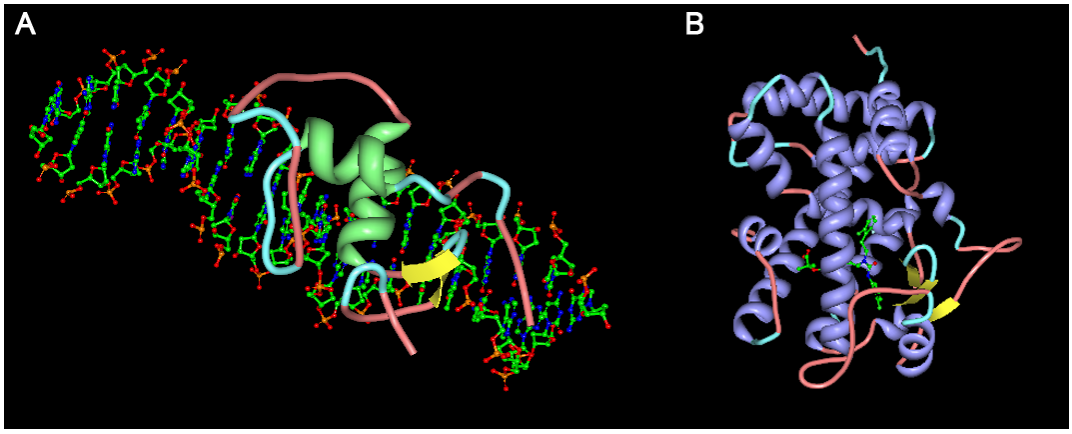

Fig 2. *3D structure of PPARδ domains.* **A.** The overall architecture of the PPARδ DNA-binding domain (amino acids 101 to 164) complexed to its cognate response element. Double stranded DNA is represented as sticks. Protein backbone is showed as ribbons colored by conformation type: helix - green, turn - cyan, coil – red, strand – yellow. **B.** 3D structure of the PPARδ ligand-binding domain (aa 210 to 476) bound to the synthetic ligand GW2433. The PPARδ backbone is represented by the purple ribbon, and GW2433 is represented with sticks, color coded as follows: carbon and chlorine – green, oxygen – red, nitrogen – blue, fluorine – white.

Models represent X-ray crystallographic structures deposited in the Protein DataBank PDB (Berman *et al.*, 2000) with accession numbers 1A6Y (A)(Zhao *et al.*, 1998) and 1GWX (B)(Xu *et al.*, 1999). For visualization the MBT Protein Workshop (Moreland *et al.*, 2005) application was used.

The LBD folds into a relatively compact structure that contains a bundle of 13 helices and a small four-stranded β-sheet surrounding a very large, Y-shaped cavity within the protein – the ligand binding site (Fig. 2B). Thanks to its size PPARs are able to bind to a broad spectrum of lower-affinity lipophilic compounds with an acidic head group generated from dietary fat or intracellular metabolism. The hydrophobic ligand-binding pocket (LBP) structure varies significantly among PPAR isotypes. The PPARδ pocket is significantly smaller, and the PPARα pocket is more lipophilic than the other two, respectively. In addition, single amino acid differences in the pockets can be major determinants of ligand isotype selectivity (Zoete *et al.*, 2007). Based on distinct LBP structure for each of PPAR isotypes, a number of highly specific synthetic ligands serving as selective PPAR agonists (e. g. L-165041 or GW501516 for PPARδ) had been developed.

Orchestrated with ligand-binding function of PPARδ LBD, allosteric alterations in the AF-2 helical domain lead to recruitment of co-activator proteins such as p300 or to the release of co-repressors, such as silencing mediator for retinoid and thyroid hormone receptors (SMRT), SMRT/HDAC (histone deacetylase) I-associated repressor protein (SHARP), or nuclear receptor co-repressor (NCoR) (Tan *et al.*, 2004a). Conformational changes upon association of LBD with co-repressors do not impair DNA-binding facilitated by the DNA binding domain of PPARδ. Thus, the isoform can repress expression of the PPARα and/or PPARγ-mediated target genes by binding to a specific DNA sequence common to all PPARs termed the peroxisome proliferator responsive element (PPRE) (Tan *et al.*, 2005). Canonically, PPAR activation is initiated by the binding of an appropriate lipid ligand to the LBD and subsequent AF-2 helix movement allowing PPAR heterodimerization with RXR. However, it has been recently reported that a high percentage of PPARs is associated with RXR *in vivo* even in the absence of ligand (Tudor *et al.*, 2007).

PPARδ remains still the most enigmatic of three isoforms. It is expressed in various cell types including adipocytes, endothelila, muscle, and colon cells. In the skin, it is expressed in basal and suprabasal epidermal keratinocytes (Icre *et al.*, 2006) and contributes to the so-called regenerative skin phenotype characterizing the late phase of wound healing (Piqueras *et al.*, 2007). PPARδ is induced by gamma interferon, stress-activated kinase, or tumor necrosis factor alpha (TNFα) via activator protein 1 (AP1)–mediated transcription. PPARδ regulates keratinocyte differentiation, blocks apoptosis (reviewed in (Tan *et al.*, 2002)), and induces angiogenesis (Madsen *et al.*, 1992). This profile of biological activity overlaps with pathological processes observed in psoriasis.

4. Aim of the study.

The nuclear hormone receptor PPARδ shows an activity profile highly consistent with a role in psoriasis and is strongly upregulated in psoriatic skin. Therefore, to characterize the potential contribution of this transcription factor to the hyperproliferation or/and dysregulated differentiation of keratinocytes, the aim of this study was: (1) to identify which of the known transcriptional pathways regulating PPARδ is active in psoriasis, (2) to identify novel PPARδ target genes in keratinocytes, and (3) to generate lentiviral construct suitable for further investigation of PPARδ function *in vivo.*