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Influence of light on aryl hydrocarbon receptor signaling and consequences in drug metabolism, physiology and disease

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Introduction: A key to understanding the biological function(s) of the aryl hydrocarbon receptor (AHR) – a xenobiotic-activated receptor – is to identify its endogenous ligand(s). The discovery of a tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole (FICZ) as an endogenous, high affinity agonist of AHR filled this knowledge gap in the context of skin physiology and pathology in response to light and opened several new directions for research on AHR.
Area covered: This paper reviews major developments in the study of light-elicited AHR signaling and its impact on drug metabolism, skin physiology and disease with a focus on the identification of AHR ligands from Trp photoproducts and the AHR-mediated UV response. This review consists of material obtained from Medline and PubMed literature searches up to May 2011.
Expert opinion: The recognition of FICZ as a potent, endogenous ligand of AHR provided a molecular link between light exposure and AHR signaling and function. The uncovering of the bifurcated signaling pathway of AHR in the mammalian UV response – that is, activation of the cytoplasmic AHR by light via FICZ leads to: i) AHR/AH response element-dependent transcription to induce CYP1A1 and ii) activation of the AHR-pp60^{src}-EGFR pathway to induce Cox-2 – put forward a working model for the multiple roles of AHR in skin function and disease that include drug metabolism, circadian oscillation, melanogenesis, inflammation, immunosuppression and cancer. Such findings suggest AHR as a therapeutic target for cancer, autoimmune dysfunction, inflammatory disease and stem cell therapy.

Keywords: AH receptor, AHR ligand, CYP1A1 induction, FICZ, tryptophan photoreaction, UV response

Expert Opin. Drug Metab. Toxicol. (2011) 7(10):1267-1293

1. AHR, an XAR with newly identified endogenous ligands

1.1 AHR and the mammalian xenobiotic response

Mammalian species have evolved with elaborate strategies known as the xenobiotic response to defend against a wide variety of chemicals from the environment. Induction of drug-metabolizing enzymes (DMEs) and drug transporters, factors that determine the fate of xenochemicals in the body, is a principle mechanism by which the body detoxifies and disposes chemicals [1]. Induction is generally controlled by a group of ligand-activated transcription factors called xenobiotic-activated receptor (XAR) [2]. XARs coordinate the transcriptional response to specific chemical signals to eliminate the chemicals, antagonize toxicity and repair damaged tissues [2,3].

10.1517/17425255.2011.614947 ISSN 1742-5255

Article highlights.

- Aryl hydrocarbon receptor (AHR) is a xenobiotic-activated receptor that mediates the adaptive and toxic responses to a wide range of environmental chemicals important for drug metabolism, toxicity and carcinogenesis.
- Recent studies with genetic models and newly identified ligands, both synthetic and naturally produced, have renewed the interest in the physiological function, endogenous ligand and therapeutic potential of the receptor.
- The studies on induction of CYP1A1 by light established AHR as the mediator for induction and led to the identification of L-tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole (FICZ) as an endogenous, high-affinity AHR agonist.
- FICZ is produced in skin cells on exposure to UV and visible lights and is rapidly metabolized in the body by CYP1A1 and other drug metabolizing enzymes.
- Activation of AHR by FICZ leads to bifurcated signaling transduction: i) translocation of the cytoplasmic AHR into the nucleus to mediate AHRE-dependent induction of CYP1A1 and ii) activation of the AHR-pp60^{src}-EGFR pathway to activate ERK1/2 and induce Cox-2, as part of the mammalian UV response to resist UV stress.
- Activation of AHR by light impacts a number of skin functions that include drug metabolism, circadian oscillation, melanogenesis, skin inflammation, immunosuppression and carcinogenesis. The findings suggest several therapeutic potentials for AHR ligands.
- Future studies should investigate the molecular interactions between FICZ and AHR, the molecular mechanisms of immunomodulation by AHR, the therapeutic applications of AHR ligands, the safety evaluation of AHR activation and CYP1A1 induction for skin cancer and toxicity with appropriate animal models, and finally the potentially broader functions of FICZ than those mediated through AHR.

This box summarizes key points contained in the article.

The aryl hydrocarbon receptor (AHR) is a basic helix loop helix (bHLH) Per-Arnt-Sim homology (PAS) transcription factor ubiquitously expressed in animal tissues and cells [4,5]. AHR was originally discovered from the observation that polycyclic aromatic hydrocarbons (PAHs) induce their own metabolism [6,7]. PAHs activate AHR to induce cytochrome P450 (CYP, P450) 1A1 that catalyzes the mono-oxygenation of PAHs, the initial step of PAH metabolism. AHR was subsequently found to mediate the induction of a range of DMEs and transporters important for the metabolism and disposition of many drugs and environmental chemicals [5]. Induction increases the rate of metabolism of xenochemicals and subsides as the chemicals are disposed. Therefore, AHR is generally considered to function as an XAR [2].

AHR gained wide recognition in the fields of chemical carcinogenesis, toxicology and environmental health due to its prominent roles in the carcinogenesis and toxicity of benzo[*a*]pyrene (BaP), a prototypical carcinogenic PAH, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), a prototypical halogenated aromatic hydrocarbon (HAH). PAHs are found in tobacco smoke, automobile exhaust and charcoal-broiled meat, whereas HAHs are persistent environmental contaminants. Induction of CYP1A1 via AHR is critical for the metabolic activation of BaP to its ultimate carcinogen, *trans*-7,8-diol 9,10-epoxide, which forms adducts and causes mutations in DNA [7]. AHR mediates most of the adaptive and adverse effects of TCDD in animals and humans, including induction of DMEs, tumor formation, developmental abnormality, endocrine disruption, immunosuppression, wasting and skin lesion [8-10]. Notably, TCDD induces persistent expression of AHR target genes due to its high affinity to AHR and resistance to metabolic breakdown in the body, accounting in part for its profound and lasting toxic effects in animals.

1.2 The canonical pathway and ligands of AHR

Genetic and molecular analyses of induction of CYP1A1 revealed a well defined, ligand-activated transcriptional pathway, known as the AHR/aryl hydrocarbon response element (AHRE) paradigm, for induction of DMEs by AHR [5,11-12]. Unliganded AHR is localized in the cytoplasm in a complex with chaperone proteins including hsp90, the AHR-interacting protein (AIP) and p23 [13-15]. Ligand binding triggers the dissociation of the complex and nuclear translocation of AHR. In the nucleus, AHR dimerizes with another bHLH-PAS protein called AH receptor nuclear translocator (Arnt) [16]. The AHR/Arnt dimer binds to specific DNA sequences termed AHRE in the enhancer regions of AHR target genes, followed by transcription of the genes, to mediate the biological effects [17]. Activated AHR in the nucleus is degraded through the ubiquitin-26S proteasome-mediated proteolysis to terminate the induction [18,19].

Well-established ligands of AHR are mostly environmental chemicals that, in addition to TCDD and BaP, include polychlorinated dibenzofurans, biphenyls and naphthalenes; 3-methylcholanthrene (3-MC); and β-naphthoflavone (βNF) (**Figure 1**) [8,20-21]. In these examples, metabolic resistance and coplanarity appear to influence AHR–ligand interaction. Metabolically stable HAHs are often more potent AHR ligands (binding affinities in the picomolar to nanomolar range) than labile PAHs and other ligands (binding affinities in the nanomolar to micromolar range), raising the possibility that, in addition to persistence, the structural determinants of metabolic stability of the ligands affect their binding affinity and efficacy for AHR.

1.3 Light and endogenous ligands of AHR

Studies of genetic models with altered AHR activities have helped uncover multiple roles of AHR in mammalian physiology, development and disease in recent years [22-28]. Moreover, new ligands with structures and properties different from those of PAHs and HAHs were identified (**Figure 1**). Although some ligands were discovered from screening chemical libraries

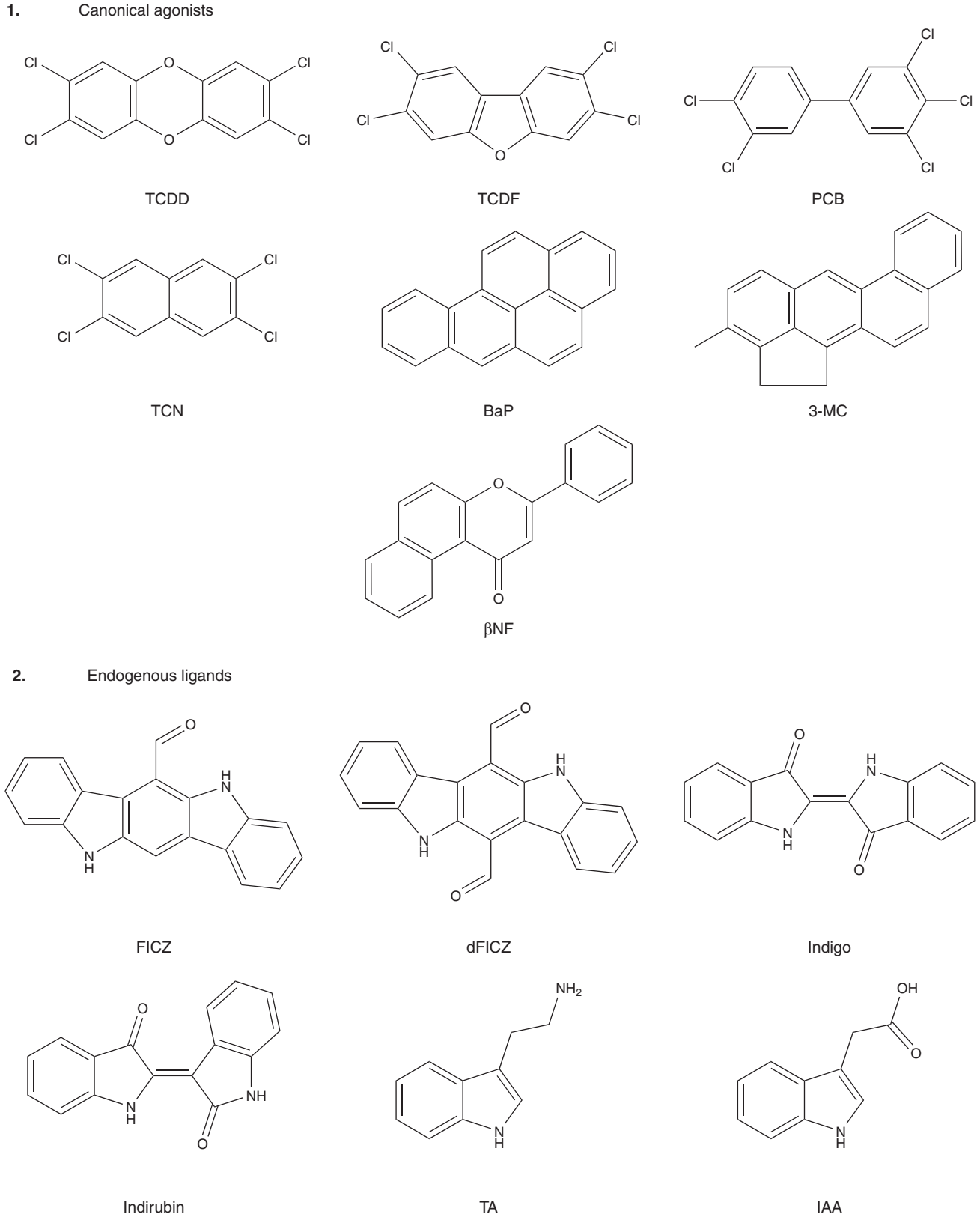


Figure 1. Canonical and newly identified ligands and modulators of AHR (continued).

[28-34], many were found from biological samples [20,21,35-47]. These findings provide new insights into the long-suspected physiological functions of AHR that are likely to be mediated through endogenous ligands.

Putative endogenous ligands of AHR include tryptophan (Trp) derivatives, arachidonic acids, steroids and heme metabolites (**Figure 1**). L-Trp is an essential amino acid in humans with physiological levels ranging from 70 to 150 μM [45]. Because of its aromaticity and indole structure, Trp is metabolized to a number of biologically active metabolites in the body, such as serotonin, melatonin and niacin; some metabolites are ligands for AHR, including kynurenine (KN), kynurenic acid, tryptamine, indole-3-acetic acid, indole-3-pyruvic acid (IPA), 1-(1*H*-indol-3-yl)-3-(3*H*-indol-3-ylidene) propan-2-one (IIPO) and 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) (**Table 1**) [37-41,45,48-51]. IPA and IIPO can also be formed from D-Trp [49,52]. Plant-derived indole-3-carbinol can condense to form AHR agonists indolo[3,2-*b*]carbazole and 3,3′-diindolylmethane (DIM) in the stomach [36], whereas agonists indigo and indirubin are produced in both plants and the human body [35]. *Malassezia furfur*, residential yeast on the human skin, converts Trp to indole metabolites including AHR agonist malassezin [53].

Among the newly identified endogenous ligands, 6-formylindolo[3,2-*b*]carbazole (FICZ) and 6,12-diformylindolo [3,2-*b*]carbazole (dFICZ) are formed from L-Trp in mammalian cells exposed to UV light [42-44]. FICZ and dFICZ bind to AHR with an affinity in the picomolar range and mediate DRE-driven induction of CYP1 genes by UV and visible lights [44]. Furthermore, analyses of light–AHR interaction through FICZ revealed a novel mechanism by which the AHR pathway interacts with the physiological response to UV radiation, that is, the mammalian UV response, to impact mammalian physiology and disease [54]. This review summarizes major developments in the study of activation of AHR by light with a focus on the identification of FICZ and the impact of light on AHR signal transduction and function in drug metabolism, mammalian physiology and disease.

2. Sunlight and the mammalian UV response

2.1 Health effects of sunlight exposure

The sunlight that reaches the surface of the Earth consists of UV (mainly UVB of wavelength 280 – 315 nm and UVA 315 – 400 nm), visible (380 – 780 nm) and infrared (700 – 10^6 nm) radiations. In addition to providing energy to fuel most of the life on Earth, sunlight is utilized for vision, circadian oscillation, vitamin D synthesis and body temperature control in mammalian species. In humans, vision is accomplished through photoreceptors in the retina that convert photons of visible light to chemical reactions and electro signals that propagate to neurons in the brain to produce vision. Moreover, interaction of the signals with circadian rhythm molecules, such as the PAS-containing circadian transcription factors, in the hypothalamic suprachiasmatic nuclei (SCN) produces the principal circadian oscillation to coordinate the daily cycles of physiology and behavior [55,56].

The skin is the largest organ and the site of most exposure to sunlight in human body. It plays a pivotal role in the physiology and disease pathogenesis related to sunlight exposure. The involvement of light in the synthesis of vitamin D_3 from 7-dehydrocholesterol in the skin was recognized a century ago when rickets, a childhood disease of vitamin D deficiency, was found ameliorated by sunlight [57]. Skin tanning, which is associated with beauty, young complexion and health in today's social norm, is a consequence of adaptive color response to light. UVA stimulates the release of melanin from melanocytes and the rapid darkening of the pigment through oxidation, whereas UVB induces the synthesis of new melanin from tyrosine in the skin, a process termed melanogenesis [58]. Melanin effectively absorbs UV light and dissipates the energy as heat to protect the skin from damage from sunlight. The skin is also the first line of protection against chemical insults from the environment. Fittingly, it is endowed with a plethora of defensive mechanisms that include scavenger molecules, detoxifying enzymes and regulatory proteins, some of which are induced on exposure to

Figure 1. Canonical and newly identified ligands and modulators of AHR. (Continued). A wide range of chemicals with diverse structures, both man-made and naturally occurring, can activate or inhibit AHR by directly binding to AHR (agonists, partial agonists and antagonists). The binding affinities of the ligands for AHR vary in the range from picomolar to micromolar. Some clearly modulate AHR activities, but their binding to AHR is weak or has not been firmly established, such as in the case of resveratrol, oltipraz and primaquine; these are only qualified as AHR modulators. Full names and references are as follows: TCDD, 2,3,7,8-tetrachorodibenzo-*p*-dioxin; TCDF, 2,3,7,8-tetrachlorodibenzofuran; PCB, 3,3′,4,4′,5-pentachlorobiphenyl; TCN, 2,3,6,7-tetrachloronaphthalene; BaP, benzo[*a*]pyrene; 3-MC, 3-methylcholanthrene; and βNF, β-naphthoflavone [8,20-21]. FICZ, 6-formylindolo[3,2-*b*]carbazole and dFICZ, 6,12-diformylindolo[3,2-*b*]carbazole [43]. Indigo and indirubin [35]. TA, tryptamine and IAA, indoleacetic acid [45]. KN, kynurenine and KA, kynurenic acid [38-40,51]. ITE, 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester [37]. PGG2, prostaglandin G2 and lipoxin A4 [46]. Equilenin [173]. Bilirubin [174]. I3C, indole-3-carbinol and ICZ, indolo[3,2-*b*]carbazole [36]. DIM, 3,3′-diindolylmethane [172]. Quercetin [175]. Resveratrol [47]. Omeprazole [176]. Oltipraz [177]. Primaquine [178]. VAF347 [33]. M50354 [34]. αNF, α-naphthoflavone [179]. CH-223191, 2-methyl-2*H*-pyrazole-3-carboxylic acid (2-methyl-4-*o*-tolylazo-phenyl)-amide [29]; TME, 6,2′,4′-trimethoxyflavone [30]; SGA360, 1-allyl-3-(3,4-dimethoxyphenyl)-7-(trifluoromethyl)-1*H*-indazole [31] and GNF351, N-[2-(3*H*-indol-3-yl)ethyl]-9-isopropyl-2-(5-methyl-3-pyridyl)purin-6-amine [32]. SR1, StemRegenin 1, 4-(2-(2-(benzo[*b*]thiophen-3-yl)-9-isopropyl-9H-purin-6-ylamino)ethyl)phenol [28].

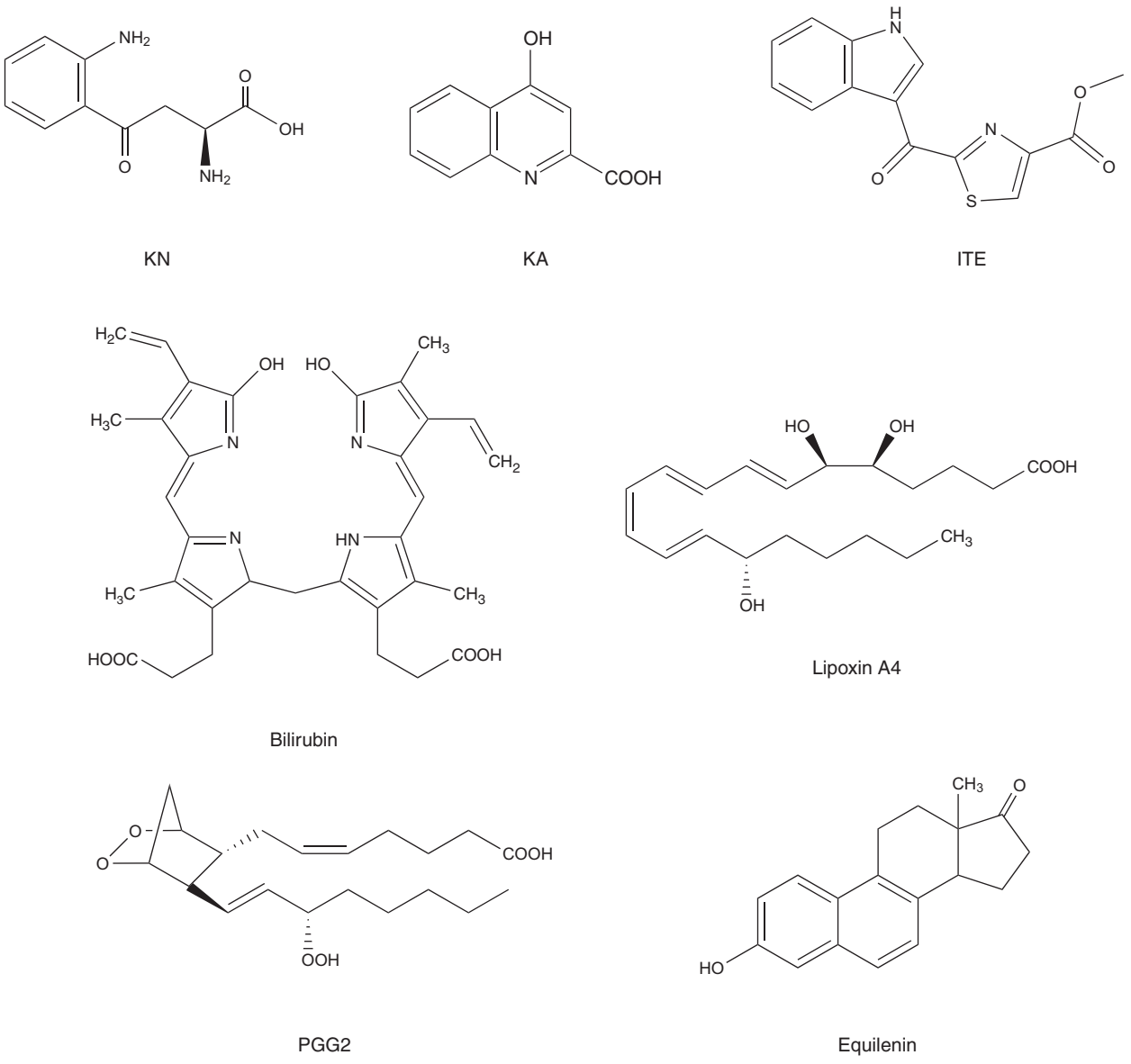


Figure 1. Canonical and newly identified ligands and modulators of AHR (continued).

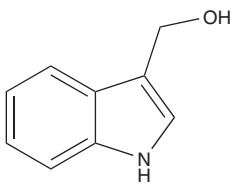
sunlight to boost protection in the skin and extracutaneous organs [59-62].

Overexposure to UV light, either from sunbath or sunlamps, can damage exposed tissues resulting in sunburn, skin aging, and malignancy in the skin or increased lens turbidity in the eyes [63-66]. UV radiation is the principal etiological factor responsible for the majority of skin cancer, now the most common type of cancer in the US with > 1 million new cases each year [67]. Moreover, the incidence of skin cancer is expected to rise because of depletion of stratospheric ozone, increased exposure to solar radiation and longer life expectancy. Sunlight exposure also causes systemic health effects. Insufficiency in sunlight exposure results in seasonal affective disorder in addition to rickets, whereas overexposure causes immunosuppression [68]. In the former cases, hormonal and neuronal signals induced by sunlight are clearly responsible for the systemic effects, whereas molecules including cytokines, phospholipids and epidermal photoproducts released locally from exposed skin interfere with immune functions to bring about systemic immunosuppression in the latter.

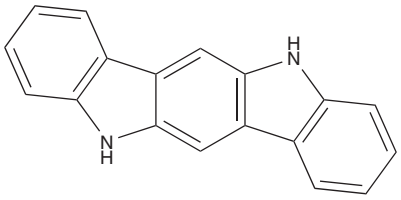
2.2 The mammalian UV response

Both the beneficial and adverse effects of sunlight exposure result from cellular reactions triggered by absorption of photons. Many of the reactions can be induced by UV light and, therefore, are collectively referred to as the mammalian UV response [69]. Some reactions are adaptive in nature, such as the induction of melanin, DNA repair, cell-cycle

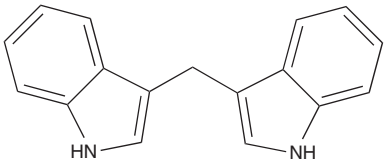
3. Ligands from edible plants



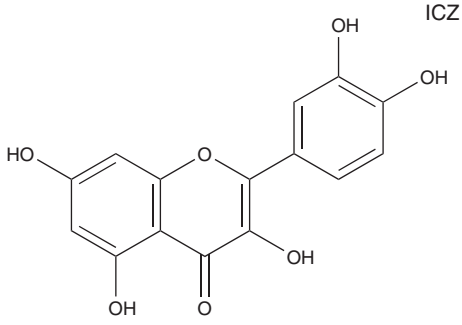
I3C



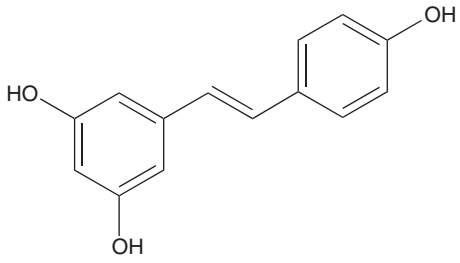
ICZ



DIM

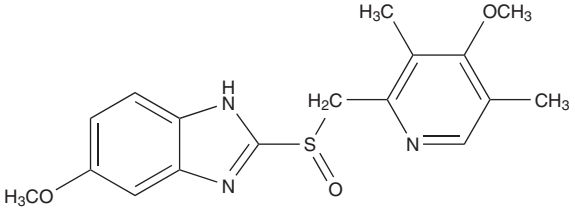


Quercetin

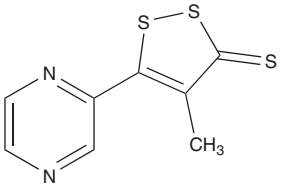


Resveratrol

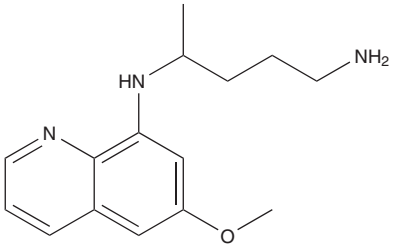
4. Therapeutic agents



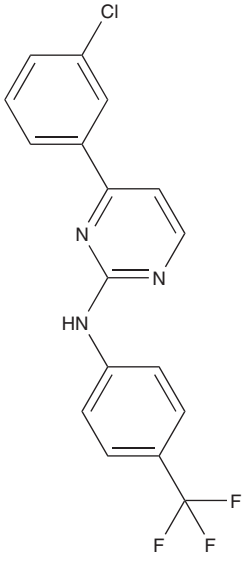
Omeprazole



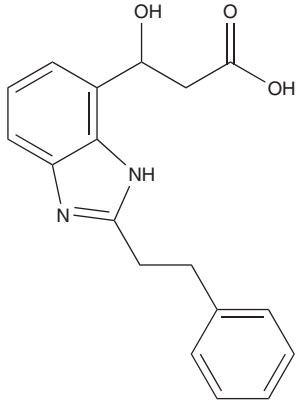
Oltipraz



Primaquine



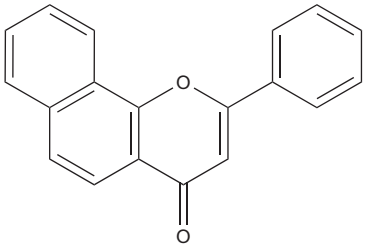
VAF347



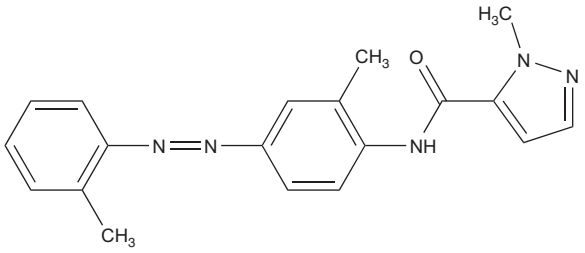
M50354

Figure 1. Canonical and newly identified ligands and modulators of AHR (continued).

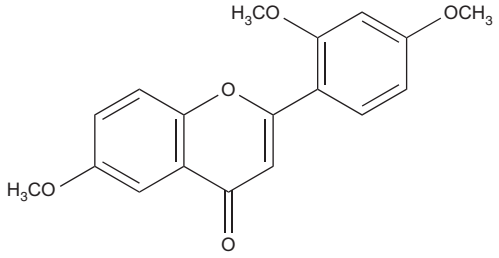
5. Antagonists



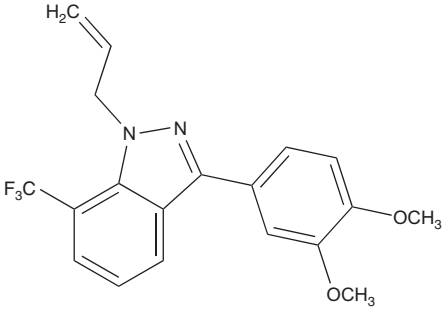
αNF



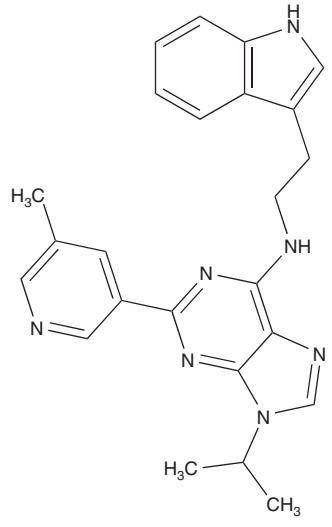
CH-223191



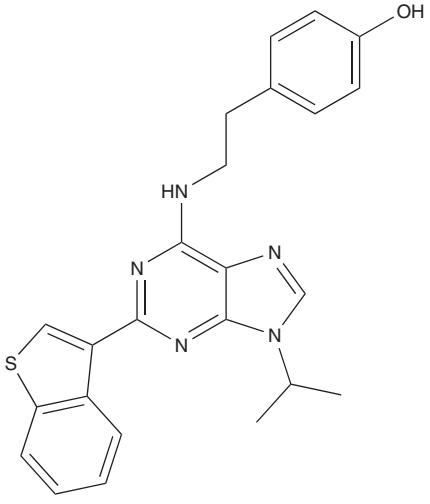
TME



SGA360



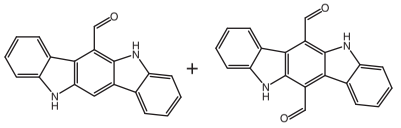
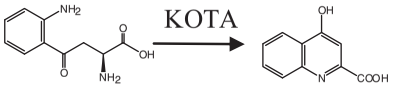
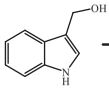
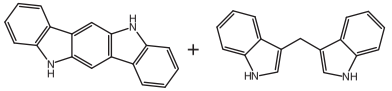
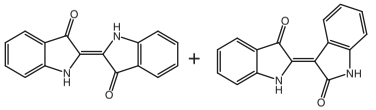
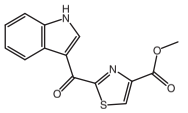
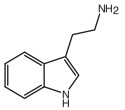
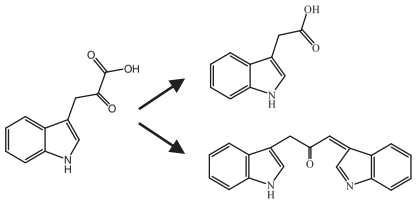
GNF351



SR1

Figure 1. Canonical and newly identified ligands and modulators of AHR (continued).

Table 1. AHR ligands derived from Trp.

Ligand	Source & Pathway	Note & Reference
FICZ dFICZ	L-Trp — UV light → 	Photoproducts; K_d: 0.07 & 0.44 nM [42,43]
KN KA	L-Trp — IDO → KF →  KOTA →	KN: ↑ toleragenic DC + T_{reg} [39,40]; KA: ↑ CYP1A1 & IL-6 [38,51]
I3C ICZ DIM	L-Trp — Plant →  Acidic → 	I3C from Cruciferous plants converts to ICZ & DIM in stomach; anti-cancer [36,174]
Indigo Indirubin	L-Trp — Plant & Human → 	Detected in human urine; EC_{50} in the range of nM to μM [35]
ITE	L-Trp — + L-Cys → Condensation & methylation → 	Isolated from porcine lung [37]; ↓ EAE by ↑ $FoxP3^+$ T_{reg}& toleragenic DC [141]
Tryptamine	L-Trp — GI microbial TDC → 	EC_{50}: 200 μM [45]
IPA IAA IIPO	L-Trp — AST & GI microbe → D-Trp — DAAO → 	IPA forms from both L-& D-Trp and converts to IAA and IIPO [45,48,49,51]
Malassezin ICZ	L-Trp — Skin yeast → 	Produced by skin yeast & cyclizes to ICZ; EC_{50}: 1.57 μM [53]

AST: Aspartate aminotransferase; Cys: Cysteine; DAAO: D-amino acid oxidase; GI: Gastrointestinal; IDO: Indoleamine 2,3-dioxygenase; KF: Kynurenine formamidase; KOTA: Kynurenine-oxoglutarate transaminase; Trp: Tryptophan.

arrest, apoptosis and inflammation, to protect the organism from detrimental outcomes. Other responses result in increased cell proliferation, tumor promotion and inhibition of immune function that account for the carcinogenic and immunosuppressive effects of UV radiation.

At a molecular level, UV light reaches into the energy range of chemical bonds (~ 6 eV for C-C bond). Energy absorbed is thus sufficient to excite chemical bonds and cause changes in molecules. Bases in DNA and RNA as well as aromatic amino acids absorb UV photons efficiently producing, for instance, cyclobutane pyrimidine dimers (CPDs) and Trp photoproducts. Absorption of UV light by molecular oxygen generates reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and lipid peroxide radicals [70,71]. ROS produced in close proximity to DNA, RNA, proteins and membrane lipids directly damage the macromolecules [72]. Superoxide anion radical is converted by superoxide dismutase to hydrogen peroxide (H_2O_2) that has a longer half-life than most ROS and, thus, is freely diffused in cells. In fact, some UV responses can be induced by treating cells with H_2O_2. Production of ROS and other radicals from UV light can be modulated by photosensitizers, such as flavins, abundantly present in the body and edible plants. In aggregate, UV radiation affects molecules of cells via direct absorption of photons or through the generation of ROS and other radicals.

2.3 Reception and signal transduction of UV response

Many responses to UV light occur similarly in different types of cells. Furthermore, UV responses partially resemble cellular responses elicited by exposure to H_2O_2, ionizing irradiation and alkylating agents. Therefore, the mammalian UV response probably represents a common program in cells for the response to UV radiation; it may also serve physiological purposes even in the absence of UV light. Indeed, exposure to UV light activates nuclear, ribosomal, plasma membrane and cytoplasmic (discussed in Section 3) signaling pathways that overlap with other cellular functions for signal recognition, transduction and effectors (**Figure 2**). UV response results in repair and survival of damaged cells, or causes cell death, inflammation and tumor formation depending on the dose of UV radiation, types of the response and the tissues and cells exposed.

2.3.1 DNA damage response

Absorption of UVB by chromosomal DNA leads to the formation of DNA photoproducts including CPDs, single strand DNA (ssDNA) and double strand breaks [73]. UV-induced ROS and lipid peroxides can also cause DNA damage. CPDs are probably an important type of DNA damage by UVB because expression of CPD photolyase reduces UVB-induced erythema, epidermal hyperplasia and apoptosis in mice [74]. However, recognition of UV-induced DNA damage in UV response is probably mediated through ssDNA. ssDNA can result from UV-induced replication and transcription arrest due to CPD formation, nucleotide and base excision repair, or DNA backbone break [73,75,76]. ssDNA formed from UV radiation is immediately covered with the ssDNA binding protein replication protein A (RPA). RPA recruits the ATR (ataxia-teleangiectasia and *R*ad3 related) and ATR-interacting protein complex [77]. ssDNA is also recognized by the MRN complex of *M*re11 (mutated in Ataxia-teleangiectasia-like disorder), *R*ad50 and *N*bs1 (mutated in human Nijmegen breakage syndrome). MRN recruits ATM (ataxia teleangiectasia-mutated), ATR and DNA-dependent protein kinase [78,79]. ATR and ATM activate checkpoint kinases Chk1 and Chk2 and downstream effecter molecules p53, JNK and histone variant H2AX. DNA damage response results in DNA repair, cell-cycle arrest or apoptosis to allow organisms to survive in the presence of DNA damage.

2.3.2 PTP oxidation and RTK signaling

Nuclear-independent signaling by UV light was first demonstrated in enucleated cells for JNK activation [80]. Protein tyrosine phosphatases (PTPs) are likely to be a primary, non-nuclear target of UVB. PTPs reduce the activities of receptor tyrosine kinases (RTKs) associated with the plasma membrane, such as EGFR, as well as other phosphotyrosine-dependent kinases, such as the stress-activated protein kinases JNK and p38, by de-phosphorylating the proteins [81]. PTPs contain a critical cysteine residue in their catalytic pockets. The cysteine is stabilized by surrounding positively charged residues (His-Cys-X-X-Gly-X-X-Arg-Ser/Thr) as a thiolate anion, but is sensitive to oxidation. On oxidation by UVB, the cysteine thiol is converted to sulfenic acid, cyclic sulfenylamide, disulfide, or irreversible products of sulfinic and sulfonic acid derivatives resulting in PTP inactivation [81-83]. Inactivation of PTP boosts the activities of RTKs and downstream signal transduction to regulate transcription factors AP-1, p53 and NF-κB. H_2O_2 and other ROS induced by UV light may mediate the oxidation of PTP cysteine thiol.

2.3.3 Ribotoxic stress

UVB or UVC can damage the 28S rRNA of ribosomes. When the affected ribosomes are engaged in translational elongation, the photoproducts of 28S rRNA activate a specific signal transduction pathway known as the ribotoxic stress response [84]. Ribotoxic stress is probably a result of direct UV damage to ribosomal RNA, as ROS does not seem to be critical. Ribotoxic stress response involves JNK and p38 as well as upstream kinase MKK4 and downstream effectors p53, AP-1 and NF-κB. The mechanism by which JNK and p38 are activated in ribotoxic stress is unclear at the present. Whether translation arrest induced by UV light itself can initiate the signal transduction in the UV response is uncertain.

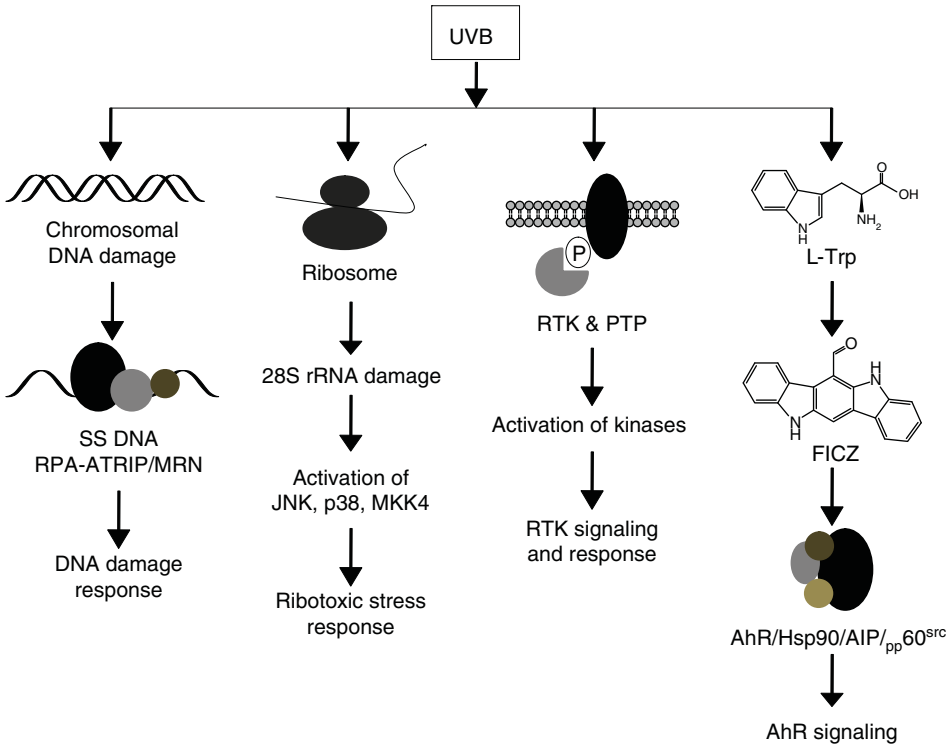


Figure 2. Reception and signal transduction of mammalian UV response. UV radiation stimulates or damages primary targets – chromosomal DNA, ribosomal RNA, PTP associated with RTK and soluble Trp – to initiate nuclear, ribosomal, plasma membrane and cytoplasmic signaling cascades of the mammalian UV response. The response promotes cell survival, or leads to apoptosis, inflammation and tumor formation, depending on the UV dose, types of UV damage, and tissues and cells exposed.

PTP: Protein tyrosine phosphatase; RTK: Receptor tyrosine kinase; Trp: Tryptophan.

3. Influence of light on AHR signaling and drug metabolism: Identification of Trp photoproducts as AHR ligands

3.1 Induction of DMEs by light

The skin expresses P450s and other DMEs, many of which are inducible [59,62,85]. Induction of CYP1 enzymes in the skin received attention as it may increase the metabolic activation of PAHs and synergize with UVB to induce cancer in the skin, such as in the Goeckerman regimen for the treatment of psoriasis [59,60]. Induction of the enzymes by light occurred in neonatal rats in which UVB radiation increased CYP1A enzyme activities in the skin measured as aryl hydrocarbon hydroxylase (AHH, 194%) and 7-ethoxyresorufin O-deethylase (EROD, 244%) [60]. Moreover, UVB synergized with crude coal tar to increase the induction (AHH 858% and EROD 1166%) that may account for increased tumorigenecity of the co-treatment compared with either UVB or crude coal tar alone [60]. Induction was subsequently demonstrated in the human skin in which exposure to UVB at four minimal erythema doses induced the mRNA and protein of both CYP1A1 and 1B1 in the epidermis [61].

Local exposure of skin to UV light affects DME expression in remote organs. Long-term UVB irradiation of skin increased hepatic CYP1A1 activity in both mice and rats [86,87]. In addition, the expression of CYP1A1 in rat liver and pituitary gland exhibited diurnal variation indicating a physiological circadian influence on CYP1A1 expression in internal organs by the daily light/dark cycle [88,89]. In humans, the peripheral blood lymphocytes showed a seasonal variation of AHH activity with maximal induced activities occurring during the late summer and early fall and minimal activity 6 months later [90]. Seasonal variation was also observed for CYP1B1 mRNA expression in human PBMCs [91]. Therefore, sunlight influences DME expression *in vivo* both locally and systemically under physiological conditions.

Studies on cultured cells provided mechanistic insights into DME induction by light. Light induction of CYP1A1 is observed in a variety of cultured cells including human HaCaT keratinocytes, human blood lymphocytes, and mouse and rat liver cells suggesting a common mechanism involving a direct effect of light for the induction [92,93]. Induction is mediated through AHR because induction occurs in wild type but not AHR-deficient mouse hepatoma or AHR knockdown HaCaT

cells [54,93]. CYP1B1 is induced by UV light in human primary keratinocytes and other cells; the induction may involve a UV-response element-like DNA enhancer in the CYP1B1 promoter in addition to AHRE [94,95]. Additionally, mild illumination of culture medium containing riboflavin induced AHH and the medium retained its inducing capacity after storage in the dark for 24 h suggesting the formation of a stable inducer (s) [92,96]. Taken together, both *in vivo* and *in vitro* studies suggest a molecular model of induction in which light – mainly UVB – induces the formation of an inducing agent(s) in the skin that circulates in the body, binds to AHR, and induces AHR-controlled DMEs in the skin and extracutaneous tissues. A key to elucidating the molecular mechanism of regulation of DMEs by light is, therefore, to identify the light-generated inducer that functions as an AHR ligand.

3.2 Trp photoreaction and AHR ligands

In addition to nuclear DNA and ribosomal RNA, several amino acids including cysteine, tyrosine and Trp absorb UV light to produce stable photoproducts. Among the amino acids, Trp is the strongest near-UV absorbing chromophore. A breakthrough in the search for the UV-generated CYP1A1 inducer was made when two Trp photoproducts from an aqueous L-Trp solution irradiated with UVB were found capable of efficiently competing with TCDD for binding in rat liver cytosolic preparations [42]. Consistent with the finding, induction of CYP1A1 mRNA by light was increased by adding additional Trp before UV irradiation, and the increased induction was AHR-dependent [93]. Conversely, induction was compromised in Trp-starved cells and was restored by adding back Trp [54]. Finally, the Trp photoproducts were shown to activate AHR to bind AHRE and induce AHRE-dependent transcription [97]. These lines of evidence support that Trp photoproducts are AHR ligands that mediate CYP1A1 induction by UV light.

By using mass spectrometry, ^{1}H NMR and ^{13}C NMR, the two Trp-derived AHR ligands were structurally resolved to be FICZ and dFICZ (**Figure 3**) [43]. FICZ and dFICZ bind to AHR with higher affinities than TCDD; the K_d values of FICZ and dFICZ were 0.07 and 0.44 nM, respectively, lower than that of TCDD (0.48 nM) [42]. UVB is the most efficient in generating FICZ from aqueous Trp, whereas visible light and UVA induce FICZ formation with lower yields. The efficiencies were increased to 40- to 400-fold higher in the presence of photosensitizer riboflavin, especially for visible and UVA lights [44]. FICZ was also detected in the cell culture medium exposed to normal laboratory light, suggesting that FICZ is responsible for the background CYP1A1 expression in cells cultured with light-exposed media [98]. Direct evidence of UV-induced formation of FICZ in cells came from a study in which HaCaT cells were Trp-starved prior to incubation with [$^{13}C_{11}{}^{15}N_2$]Trp and UVB irradiation. Under these conditions, ~ 80 pM of labeled FICZ was detected from extracts of the cells by HPLC-MS-MS [54].

Although FICZ and dFICZ bind AhR tightly, the inducers only cause transient induction of CYP1A1 due to their rapid metabolism in cells [99-102]. Systematic analyses of FICZ metabolism with rat and human liver preparations, recombinant human enzymes, and specific inhibitors revealed that FICZ is an exceptionally good substrate of CYP1A1, 1A2 and 1B1 giving rise to a range of hydroxylated metabolites that are remarkably good substrates for sulfotranferases 1A1, 1A2, 1B1 and 1E1 (**Figure 4**) [44,101-102]. Importantly, stable sulfoconjugates of phenolic metabolites of FICZ, such as 8-SO_4-FICZ, were found in human urine samples, indicating FICZ is an endogenous inducer of CYP1A enzymes and an endogenous substrate for the DMEs in humans [44].

3.3 Induction of CYP1A1 by FICZ: a molecular model of cytoplasmic UV response

In addition to FICZ, a number of endogenously derived compounds have been shown to bind AHR and induce CYP1A1, including metabolites of indoles, Trp, heme, steroids and arachidonic acids (**Figure 1**, **Table 1**). Several lines of evidence support FICZ as the physiological ligand of AHR for induction of DMEs by light. First, light-induced expression of CYP1A1 is mediated through AHR/AHRE signaling *in vitro* and in intact animals [54,97]. Second, FICZ is formed efficiently by photolysis of Trp on exposure to UV and visible lights [42,44,54,98]. Third, stable metabolites of FICZ are present in human urine samples [44]. Fourth, FICZ exhibits greater affinity for AHR than most of the other ligands of AHR found endogenously [42,46]. Finally, FICZ activates AHR/AHRE signaling and induces AHR target genes efficiently but transiently due to its rapid metabolism by CYP1 enzymes and other DMEs that are induced [44]. In this manner, induction of CYP1A1 by light through FICZ and AHR follows an auto-feedback regulation characteristic of many physiological signaling pathways. Therefore, generation of FICZ from Trp through photoreaction in the cytoplasm and the subsequent activation of AHR and induction of CYP1A1 represent a physiological response to UV light that regulates the chemical homeostasis in the skin and other organs through induction of DMEs. From this prospect, the light-activated FICZ-AHR signaling joins the DNA damage response, the PTP/RTK signaling and the ribotoxic response as part of the mammalian UV response – that is, the cytoplasmic UV response – to resist UV stress (**Figures 2** and **5**).

4. Influence of light on AHR function in physiology and disease

4.1 The AHR-pp60^{src}-EGFR pathway: extending the cytoplasmic UV response to cell membrane

UVB induces the internalization and activation of EGFR [103,104], a critical step in the multiple signaling pathways elicited by UVB. These pathways involve MAPKs (ERK, p38 and JNK) and their downstream AP-1 transcription

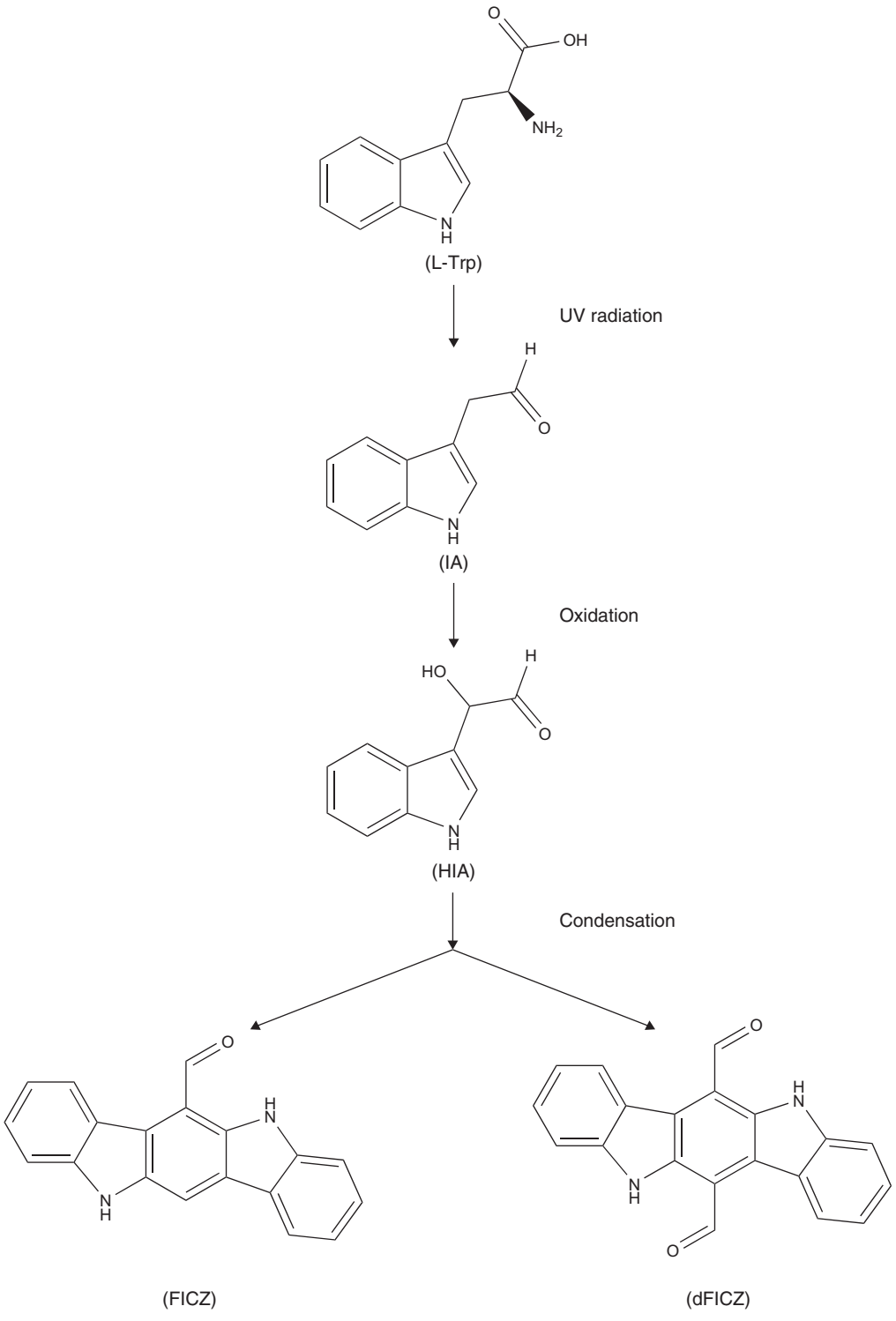


Figure 3. Photoproduction of FICZ and dFICZ from Trp. The proposed reactions of photoproduction of AhR ligands FICZ and dFICZ from L-Trp were based on information from [43].

dFICZ: 6,12-Diformylindolo[3,2-*b*]carbazole; FICZ: 6-Formylindolo[3,2-*b*]carbazole; HIA: 2-hydroxy-2-(1*H*-indol-3-yl)acetaldehyde; IA: 2-(1*H*-indol-3-yl)acetaldehyde; Trp: Tryptophan.

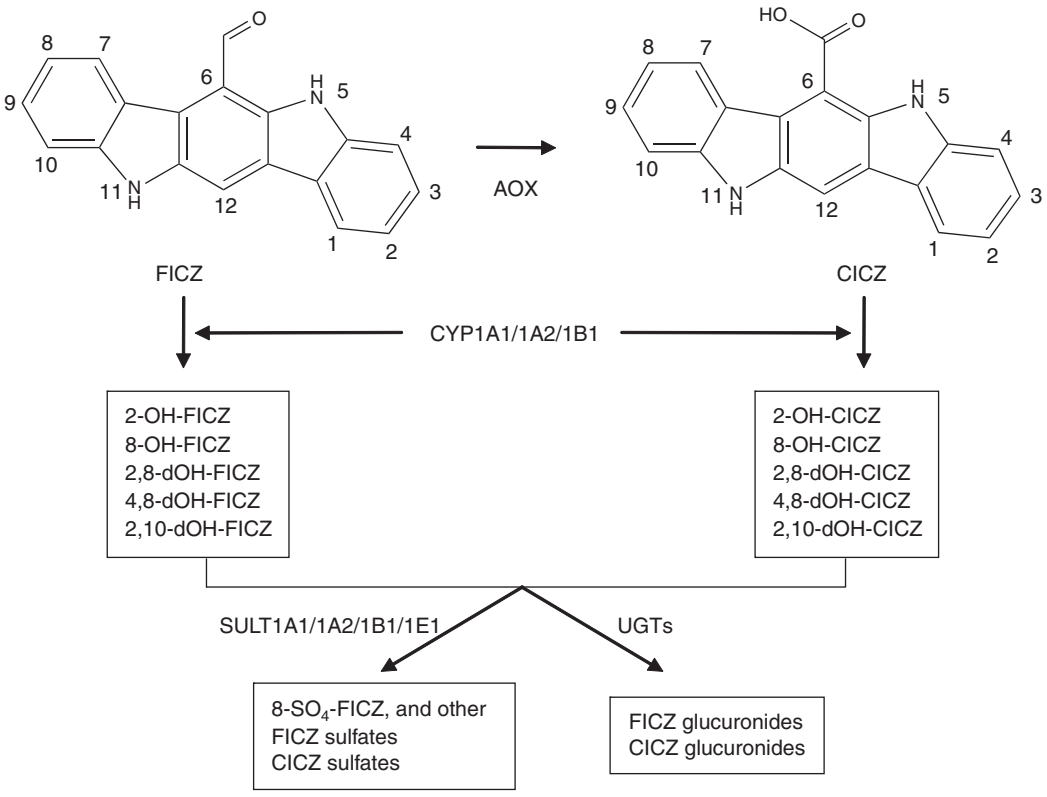


Figure 4. Metabolism of FICZ. FICZ is rapidly hydroxylated by CYPs 1A1, 1A2 and 1B1 at multiple positions. Hydroxylated metabolites are converted by SULTs and UGTs to sulfate or glucuronide conjugates, respectively.
The figure was based on information from [44,101,102].
AOX: Aldehyde oxidase; CICZ: Indolo[3,2-*b*]carbazole-6-carboxylic acid; FICZ: 6-Formylindolo[3,2-*b*]carbazole; SULT: Sulfotransferase; UGT: UDP-glucuronosyltransferase.

factors, AKT, PKC and PKA, many of which are implicated in UVB-induced skin inflammation and photocarcinogenesis [103]. On the other hand, FICZ stimulates the internalization of EGFR leading to phosphorylation of ERK1/2 and induction of downstream Cox-2 [54]. Activation of EGFR by FICZ is AHR-dependent because knockdown of AHR using AHR-specific shRNA inhibited the internalization and activation of EGFR; moreover, induction of Cox-2 mRNA by UVB in mouse skin was blunted in AHR-deficient mice similarly to the induction of CYP1A1. Therefore, FICZ mediates the internalization and activation of EGFR by light through an AHR-dependent pathway.

A rational question raised from the observation was how does the activation of AHR by FICZ in the cytoplasm influence EGFR in the plasma membrane? It is known that the proto-oncogenic tyrosine kinase c-src ($_{pp}60^{src}$) is associated with the activation of EGFR by UVB [105]. $_{pp}60^{src}$ Also interacts with unliganded AHR in the cytoplasm, and activation of AHR by agonists, such as TCDD, dissociates $_{pp}60^{src}$ from AHR leading to activation of the kinase [106]. Furthermore, inhibition of $_{pp}60^{src}$ with c-src inhibitor PP2 in HaCaT cells exposed to UVB or FICZ mimics the effects of AHR silencing, blocking UVB or FICZ-induced EGFR internalization and subsequent ERK phosphorylation and Cox-2 induction, suggesting that signaling from AHR to EGFR for ERK activation and Cox-2 induction in the response to light is mediated through c-src ($_{pp}60^{src}$) [36].

Together, these findings unraveled a novel AHR-$_{pp}60^{src}$-EGFR signaling pathway, through which the cytoplasmic UV response elicited by FICZ via AHR is extended to the plasma membrane-associated signal transduction (**Figure 5**) [54,107]. As discussed below, this bifurcated signal transduction of AHR in the response to light has significant impact on skin physiology, immune regulation, cancer, toxicity and drug therapy, in addition to drug metabolism. From a mechanistic point of view, it is conceivable that, even though not all AHR-mediated skin functions are mediated through this bifurcated signaling pathway, many of them may involve direct transcription and/or signal transduction analogous to CYP1A1 induction and AHR-$_{pp}60^{src}$-EGFR signaling.

4.2 Circadian oscillation

The sunlight influences mammalian circadian oscillation observed in many physiological functions, such as behavior, metabolism, cell growth and immune function [108-114]. Circadian oscillation is regulated by auto-regulatory

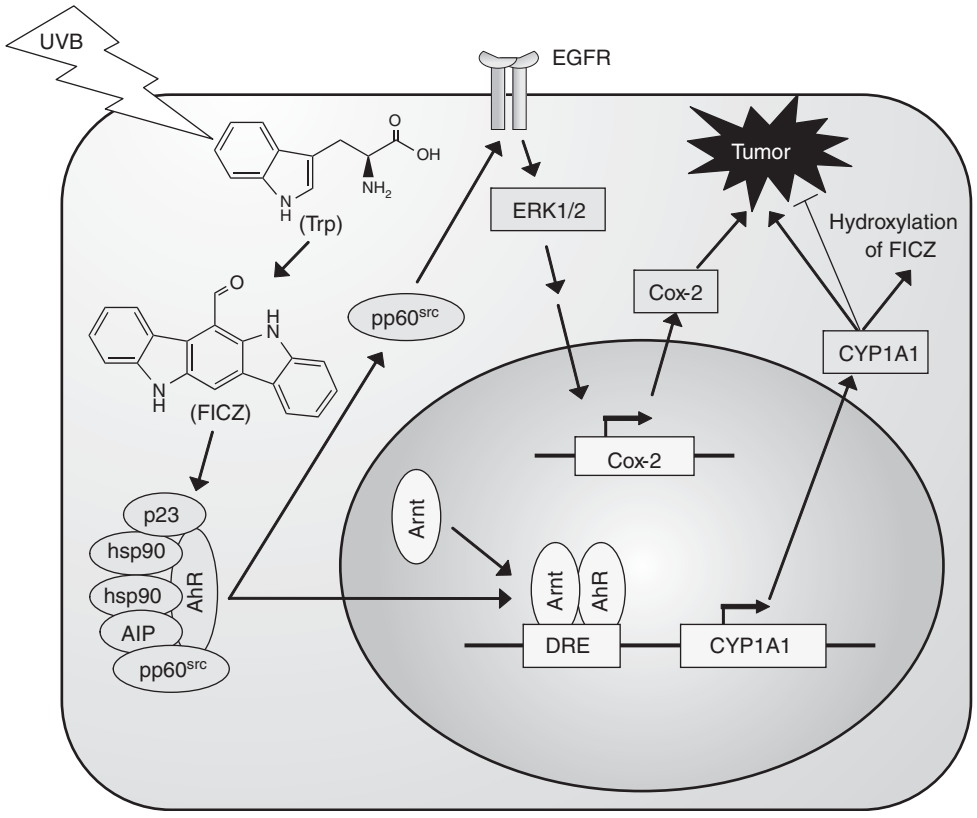


Figure 5. Bifurcated AHR signaling in UV response and carcinogenesis. Unliganded AHR resides in the cytoplasm in a complex that contains hsp90, AIP, p23 and pp60^{src}. L-Trp efficiently absorbs UV photons and is oxidized to form FICZ. FICZ binds to AHR with a high affinity leading to dissociation of AHR from associated proteins. Activated AHR enters the nucleus and heterodimerizes with Arnt to mediate the transcription of CYP1A1. CYP1A1 in turn metabolizes FICZ to reduce and terminate induction. Release of pp60^{src} from the cytoplasmic AHR complex activates c-src to cause the internalization and activation of EGFR and consequently, the activation of ERK1/2 and induction of Cox-2. Induction of CYP1A1 may increase the metabolic activation or detoxification of carcinogens to promote or inhibit carcinogenesis in the skin and other organs. Induction of Cox-2 increases the production of PGE_2 that promotes tumor formation by suppressing immune functions and by inhibiting UVB-induced apoptosis. AHR also affects photocarcinogenesis by inducing melanogenesis, inhibiting inflammation, and modulating T_H and DC development, the molecular bases of which are unclear but may involve direct transcription and signal transduction by AHR analogous to CYP1A1 induction and AHR-pp60^{src}-EGFR signaling.

AIP: AHR-interacting protein; Arnt: AH receptor nuclear translocator; DC: Dendritic cell; FICZ: 6-Formylindolo[3,2-*b*]carbazole; Trp: Tryptophan.

transcriptional feedback loops containing both positive and negative components [55]. The central pacemaker of circadian rhythm is located in SCN of the hypothalamus. The circadian clock is corrected to the 24 h rhythm by the light/dark cycle to synchronize the phases of both central and peripheral clocks in tissues [55]. Circadian oscillation of gene expression in the skin can be modulated by light directly. Central and peripheral circadian oscillators can also function independently of light/dark and respond to endogenous and environmental stimuli such as feeding behavior and environmental chemicals.

The basic cogs of circadian oscillator include five PAS-domain-containing proteins: period (PER)-1, -2 and -3, clock (CLK), and brain muscle ARNT-like protein 1 (BMAL1, ARNT3, MOP3) [114]. The PAS domain and the bHLH motif mediate heterodimerization among the PAS proteins to regulate transcription of target genes. BMAL1 and CLK appear to play a central role in the regulation of circadian oscillation. In this role, BMAL1 and CLK form a heterodimer that binds to E-box DNA recognition sequences to drive transcription of circadian-regulated genes including Per1 and cryptochrome (Cry) [108,115]. PER and CRY in turn form various complexes to inhibit BMAL1/CLK-mediated transcription including the transcription of their own. Daily variation of BMAL1 expression can reach > 10-fold [116].

BMAL1 shares a high sequence similarity with ARNT in the bHLH-PAS domain (44.3%) [117]. On the other hand, the intron/exon splice pattern around bHLH/PAS in *Bmal1* closely resembles that of *Ahr* suggesting that *Bmal1* and *Ahr*

are derived from a common ancestral gene [118]. Although AHR does not appear to heterodimerize with BMAL1 [119], the close relationship of BMAL1 to ARNT and AHR in sequence and origin suggests certain levels of interaction between AHR signaling and mammalian circadian regulation.

Both AHR and ARNT proteins exhibit diurnal changes in rat liver, lungs and thymus with the degree of amplitude approximately two to threefold [120]. The mRNA expression of AHR, however, appears to cycle throughout the day with a single peak of about two to threefold [89,121]. The reason for this differential circadian expression pattern between AHR mRNA and protein is unclear. In SCN, daily oscillation of AHR, Arnt and CYP1A1 expression was observed [89,121]. Circadian oscillation of AHR expression was possibly controlled by circadian oscillators because disruption of Per1 and Per2 altered AHR signaling in mammary gland, which correlated with higher induction of CYP1A1 and 1B1 in Per knockout mice compared with wild type [122].

Targeted disruption of *Ahr* in mice does not appear to cause apparent abnormalities in the circadian rhythm under a normal light/dark cycle, possibly due to redundant and robust circadian regulation in mammals. However, treatment with TCDD resulted in a significant reduction in the amplitude of light-induced phase delay in mouse activity [121]. This observation supports the notion that activation of AHR may interfere with the ability of the circadian oscillators to adjust to alterations in environmental lighting. Disruption of circadian rhythm by AHR agonists is also observed in other systems. Activation of AHR by TCDD disrupted the diurnal changes of cell number of Lin(-)Sca1(+)cKit(+) (LSK) bone marrow cells and myeloid and erythroid precursor cells contributing to the suppression of immune function by TCDD [123]. In the above two cases, disruption of circadian oscillation by TCDD is associated with altered expression of CLK genes indicating a direct role of AHR in the regulation of circadian oscillator gene expression. The expression of *Bmal1* and *Per1* in mouse liver was altered in the first case [121], whereas the expression of *Per1* and *Per2* mRNA in bone marrow cells and myeloid and erythroid precursors was changed in the latter [123].

Administration of FICZ or Trp photoproducts to C57BL/6J mice dose-dependently induced c-fos mRNA, a commonly used marker for light response, in an AHR-dependent manner [124]. In SCN2.2, an immortalized rat SCN cell line, FICZ altered the circadian expression of *Per1*, *Cry1* and *Cry2*. FICZ also inhibited glutamate-induced phase shifting of the mouse SCN electrical activity rhythm. The findings revealed a potential for Trp photoproducts to modulate light-dependent regulation of circadian rhythm by triggering AHR signaling [124].

Together, these findings reveal a mutual regulation between the AHR pathway and mammalian circadian oscillators to integrate signals from sunlight through FICZ or from the environment through exogenous AHR ligands.

4.3 Melanogenesis and skin tanning

Melanogenesis is a key adaptive response to protect skin cells against UVB-induced DNA damage. On UVB stimulation, melanocytes synthesize melanin from tyrosine in a specialized organelle, the melanosomes, through a complex process involving signal transduction, transcription and enzymatic reactions. Melanin released from melanocytes accumulates in surrounding keratinocytes where it forms a cap around the nucleous to protect against UV-damage to DNA. Increased melanogenesis (mainly by UVB) and the release and darkening of melanin (mainly by UVA) cause skin tanning.

Activation of AHR is associated with melanogenesis. Exposure to TCDD resulted in hyperpigmentation in addition to chloracne and porphyria cutanea tarda in the human skin [125]. Accidental mass-poisoning from cooking oil contaminated with polychlorylated biphenyls caused increased skin and gingival pigmentation in the patients, and dark pigmentation of head, face and genital in children ('cola-colored babies') born to exposed mothers [126-128]. In pityriasis vesicolor, a human skin disease caused by infection of the yeast *M. furfur*, the affected skin fluoresces under UV light and depigmentation appears in the end stage due to interrupted melanin synthesis. Secondary metabolites, such as the AHR ligand malassezin, from the yeast potentially inhibit skin tyrosinase and cause depigmentation [53].

The role of AHR in melanogenesis was directly analyzed in cell and animal models [129,130]. Several conclusions were made from the studies: i) skin melanocytes express functional AHR; ii) UVB-induced skin tanning requires the AHR in melanocytes but not keratinocytes; iii) UVB increased pigmentation, melanocyte density and melanocyte differentiation in an AHR-dependent manner; iv) AHR controls the expression of pigmentation and melanocyte differentiation-related genes via AHRE-dependent transcription including stem cell factor-1 and c-kit; and v) TCDD may induce tyrosinase and tyrosine-related protein 2 in melanocytes to increase melanin synthesis parallel to activation of the AHR signaling pathway, although a negative result was found in a separate study. Overall, the results linked AHR signaling to the physiological pigmentation of the skin induced by solar UVB radiation for protection against UV radiation.

4.4 Inflammation: interaction of AHR with the NF-κB pathway

Inflammation plays a unique role in UV and non-UV-induced skin lesions including cancer [131,132]. PAHs are known to induce skin inflammation. Persistent activation of AHR by TCDD causes a chronic and generalized inflammatory response in the skin involving macrophage proliferation and neutrophilia, a pathological skin condition termed chloracne [8,133]. Expressing a constitutively active form of AHR in the skin in a transgenic mouse model resulted in atopic dermatitis-like skin inflammation with itching [134]. The lesion was accompanied with inflammation and immunological imbalance. Together, these findings support the notion that

activation of the AHR signaling pathway indeed modulates skin inflammatory lesions.

NF-κB is known to play a critical role in controlling the inflammatory process in the skin. NF-κB protects the proliferative epidermis from the deleterious effects of UVB by regulating the expression of inflammatory cytokines and other mediators [135]. Dysfunction of NF-κB is associated with a variety of skin inflammatory lesions and cancer [135]. Moreover, AHR and NF-κB appear to mutually regulate each other in the skin and other organs [136,137]. UVB-induced expression of CYP1A1 involves a crosstalk between AHR and NF-κB in which UVB initially inhibits CYP1A1 expression by activating NF-κB leading to a longer half-life of FICZ and, consequently, induction of CYP1A1 for a longer time [136]. Inhibition of NF-κB alleviates the initial inhibition of CYP1A1 expression by UVB. However, to what extent the mutual regulation between AHR and NF-κB contributes to UVB-induced skin inflammatory lesions remains an open question. Nevertheless, the finding that activation of AHR by UVR influences skin inflammation opens new avenues of research on inflammatory disease and chemical toxicity in the skin, an area that remains poorly understood. Because keratinocyte-generated soluble inflammatory mediators can influence inflammation in other organs, the impact of AHR on inflammation is likely to go beyond the skin.

4.5 Immunosuppression

UV exposure suppresses a variety of immune reactions, including tumor immunity, contact hypersensitivity to chemical haptens and delayed type hypersensitivity (DTH) to protein and microbial antigens [138]. Chronic exposure to UV radiation induced tumors in mice; when transplanted, the UV-induced tumors grew progressively only if the recipient mice were immunosuppressed or have received a subcarcinogenic dose of UV radiation [139]. These findings suggest that UV radiation has two effects: skin cancer induction and immune suppression; both are necessary for the tumor to develop in UV-irradiated skin. The findings support the notion that immunosuppression is a major risk factor for skin cancer induction by UV light. In the case of DTH, a single exposure to UV radiation at doses that are easily obtained during normal recreational and occupational exposure suppresses DTH to a variety of microbial antigens, raising the concern that immunosuppression by sunlight may depress the immune response to infection as well as the protection from previous vaccination [138].

The mechanism(s) by which sunlight inhibits systemic immune functions is complex, involving the production of an array of skin-derived immunosuppressive mediators and modifiers, such as platelet-activating factor, PGE_2, cis-urocanic acid, histamine, IL-4 and IL-10. A cascade of events by the soluble factors is activated to suppress IL-12 production from dendritic cells (DCs) leading to immune suppression [138]. Transcriptional upregulation of Cox-2 by UVB is required for increased PGE_2 production and subsequent signaling events.

The impact of AHR on immune development and function was well recognized from studies of TCDD and other AHR ligands. TCDD has multiple immunosuppressive effects in animals, including thymic involution, decreased host resistance to pathogens and tumors, suppressed fetal lymphocyte development and maturation, and suppressed adaptive immune responses (antibody production, DTH and cytotoxic T lymphocyte). Recent studies with AHR knockout models and newly-identified AHR ligands provided significant insights into the mechanism by which AHR regulates immune functions through DCs and T_H cells. DCs and subsets of T_H cells characterized by the unique expression of cytokines and 'master regulator' transcription factors play critical roles in immune regulation as well as the pathogenesis of inflammatory, autoimmune, allograft, allergic and infectious diseases. AHR participates in the development and function of $CD4^+CD25^+FoxP3^+$ regulatory T cells (T_{reg}), IL-17-producing helper T cells (T_H17), type 1 regulatory T cells (Tr1) and DCs [33,34,39,40,140-147]. AHR does not appear to be essential for the differentiation of the immune regulatory cells, but activation of AHR significantly modulates the development of the cells. Furthermore, the effect of AHR activation on the cells appears to be ligand-dependent. TCDD stimulated the generation of T_{reg} *in vitro* and *in vivo* and suppressed experimental autoimmune encephalomyelitis (EAE); FICZ interfered with T_{reg} cell development, boosted T_H17 cell differentiation and increased the severity of EAE in mice; another endogenous AHR agonist ITE induced $FoxP3^+$ T_{reg} and tolerogenic DC and suppressed EAE; finally, AHR antagonist CH-223191 repressed the development of T_H17 and the production of IL-17 and IL-22 [140-143]. These findings raised the possibility that the beneficial effects of AHR on T_H cells and DCs through natural or synthetic ligands that are less toxic than TCDD can be exploited for drug development against autoimmune, allograft and allergic diseases.

AHR may participate in UV light-induced immunosuppression via two mechanisms. First, activation of the AHR-$pp60^{src}$-EGFR pathway by light induces the mRNA and protein expression of Cox-2, which increases the production of PGE_2. PGE_2 in turn activates its downstream signal transduction that ultimately targets DCs for immunosuppression. Second, activation of AHR by light modulates the development of T_{reg}, T_H17, Tr1 and DC and, thereby, alters immune functions. Indeed, treatment with FICZ suppressed T_{reg} development but augmented T_H17 generation and Tr1 differentiation in mice [140,143,147]. The molecular mechanism by which AHR regulates the differentiation of the immune regulatory cells remains largely unclear. In the case of Tr1, AHR interacted with c-Maf to promote the differentiation of Tr1 induced by IL-27; AHR bound to c-Maf and promoted transactivation of *Il10* and *Il21* promoters for the generation of Tr1 and, consequently, amelioration of EAE in mice [147]. In human cells, AHR activation promoted the differentiation of $CD4^+FoxP3^-$ T (Tr1-like) cells through granzyme B and induced $Foxp^+$ iT_{reg} cells in the presence of TGF-β through Smad1 and Aiolos [148].

4.6 Cancer

Skin cancer is a major health risk from UV exposure. Information on a direct involvement of AHR in photocarcinogenesis is lacking. From the above discussion, it is conceivable that activation of AHR by light has both inhibitory and promoting effects on cancer. AHR may influence tumor formation induced or promoted by UV radiation through several mechanisms: i) induction of CYP1A1 and other DMEs via AHR-mediated transcription (Section 3.1, **Figure 5**); ii) induction of Cox-2 via the AhR-pp60^{src}-EGFR pathway (Section 4.1, **Figure 5**); iii) suppression of tumor immunity by inducing Cox-2 and by altering the balance among T_H cells and DCs (Section 4.5); iv) interaction with the NF-κB signaling pathway to influence skin inflammation (Section 4.4); and v) induction of melanogenesis to protect against UVB-induced DNA damage (Section 4.3).

The influence of induction of CYP1A1 and other DMEs by light on UV- and non-UV-induced cancers is several-fold. First, induction of CYP1A1, 1A2 and 1B1 by light may increase the metabolic activation of co-exposed carcinogens, such as BaP from smoking and aflatoxin B1 from contaminated food, to ultimate carcinogens, causing mutations in the skin and other tissues. In one example, exposure to UVB or FICZ sensitized keratinocytes to DNA adduct formation by BaP; increased adduct formation correlated with induction of CYP1A1 and 1B1; whereas, treatment with AHR antagonist αNF, which suppressed induction of the CYPs by UVB or FICZ, blocked the sensitization for DNA adduct formation [149]. Second, induction of CYP1A1, 1A2 and 1B1 increases the metabolic clearance of carcinogens, giving rise to protection against the carcinogenic and toxic effects of the carcinogens. Metabolic protection by CYP1 enzymes has been observed in animal models, in which metabolic clearance of carcinogens at the port of entry outweighed metabolic activation at target organs to reduce cancer incidence and toxicity in intact animals [7,150]. Third, other DMEs and transporters controlled by AHR also influence chemical carcinogenesis by modulating the metabolism and disposition of carcinogens. These include CYPs (2S1 and 2A5), glutathione S-transferase A1, UDP-glucuronosyltransferases (1A1, 1A6 and 1A9), NAD[P]H:quinone oxidoreductase 1, aldehyde dehydrogenase 3A1 and multi-drug resistance proteins (2, 3, 5 and 6) [5]. Fourth, P450s participate in the metabolism of arachidonic acid and its derivatives that are associated with skin cancer; induction of P450s and other DMEs by light may impact on skin carcinogenesis by influencing the metabolism of Cox-2 substrates and products [151]. Taken together, the balance between DMEs that bioactivate carcinogens and those that detoxify the chemicals determines the ultimate fate of carcinogens and, hence, the outcome in photocarcinogenesis. Therefore, both *in vitro* and whole animal studies are needed for safety evaluation of AHR activation and CYP1 induction in skin carcinogenesis [7].

Cox-2 is another important target of AHR in the crossroad of photocarcinogenesis and AHR signaling. Cox-2 converts arachidonic acids to prostaglandins and has been implicated in skin carcinogenesis and colon cancer [152]. Cox-2 is highly regulated and is induced by UVB through the AHR-pp60^{src}-EGFR pathway. Induction of Cox-2 by light may play a role in UVB photocarcinogenesis. Cox-2-deficient mice showed reduction of UVB-induced skin tumor formation, whereas overexpression of Cox-2 in the skin increased UVB photocarcinogenesis, establishing a requirement for Cox-2 in the development of skin tumors [153]. Cox-2 inhibited UVB-induced apoptosis in mouse skin; conversely, loss of Cox-2 in mice increased UVB-induced epidermal apoptosis [154,155]. In both scenarios, the anti-apoptotic effects of Cox-2 involved the cytoprotective activity of the major metabolite PGE_2 and its receptors [155]. Induction of Cox-2 also suppressed tumor immunity through PGE_2, which is a prerequisite for the development of UVB-induced tumors in the skin and for the transplantation of UVB-induced skin cancer [68].

5. AHR as a target for drug development

Although a role of AHR in human disease has long been suggested from the remarkable adverse effects of TCDD and other ligands, the interest in utilizing AHR as a drug target was cultivated only in recent years. The rapid expansion of the repertoire of recognized AHR ligands, both synthetic and naturally occurring, provided new opportunities for exploring the therapeutic potentials of AHR ligands. A strikingly divergent spectrum of biological effects emerged from these ligand–AHR interactions. Notably, some of the new AHR agonists and antagonists exhibit beneficial effects that can be exploited for drug development with low toxicity [2,133,156].

5.1 Anti-immune and anti-inflammation

DCs play an obligatory role in the initiation and maintenance of immune response by providing help to naive T cells to develop into effecter T_H cells. AHR has been shown to block DC function to generate pro-inflammatory T_H in an agonist-dependent manner [33,133]. Inhibition of DC by AHR was responsible for the anti-inflammatory activity of a novel, low molecular mass, anti-allergic drug candidate VAF347 (**Figure 1**). VAF347 activated AHR to inhibit DC expression of IL-6, CD86 and HLA-DR, three relevant molecules for pro-inflammatory T_H development *in vitro* and *in vivo*, and thereby, inhibited allergic lung inflammation in a mouse model [33,133]. A water-soluble derivative of VAF347 (VAG539) promoted long-term graft acceptance and active tolerance in mice transplanted with MHC-mismatched pancreatic islet allografts. In this case, VAG539 activated AHR to induce islet allograft-specific tolerance through both direct and DC-mediated effects on T_{reg} survival and function [146].

The therapeutic potentials of regulating the development of $CD4^+CD25^+FoxP3^+$ T_{reg}, $FoxP3^-$ Tr1 and T_H17 cells by AHR were illustrated in several models of autoimmune pathology. Activation of AHR by TCDD stimulated T_{reg} but inhibited T_H17 development, thereby reducing the immunopathology of

EAE, experimental autoimmune uveoretinitis and spontaneous autoimmune diabetes [140-146]. TCDD also promoted the differentiation of mouse FoxP3$^-$ Tr1 and human FoxP3$^-$ Tr1-like and FoxP$^+$ iT$_{reg}$ cells [147,148]. Activation of AHR by FICZ appeared to interfere with T$_{reg}$ development, but stimulate T$_H$17 differentiation to generate a pro-inflammatory autoimmune potential, resulting in accelerated onset and increased pathology of EAE in mice [140,143]. On the other hand, FICZ also stimulated the development of Tr1, which dampens inflammation and immunopathology [147]. AHR agonist ITE suppressed EAE both by directly inducing the differentiation of FoxP3$^+$ T$_{reg}$ and by inducing tolerogenic DCs that also support FoxP3$^+$ T$_{reg}$ development [141]. Activation of AHR by KN negatively regulated DC immunogenicity and skewed the differentiation of naive T cells to T$_{reg}$ from T$_H$17 cells [39,40]. Inhibition of AHR by antagonist CH-223191 attenuated T$_H$17 development *in vivo* and the subsequent secretion of IL-17 and -22, further supporting a role of AHR in the regulation of T$_H$17 [142]. Taken together, AHR regulates T$_{reg}$, Tr1, T$_H$17 and DC differentiation in ligand- and context-dependent manners, providing unique targets for therapeutic immunomodulation.

M50367 is an orally active anti-allergic agent. M50367 skewed the T$_H$1/T$_H$2 balance toward T$_H$1 dominance by suppressing naive T$_H$ cell differentiation into T$_H$2 cells, and inhibited the production of plasma IgE and pulmonary eosinophilia, resulting in the suppression of airway hyperresponsiveness in murine models of atopic asthma [157]. The anti-allergic action of M50367 was mediated through its metabolite M50354 (**Figure 1**). M50354 activates AHR to suppress GATA-3 expression in T$_H$ cells and modulates the T$_H$1/T$_H$2 balance, thereby inhibiting atopic asthma [34].

5.2 Anti-estrogen for treatment of hormone-dependent cancer

TCDD is anti-estragenic, inhibiting 17β-estradiol (E2)-induced mammary and uterine tumors in rats [156]. TCDD inhibits E2-induced response in breast and endometrial cancer cell lines through a complex inhibitory AHR-estrogen receptor (ER) crosstalk. 6-Alkyl-1,3,8-trichlorodibenzofurans and substituted diindolylmethanes are two classes of selective AhR modulators (SAhRMs) that are relatively nontoxic but exhibit anti-estrogen and anticancer activities similarly to TCDD. The SAhRMs inhibit ER-positive and -negative mammary tumor growth and synergize with tamoxifen in inhibiting breast cancer growth and blocking tamoxifen-induced estrogenic activity in the uterus. In addition, SAhRMs inhibit AHR-androgen receptor crosstalk. Therefore, the SAhRMs are potential drug leads for anticancer therapy against hormone-dependent cancers, in particular, in combined therapies with tamoxifen and other ER modulators for breast cancer [156]. SAhRM DIM is also a potent modulator of the innate immune response system and is being developed for treatment of cervical dysplasia linked to human papilloma viral infection [158]. The role of AHR in the immune-modulating and antiviral activities of DIM is currently unclear.

5.3 Inhibition of CYP1A1 induction and metabolism

Resveratrol (trans-3,4′,5-trihydroxystilbene, **Figure 1**) is a phytoalexin synthesized by a variety of plants, such as the grape skin, in response to injury, UV radiation and fungal infection. Resveratrol demonstrated a number of biological activities *in vitro* and *in vivo*, including chemoprevention and chemotherapy against cancer. Resveratrol inhibited tumorigenesis in a mouse skin cancer model and the development of preneoplastic lesions in mouse mammary glands in culture. In both cases, 7,12-dimethylbenz[α]anthracene (DMBA, a PAH) was used as the carcinogen [159]. Resveratrol repressed events associated with the three major stages of carcinogenesis, that is, initiation, promotion and progression of tumors.

Resveratrol antagonizes the transcription of several DME genes regulated by AHR [47,160]. This action was verified in different cell types with different inducers, such as TCDD, DMBA and BaP. Molecular characterization of inhibition of CYP1A1 induction revealed that resveratrol may block several steps of AHR signaling; whether it directly competes with AHR agonists for AHR binding remains controversial [47,160-161]. Inhibition of CYP1A1 and CYP1B1 gene expression by resveratrol correlated with reduced BaP-DNA adduct formation in human bronchial epithelial cells [162]. Resveratrol decreased estrogen metabolism and blocked formation of estrogen-DNA adducts in breast cancer cells treated with TCDD/estradiol, and suppressed TCDD/estradiol-induced cell transformation [163]. Resveratrol also directly inhibits CYP1A1 in microsomal human liver preparations and in microsomes isolated from BaP-treated human hepatoma cells [164,165].

5.4 Potentials for stem cell therapy

AHR is expressed in hematopoietic stem cells (HSCs) [28,166]. Activation of AHR by TCDD decreased the reconstitution activity of LSK bone marrow stem cells and disrupted the diurnal changes of LSK, myeloid precursor and erythroid precursor cells in the bone marrow [123,167]. In addition to induction of DMEs, AHR regulates a number of signaling pathways that control hematopoiesis including HES-1, c-MYC, C/EBP-, Pu.1-, β-catenin-, CXCR4- and STAT5-dependent processes, strongly implicating AHR in HSC biology [26].

The clinical application of HSCs is often limited by the capacity to expand HSCs *ex vivo*. Unbiased screening with human HSCs identified StemRegenin 1 (SR1, **Figure 1**), a purine derivative that promoted the *ex vivo* expansion of human CD34$^+$ HSCs, that is, undifferentiated HSCs [28]. SR1 increased HSC expansion by 50-fold and the expansion of HSCs that retain the ability to engraft immunodeficient mice by 17-fold. SR1 dose-dependently inhibited TCDD-induced expression of CYP1B1 mRNA in HSCs by directly binding to AHR. SR1 also inhibited induction of a human AHR-dependent luciferase reporter gene with an IC$_{50}$ at 127 nM. Finally, expansion of CD34$^+$ HSCs by SR1 required functional AHR. Therefore, SR1 increased the number of HSCs by direct binding and inhibiting AHR. These findings

potentially facilitate the clinical use of HSC 'transplant' therapy [28].

In aggregates, AhR ligands including FICZ have therapeutic potentials for a range of disease conditions. Given the roles of AhR in skin photocarcinogenesis, inflammation, immunosuppression and aging, it is tempting to speculate that AhR agonists or antagonists can be exploited for therapy against human skin diseases including skin aging, skin cancer, and autoimmune and chronic inflammatory skin lesions such as psoriasis, which are influenced by light and AHR.

6. Other effects of FICZ

FICZ affects a range of signaling pathways, some of which appear not to be dependent on AHR. The long interspersed nucleotide element 1 retrotransposition (L1-RTP) is responsible for certain genome shuffling in humans. L1-RTP generates unique expression profiles of genes depending on the integration sites of newly synthesized L1 DNA. FICZ was found to induce L1-RTP [168]. Interestingly, FICZ-induced L1-RTP required ARNT1 but not AHR. Induction also involved MAPK and CREB signaling. It was hypothesized that another protein serves as a sensor for FICZ and dimerizes with ARNT1 to mediate L1-RTP, because ARNT1 itself does not bind a ligand and generally heterodimerizes with a partner protein to regulate gene transcription. A toxicogenomic study suggested that the circadian bHLH-PAS protein PER is a potential candidate for this protein [169].

Liver X receptors (LXRs) regulate lipid and cholesterol metabolism in mammals. FICZ was shown to activate human LXRα and β with EC_{50} values of 3.1 and 0.99 μM, respectively, which are lower than those of recognized LXR endogenous ligands oxysterols [170]. The physiological function of activation of LXR by FICZ remains to be elucidated.

The above examples suggest that FICZ elicits a range of signaling events that go beyond AHR signaling in the response to light [171]. It is plausible to speculate that FICZ modulates the transcriptomic program of the mammalian UV response by activating a number of XARs, which include, but are not limited to, AHR, LXR and the XAR for L1-RTP, to resist UV stress.

7. Conclusion

Light impacts the many aspects of mammalian physiology and disease by interacting with photon-absorbing molecules, such as Trp, in the skin. Although known to be a xenobiotic receptor, AHR has long been suspected to have endogenous functions in multiple organs including the skin. The identification of Trp photoproduct FICZ as a potent, endogenous ligand of AHR provided a molecular link between light exposure and AHR signaling and function. In this prospect, AHR plays a critical role in sensing and transducing light signal to initiate the previously poorly-understood cytoplasmic UV response. The uncovering of the bifurcated signaling pathway of AHR in response to light – that is, activation of AHR/AHRE-dependent transcription to induce CYP1A1 and other AHR target genes, and activation of the AHR-pp60^{src}-EGFR pathway to stimulate ERK1/2 signaling for induction of Cox-2 – put forward a working model for the multiple roles of AHR in skin function and disease, including drug metabolism, circadian oscillation, melanogenesis, inflammation, immunosuppression and cancer. These findings also suggest therapeutic potential of modulating the AHR signaling pathway for the treatment of cancer, inflammatory and immune-related diseases in the future.

8. Expert opinion

Induction of DMEs in response to chemical insults is an adaptive response found in most organisms. Induction of CYP1A1 through AHR is the most-well studied. Indeed, the research on the induction has largely increased our understanding of how the body regulates drug metabolism to maintain chemical homeostasis [41-44,54,98-102,129,130]. On the other hand, most of the known substrates for CYP1A1 and ligands for AHR are xenobiotics, such as drugs, environmental chemicals and dietary constituents. Their endogenous substrates/ligands and physiological functions remain elusive for the most part.

The studies led by Rannug and associates on light-induced expression of CYP1A1 in the skin appear to have filled this knowledge gap in the context of skin physiology and disease in the response to light. In this vista, several major advancements were made, including: i) establishing AHR as the mediator of CYP1A1 induction by light, ii) identifying and elucidating the structure of Trp photoproduct FICZ as the inducing agent, iii) establishing FICZ as a potent and physiological ligand of AHR and iv) establishing FICZ as an endogenous substrate of CYP1A1. These findings put forth a physiological framework for light-induced expression of CYP1A1 in which a sequence of auto-regulatory loop is followed such that induction is appropriate to the intensity, duration and frequency of exposure to light. Furthermore, the studies uncovered a bifurcated signaling pathway of AHR that is activated by light through FICZ and transduces the signal to both the nucleus and the plasma membrane (**Figure 5**). In this manner, light activation of the cytoplasmic AHR joins the nuclear, ribosomal and cell membrane signaling pathways of the mammalian UV response to influence the chemical homeostasis, circadian rhythm, melanogenesis, inflammation, immune response, and carcinogenesis in the skin and extracutaneous organs.

The findings on FICZ–AHR interaction and AHR-mediated cytoplasmic UV response raised several issues for future studies. One issue concerns with ligand-binding to AHR. Apparently, the structures of known ligands of AHR including Trp photoproducts and their effects on AHR activities are more diverse than previously anticipated (**Figure 1**). From a structural point of view, this implies that the binding pocket of AHR for its ligands is rather promiscuous, or exhibits

high plasticity on ligand-binding, such that it can both accommodate ligands with diverse structures and elicit differential responses to specific ligands. In this regard, the finding that some ligands selectively modulate AHR activities to boost the beneficial effects but avoid the toxic outcomes of AHR activation is welcome news for drug development. FICZ binds AHR with a higher affinity than that of TCDD but induces CYP1A1 only transiently due to its rapid metabolism by CYP1A1 and other DMEs. This observation appears to contradict a previous belief that stable inducers, such as TCDD, generally have higher affinities for AHR than metabolically liable inducers, such as BaP and 3-MC. Understanding how FICZ interacts with AHR may provide new insights into the structural, chemical and physical properties that determine the affinity, efficacy and biological effects of AHR ligands.

How AHR activation impacts UV and non-UV-induced carcinogenesis in the skin is a complex issue that involves multiple signaling pathways and functions, both protective and tumor promoting. In the example of CYP1 induction, the induction increases both the activation and detoxification of carcinogens, resulting in increased DNA adduct formation and mutation in cultured cells on one hand, but reduced toxicity and cancer in some animal models on the other. Therefore, both *in vitro* and *in vivo* models are needed for safety evaluation of AHR activation and CYP1 induction by light.

Light has systemic effects in the body, such as circadian oscillation and immune suppression. Because AHR mediates the circadian and seasonal variations in the expression of CYP1A1 and other DMEs and influences immune reactions in internal organs, it is rational to speculate that FICZ or other light-induced ligands of AHR are released from the skin and travel to extracutaneous organs to mediate AHR-dependent UV response in internal organs influencing, for instance, circadian oscillation of DMEs and immune functions.

The therapeutic potentials of the findings can be inferred from two aspects. First, although much remains to be learned from AHR-mediated UV response, it is rational to believe that interfering with the signaling events of AHR in the skin can be exploited for drug therapy. AHR ligands exhibit a broad spectrum of activities ranging from full agonists to antagonists or ligands that only modulate some of the AHR functions. Several AHR ligands that selectively increase the beneficial effects or inhibit the adverse activities of AHR have been shown effective in certain disease models. Second, FICZ itself can be a drug lead for therapy and other biological applications due to its high affinity for AHR, endogenous production, fast clearance and unique biological activities. Indeed, AHR activation by FICZ has been shown to interfere with T_{reg} cell development, boost T_H17 and Tr1 differentiation, and impact the onset and severity of EAE in mice. Molecular understanding of FICZ–AHR interaction and its biological effects should facilitate AHR-based drug therapy.

As the list of functions affected by FICZ expands quickly, more signaling molecules and pathways are implicated in FICZ action and some are independent of AHR. Thus, FICZ affects a broader range of skin physiology than those mediated through AHR in the response to light. Continued research to elucidate the function and mechanism of action of FICZ in UV and other responses is expected to have productive and influential outcomes.

Addendum

After initial online publication of this article, a new study was published reporting the identification and characterization of a new tryptophan photoproduct as an AhR activator. This and a previous study from the same laboratory pointed out that exposure of aqueous tryptophan to light that passed through glass windows can result in the production of multiple photoproducts, some of which are potential AhR agonists in addition to FICZ and dFICZ [180,181].

Declaration of interest

This research was supported by the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. The author declares no other conflicts of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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