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Review

Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways

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ABSTRACT

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a widespread environmental pollutant, which causes a variety of severe health effects, e.g. immunosuppression, hepatotoxicity, and carcinogenesis. The main mediator of TCDD toxicity is the arylhydrocarbon receptor (AhR), which, upon activation, translocates into the nucleus and enforces gene expression. Since most of the pleiotropic effects caused by TCDD are associated with alterations in cell growth and differentiation, the analysis of the interference of the AhR with factors controlling these cellular functions seems to be a promising target regarding the prevention and treatment of chemical-provoked diseases. Cell growth and differentiation are regulated by numerous growth factors and cytokines. These multifunctional peptides promote or inhibit cell growth and regulate differentiation and other cellular processes, depending on cell-type and developmental stage. They are involved in the regulation of a broad range of physiological processes, including immune response, hematopoiesis, neurogenesis, and tissue remodeling. The complex network of growth factors and cytokines is accurately regulated and disturbances of this system are associated with adverse health effects. The molecular mechanisms by which the AhR interferes with this signaling network are multifaceted and the physiological consequences of this cross-talk are quite enigmatic. The investigation of this complex interaction is an exciting task, especially with respect to the recently described non-genomic and/or ligand-independent activities of AhR. Therefore, we summarize the current knowledge about the interaction of the AhR with three cytokine-/growth factor-related signal transducers – the epidermal growth factor (EGF) family, tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) – with regard to pathophysiological findings.

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Abbreviations: AhR, arylhydrocarbon receptor; AIP, AhR interacting protein; ARNT, AhR nuclear translocator; Apaf1, apoptotic protease-activating factor 1; AREG, amphiregulin; bHLH, basic helix–loop–helix; B(*a*)P, benzo(*a*)pyrene; CDK, cyclin-dependent kinase; Cdkn, CDK inhibitor; COX-2, cyclooxygenase-2; CREB, cAMP-responsive element binding protein; CYP, cytochrome P450; E2F, elongation 2 factor; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; EREG, epiregulin; ERK, extracellular signal-regulated kinase; FICZ, 6-formylindolo[3,2-*b*]carbazole; hsp90, 90 kDa heat-shock protein; IL, interleukin; LTBP, latent TGF-β binding protein; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NF, naphtoflavone; PAH, polyaromatic hydrocarbon; PAI, plasminogen activators inhibitor; PAS, Per-ARNT-Sim; PKA, protein kinase A; RB, retinoblastoma; SARA, Smad anchor of receptor activation; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TβR, TGF-β receptor; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumor necrosis factor; UVB, ultraviolet-B; XRE, xenobiotic-responsive element.

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Contents

1. Introduction

Halogenated polyaromatic hydrocarbons (PAHs) like dibenzo-*p*-dioxins, dibenzo-*p*-furans and polychlorinated biphenyls are widespread industry- and combustion-derived environmental pollutants, which embody an alerting risk potential for human health. Among them, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent toxicant, causing immunological abnormalities, teratogenic and carcinogenic effects, wasting syndrome, alterations in the endocrine system and hepatotoxicity in several species [1]. In humans the most conspicuous effect of TCDD intoxication is chloracne, caused by alterations in differentiation and proliferation of epidermal cells. The fact that several others of the mentioned adverse effects of TCDD, for example its tumor promoting property, are also based on modified patterns in differentiation and proliferation, strengthens the idea that TCDD influences growth factor-dependent cellular pathways.

Growth factors and cytokines are peptide regulatory factors which are essential for cell fate and function, since they are involved in all aspects of cell differentiation, survival, proliferation, senescence and apoptosis [2]. Growth factors and cytokines are expressed and secreted by almost all tissues, even though cytokines were originally defined as signaling peptides of the hematopoietic and immune system, respectively. Growth factors and cytokines act in autocrine and paracrine loops and exert multiple actions on various, sometimes common, target structures. One main function of cytokines and growth factors is the activation of transcription factors, which modulate the cellular gene expression pattern and subsequently alter cell function [2]. For these actions specific membrane receptors are required. Numerous *in vivo* and *in vitro* studies reported about TCDD-mediated modifications of growth factor and cytokine signaling. For instance, changes in transforming growth factor (TGF)-α, TGF-β, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-6, IL-8 and interferon-γ gene expression rates were noted upon TCDD exposure [3–6]. However, even if the underlying molecular mechanisms for this transcriptional modulation of cytokines and growth factors are not fully understood, it is broadly accepted that the arylhydrocarbon receptor (AhR), at least in part, is involved in the regulation of these TCDD-induced changes in gene expression [7–9].

The AhR is a chemosensor for halogenated PAHs and related xenobiotics, which belongs to the basic helix–loop–helix Per-ARNT-Sim (bHLH/PAS) protein superfamily. In its non-activated form, the cytosolic AhR is bound to several chaperones like heat-shock protein 90 (hsp90), p23 and the AhR interacting protein (AIP) [1]. Upon ligand binding, the AhR translocates into the nucleus, dimerizes with ARNT (AhR nuclear translocator) and enforces gene transcription via binding to xenobiotic-responsive elements (XREs) within the promoter sequences of target genes. The AhR gene battery consists of genes encoding for phase I and II drug metabolizing enzymes, with cytochrome P450 (CYP) 1A1 as the most prominent one, as well as for proteins involved in regulation of cell growth and differentiation [1]. Another AhR target gene is the AhR repressor, a regulatory component of AhR signaling, which is capable of blocking XRE-mediated gene expression in a competitive way.

In this review article we summarize the current knowledge about the molecular interaction of the activated AhR molecule with several cytokine- and growth factor-driven signaling pathways. The main focus is on the interference of the AhR with TGF-β, TNF-α, the family of epidermal growth factors (EGF) and their respective receptors, since these molecules are closely associated with our recent research interests. Early milestones and scientific strategies as well as today's open questions regarding this complex field of AhR biology are discussed.

2. Interference of AhR-dependent pathways with the EGF receptor (EGFR) and its ligands

EGF and the group of related (EGF-like) growth factors are extracellular protein ligands for specific cell-surface receptors, called the ErbB family, which convert extracellular signals into biological responses [10,11]. The common structural motifs of the ErbB proteins are an extracellular ligand-binding domain, a hydrophobic transmembrane domain and a cytoplasmic domain harboring tyrosine kinase activity [11]. The probably most prominent ErbB receptor is ErbB-1, also known as epidermal growth factor receptor (EGFR). The EGFR is activated via binding of EGF or related growth factors, e.g. transforming growth factor-α (TGF-α), amphiregulin (AREG), epiregulin (EREG), heparin-binding EGF-like growth factor and others [12]. Ligand-binding leads to homo- or heterodimerization of the EGF-/ErbB receptors, resulting in the stimulation of intrinsic tyrosine kinase activities coupled with the autophosphorylation of several tyrosine residues within the cytoplasmic receptor domain [13]. These phosphorylated residues serve as docking sites for adaptor-like signaling molecules which directly activate major signal transduction pathways, thus translating a given signal into a biological response. Subsequently, EGF-induced signaling gets attenuated via

internalization of the growth factor molecules [14]. The EGFR and its downstream pathways are involved in the regulation of several normal cellular processes like cell proliferation, differentiation, and migration. Its expression rate is abnormally high in the majority of human epithelial cancers, where auto- and paracrine EGFR activation appears to be critical for tumor progression and pathogenesis [15].

Exogenous EGF administration causes severe biological effects, for instance induction of fatty liver [16], inhibition of palatal fusion [17] and promotion of skin tumorigenesis [18]. Interestingly, the toxic effects provoked by TCDD intoxication display striking similarities with regard to the EGF-caused adverse endpoints [19–22], indicating that TCDD and EGF affect the same cellular target structures. The first hints for a direct interference of TCDD with EGF-triggered signaling pathways came from a pioneering work done by Matsumura and co-workers, who compared the biological consequences of TCDD versus EGF administration in a neonate mouse model. The authors noticed similar changes in several pathological endpoints: TCDD and EGF exposed animals showed an earlier eyelid opening, a premature tooth eruption and a reduction of body and thymus weight [23]. At a cellular level, the authors observed enhanced protein kinase activities accompanied by a decreased binding capacity of radiolabeled EGF in hepatic plasma membranes of several rodent species [23]. Since TCDD neither changes the binding affinity of EGF to its receptor nor binds to the EGFR itself, the described decline in EGF-binding seems to be due to a reduced EGFR content on the cell membrane. This remarkable finding has indicated for the first time, that TCDD somehow is capable of counterfeiting EGF-mediated signaling, which might be one explanation for the pleiotropic effects caused by dioxin exposure. Several *in vivo* and *in vitro* follow-up studies revealed that TCDD exposure increases protein kinase activities, particularly those of protein kinase C and protein tyrosine kinases. In consensus with this data, the investigators observed an enhanced protein phosphorylation within the plasma membrane fraction [24–26]. Further studies revealed that these TCDD-induced changes were also detectable in pancreatic tissue and adipocytes and thus were not restricted to hepatocytes [27,28]. Each of these reports confirmed the TCDD-induced reduction of EGF-binding in the cell membrane. The given logical explanation for this phenomenon was that TCDD treatment led to an intracellular activation of the EGFR in an EGF-like fashion: via stimulation of protein kinases which subsequently phosphorylate specific tyrosine residues within the EGFR molecule [29,30]. In this regard, it is worth mentioning that TCDD exposure led to alterations in the cellular levels of TGF-α, a known EGFR-ligand. In human keratinocytes TCDD enhanced the expression of TGF-α in a post-transcriptional fashion which might be the reason for the down-regulation of the EGFR [31,32]. In contrast to these *in vitro* studies it was reported that TCDD decreased the expression of TGF-α during palatogenesis [33]. Alterations in EGF and TGF-α levels as well as changes in TGF-β signaling are main influencing factors during TCDD-induced teratogenesis [34,35]. However, a first indication for a direct involvement of the AhR during TCDD-evoked activation of the EGFR arose by a comparison of the influence of TCDD on the EGF-binding capacity in dioxin-responsive versus nonresponsive mouse strains [30]. The EGF-binding capacity in the hepatic plasma membrane of the nonresponsive animals was considerably less affected than in the samples of the dioxin-sensitive mice, implying an involvement of the AhR gene locus during TCDD-triggered modulation of the membranous EGFR molecules [30,36]. Analyses in nuclear-free and cell-free systems support the notion of a second AhR-dependent signaling pathway, distinct from the classical XRE-driven mode of target gene activation [37]. The identification of the non-receptor tyrosine kinase c-src ($pp60^{src}$) as associated component of the cytosolic AhR complex has closed the gap between EGFR stimulation and ligand-activated AhR pathway [38,39]. The soluble c-src kinase is able to activate the EGFR via phosphorylation of two specific tyrosine residues, resulting in receptor dimerization and initiation of downstream signaling [40,41]. As illustrated in Fig. 1, ligand binding of the cytosolic AhR leads to the dissociation of the multiprotein complex and the subsequent release of c-src in the cytoplasm [39,42]. The c-src kinase translocates to the cell membrane where it can interact with the EGFR in a bidirectional fashion: c-src can bind, phosphorylate and thereby activate the EGFR, and *vice versa* [43]. The relevance of c-src in TCDD toxicity was figured out by the usage of c-src-deficient mice. TCDD-intoxicated c-src knock-out animals displayed reduced triglyceride accumulation in the liver, glycogen depletion, down-modulation of phosphoenolpyruvate carboxykinase and reduced hepatotoxic effects as most pronounced differences in comparison to c-src proficient animals [44–46]. In addition, dioxin-induced transcriptional up-regulation of growth factors like TGF-α and platelet derived growth factor observed in C57BL/6 mice, was attenuated in c-src-deficient animals [47]. The important role of the c-src kinase in TCDD-induced cellular alterations was also shown in an epithelial breast cancer cell line (MCF10A), where TCDD was capable to antagonize insulin-driven cell growth and differentiation. By means of pharmacological inhibition the authors could show that these antagonizing properties of TCDD were mediated through a c-src-dependent activation of the extracellular signal-regulated kinases 1/2 (ERK1/2) [48]. Controversially, an earlier study has reported that the AhR ligands benzo(*a*)pyrene (B(*a*)P) and TCDD were able to mimic insulin-like growth factor signaling pathways in MCF10A cells, resulting in a stimulation of cell growth [49]. However, these and several other studies revealed that exposure to TCDD and related xenobiotics like B(*a*)P or hexachlorobenzene results in the activation of mitogen-activated signaling pathways, most of which are downstream targets of the EGFR [50–52]. Thus, this complex three-way connection between AhR, c-src and EGFR may represent one of the most important upstream signaling complexes regarding dioxin toxicity.

In addition to the role in response towards AhR-activating xenobiotics, a study done by Fritsche et al. [53] revealed that this interaction of AhR and EGFR is of physiological relevance during ultraviolet-B (UVB) exposure of skin. UVB radiation induces two major signaling routes in mammalian cells. The first one is the generation of DNA photoproducts within the nucleus; the second is characterized by the activation of cell-surface receptors. Fritsche et al. identified the amino acid tryptophan as the responsible chromophore for that pathway. UVB irradiation of human HaCaT keratinocytes leads to the intracellular formation of 6-formylindolo[3,2-*b*]carbazole

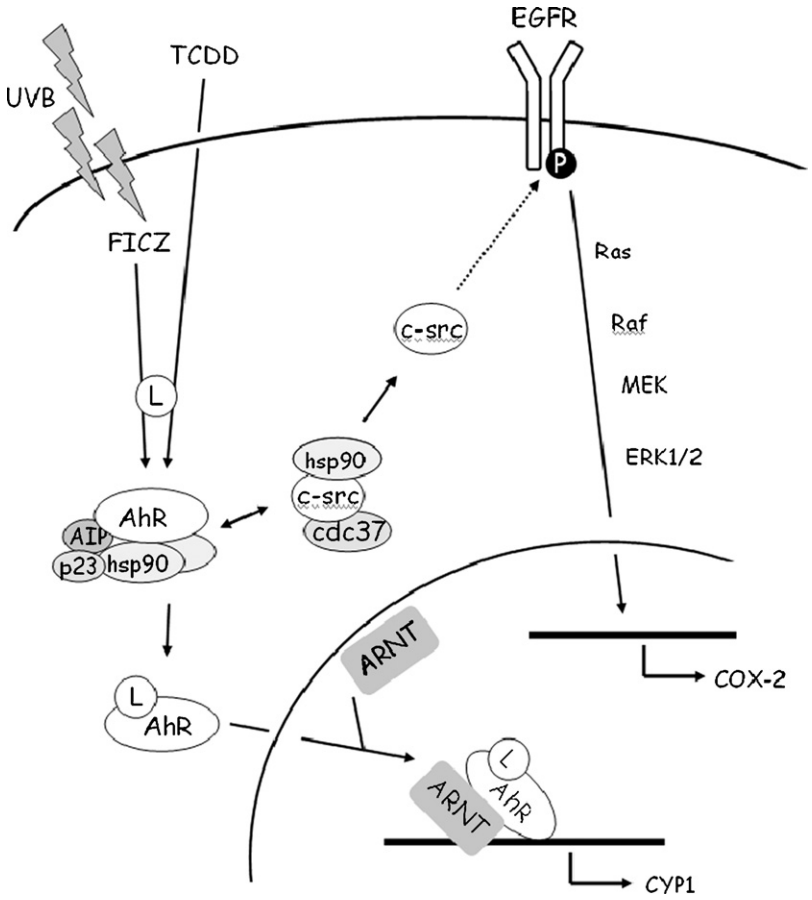

Fig. 1 – Cross-talk of AhR and EGFR signal transduction. Binding of a ligand, e.g. TCDD or the UVB-photoproduct FICZ, to the AhR leads to the dissociation of the cytosolic multiprotein complex. Subsequently, two cellular signaling events are initiated: the first one is the translocation of the AhR in the nucleus where it dimerizes with ARNT and binds to XREs in the enhancer region of target genes, like CYP1, to enforce their transcription; the second is the stimulation of c-src release from the cytosolic cdc37/hsp90/c-src complex upon which c-src translocates to the cell membrane and phosphorylates the EGFR. This results in the activation of MAPK-triggered signal transduction, resulting in an enhanced expression of target genes, like COX-2.

(FICZ), a condensation product of tryptophan molecules. FICZ is a high affinity ligand for the AhR which leads to a rapid nuclear translocation of the AhR upon its UVB-induced generation [53]. Beside the transcriptional induction of CYP1A1, AhR activation leads to a c-src-dependent stimulation of EGFR and its downstream target ERK1/2, which subsequently induces cyclooxygenase-2 (COX-2) expression [53]. These *in vitro* results were confirmed by studies on AhR-deficient animals, which displayed a lack of CYP1A1 and COX-2 mRNA induction upon UVB-stress. Since both, CYP1A1 and COX-2, are involved in skin carcinogenesis [53–55], the AhR seems to be an important regulatory player within the global UVB-stress response.

In a recent publication, it was reported that TCDD treatment of human THP-1 macrophages causes a transcriptional up-regulation of TNF-α [56]. The TCDD-stimulated increase in TNF-α mRNA was due to the activation of the EGFR and its downstream target ERK1/2. Interestingly, it was possible to block TCDD-induced TNF-α expression by co-treating the macrophages with α-naphtoflavone (α-NF), a classical AhR antagonist, but not by using the known c-src inhibitor PP2 [56]. Thus, there might be other pathways involved in connecting the ligand-dependent AhR activation to the EGFR signaling cascade. As shown in Fig. 2, one possible link might be cAMP-driven protein kinase A (PKA) signaling, which is known to regulate the transcription of the EGFR-ligand AREG [57]. Moreover, exposure of human oral epithelial cells to tobacco smoke extract led to a stimulation of cAMP/PKA signaling and activation of cAMP-responsive element binding proteins (CREB), resulting in an enhanced mRNA expression of AREG [58]. Astonishingly, pre-treatment with α-NF abrogated the tobacco smoke-induced AREG induction. In addition, a strong AREG up-regulation in response to exogenous FICZ confirmed the AhR-dependency of AREG expression [58]. Since PKA is known to initiate EGFR and its downstream signal transduction [59], this signaling step may represent a c-src-independent connection between AhR and EGFR pathways. In mice and murine cell lines, TCDD induced AREG and EREG gene expression in an AhR/XRE-dependent manner [60,61]. Perdew and co-workers [61] identified a functional XRE within the murine EREG promoter, which was not retrievable in the homologues sequence of the human gene. However,

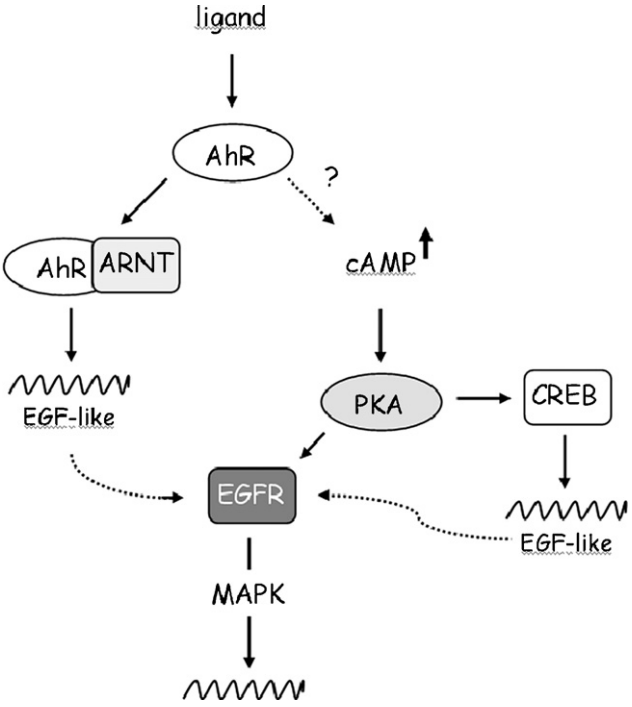

Fig. 2 – Putative c-src-independent signaling routes for an AhR-mediated activation of the EGFR. Ligand-dependent activation of the AhR can result in the initiation of two c-src-independent signaling steps both of which can potentially initiate the EGFR and its downstream signaling. First, the activation of the classical AhR/ARNT pathway results in an enhanced expression of genes encoding EGF-like proteins which can directly activate the EGFR. Second, the activation of the AhR leads to an increase in intracellular cAMP via a yet unknown mechanism. This stimulates PKA molecules which either directly phosphorylate the EGFR or activate cAMP-responsive element binding proteins (CREB), again resulting in a transcriptional induction of EGFR ligands.

based on these data, one could speculate that TCDD-induced up-regulation of EGFR-ligands like EREG and AREG may cause a delayed autocrine activation of the EGFR, which would also explain an AhR-mediated activation of EGFR in presence of pharmacological c-src inhibitors (Fig. 2).

To make the story quite more complex, a recent report identified the kinome chaperone cdc37 as a central player in AhR/c-src cross-talk [62]. As shown in Fig. 1, cdc37 builds a cytosolic protein complex with hsp90 and c-src, which gets activated by the TCDD-stimulated AhR/hsp90/AIP complex via a yet unknown mechanism. The core statement of this study is that c-src is rather part of a co-localized chaperone complex than an integral component of the AhR multiprotein complex. However, since cdc37 antisense treatment was able to suppress early TCDD-induced ERK1/2 activation [62], the investigation of the functional importance of this molecule during TCDD-evoked toxicity will be an exciting future task.

In conclusion, the published reports on the interference of the AhR with EGFR-dependent signaling cascades reveal an important role of this cross-talk during normal embryonic development as well as during exposure to environmental stressors like TCDD and UVB radiation, respectively. Since both AhR and EGFR are highly up-regulated in several types of tumors, e.g. breast and lung carcinomas [11,15,63,64], the elucidation of the molecular details of this interaction remains a promising issue for future research activities, especially with regard to the development of new preventive and therapeutic strategies against cancer diseases.

3. Cross-talk between TNF-α- and AhR-driven signal transduction

The multifunctional cytokine TNF-α was described for the first time by the physician William B. Coley nearly a century ago. Decades later two cytotoxic factors produced by lymphocytes and macrophages were characterized on molecular level: lymphotoxin and TNF-α [65]. This comparison revealed striking similarities of both signaling molecules regarding structure and biological function. Today the search term ''tumor necrosis factor'' results in nearly 90,000 hits in PubMed, pointing to the enormous interest in this molecule. TNF-α is a proinflammatory cytokine that is produced mainly by macrophages and also by neuronal, endothelial and mast cells, lymphocytes and fibroblasts [66]. This cytokine is released in response to several stress signals like bacterial toxins, parasite invasion and chemical exposure [66]. The released form is a homotrimer (sTNF) that is cleaved from a homotrimeric type II transmembrane protein (mTNF) by the metalloprotease TNF-α converting enzyme [67,68]. TNF-α signaling is mediated by two transmembrane receptors: TNF receptor 1 and 2 (TNF-R1/2), respectively. TNF-R1 is constitutively expressed in most cells, whereas TNF-R2 is primarily found on the surface of immune cells. Both forms of TNF-α, the soluble and the membrane-bound, can bind to these receptors, resulting in caspase-mediated apoptosis, activation of JNK or p38/MAPK cascades as well as stimulation of NF-κB-triggered signaling [69]. TNF-α is involved in the apoptosis of tumor cells, regulation of the inflammatory response and other functions of the immune system [65,69,70]. In addition TNF-α is considered to be a key player in the development of septic shock [65]. Prolonged exposure to low concentrations of TNF-α can result in cachexia, often found in tumor patients [69]. Interestingly, intoxication of guinea pigs with TCDD caused the same adverse effect [71,72], leading to the assumption that TNF-α plays an important role in acute dioxin toxicity. Moreover, this finding points to an interference of TNF-α-mediated and AhR-dependent signaling events. Exposure of mice to different amounts of TCDD led to hypersensitivity towards endotoxin, an effect which was due to a dose-dependent increase of TNF-α serum levels [73]. Treatment with TNF-α antibodies was able to diminish these symptoms associated with acute TCDD toxicity [74]. This effect is probably mediated by macrophages. It was shown that peritoneal macrophages from mice stimulated with lipopolysaccharide had an increased TNF-α production upon treatment with TCDD [75]. A contribution of the AhR can be postulated, however this is cell-type dependent as discussed in the following [76,77]. Recently, it was reported that TCDD-induced TNF-α production in differentiated THP-1 human

macrophages is mediated by the AhR [56]. As mentioned above, treatment with the AhR antagonist α-NF was able to suppress TCDD-induced TNF-α expression, which was also true for treatment with genestein, a general inhibitor for protein tyrosin kinases, and the specific EGFR blocker PD153035. However, the c-src inhibitor PP2 had no effect on TCDD-induced TNF-α expression [56]. Independently from that observation, an early EGFR phosphorylation (5 min) was noted in response toward TCDD treatment. The usage of the MEK-ERK inhibitor PD98059 also prevented TNF-α induction, whereas JNK- and p38/MAPK inhibitors showed no effects. These results suggest that TNF-α expression in differentiated THP-1 macrophages is AhR-dependent and involves the activation of EGFR and ERK. In contrast, treatment of primary human macrophages with AhR antagonists had no significant effect on the B(*a*)P-induced TNF-α expression [78]. As proposed by Cheon et al., the key element in this signaling cascade is most probably an antecedent sensitization of macrophages by a mitogenic signal. Promonocytic THP-1 cells were treated with the phorbol ester PMA to stimulate macrophage differentiation, an attendance which might present a pre-sensitization step. Co-treatment of RAW 264.7 macrophages with B(*a*)P and carbon black, MAPK-activating particles, resulted in an enhanced TNF-α expression, whereas B(*a*)P treatment alone had no effect on TNF-α expression rate [79].

Contrariwise, it has also been reported that the AhR signaling cascade is affected by TNF-α. Primary hepatocytes that were co-treated with TNF-α showed a significant repression of β-NF-induced CYP1A mRNA expression [80]. This observation was confirmed by gene activity assays performed with a CYP1A1 promoter-driven luciferase reporter plasmid [81]. Upon TNF-α exposure of transiently transfected Hepa1c1c7 cells, a significant dose-dependent reduction of the TCDD-mediated increase in luciferase activity was observed. A similar result was achieved by co-transfection of a RelA (p65)-encoding expression vector. NF-κB is a known inducible target of TNF-α signaling which also interacts with several other transcription factors [82]. Gel shift analyses revealed a physical interaction between AhR and RelA. In a follow-up study, Ke et al. [83] demonstrated that the TNF-α-mediated suppression of CYP1A1 promoter activity is retained by co-treatment with the chemical NF-κB inhibitor pyrrolidine dithiocarbamate or a NF-κB repressor (dominant negative IκBα) plasmid, respectively. Although these data point to an interaction between AhR and NF-κB during TNF-α-mediated repression of TCDD-induced CYP1A1 transcription, the authors could show that the binding pattern of the AhR/ARNT dimer to the XRE was not affected. Thus, it is questionable if the interaction between AhR and NF-κB is responsible for the inhibition of CYP1A1 expression. This leads to the anticipation that the effects of TNF-α on AhR target gene expression are located downstream of AhR/ARNT promoter binding. However, a TCDD-mediated suppression of TNF-α-induced NF-κB/RelA activation was observed in dendritic cells [84]. The authors speculated that this suppression resulted from an association between the AhR and RelA. In regard to the TNF-α-mediated repression of TCDD-induced CYP1A1 expression, the interaction of AhR and NF-κB with transcriptional co-regulators, like p300/CBP and SRC-1, was investigated. It is known that both co-regulator molecules are involved in the modulation of the AhR as well as the NF-κB signal transduction pathway [85,86]. In further activity assays it was proved that p300/CBP and SRC-1 are capable to reverse the RelA-mediated suppression of TCDD-induced CYP1A1 promoter activity. This finding suggests that the AhR and RelA compete for common co-factors (Fig. 3). The co-regulators p300/CBP and SRC-1 are known to possess intrinsic histone acetyltransferase activities. Ke et al. showed that TNF-α inhibited the acetylation of histone H4 at the TATA-box motive adjacent to the AhR/ARNT binding region within the CYP1A1 enhancer. A possible underlying mechanism is a NF-κB-mediated inhibition of histone H4 acetylation at the CYP1A1 promoter and thereby a block of gene expression [83]. Interestingly, it was reported that AhR agonist-induced expression of CYP1B1 in rat liver epithelial WB-F344 cells and hepatic stellate cells is further enhanced by co-treatment with TNF-α [87,88]. However, even if the underlying mechanism is still unknown, these results are contrary to the observations discussed above. Umannova et al. [88,89] reported that TNF-α is able to amplify the effects of AhR ligands on deregulation of cell proliferation. In addition, TNF-α may mediate both suppression and potentiation of the AhR response based on the nature of the target gene. TNF-α exposure led to an enhanced expression of CYP1B1, while it simultaneously suppressed the B(*a*)P-induced CYP1A1 expression [88,89]. This contrary regulation of CYP1 isoforms was accompanied with an increase in DNA adduct formation and an enhancement of B(*a*)P-induced apoptosis. These effects were diminished by co-treatment with fluoranthene, an inhibitor of CYP1B1. These results imply that TNF-α enhances the genotoxic potential of PAHs via up-regulation of CYP1B1 [88,89].

In summary, the cross-talk between TNF-α and AhR-dependent signaling cascades remains a complex, cell-type specific regulated process. The underlying molecular details of this interaction are quite unclear, even if some aspects are uncovered. The activated AhR can modulate TNF-α expression in an EGFR-dependent manner. This, in turn, leads to an enforcement of intracellular proinflammatory signals yielding in a proliferative response, a signaling event which might benefit the tumor promoting properties of TCDD and other dioxins. Since both, TNF-α and EGFR, are involved in the pathogenesis of UV-induced skin inflammation and tumorigenesis [90,91], it is tempting to speculate that the UVB-activated AhR plays a connective role in this context. In addition, the synthesized TNF-α is capable to modulate AhR-regulated genes and thus enhances or represses drug metabolism. This might alter the half-life of drugs and thereby the efficiency of therapeutic strategies. Moreover, the TNF-α-mediated changes in the P450 monooxygenase system might play a crucial role in the activation of genotoxic chemicals like B(*a*)P. Thus, this dynamic bidirectional interplay between the AhR pathway and TNF-α cytokines may represent an important aspect during TCDD-evoked inflammatory and carcinogenic effects, respectively.

4. Interaction of the TGF-β family with the AhR pathway

The mammalian transforming growth factor family represents a large group of multifunctional cytokines that control

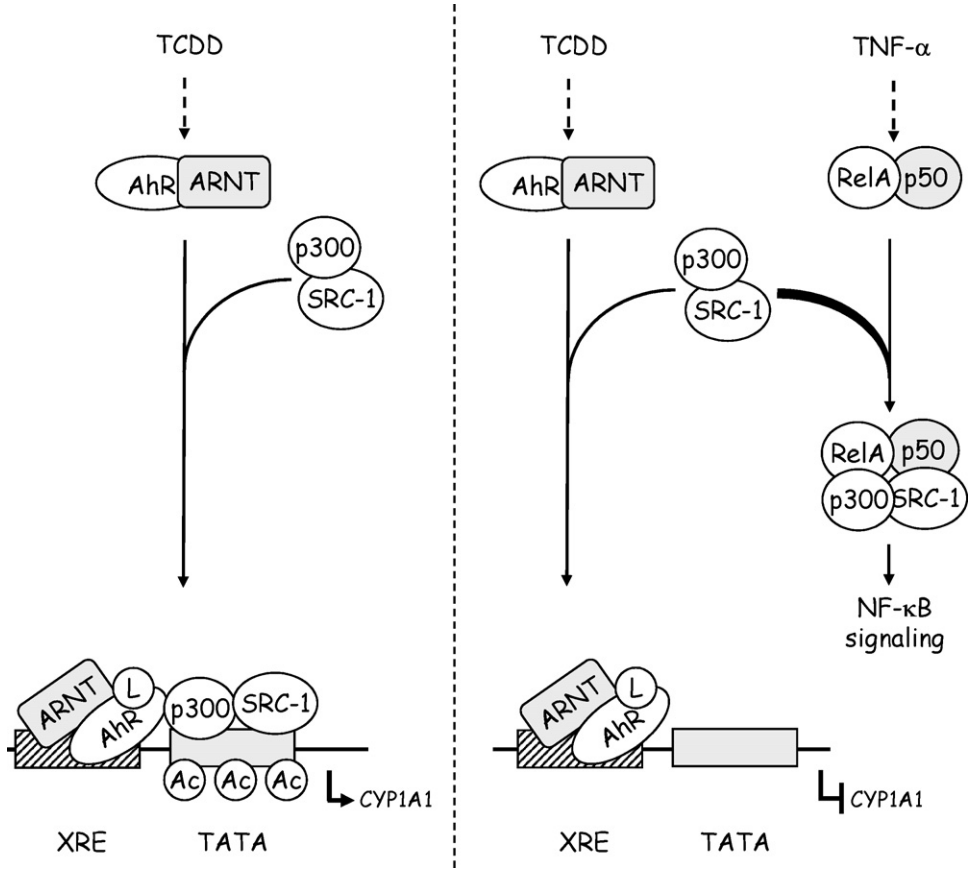

Fig. 3 – Potential mechanism of TNF-α-mediated suppression of TCDD-triggered CYP1A1 transcription. The TCDD-activated AhR/ARNT dimer binds to the XRE motifs of the CYP1A1 gene and subsequently recruits the co-activators p300/CBP and SRC-1 to the TATA-box. Due to the intrinsic histone acetyltransferase activities of these co-factors the local chromatin structure turns into a more accessible state, leading to an increased CYP1A1 transcription. TNF-α-mediated activation of NF-κB signal transduction leads to a competitive recruitment of p300/CBP and SRC-1 by the RelA subunit, which can lead to an impaired activation of AhR-dependent transcription. The described mechanism works of course in a bidirectional fashion. The final outcome depends on the predominance of one of both stimuli.

various cellular functions, like tissue homeostasis and repair, cell growth, apoptosis and immune response. It consists of three TGF-β isoforms, activin, and the bone morphogenetic proteins. The TGF-β isoforms (TGF-β_1, TGFβ_2, TGFβ_3) are important inhibitors of epithelial cell growth and are prominent players in cancer, cardiovascular diseases and immune response [92,93]. In this context TGF-β has recently attracted much attention, because it seems to resemble the AhR into a critical differentiation factor which triggers the differentiation of naive $CD4^+$ T cells into T_{reg} and T_H17 cells in a ligand-specific manner [94,95]. The persistent, long-acting AhR agonist TCDD induced functional T_{reg} cells that suppressed experimental autoimmune encephalitis [94]. In contrast, the short-acting endogenous agonist FICZ pushed the differentiation of naive T cells into T_H17 cells, resulting in an increased severity of autoimmune encephalitis *in vivo* [94,95]. However, in another study such a ligand-dependent effect on T-cell differentiation was not observed [96]. Nevertheless, these studies reported that AhR gene expression in T_H17 cells was highly up-regulated during cell differentiation, which was induced by co-treatment with TGF-β_1 and IL-6. This result suggests a co-operative activity of both cytokines on AhR gene regulation.

The first hint for a functional role of TGF-β as a putative downstream mediator of AhR signaling came from a teratogenicity study, showing that TCDD led to a reduction of TGF-β_1 expression in palate shelves and thereby blocks shelve fusion [20,97]. Treatment of human keratinocytes with TCDD resulted in a suppression of TGF-β_2 transcription [32]. Contrariwise, TCDD exposure of MCF-7 breast cancer cells led to an enhanced expression of TGF-β_3 [4], indicating a cell-specific effect of the activated AhR protein on the TGF-β system. On the other hand, it was reported that in A549 lung carcinoma cells TGF-β is able to down-modulate both, AhR-regulated phase I enzymes as well as mRNA expression of the AhR itself [98]. It was suggested that these effects are independent of each other, since the time course of down-regulation was similar for both genes. Thus, it seems that the activated AhR is able to modulate TGF-β expression and *vice versa*. Puga and co-workers [99] characterized the expression rates of several TGF-β genes as well as of the AhR in diethylnitrosamine-induced liver tumors from mice with a liver-specific ablation of the retinoblastoma protein (RB). In RB-positive liver tumor samples, Cdkn2d and TGF-β_1 were repressed, whereas the expression levels of Cdkn2c, TGF-β_2, TGF-β_3 and plasminogen

activator inhibitor (PAI)-1 were enhanced. In contrast to that, samples from RB-negative tumor cells only displayed an increased expression of Cdkn2c and TGF-β_3. The transcription of the AhR was significantly reduced in all tumors, supporting our hypothesis for a strong link between TGF-β signaling and the AhR pathway. A further evidence for an interaction of TGF-β and AhR signaling cascades was obtained by AhR gene targeting studies, showing that in livers of AhR-deficient animals the latent TGF-β binding protein-1 (LTBP-1) mRNA and protein was overexpressed in the fibrotic region [100]. The LTBP-1 protein has an important function in the activation of latent secreted TGF-β. The interaction of the AhR with the TGF-β/LTBP-1 system is reviewed intensely in another article of this special issue. Therefore, in the following we will focus on the impact of TGF-β on the AhR pathway. The diversity of TGF-β responses is elicited by two types of receptors (TβR-I and TβR-II), which transmit TGF-β signals via their serine/threonine kinases activities [92,93,101]. Briefly, TGF-β binding induces the clustering of both receptors. TβR-II acts as a co-activator of TβR-I resulting in activation of the receptor kinase. Subsequently, adaptor proteins like SARA (Smad anchor of receptor activation) mediate the recruitment of the intracellular downstream mediators Smad2 and Smad3 to the TβR-I, which subsequently phosphorylates Smad2/3 (Fig. 4). The activated Smads2/3 recruit Co-Smads, like Smad4. This assembly is essential for the initiation of TGF-β-induced transcriptional responses. The Smad complex enters the nucleus and recognizes target sequences (e.g. 5′-CACAG-3′) located within the regulatory region of target genes. The interaction with this sequence is common for the mammalian TGF-β family and is of relatively low affinity, which means that DNA-binding co-factors must be recruited to evoke the specific gene responses. Several of such co-activators were identified, including FAST-1 [102], which has no intrinsic transactivation activity, FRE3 and p300/CREB [103–105] which bear histone acetyltransferase activities. Known co-repressors of TGF-β signaling are TIGF, cSki, SnoN [103,106] and Evi-1 [107]. The cellular equipment with these co-factors seems to define the kinetic and mode of TGF-β action, especially on AhR expression. For example, studies on high TIGF expressing A549 cells revealed that TGF-β_1 is capable of blocking AhR transcription, whereas in human HepG2 hepatocarcinoma cells, which express similar levels of TGIF and p300, TGF-β_1 stimulated the synthesis of AhR mRNA [108]. The detailed analysis of the transcriptional activity of TGF-β_1 on the human AhR gene locus identified a functional Smad binding site within the 5′-flanking region. The functional importance of this Smad binding sequence was verified by site-directed mutagenesis, electrophoretic mobility assays and ectopic overexpression of Smad2/3 and Smad 4 [108]. Thus, these findings strongly support the recent observations that TGF-β_1 can enhance the AhR during naive T-cell differentiation.

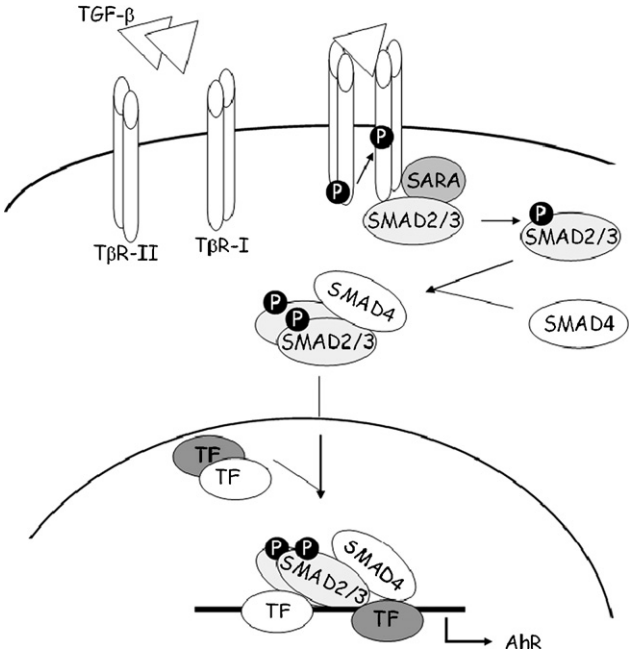

Fig. 4 – Transcriptional regulation of AhR expression via the classical TGF-β pathway. TGF-β binds to the TβR-II receptor and subsequently activates the TβR-I receptor which leads to the formation of a hetero-tetrameric complex. The activated TβR-I receptor recruits and phosphorylates receptor-specific Smads (here Smad2/3), a process which is mediated by the adaptor protein SARA. The phosphorylated Smad2/3 dimer recruits common Smad4 to build a protein complex which translocates into the nucleus, where it recruits further transcription factors (TF) and co-factors to modulate the expression of target genes, like the AhR.

Since the interplay between TGF-β signaling and the AhR pathway is only hardly understood, in the following part we will discuss some aspects of the TGF-β/AhR cross-talk regarding toxicologically relevant aspects. Interestingly, both signal transducers have common cellular target structures, e.g. the cell cycle control machinery. A number of studies have explored the role of the AhR in the cell cycle control, and details of this AhR action are reviewed by Elferink [109] as well as in other chapters of this AhR special issue. A key player in the control of cell cycle checkpoints is the tumor suppressor protein RB that gets functionally inactivated by CDK-mediated phosphorylation. Phosphorylated pRB binds to elongation 2 factor (E2F)-1 and E2F-3, which are the major forms of pRB complexes in actively cycling cells. Hyperphosphorylated RB was found to bind to the AhR molecule. This interaction resulted in a repression of E2F-dependent gene expression, which is essential to enter the DNA replication phase. Recently, it was reported that the AhR can directly interact with E2F-1 transcription factors in an RB-independent fashion, which are important regulators of proapoptotic genes like apoptotic protease-activating factor 1 (Apaf1) and p73 [110]. Apaf1 is a part of the Caspase 9/apoptosome complex, and the binding of AhR/E2F-1 to the Apaf1 promoter resulted in a repression of transcription of the respective gene. From this finding the authors proposed that the activated AhR can function in an oncogene-like fashion. Interestingly, the RB protein was identified as an important target molecule of TGF-β signaling, that is involved in TGF-β-mediated growth suppression [111]. This action of TGF-β is associated with a decreased G1 cyclin-dependent kinase activity and maintenance of RB in a lower phosphorylation status. TGF-β can down-regulate pRb-E2F-1 and pRb-E2F-3 complexes as a result of inhibition of E2F-1 and E2F-3, and the loss of functional RB is

known to induce apoptosis. Although many factors are involved in TGF-β-mediated deregulation of RB, it appears that E2F-1 molecule is a key regulator in this pathway and its activation initiates TGF-β-induced apoptosis [112,113]. Thus, E2F-1 is a common target molecule for the AhR and the TGF-β pathways, and a modulation of these pathways by endogenous or exogenous factors could decide about cellular fate: survival or death.

Besides its functional role in cell cycle control TGF-β is also involved in the regulation of degradation and remodeling of the extracellular matrix (ECM). The ECM is built up by the plasminogen and matrix metalloproteinase (MMP) enzyme system which regulates ECM composition, and thus, cell adhesion, cell migration and wound healing. Details of the fine-tuned interaction of the plasminogen–MMP system are reviewed in refs. [114,115]. Plasminogen is secreted as an inactive pro-enzyme which gets proteolytically cleaved into plasmin by urokinase plasminogen activators. The activated enzyme degrades fibrin and activates MMP and latent TGF-β, respectively. The activity of plasmin is inhibited by PAIs. MMP are a group of at least 23 extracellular proteases that are secreted into the ECM. The proteolytically activated enzymes degrade ECM components including collagen (MMP-1, -8, -13) and gelatine (MMP-2, -9). The action of MMP is regulated by the tissue inhibitors of metalloproteinase (TIMP). TGF-β possesses a key function within this complex system, since it stimulates the expression of PAI-1 and TIMP-1 and suppresses the transcription of plasminogen activators. Disturbances of this complex pathway by endogenous and exogenous factors, like TCDD and related compounds, might result in the destruction of tissue architecture, inhibition of cell growth, apoptosis, cell migration, cell–cell communication and in promotion of tumorigenesis.

TGF-β has a fundamental role in regulation of skin homeostasis. The majority of information about its function in ECM modulation was obtained from studies dealing with wound healing or consequences of UV radiation. UV light is a well-known exogenous stressor that causes destruction of the ECM and, thus, induces wrinkle formation and skin carcinogenesis. TGF-β is expressed in epidermal and dermal cells and UV seems to block TGF-β signaling via down-regulation of TβR-II, a decrease in TGF-β_1 level or via induction of Smad7, which exerts inhibitory effects on TGF-β signaling. The UV effects are accompanied by a reduction of collagen synthesis and an enhanced expression of MMP-1 and MMP-3 [116,117]. However, the involvement of the AhR in these UV responses on ECM still remains an open question. On the other hand, it has to be considered that UV radiation induces reactive oxygen species that, in turn, initiate cellular signaling pathways, e.g. the AP-1 system. As already mentioned above, UVB radiation induces the intracellular formation of the AhR ligand FICZ. Therefore, one could speculate that some of the described UV-evoked effects, like the transcriptional induction of PAI-1 [118], a known TCDD-sensitive gene, may be mediated by the AhR molecule.

The importance of TGF-β signaling was demonstrated by classical initiation–promotion protocols. Initiation was induced by exposure of skin to the AhR ligand 7,12-dimethylbenz[a]anthracene, whereas promotion was conducted by continuous treatment with the phorbol ester TPA. This treatment protocol resulted in a dramatic loss of TGF-β in the affected skin, especially in papillomas. The authors conclude that the loss of TGF-β is a highly risk factor for malignant tumor progression [119]. In a similar study, performed on Smad3-deficient animals, the mice turned out to be resistant towards chemical-induced skin tumors. Thus, Smad3 seems to be necessary for TPA-mediated tumor promotion [120]. Interestingly, likewise TGF-β_1 the phorbol ester TPA is also a cell-specific regulator of AhR transcription [121].

A clear evidence for the integration of the AhR in the regulation of ECM was obtained by studies in a zebra fish model. Intoxication of adult fishes or larvae with TCDD resulted in a block of regenerative growth of amputated caudal fins [122], indicating that activation of the AhR is a critical factor in tissue remodeling. Studies in mammalian cell lines showed that TCDD is able to elicit various effects on the ECM system. TCDD enhanced the expression of MMP-1, MMP-2, MMP-9, MMP-13 and TIMP-3, respectively [123–125]. However, the involvement of TGF-β was not investigated in these studies.

In summary, there are several hints for an involvement of both AhR and TGF-β in ECM pathogenicity as well as in the impairment of cell cycle control enforced by environmental stressors. Therefore, the elucidation of the molecular interplay between TGF-β signaling and the AhR pathway will provide a more detailed insight into the pleiotropic responses induced by TCDD and related environmental pollutants.

5. Concluding remarks

Cytokines and growth factor are implicated in many physiological and pathological processes. Disturbances of the balance of their orchestra of interaction could provoke disastrous consequences for cell and tissue homeostasis, respectively. As discussed, the AhR and its ligands are capable of interfering in manifold ways with cytokine and growth factor signaling cascades. The clarification of the underlying mechanisms will provide new opportunities to develop preventive and therapeutic strategies. For instance, the interference of the AhR with EGFR signaling has an important function in maintaining normal epithelial development, but an enhanced activation of this cross-talk by UVB-stress could lead to dangerous consequences for the skin. Therefore, the inhibition of this AhR/EGFR interaction by specific AhR inhibitors may be a helpful tool in the defense against UVB-induced skin damages. With regard to TGF-β, there are growing evidences for a close link between that cytokine and the AhR pathway. TGF-β controls the AhR transcription in a cell-specific manner. However, neither the physiological nor the toxicological importance of this action of TGF-β is understood precisely. Thus, the question arises whether TGF-β-modulated AhR expression is friend or foe? Besides their interesting interaction in regulation of T-cell development, the molecular mechanisms of their interplay with respect to cell cycle control and ECM remodeling are quite enigmatic, and thus an open field for future research. The elucidation of the molecular details of AhR and cytokine/growth factor cross-talk will contribute to a better understanding of the pleiotropic effects caused by TCDD and other environmental

chemicals. With this article we hope to further stimulate future research on this fascinating field of AhR biology.

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