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Skin response to a carcinogen involves the xenobiotic receptor pregnane X receptor

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Abstract

Skin is in daily contact with potentially harmful molecules from the environment such as cigarette smoke, automobile emissions, industrial soot and groundwater. Pregnane X receptor (PXR) is a transcription factor expressed in liver and intestine that is activated by xenobiotic chemicals including drugs and environmental pollutants. Topical application of the tumor initiator 7,12-dimethylbenz(a)anthracene (DMBA) enhances *Pxr*, *Cyp1a1*, *Cyp1b1* and *Cyp3a11*, but not *Ahr* expression in the skin. Surprisingly, DMBA-induced *Pxr* upregulation is largely impaired in Langerin⁺ cell-depleted skin, suggesting that DMBA mainly triggers *Pxr* in Langerin⁺ cells. Furthermore, PXR deficiency protects from DNA damage in epidermal cells but to a lesser extent than aryl hydrocarbon receptor (AHR) deficiency. Interestingly, skin exposure to low doses of DMBA induces migration of PXR-deficient but not of wild-type and AHR-deficient Langerhans cells (LCs). PXR-humanized mice show a marked increase in DNA damage to epidermal cells after topical application of DMBA, demonstrating relevance of these findings in human tissue. This is the first report suggesting that carcinogens might trigger PXR in epidermal cells, particularly in LCs, thus leading to DNA damage. Further studies are required to better delineate the role of PXR in cutaneous carcinogenesis.

Keywords

DNA damage; H2AX; Langerhans cells; pregnane X receptor; skin

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Conflict of interests

The authors have declared no conflicting interests.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Introduction

The environment contains various pollutants coming from cigarette smoke, automobile emissions, industrial soot and groundwater. Skin is in daily contact with such potentially harmful molecules. Repeated exposure to such chemicals is implicated in skin carcinogenesis. Skin tumors are initiated by contact with carcinogens such as polycyclic aromatic hydrocarbons (PAHs), which induce DNA damage and gene mutations in keratinocytes and epidermal stem cells (1–3). DNA damage results in bio-activation of chemicals into electrophilic intermediates via cytochrome P450 including CYP1B1, CYP1A1, CYP1A2 and CYP3A4 (4–6). Recent evidence showed that CYP3A59 might also be involved (7). Several cytochrome P450 enzymes are under the transcriptional control of the aryl hydrocarbon receptor (AHR) and/or the pregnane X receptor (PXR) (5,8). PXR, a member of the nuclear receptor superfamily, is primarily expressed in the liver and the gastrointestinal tract where it regulates the transcription of genes involved in all phases of endo- and xenobiotic transport and metabolism and thus prevents toxic accumulation of metabolites within cells (9). PXR controls not only the expression of cytochrome P450 enzymes, but also controls the expression of multiple drug resistance transporters (MDR1/ABCB1), among many other genes (9,10). PXR is activated by a broad range of endogenous compounds, including steroids, bile acid intermediates and a wide variety of drugs including antibiotics, glucocorticoids and carcinogens (4,6,10,11). Quantitative PCR experiments revealed low but consistent levels of *Pxr* mRNA in immune cells including T and B lymphocytes in both humans and mice (12–15). We previously showed that PXR is expressed in activated T lymphocytes and modulates T lymphocyte function (15).

Skin is a highly immunological organ where Langerhans cells (LCs) are pivotal dendritic cells (DCs) modulating cutaneous immunity (16,17). Located at the interface between the body and its environment, LCs efficiently capture antigens that are presented to skin-resident memory T cells or to naive T cells after migration to the regional lymph nodes (16,17). In the epidermis, chemicals including carcinogens can covalently bind to proteins and become haptens able to induce LC maturation and migration to regional lymph nodes (18). LCs, beside other epidermal cells, can uptake and metabolize PAHs such as DMBA (19). Migration of DMBA-activated LCs was previously reported to be increased (20) or unchanged (21), depending on the dose of DMBA. Moreover, LCs would possess an innate ability to metabolize DMBA via CYP1B1 and to transfer activated DMBA metabolites to keratinocytes, hereby promoting DNA damage (19). Thus, LCs may contribute to tumor initiation via non-immunological properties (19). However, this needs to be further investigated.

Both, PXR and AHR are activated by xenobiotics (22) and are likely involved in the development of squamous cell carcinoma (SCC) via the regulation of CYP3 and CYP1 enzymes, respectively (23). In skin, the procarcinogenic effects of CYP enzymes might not only be mediated via generation of deleterious metabolites from xenobiotics but also via generation of by-products such as reactive oxygen species (24,25). Because PXR-dependent CYPs, similar to AHR-dependent CYPs, are involved in chemical bio-activation and because

PXR and AHR share target genes (8), we explored the role of PXR in the skin after topical application with a carcinogen.

Methods

Reagents and antibodies

7,12-dimethylbenz(a)anthracene (DMBA) was purchased from Sigma Chemical (St Louis, MO, USA). Anti-PXR antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Directly labelled primary monoclonal mouse antibodies (mAb) specific for CCR7 and CD11c were purchased from BD Pharmingen (San Diego, CA, USA). Biotinylated anti-CD103 and anti- β tubulin were purchased from BD Pharmingen. Directly labelled mAb against mouse Langerin (clone 929F.3) was purchased from Dendritics (Lyon, France) and used after permeabilization with Cytofix/Cytoperm kit (BD Biosciences, San Diego, CA, USA), according to the manufacturer's instruction. Anti-mouse γ -H2AX antibody was purchased from Cell Signaling (Danvers, MA, USA), anti-mouse 53BP1 from Thermo Scientific (Waltham, MA, USA) and anti-rabbit Alexa Fluor 594 and 680 Ab from Invitrogen (Carlsbad, CA, USA). IRDye[®]800 anti-mouse Ab was purchased from Rockland Inc. (Gilbertsville, PA, USA). Recombinant PXR protein was purchased from Active Motif (Carlsbad, CA, USA).

Animals

Mice of the inbred strain C57BL/6 were purchased from Charles River Laboratories (Sulzfeld, Germany). *Pxr*^{-/-} (26), transgenic mice humanized for *hPxr* and its target gene *Cyp3a4* (Tg-3A4/hPXR) (27) and *Ahr*^{-/-} (28) mice were described previously. Langerin-DTR-EGFP knock-in mice expressing a diphtheria toxin receptor (DTR) and enhanced green fluorescent protein (EGFP) under the murine Langerin promoter in LCs and in Langerin⁺ dermal and lymph node DCs were bred on a C57BL/6 background as described previously (29). Langerin-DTR mice were injected intraperitoneally with diphtheria toxin (DT) or PBS at day -2 (0.5 μ g/mouse). Animal experimentation was in accordance with applicable standards and approved by the Institutional Animal Experimentation Committee at the Austrian Ministry of Science and Research (BMWF-66.011/0135-II/3b/2011).

Immunocytochemical analysis

Detection of γ H2AX in paraffin-embedded sections was facilitated by antigen retrieval in citrate buffer pH = 6. Cytospins with epidermal cells were fixed in 4% formaldehyde for 15 min at room temperature. Incubation with anti- γ H2AX or isotype control was performed overnight at 4°C after blocking with 10% goat serum for 1 h at room temperature. Antibody binding was visualized using anti-rabbit Alexa Fluor 594 Ab. Antibodies were diluted in 2% goat serum or BSA containing 0.3% or 0.5% Triton X-100. Cytospins were counterstained with anti-MHC class II-FITC mAb. DAPI was used to counterstain cell nuclei. γ H2AX⁺ cells in paraffin-embedded sections were counted with a calibrated grid (7–10 fields per sample). Epidermal sheets were prepared by separation with ammonium thiocyanate (Merck, Westchester, PA, USA), fixed with acetone and stained with anti-MHC class II-FITC mAb (30). Staining was visualized with a 10 $\times$, 40 $\times$ or 100 $\times$ objective using an Olympus BX60 epifluorescence microscope (Hamburg, Germany).

In vivo migration assay

Mouse ears were topically treated with 0.5 mg/ml DMBA (0.05%) in acetone, and 2 days later, cells from auricular lymph nodes were prepared as reported earlier and counted in the haemocytometer. Cell suspensions were then stained and analysed by flow cytometry. Emigrated LCs were defined as Langer-in⁺ CCR7⁺ CD11c⁺ CD103⁻ cells according to the literature (31).

Quantification of gene expression

Total RNA was extracted from mouse ears using TRIZOL (Gibco BRL, Life Technology, Vienna, Austria). Random-primed cDNA was prepared (Superscript II RNase H – reverse transcriptase; Life Technologies, Vienna, Austria) from total RNA. Genomic DNA was removed from samples by DNase treatment (Ambion, Austin, TX, USA). Quantitative polymerase chain reaction (PCR) analysis was performed by real-time PCR (real-time PCR detection system CFX96; Bio-Rad, Vienna, Austria) using the Brilliant III Ultra-Fast Quantitative PCR kit from Agilent technologies (Vienna, Austria). Sequences for probes and primers specific for TATA binding protein (*Tbp*), *Ahr*, *Cyp3a11*, *Cyp1a1*, *Cyp1b1*, *Cyp3a4*, *H2ax*, *Pena*, *Mdr1*, *Ccr7* and *Pxr* mRNA molecules were selected using Primer Express software (Applied Biosystems, Foster City, CA, USA) and synthesized by Microsynth (Balgach, Switzerland) or purchased from Applied Biosystems.

Western blot analysis

Skin was lysed in modified RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, pH = 7.4) in the presence of protease inhibitors (Pierce, Rockford, IL, USA). Endogenous proteins (100 µg) were detected with primary and secondary antibodies as listed above. Blots were then scanned with a LI-COR Biosciences analyzer. Intensity of bands was analysed with the Odyssey software (Lincoln, NE, USA).

Statistical analysis

All experiments were performed multiple times with *n* representing the number of mice per group. Statistical analyses were performed using the GraphPad Prism software (San Diego, CA, USA). Results are expressed as mean ± SEM and analysed for statistical significance as appropriate. *P*-values are indicated in the figures.

Results

PXR is upregulated in skin after topical application of DMBA

Because PXR is activated by xenobiotic chemicals, we hypothesized that PXR might be upregulated by carcinogens such as DMBA in the skin. Accordingly, we found that, 48 h after topical application of DMBA, skin expression of *Pxr* and *Cyp1a1* mRNAs is markedly increased. In contrast, *Cyp1b1* and *Cyp3a11* mRNAs are only moderately increased (Fig. 1a) and multiple drug resistance transporters *Mdr1/ABCB1* and *Ahr* mRNAs are not altered (Fig. 1a). We confirmed the upregulation of PXR at the protein level in the DMBA-treated skin (Fig. 1b and Figure S1). Of note, the DMBA-mediated increase in the expression of

Cyp1a1 and *Cyp1b1* mRNAs is not abolished in *Pxr*^{-/-} mice consistent with the major role of AHR in regulating these genes (Figure S2). Time course of gene expression after a single topical application of DMBA shows that *Pxr* mRNA expression increases with time, whereas *Ahr* mRNA levels are not altered (Fig. 1c). Expression of *Cyp1b1* and *Cyp1a1* mRNAs peaks at 4 h, whereas expression of *Cyp3a11* mRNA peaks at 24 h (Fig. 1d). DMBA induces a decrease in *Mdr1/ABCB1* mRNA expression of 34% at 24 h (data not shown). Because DMBA cannot be applied to human skin, we circumvent this problem by utilizing mice humanized for PXR and CYP3A4 (Tg-3A4/hPXR) topically treated with DMBA. We found that mRNA levels of *Cyp1a1* and *Cyp1b1* are increased in the skin of Tg-3A4/hPXR mice topically treated with DMBA, while *Ahr*, *Cyp3a4*, *Mdr1/ABCB1* and *Pxr* mRNAs are not altered (Fig. 1e). In contrast, protein levels of PXR increase after topical application of DMBA (Fig. 1f and Figure S1). These results show that expression of *Pxr* is triggered by topical application of DMBA and suggest a role of PXR in inducing DNA damage to epidermal cells.

PXR is involved in DNA damage to epidermal cells after topical application of DMBA

We next investigated the effects of PXR deficiency on the capacity of topically applied DMBA to induce DNA damage in epidermal cells. γ H2AX is recruited to DNA double-strand breaks and to DNA-PAH adducts (32). After topical treatment with DMBA, DNA damage assessed by γ H2AX staining is induced in *Pxr*^{+/+} mice in basal keratinocytes and in cells from hair follicles but not in *Pxr*^{-/-} mice (Fig. 2a,b). Of note, DNA damage is also observed in LCs as illustrated in Figure S3a. Similarly, *Ahr*^{-/-} mice exhibit reduced numbers of γ H2AX⁺ epidermal cells when compared to littermate controls after topical application of DMBA (Fig. 2a,c). At the transcriptional level, expression of *H2AX* is largely decreased in *Ahr*^{-/-} and *Pxr*^{-/-} mice when compared to littermate controls (Fig. 2d). 53BP1 and PCNA are involved in cellular responses to DNA damage (33,34). We found that 53BP1 (Figure S3b) and *Pcna* (Figure S3c) increases in the skin of *Pxr*^{+/+} mice treated with topical application of DMBA. In contrast, DMBA treatment does not increase 53BP1 and *Pcna* in *Pxr*^{-/-} mice when compared to vehicle-treated mice. Thus, these results show that PXR deficiency largely protects epidermal cells from DNA damage. In contrast, numbers of γ -H2AX⁺ keratinocytes in the skin of PXR-humanized mice topically treated with DMBA are markedly increased when compared to vehicle-treated mice [fivefold increase in Tg-3A4/hPXR mice (Fig. 2e) versus 1.6-fold in control mice (Fig. 2b)]. Hence, PXR participates in response of epidermal cells to DNA damage after exposure to a potentially harmful molecule.

PXR upregulation mainly occurs in Langerin⁺ skin DC after topical application of DMBA

Langerhans cells have previously been shown to be involved in DMBA-mediated DNA damage in skin (19). We therefore evaluated the contribution of LCs to the upregulation of PXR after topical application of DMBA. Mice depleted of Langerin⁺ cells including LCs and topically treated with DMBA show a decrease in *Pxr* mRNA induction in the skin (Fig. 3a). In contrast, expression of *Cyp1a1*, *Cyp1b1*, *Cyp3a11*, *Mdr1/ABCB1* and *Ahr* mRNAs is not significantly altered by the depletion in Langerin⁺ cells (Fig. 3a,b and data not shown). Quantitative PCR reveals that *Pxr* mRNA is expressed in highly purified human LCs to a higher extent than in mouse and human epidermis and keratinocytes (Fig. 3c). In contrast to

human LCs, isolation of sufficient amounts of highly purified and viable mouse LCs is barely achievable and renders measurements of *Pxr* mRNA expression in mouse LCs by quantitative PCR impossible. Immunohistochemistry shows positive staining for PXR in both the nucleus and the cytoplasm of mouse LCs (Fig. 3d). Thus, LCs express PXR and DMBA triggers *Pxr* mainly in Langerin⁺ DCs including LCs.

PXR deficiency promotes LC migration after topical application of DMBA

Doses over 0.1% DMBA induce LC migration to regional lymph nodes to putatively initiate a primary cutaneous immune response (20). At lower doses, DMBA is metabolized by LCs located in the epidermis which promotes DNA damage to epidermal cells (19). We topically applied 0.05% DMBA, a dose which is classically used for tumor initiation, to test the ability of PXR deficiency to render LCs more susceptible to carcinogen-mediated activation and migration to regional draining lymph nodes. We found that *Pxr*^{-/-} mice exhibit decreased numbers of LCs in the epidermis (Fig. 4a) and increased numbers of LCs in skin draining lymph nodes (Fig. 4b and Figure S4) after topical application of low dose of DMBA when compared to vehicle-treated *Pxr*^{-/-} mice. These effects are accompanied by an increase in *Ccr7* mRNA expression in the skin of *Pxr*^{-/-} mice but not in the skin of wild-type mice (Fig. 4c). In line with this result, numbers of LCs in the epidermis (Fig. 4a) and numbers of LCs in skin draining lymph nodes (Fig. 4b and Figure S4) remain unchanged in wild-type mice, regardless of treatment. There is a 2.1-fold increase in epidermal *Ccr7* mRNA expression in *Pxr*^{-/-} mice, but not in littermate controls (0.8-fold, data not shown). In contrast, AHR deficiency does not promote LC migration after topical application of DMBA (Fig. 4a,b). Accordingly, *Ccr7* mRNA expression is not induced in the skin of *Ahr*^{-/-} mice after topical application of DMBA (Fig. 4c). Thus, these results show that PXR deficiency promotes LC migration which is accompanied by an upregulation of *Ccr7* even after topical application of low-dose DMBA. Presence of LCs in the epidermis actively participates in the induction of DNA damage to surrounding keratinocytes (19). Therefore, promotion of LC emigration from epidermis by PXR deficiency might be involved in the decrease of DNA damage to epidermal cells observed in PXR-deficient skin after topical application of DMBA (Fig. 2).

Discussion

We here identify PXR as complementary to AHR for regulating the induction of DNA damage in epidermal cells following skin exposure to DMBA (Fig. 2). Whereas PXR deficiency leads to reduced amounts of DNA damage, transgenic expression of human *Pxr* in mice leads to a marked increase in DNA damage in epidermal cells after topical application of DMBA (Fig. 2). PXR is upregulated after topical application of DMBA, and this upregulation mainly occurs in Langerin⁺ skin DCs including LCs (Fig. 3). Because LCs are involved in DNA damage to surrounding keratinocytes after topical treatment with DMBA, promotion or blockage of LC migration upon PXR downregulation or activation might interfere with the cutaneous response to carcinogens. In contrast, although AHR deficiency markedly decreases DNA damage to epidermal cells, it does not alter LC migration and *Ahr* expression is not affected by depletion of Langerin⁺ skin DCs after topical application of DMBA. Hence, in keratinocytes, PXR might be less important than AHR for DNA damage

after topical treatment with DMBA. Instead, PXR might be involved in the response of LCs to a carcinogen such as DMBA.

Skin exposure to environmental pollutants and pesticides increases the risk of developing non-melanoma skin cancer after adjustment for age, skin and hair colour, mother's ethnic origin, and sunlight exposure (35,36). PAHs in cigarette smoke, extracts of emissions and indoor air particles, smoky coal combustion, cooking oil fumes and chimney soot require metabolic activation to induce DNA damage. Notably, in epidermal stem cells, these compounds can persist for many years and then give rise to skin tumors (37). PXR is activated by a broad range of endo- and xenobiotics including PAHs potentially inducing carcinogenesis (4,6,38). PXR, similar to AHR, controls the expression of phase I drug-metabolizing enzymes capable of initiating toxicity by metabolically activating xenobiotic substrates able to cause toxicity and oxidative stress, and induction of phase II enzymes involved in detoxification; PXR also regulates several xenobiotic transporters (22). Moreover, PXR and AHR are involved in the development of squamous cell carcinoma via the regulation of CYP3 and CYP1 enzymes, respectively (23). We found that expression of *Pxr* and *Cyp1a1* mRNAs is markedly increased. In contrast, *Cyp3a11* and *Cyp1b1* mRNAs are only moderately increased 48 h after topical application of DMBA. Use of *Pxr*^{-/-} mice topically treated with DMBA shows that expression of *Cyp1a1* and *Cyp1b1* is independent of PXR in the skin (8,9,39). A time course of gene expression after topical application of DMBA shows that *Pxr* expression is already increased 24 h after treatment, whereas expression of *Ahr* is never altered. Expression of cytochrome P450 mRNAs, that is *Cyp1a1*, *Cyp3a11* and *Cyp1b1*, peaks 4–24 h after stimulation with DMBA, exhibiting a similar pattern of expression, yet different from their upstream transcription factors, PXR and AHR. Relevance of our findings for humans is proven by experiments in humanized mice expressing the human *Pxr* and its downstream gene, namely *Cyp3a4*. When humanized mice are topically treated with DMBA, PXR is increased at the protein level. Thus, DMBA is able to trigger PXR in the skin. In line with our results, previous work has shown that the epidermis exhibits low basal expression of *Cyp3a4* (40) and *Pxr* (41) and high expression of phase II enzymes, some of which are under the control of PXR (42). Thus, epidermal cells might be major sites of xenobiotic metabolism (41,42) where PXR might play a regulating role. The skin produces PXR ligands such as pregnenolone potentially via CYP11A1 (43). Thus, PXR might also exhibit physiological functions in the skin which remain to be elucidated.

Interestingly, we found that PXR deficiency protects keratinocytes from DNA double-strand breaks after exposure to a single topical application of DMBA, but to a lesser extent than AHR deficiency. Thus, PXR might contribute to the deleterious effects of DMBA on DNA. This hypothesis is supported by prior studies showing that PAH-DNA adducts are present in CYP1B1-deficient, CYP1A2-deficient or AHR-deficient mouse epidermis (2). Moreover, CYP1A1/CYP1B1 double knockout mice develop squamous cell carcinoma (7), and PXR and CYP3A are activated by various PAHs (4,6). Interestingly, despite undetectable CYP1A1 and low or undetectable CYP1B1 in bronchoalveolar macrophages from smokers and non-smokers, high levels of PAH-DNA adducts are detected in samples from smokers with high levels of CYP3A4 (44). Accordingly, DNA damage in basal keratinocytes is dramatically increased (fivefold) in PXR-humanized mice topically treated with DMBA.

Thus, PXR activation in epidermal cells might be deleterious, whereas PXR deficiency might be protective after topical contact with carcinogens.

We show here that PXR is expressed in human and mouse LCs and that Langerin⁺ skin DCs are required for the DMBA-mediated upregulation of *Pxr*, but are dispensable for the induction of *Cyp1b1* and *Cyp1a1*. Thus, DMBA might preferentially trigger AHR in keratinocytes and PXR in Langerin⁺ skin DCs including LCs. DMBA is known to be captured and processed by LCs (45), and LC migration after DMBA application depends on the dose of carcinogen applied to the skin (20,21). We found that DMBA, at low dose, provokes LC migration to skin draining lymph nodes in *Pxr*^{-/-} mice but not in *Ahr*^{-/-} mice or control mice. In *Pxr*^{-/-} mice, LC migration correlates with increased expression of *Ccr7*. Thus, *Pxr*^{-/-} LCs are more prone to migrate to skin draining lymph nodes in response to a carcinogen. *Pxr*^{-/-} LCs might exhibit a reduced capacity to activate DMBA and consequently a bigger capacity to transform DMBA into a hapten able to induce their maturation and migration to regional lymph nodes.

The mechanism by which PXR regulates DNA damage in keratinocytes remains to be elucidated. However, we speculate that reduced DNA damage observed in the epidermis of PXR-deficient mice topically treated with DMBA might result from increased migration of *Pxr*^{-/-} LCs. LCs isolated from squamous cell carcinoma induce a strong Th1 immune response (46) and migration of DCs including LCs is important for inducing immunity in skin tumors because it is a prerequisite for T-cell priming. CD8⁺ T cells play a protective role in skin tumor development (47), suggesting that LC migration to the skin draining lymph node where they prime cytotoxic T cells is important for elimination of damaged epidermal cells. However, reduced DNA damage and increased LC migration in the context of PXR deficiency occur 48 h after topical treatment with DMBA, thus precluding a major role of adaptive immunity. Hence, a possible explanation would be that *Pxr*^{-/-} LCs exhibit impaired DMBA metabolism which promotes their migration to skin draining lymph nodes as a result of increased DMBA haptenization. In turn, if LCs are no longer present in the skin, their capacity to transfer DMBA metabolites to surrounding keratinocytes might be reduced. In contrast, presence of activated PXR in LCs might contribute to the accumulation of damaged cells in the skin via downregulation of LC migration. Furthermore, AHR deficiency reduces DNA damage without affecting LC migration and *Ccr7* expression, suggesting that AHR and PXR signal via distinct pathways.

In conclusion, the present study is the first report showing a novel role for PXR in the skin. PXR might contribute to chemical metabolism mainly in LCs and might exhibit complementary functions with another important receptor for many chemicals including carcinogens, namely AHR. Because PXR is activated by a broad range of chemicals, drugs and pollutants, this work might initiate further research aiming at a better understanding of pollutant effects on human skin. Moreover, synthetic PXR antagonists may provide novel possibilities for reducing the negative impact of chemicals on DNA integrity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

Pregnane X receptor (PXR) is upregulated in the skin after topical application of DMBA. Mice were topically treated with 0.05% DMBA on right ears and with acetone on left ears. (a) Quantitative PCR showing expression of *Pxr*, *Cyp3a11*, *Ahr*, *Cyp1a1*, *Cyp1b1* and *Mdr1/ABCB1* in mouse skin 48 h after a single topical application of DMBA, $n = 10-19$. (b) Western blot shows PXR and β -tubulin in the skin of mice 48 h after topical application of DMBA. Histogram shows paired-PXR (acetone versus DMBA) expression relative to β -tubulin. Data were analysed with a paired *t*-test, $n = 5$. Time course of *Pxr* and *Ahr* expression (c) and of *Cyp3a11*, *Cyp1a1* and *Cyp1b1* expression (d) assessed by quantitative PCR, $n = 4-18$. (e) Histogram shows expression of *Pxr*, *Cyp3a4*, *Ahr*, *Cyp1a1*, *Cyp1b1* and *Mdr1/ABCB1* assessed by quantitative PCR in the skin of Tg-3A4/hPXR-humanized mice 48 h after a single topical application of DMBA or acetone, $n = 6-12$. (f) Western blot shows PXR and β -tubulin in the skin of humanized mice 48 h after a single topical application of DMBA. Human recombinant PXR (rec. PXR) serves as a positive control. The histogram shows paired-PXR (acetone versus DMBA) expression relative to β -tubulin. Data are pooled from multiple independent experiments. Data were analysed with a paired *t*-test, $n = 5$. (a, c, d, e) Results are shown as fold increase comparing gene expression in DMBA- and acetone-treated skin in the same animal.

Figure 2.

Pregnane X receptor (PXR) is involved in DNA damage to epidermal cells after topical application of DMBA. Mice were topically treated with 0.05% DMBA on right ears and with acetone on left ears. Analyses were carried out 48 h after treatment. (a) Representative pictures showing immunostaining for γ -H2AX in $Pxr^{+/+}$ versus $Pxr^{-/-}$ (left panel) and in $Ahr^{+/+}$ versus $Ahr^{-/-}$ (right panel) mouse skin treated with DMBA or acetone. Histograms show percentages of γ -H2AX⁺ $Pxr^{+/+}$ versus $Pxr^{+/+}$ ($n = 7-8$) (b) and $Ahr^{+/+}$ versus $Ahr^{-/-}$ ($n = 5$) (c) keratinocytes in mouse skin treated with DMBA or acetone. (d) Expression of

H2AX assessed by quantitative PCR in *Pxr*^{-/-}, *Ahr*^{-/-} and wild-type mouse skin treated with DmBa or acetone, *n* = 4–10. Results are shown as fold increase comparing gene expression in DMBA- and acetone-treated skin in the same animal. (e) Representative pictures and histogram show percentages of γ -H2AX⁺ cells in Tg-3A4/hPXR-humanized mouse skin after topical application of DMBA or acetone, *n* = 5. Nuclei were counterstained with DAPI. Magnification $\times 40$. Data are pooled from multiple independent experiments. Data were analysed with a Student's *t*-test. N.S., not significant.

Figure 3.

Pregnane X receptor (PXR) upregulation mainly occurs in Langerin⁺ skin dendritic cells (DCs) after topical application of DMBA. Mice were topically treated with 0.05% DMBA on right ears and with acetone on left ears. Quantitative PCR showing expression of *Pxr* and *Ahr* (a) and of *Cyp1a1* and *Cyp1b1* (b) in mouse skin depleted (—) or not (+) of Langerin⁺ skin DCs 0, 24 and 48 h after a topical application of DMBA, $n = 4-19$. Results are shown as fold increase comparing gene expression in DMBA- and acetone-treated skin in the same animal. Data are pooled from multiple independent experiments. (c) Quantitative PCR showing *Pxr* expression in mouse epidermis ($n = 10$) and second-passage keratinocytes ($n = 8$) and in human epidermis ($n = 4$), second-passage human keratinocytes ($n = 4$) and highly purified freshly isolated human Langerhans cells (LCs) ($n = 2$). (d) Representative pictures show PXR immunostaining in mouse immature LCs. Mouse LCs were counterstained with anti-MHC class II-FITC mAb and nuclei with DAPI. Magnification $\times 100$.

Figure 4.

PXR deficiency promotes Langerhans cell (LC) migration after topical application of DMBA. Mice were topically treated with 0.05% DMBA on right ears and with acetone on left ears. (a) Histogram and representative pictures show numbers of LCs in epidermal sheets of *Pxr*^{+/+} versus *Pxr*^{-/-} ($n = 14$) and of *Ahr*^{+/+} versus *Ahr*^{-/-} ($n = 5$) treated with DMBA or acetone. (b) The histogram shows emigrated LCs in the skin draining lymph nodes of *Pxr*^{+/+} versus *Pxr*^{-/-} and of *Ahr*^{+/+} versus *Ahr*^{-/-} treated with DMBA or acetone, $n = 5-9$. (c) Expression of *Ccr7* assessed by quantitative PCR in *Pxr*^{+/+} versus *Pxr*^{-/-} and in

Ahr^{+/+} versus *Ahr*^{-/-} mouse skin treated with DMBA or acetone, *n* = 9–12. PXR, pregnane X receptor.

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