



# Antioxidative Phytochemicals Accelerate Epidermal Terminal Differentiation via the AHR-OVOL1 Pathway: Implications for Atopic Dermatitis

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**Aryl hydrocarbon receptor (AHR) is a chemical sensor that is expressed abundantly in epidermal keratinocytes. Oxidative AHR ligands induce the production of reactive oxygen species. However, antioxidant AHR ligands inhibit reactive oxygen species generation via activation of nuclear factor-erythroid 2-related factor-2, which is a master switch for antioxidative signalling. In addition, AHR signalling accelerates epidermal terminal differentiation, but excessive acceleration by oxidative ligands, such as dioxins, may induce chloracne and inflammation. However, antioxidative phytochemical ligands induce the beneficial acceleration of epidermal differentiation that repairs skin barrier disruption. The upregulated expression of differentiation molecules, such as filaggrin, is mediated via the AHR-OVOL1 axis. This AHR-OVOL1 system is capable of counteracting skin barrier dysfunction in T-helper type 2-shifted inflammation. This article reviews the dynamic and multifaceted role of AHR in epidermal biology and discusses the potential use of antioxidative phytochemical ligands for AHR in inflammatory skin diseases, such as atopic dermatitis.**

**Key words:** antioxidative phytochemicals; filaggrin; aryl hydrocarbon receptor; nuclear factor-erythroid 2-related factor-2; OVOL1; atopic dermatitis.

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Atopic dermatitis (AD) is characterized by chronic itch, cutaneous inflammation and dry skin with epidermal barrier dysfunction (1–3). Since the discovery of T-helper type 1 (TH1) and TH2 immune regulation by Mosmann et al. (4), the TH2-polarized immune response has been thought to be a cardinal driver in allergic diseases including AD (5–9). This notion has been proven because blockade of TH2-derived interleukin (IL)-4 and IL-13 signalling by a specific anti-IL-4 receptor antibody, dupilumab, successfully improves skin inflammation in patients with AD (10–12). In addition, TH2-derived IL-31 is a potent itching-scratching inducer, and the administration of anti-IL-31 receptor antibody, nemolizumab, improves atopic itching in patients with AD (13–16).

## SIGNIFICANCE

Aryl hydrocarbon receptor (AHR) is a chemical sensor activated by external and internal ligands. Oxidative AHR ligands induce the production of reactive oxygen species. However, antioxidative phytochemical AHR ligands inhibit ROS generation via activation of nuclear factor-erythroid 2-related factor-2, which is a master switch for antioxidative signalling. In addition, AHR signalling upregulates filaggrin expression via OVOL1 transcription factor and accelerates epidermal terminal differentiation. This AHR-OVOL1 system is capable of counteracting the skin barrier dysfunction in T helper type 2-shifted inflammation. Therefore, antioxidative phytochemicals targeting AHR are potentially beneficial for barrier-disrupted skin diseases, such as atopic dermatitis.

Genome-wide association studies in different ethnicities have revealed at least 19 susceptible genes, including filaggrin (*FLG*), OVO-like 1 (*OVOL1*) and *IL4/IL13* (17–22). AD exhibits heterogeneous clinical and laboratory manifestations influenced by genetic, environmental and social factors (2, 5, 23–26). However, xerosis or dry skin due to skin barrier disruption is the most frequent clinical sign in AD (23, 25).

Skin barrier maturation is accomplished by sequential and coordinated expression of various terminal differentiation proteins, such as FLG and loricrin (LOR) (27). In accordance, FLG and LOR expression levels have been reported to be reduced in lesioned and non-lesioned skin in AD (28–30). Loss-of-function mutations of *FLG* have been demonstrated in some patients with AD, ranging from 10% to 50% in the Northern European and Asian AD population (31–34). Ichthyosis vulgaris is also known to be caused by the loss-of-function mutation of *FLG* (35). This may explain why AD is significantly comorbid with ichthyosis vulgaris (25, 31). However, *FLG* mutations are not found in all patients with AD, and they are less common in Southern Europeans (36) and are even absent in some African countries (37, 38). A humid atmosphere may reduce the contribution of *FLG* mutations to the onset of AD (39).

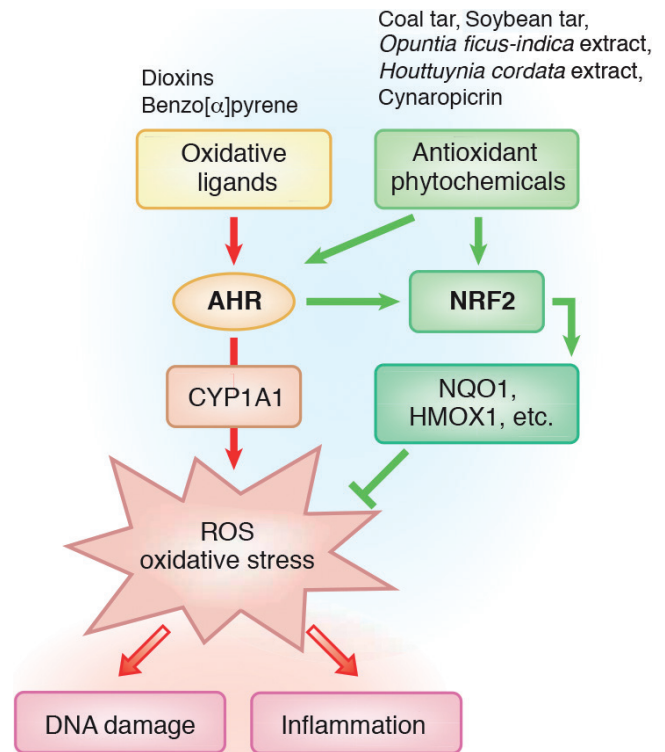
Of note, TH2-derived cytokines, IL-4 and IL-13, inhibit FLG and LOR expression (29, 30, 40–42). IL-31 also downregulates FLG and LOR expression (43).

Therefore, TH2-polarized inflammatory milieu in AD may be more influential in the downregulation of FLG expression compared with genetic mutations. In line with this notion, topical steroids significantly improve clinical inflammatory signs and normalize transepidermal water loss in lesional AD skin with the upregulation of *FLG* and *LOR* expression (44). These improvements are associated with the downregulation of the TH2 (IL-13 and IL-31) signature (44).

Given that the expression levels of FLG and LOR are associated with improvement in AD, strategies to block IL-4-mediated FLG (and LOR) downregulation may be beneficial in treating AD. Although the mechanisms to enhance FLG expression have not been fully understood, recent studies by us and other groups have revealed that aryl hydrocarbon receptor (AHR) signalling plays an essential role in upregulating the expression of FLG and other differentiation-related molecules (29, 30, 45–47). Notably, a plethora of antioxidative phytochemicals work as AHR agonists and restore the IL-4-mediated FLG downregulation (41, 42, 48, 49). These findings have unveiled the underlying mechanisms of how traditionally used antioxidant herbs and phytochemicals work well in maintaining healthy skin and in preventing atopic dry skin. This article focuses on the regulatory mechanisms of AHR/FLG signalling by exogenous antioxidative phytochemicals operating in host epidermal keratinocytes.

## REGULATORY ROLE OF ARYL HYDROCARBON RECEPTOR IN OXIDATIVE STRESS

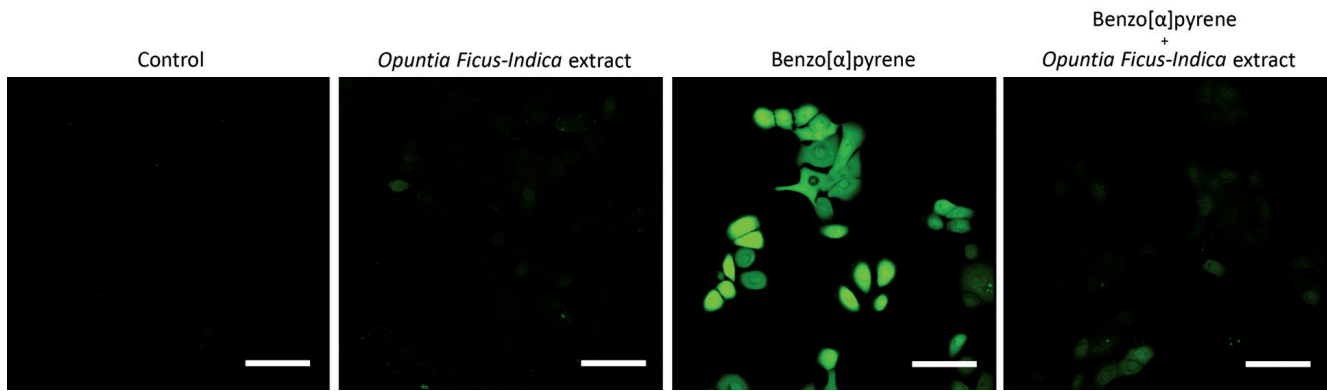
Skin cells, such as keratinocytes, harbour abundant AHR, which exerts multi-functional effects on skin homeostasis and pathology (50, 51). AHR was originally discovered as a cytosolic chemical sensor and transcription factor for halogenated and non-halogenated polycyclic aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[ $\alpha$ ]pyrene (50–52). However, in addition to dioxins and benzo[ $\alpha$ ]pyrene, AHR is a promiscuous receptor, binding with a wide range of affinities to structurally diverse low-molecular-weight chemicals including various phytochemicals (e.g. apigenin, quercetin and cynaropicrin) and tryptophan photoproducts after ultraviolet irradiation (50, 53–56). Most AHR ligands are very hydrophobic; these ligands enter target cells via diffusion and bind to cytosolic AHR. Upon ligand binding, cytosolic AHR undergoes nuclear translocation and binds to its specific DNA recognition site, namely, the xenobiotic-responsive element or dioxin-responsive element, and mediates numerous biological and toxicological effects by inducing the transcription of various AHR-responsive genes, such as cytochrome P4501A1 (*CYP1A1*) (50, 51, 53) (Fig. 1). In addition to its physiological role in the detoxification of polycyclic aromatic compounds, the activity of the *CYP1A1* enzyme can be deleterious, because it generates mutagenic metabolites



**Fig. 1. Oxidative ligands for aryl hydrocarbon receptor (AHR), such as dioxins and benzo[ $\alpha$ ]pyrene, upregulate the expression of their metabolizing enzyme, cytochrome p450 1A1 (*CYP1A1*). The metabolizing process generates reactive oxygen species (ROS). The oxidative stress results in DNA damage and inflammation. Antioxidative phytochemical ligands, such as coal tar, soybean tar, *Opuntia ficus-indica* extract, *Houttuynia cordata* extract and cynaropicrin, bind to AHR and induce *CYP1A1* upregulation. However, they are also good activators of antioxidant master transcription factors, namely, nuclear factor-erythroid 2-related factor-2 (NRF2). The activation of NRF2 enhances the expression of NAD(P)H: quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HMOX1) and inhibits generation of ROS.**

and reactive oxygen species (ROS) in keratinocytes (50–53). Extensive studies on the function of Ahr using *Ahr*-deficient mice have demonstrated that Ahr is responsible for most, if not all, of the toxic effects caused by TCDD (50, 51, 53).

Ligands for AHR are divided into at least 2 groups, oxidative and antioxidative ligands (51). Oxidative ligands, such as TCDD and benzo[ $\alpha$ ]pyrene, induce robust ROS generation in keratinocytes via AHR activation (50, 52). Antioxidative phytochemical ligands, such as coal tar, soybean tar, cynaropicrin, *Opuntia ficus-indica* extract, *Houttuynia cordata* extract, *Bidens pilosa* extract and *Galactomyces* fermentation, filtrate activate AHR and upregulate *CYP1A1* expression, but inhibit ROS generation via the activation of an antioxidant master transcription factor, nuclear factor-erythroid 2-related factor-2 (NRF2) (30, 41, 42, 49, 56–62) (Fig. 1). Oxidative ligands also induce the compensatory activation of NRF2 (63), but the generation of ROS may overwhelm the NRF2-mediated antioxidant activity. Activation of NRF2 induces the transcription of antioxidant enzymes, such as NAD(P)H: quinone oxidoreductase 1 (NQO1)



**Fig. 2.** The production of reactive oxygen species (ROS) is weak in (A) control and (B) *Opuntia ficus-indica* extract-treated keratinocytes. (C) Benzo[α]pyrene induces robust production of ROS. (D) However, benzo[α]pyrene-induced ROS production is cancelled in the simultaneous presence of *Opuntia ficus-indica* extract. Scale bar=50 μm.

and heme oxygenase-1 (HMOX1), which are the key molecules in achieving antioxidant activity in keratinocytes (58, 59, 62, 64, 65). In line with this notion, ultraviolet B radiation-induced ROS production and sunburn reaction was inhibited via the NRF2/HMOX1 pathway (66, 67).

The bidirectional regulation by AHR in oxidative/antioxidant activity is very functional. For instance, benzo[α]pyrene induces AHR-mediated oxidative stress which is efficiently inhibited by antioxidative phytochemicals via AHR-NRF2 activation (41, 49, 56, 57) (**Fig. 2**). The antioxidative phytochemical ligands for AHR are also active in counteracting oxidative stress induced by tumour necrosis factor-α (41, 49, 56, 57).

Persistent overactivation of AHR by TCDD and other dioxin-related compounds induces prolonged oxidative stress and may cause chloracne (51, 68, 69). The generation of ROS is also involved in the pathogenesis of contact dermatitis, AD and psoriasis (70–72). Therefore, antioxidative AHR agonists may be beneficial for the treatment of oxidative inflammatory skin diseases. In this context, recent clinical trials have revealed that a natural antioxidative AHR agonist, tapinarof, improves skin lesions of AD and psoriasis in topical use (73–76).

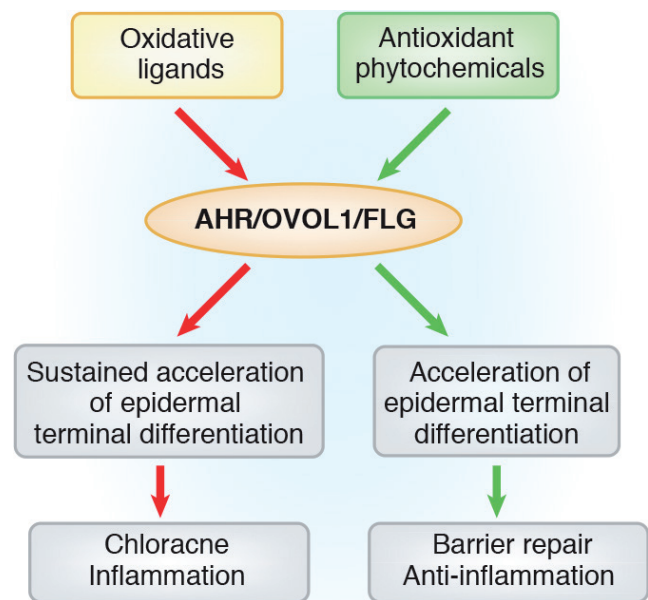
### ARYL HYDROCARBON RECEPTOR SIGNALING ACTIVATES FILAGGRIN AND LORICRIN EXPRESSION IN KERATINOCYTES

Another intriguing aspect of AHR is its promoting capacity of epidermal terminal differentiation by upregulating FLG, LOR and other differentiation-related molecules (30, 41, 42, 49). These results coincide with the findings that nuclear translocation of AHR is observed in parallel with the terminal differentiation of keratinocytes, and that AHR antagonists have impaired terminal differentiation (77). In parallel, both *Ahr*-deficient and *Ahr*-transgenic mice reveal an abnormality in keratinization (78, 79).

Both oxidative and antioxidative AHR ligands induce the coordinated upregulation of FLG, LOR, hornerin and other differentiation-related molecules (41, 42, 47, 49,

77, 80) (**Fig. 3**). Sustained overactivation of AHR by oxidative TCDD induces exaggerated and accelerated terminal differentiation, which may cause chloracne and hyperkeratosis of the epidermis (47, 80). The oxidative AHR ligands, such as TCDD and benzo[α]pyrene, also induce the keratinocytes to produce proinflammatory cytokines (52, 56, 81).

On the other hand, antioxidative AHR ligands reduce the production of proinflammatory cytokines from keratinocytes (30, 56, 77, 82) and restore the impaired epidermal barrier function in association with FLG upregulation (30, 77, 83, 84). In addition, TH2 cytokine-mediated inhibition of FLG and LOR expression is res-



**Fig. 3.** Both oxidative and antioxidative ligands for AHR accelerate epidermal terminal differentiation by upregulating the expression of filaggrin (FLG) and other differentiation molecules via the AHR/OVOL1 signalling pathway. Oxidative AHR ligands are hardly metabolized by CYP1A1 metabolizing enzyme and are retained in the body for a long time. The sustained acceleration of epidermal terminal differentiation may cause chloracne and other dioxin-related hazards. However, the acceleration of epidermal terminal differentiation mediated by antioxidative phytochemicals is beneficial for repairing barrier disruption.

cued via AHR activation by antioxidative phytochemicals (29, 30, 41, 42).

## ARYL HYDROCARBON RECEPTOR ACTIVATION RESCUES TH2-MEDIATED INHIBITION OF OVOL1-FLG AXIS

A previous study revealed that the *FLG* gene contains at least 2 xenobiotic-responsive elements where ligated AHR binds (47). Mutations in these sites abrogate AHR-mediated *FLG* expression (47). In addition to this direct AHR-*FLG* regulation, we have recently demonstrated a crucial involvement of OVOL1 in the AHR-*FLG* pathway (29, 48) (Fig. 3). OVOL1 is a transcription factor profoundly related to epithelial differentiation (29, 85, 86) and is highlighted as one of the susceptible genes in AD (19, 22). Abrasion or overexpression of OVOL1 results in the downregulation or upregulation of *FLG* expression, respectively (29). The activation of AHR by soybean tar Glyteer induces cytoplasmic to nuclear translocation of OVOL1, and this OVOL1 activation results in *FLG* upregulation (29). IL-4 inhibits *FLG* expression by blocking the cytoplasmic to nuclear translocation of OVOL1, and the IL-4-induced blockade of OVOL1 translocation is abrogated by AHR activation (29). Thus, AHR ligation rescues IL-4-mediated inhibition of the OVOL1-*FLG* axis (29). Importantly, the AHR-mediated *LOR* expression is also mediated by OVOL1 (48). These results stress the importance of AHR signalling in upregulating *FLG* expression directly or indirectly via OVOL1. Moreover, AHR signalling is effective in counteracting the IL-4-mediated barrier dysfunction in AD (30).

## CONCLUSION

The topical application of some AHR agonists reduces inflammatory skin reactions and restores skin barrier function in mice and humans (76, 84, 87). The beneficial effects of AHR ligation are related, at least in part, to its upregulation capacity of *FLG*, *LOR* and other differentiation-related genes in epidermal keratinocytes, as well as anti-inflammatory properties. In addition, some AHR agonists, including various phytochemicals, work as antioxidants via AHR-NRF2 activation. Considering that oxidative stress is exceeded in inflammatory skin conditions, antioxidative AHR agonists are particularly promising in drug development for AD in which TH2-inflammation, barrier disruption and oxidative stress are intermingled.

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