Expression of dioxin-binding Ah receptor in human skin is strictly correlated with the keratinocyte terminal differentiation

Andrei A. Panteleyev*, Reinhard Wanner**, Vladimir S. Roumak***, Angela M. Christiano*, and Thomas Rosenbach****

*Dept of Dermatology, CPS, Columbia University, New York, NY
**Inst. of Molecular Biology & Biochemistry, Free University, Berlin
***Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow
****Dept of Dermatology, Virchow Clinic, Humboldt University, Berlin

Introduction

The aromatic hydrocarbon (Ah) receptor is a basic mediator of toxic effects of 2,3,7,8-tetrachlorodibenso-*p*-dioxin (TCDD), which appeared to be one of the most dangerous environmental contaminants. Ah-receptor is a ligand-activated transcription factor that belongs to the helix-loop-helix superfamily of DNA-binding proteins (1). The bound receptor regulates the transcription of specific genes involved in xenobiotic metabolism, with cytochrome p450 1A1 and 1A2 the most extensively studied examples. In addition, in primary and transformed keratinocyte cell lines Ah-receptor has been shown to mediate the TCDD action upon several genes encoding for growth- and differentiation-regulating proteins such as epidermal growth factor receptor, plasminogen activator inhibitor-2, interleukin-1b, transforming growth factors α and β_2 (2,3,4,5). In vivo, in the skin of *hairless* mouse mutants with suppressed activity of *hr* gene, TCDD action dramatically disturbs the balance between apoptosis, proliferation, and differentiation in hair follicle and epidermal keratinocytes (6). Presumably, the same molecular effects underlie the TCDD-induced skin disorders including chloracne, which is most consistent and commonly observed clinical sign of TCDD-intoxication in humans and is characterized by altered patterns of keratinocyte differentiation (7,8).

Although the Ah-receptor achieved notoriety as a mediator of TCDD toxic action in humans and animals, studies in Ah-receptor deficient knock-out mice support the view that this

intracellular protein and its unknown endogenous ligand are the intrinsic regulators of many critical life functions, particularly those associated with development and cell growth (9). Cellular processes in skin may also be governed by this mechanism, and the findings that the expression of the Ah-receptor in cultured keratinocytes correlates with the state of cell differentiation are in line with this assumption (10). Moreover, the dioxin receptor interacts with the *hr* (*hairless*) gene which encodes a presumptive transcription factor and plays an essential role in murine (11) and human (12) skin physiology.

In order to demonstrate a possible role of the Ah-receptor in the regulation of normal and pathophysiological skin function *in vivo*, we studied the AhR mRNA expression in normal and lesional (psoriasis) human skin using nonradioactive *in situ* hybridization. Psoriasis was chosen as one of the most common dermatosis (1-3% of general population) associated with an abnormal keratinocyte differentiation pathway (13).

Material and methods

AhR riboprobe preparation

Total RNA was isolated from HaCaT-cells and transcribed into a cDNA, using an RT-PCR kit (Stratagene, Heidelberg, FRG). For the RT-PCR of AhR (ligand binding subunit), the primers were nucleotides 2699 to 2718 and 2925 to 2944 (accession number L19872) at 0.5 µM. The obtained AhR cDNA fragment was gel-purified, ligated into a pGEM-T vector (Promega, Madison, WI, USA), and amplified in Epicurian Coli XL2-Blue ultracompetent cells (Stratagene, La Jolla, USA). Plasmids were prepared using Qiagen plasmid mini-kit (Qiagen, Studio City, USA) and the identity of the insert was evaluated by sequencing.

Aliquots of the plasmids enclosing AhR specific cDNA were linearised with *Pst*I or *Sph*I restriction enzymes (Boehringer-Mannheim, Mannheim, FRG). Digoxigenin-labeled cRNAs were prepared *in vitro* with 1µg of the plasmid using the "DIG RNA Labeling Kit" (Boehringer-Mannheim, Mannheim, FRG). Sp6 and T7 RNA polymerases were used to produce sense (background control) and antisense probes according to the kit protocol.

Skin samples

Psoriatic skin samples were obtained by punch biopsy from 3 patients with obviously diagnosed plaque psoriasis. Control skin samples were obtained from perioperational melanoma excision in patients without any psoriasis history. The tissue was fixed in 4% paraformaldehyde in PBS and embedded in paraffin (JUNG-Histowax; Reichert-Jung, Heidelberg, FRG) according to standard procedures. Sections of 5µm were mounted on silane-coated glass slides and prepared for in situ hybridization as described (6).

In situ hybridization

In situ hybridization was performed as previously described (6) with 50 ng of cRNA per section for 17h at 50°C. The final detection of hybridization signal was performed by incubation of slides in nitroblue tetrazolium and ß-chloroindolylphosphate solution (Boehringer-Mannheim, Mannheim, FRG) for 16-20 h in complete darkness at room temperature. To examine the property of prepared sections, we have performed a control hybridization with a probe for c-*fos* gene, whose expression patterns in normal and psoriatic skin are established (14).

Results and Discussion

According to results obtained in normal human epidermis, Ah-receptor transcripts were detected in suprabasal cells only and clearly increased in the upper spinous cell layers, corresponding to the localization of differentiating keratinocytes. The basal cell layer represented by proliferating keratinocytes did not contain the Ah-receptor transcripts at a level detectable by a hybridization. The differentiation-correlated up-regulation of Ah receptor expression in normal skin suggests the implication of this transcription factor in normal keratinization process in human epidermis.

Ah receptor expression level in psoriatic skin was several fold lower than in normal skin. The gradual differentiation-associated increase of Ah receptor expression shown in normal epidermis, was suppressed in all psoriatic samples studied. In lesional skin, very weak positive staining denoting Ah receptor mRNA was evenly spread over the epidermis and only the uppermost spinous cell layer just beneath the stratum corneum showed an increase of Ah receptor expression. This thin cell layer represents the differentiating keratinocyte population in the psoriatic skin.

A dominant feature of psoriasis is the dramatic increase in keratinocyte growth kinetics resulting in thickening of the epidermis and rapid epidermal turnover. This hyperproliferative state is common to epidermal wound healing, but the origin of psoriasis is still unknown and numerous potential pathogenesis pathways have been suggested (14). Our finding that Ah receptor expression decreased in the differentiation-deficient psoriatic condition is in line with the current concept that lack of expression of some differentiation-associated proteins is one aspect of the hyperproliferative nature of this disease (14) and also supports the suggestion that AhR might be implicated in regulation of normal and pathological skin physiology.

These results demonstrate that AhR transcript level is also linked to keratinocyte differentiation *in vivo*. According to recent studies in AhR-deficient knock-out mice, this intracellular protein is the intrinsic regulator of many critical life functions, especially those

associated with development and cell growth (9). The differentiation-related up-regulation of Ah receptor expression in normal skin suggests a role for this transcription factor in keratinocyte physiology *in vivo*. Moreover, active expression of the dioxin receptor in human skin may underlie its sensitivity to dioxin toxic action, which results in chloracne (15) and other epithelial consequences of dioxin toxicity.

References

1. Dolwick KM, Schmidt JV, Carver LA, Swanson HJ, Bradfield CA; *Mol. Pharmacol.* **1993**, 44, 911
2. Madhukar BV, Brewster DW, Matsumura F; *Proc. Nat. Acad. Sci.* USA **1984**, 81, 7407
3. Sutter TR, Guzman K, Dold KM, Greenlee WF; *Science* **1991**, 254, 415
4. Gaido KW, Maness SC, Leonard LS, Greenlee WF; *J. Biol. Chem.* **1992**, 267, 24591
5. Okey AB, Riddick DS, Harper PA; *Toxicol. Lett.* **1994**, 70, 1
6. Panteleyev AA, Thiel R, Wanner R, Zhang J, Roumak VS, Paus R, Neubert D, Henz BM, Rosenbach T; *J. Invest. Dermatol.* **1997**, 108, 330
7. Crow KD; *Trans. St. Johns Hosp. Dermatol. Soc.* **1970**, 56, 77
8. Taylor JS; *Cutis* **1974**, 13, 585
9. Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SST, Kimura S, Nebert DW, Rudikoff S, Ward JM, Gonzalez FJ; *Science* **1995**, 268, 722
10. Wanner R, Brömmer S, Czarnetzki BM, Rosenbach T; *Biochem. Biophys. Res. Com.* **1995**, 209, 706
11. Panteleyev AA, van der Veen C, Rosenbach T, Sokolov VE, Müller-Röver S, Paus R; *J. Invest. Dermatol.* **1998** (in press)
12. Ahmad W, ul Haque MF, Brancolini V, Tsou HC, ul Haque S, Lam HM, Aita VM, Owen J, deBlaquiere M, Frank J, Cserhalmi-Friedman PB, Leask A, McGrath JA, Peacocke M, Ahmad M, Ott J, Christiano AM; *Science* **1998**, 279, 720
13. Weinstein GD, Ross P, McCullough JL, Colton A; In: *Psoriasis: cell proliferation*, Eds. Wright NA, Camplejohn RS, Churchill Livingstone, **1983**, pp 189-208
14. Dubertret L (Ed.); *Psoriasis*, **1994**, ISED, Italy, 274pp.
15. Schultz KH; *Arch. Klin. Exp. Dermatol.* **1957**, 206, 589